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Toxicity of tin on nitrogen fixing Cyanobacteria

B. R. Bamniya✉ and P. Sharma

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Abstract

In the present study the effect of Tin 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 5 ppm to 55 ppm concentrations of Tin. Heterocyst frequency is calculated after growth period of 18 days. *Nostoc muscorum* have been found to tolerate a high concentration of the test metal and *Aulosira fertilissima* found to be most sensitive towards Tin. 20 and 30 ppm concentration of Tin found to be toxic for test organisms. Complete growth inhibition occurs at 50 ppm. Heterocyst frequency increases with the increasing concentrations of Tin.

Keywords: *Cyanobacteria*, *Toxicity*, *Heavy Metals*, *purifying alga*, *nitrogen fixing*

Introduction

Metals including heavy metals are naturally occurring essential components of global ecosystem. These are required by living organisms for various metabolic processes but higher concentrations are toxic to animals, plants including planktonic algae (Cheung *et al.* , 2002; Chang *et al.*, 1996; HazDat, 2003; Fargasova 1994; Martin and Holdich, 1986; Kick *et al.* ,1971). The biological importance of any heavy metal is simply the function of its solubility under physiological conditions. Similarly, its toxicity depends on affinity to sulfur and its interaction with macro-bio-elements. 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, 1987).They are endowed with property to cope up stressful conditions such as water polluted with heavy metals. Although a considerable amount of information is available on metal interaction effects on eukaryotic algae (Strarodub *et al.* 1987; Pabbi and Singha 2006; Sarada and Rengasamy 2006; Chu and Lin 1997) but comparable information on cyanobacteria is lacking. Tin occurs in trace amounts in natural waters. But now a days higher inorganic tin concentrations are getting entry into water by industrial discharges, tributyltin use, disposal of metal cans having tin coatings, smelting and refining processes, waste incineration *etc* (Byrd and Andreae, 1986; Senesi *et al*., 1999). Although many studies have been done regarding toxicity of Tin on various organisms but limited work has been done on cyanobacteria (Boogaard *et al*., 2003; White *et al.* 1999; Thomulka and Lange 1996; Han and Cooney, 1995). With such considerations taken into account, a study was proposed to observe the toxic effect of Tin on *Nostoc muscorum*, *Anabaena doliolum* and *Aulosira fertilissima* with reference to total growth, total carbohydrate, proteins, amino acid content and heterocyst frequency.

Author's Address
Department of Environmental Sciences,
Mohan Lal Sukhadia University, Udaipur, Rajasthan (India).
E-mail:brbamniya@yahoo.co.in ✉
preetiamar2k3@yahoo.com

Materials and Method

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 $SnCl_2$ was prepared and it was then diluted with sterile distilled water to get concentrations ranging between 5 to 55 ppm. Experiments were carried out in triplicate in 125 × 25 mm test tubes with a total volume of 15 ml (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 665 nm 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 and Sridhar , 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 indicated highly significant values (Tables. 1-5).

Results and Discussion

The results indicate that the behavior of all the four parameters show a similar trend towards Tin toxicity except heterocyst frequency. These show a considerable increase upto 5 ppm showing stimulatory effect of Tin as a nutrient. Growth responses of *Nostoc muscorum* in presence of Tin from 0 to 55 ppm indicate that metal ion at trace concentrations is not much toxic to organisms. A considerable increase in total growth upto 5 ppm observed shows stimulatory effect of Tin as a nutrient. But a gradual decrease observed and the values found to be more than control upto 20 ppm. Value lower than the control was observed after 25 ppm. Beyond this level growth inhibition of the organism was not so sharp and the trend continued upto 35 ppm of its concentration and inhibition was significant at 40 ppm which continued upto 55 ppm concentration of the metal (Table.1, Fig.1).

Table. 1 Effects of various concentrations of Sn^{2+} on total growth (ppm)

Concentration (ppm)	*Nostoc muscorum*	*Anabaena doliolum*	*Aulosira fertilissima*
Control	0.420	0.306	0.135
5	0.519	0.490	0.411
10	0.510	0.466	0.292
15	0.497	0.317	0.218
20	0.457	0.292	0.166
25	0.406	0.265	0.106
30	0.345	0.065	0.061
35	0.332	0.041	0.050
40	0.047	0.029	0.022
45	0.034	0.009	0.013
50	0.022	0.007	0.007
55	0.001	0.001	0.003
SEM^{+-}	0.0043	0.0031	0.0020
CD (5%)	0.0127	0.0091	0.0058
CD (1%)	0.0172	0.0124	0.0078
CV	2.52	2.85	2.78

ANOVA for Total growth (ppm) under the influence of Sn^{2+}

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	**SS**	**MSS**	**DF**	**SS**	**MSS**	**DF**	**SS**	**MSS**
Conc.	11	1.46	0.132584*	11	1.13	0.103047*	11	0.55	0.049696*
Error	24	0.00	0.0000567	24	0.00	0.0000295	24	0.00	0.0000118
Total	35			35			35		

(* Significant)

In terms of total carbohydrate content there was significant increase at 5 ppm level but the values were indicative of growth promotion upto 15 ppm. A value lower than control was observed at 20 ppm showing toxic effect of Tin. Beyond this level decrease occurred gradually upto 55 ppm (Table.2). Protein content on the contrary indicated very interesting trend and the protein values were found higher than control upto 25 ppm. Beyond these concentrations, it decreased upto highest concentration studied (Table.3).

Table 2. Effects of various concentrations of Sn^{2+} on Total carbohydrate (ppm)

Concentration (ppm)	*Nostoc muscorum*	*Anabaena doliolum*	*Aulosira fertilissima*
Control	16.62	15.22	12.53
5	19.36	19.44	26.17
10	18.51	18.21	22.91
15	17.84	17.37	21.64
20	11.07	13.07	20.71
25	9.88	14.88	17.66
30	5.07	8.77	6.79
35	3.52	1.36	1.91
40	2.72	0.98	0.06
45	2.79	0.07	0.01
50	0.78	0.01	0.00
55	0.06	0.00	0.00
SEM^{+-}	0.1622	0.1650	0.2102
CD (5%)	0.4735	0.4815	0.6136
CD (1%)	0.6417	0.6526	0.8315
CV	3.12	3.13	3.35

ANOVA for Total carbohydrate (ppm) under the influence of Sn^{2+}

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	**SS**	**MSS**	**DF**	**SS**	**MSS**	**DF**	**SS**	**MSS**
Conc.	11	1832.21	166.5644*	11	2155.80	195.982*	11	3629.91	329.9917*
Error	24	1.90	0.078962	24	1.96	0.081655	24	3.18	0.132573
Total	35			35			35		

(* Significant)

Table 3. Effects of various concentrations of Sn^{2+} on Total protein (ppm)

Concentration (ppm)	*Nostoc muscorum*	*Anabaena doliolum*	*Aulosira fertilissima*
Control	13.99	13.54	18.73
5	16.43	16.64	47.51
10	16.19	16.12	45.01
15	15.44	16.04	25.09
20	14.54	14.21	22.22
25	14.39	7.08	19.93
30	6.48	3.01	7.22
35	1.29	0.79	1.28
40	0.31	0.48	0.09
45	0.27	0.08	0.01
50	0.06	0.02	0.00
55	0.01	0.00	0.00
SEM^{+-}	0.1488	0.1432	0.3254
CD (5%)	0.4342	0.4179	0.9498
CD (1%)	0.5885	0.5663	1.2872
CV	3.11	3.38	3.62

ANOVA for Total protein (ppm) under the influence of Sn^{2+}

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	**SS**	**MSS**	**DF**	**SS**	**MSS**	**DF**	**SS**	**MSS**
Conc.	11	1817.19	165.1993*	11	1783.28	162.1165*	11	9882.29	898.3899*
Error	24	1.59	0.066403	24	1.48	0.0615	24	7.62	0.317699
Total	35			35			35		

(* Significant)

Total amino acid content of the organism however, were higher than control upto 20 ppm then declined on gradual basis with lowest values at 45 ppm (Table.4). In *Anabaena doliolum,* growth observed only upto 55 ppm in presence of Tin. Same trend was exhibited where chlorophyll absorbance increase in comparison to control (highest level at 5 ppm) where definitely the increase in absorbance was recorded upto 20 ppm then decreased gradually. In terms of total growth there was a increase in comparison to control only upto 15 ppm and then reduction continued slowly and significantly as reduction in its contents occurred beyond 35 ppm of Tin (Table-1,). However, total carbohydrate, amino acid and protein content increased in presence of Tin upto 15-20 ppm bearing highest values and beyond that it decreased sharply which continued upto the 55 ppm of its concentration (Table.2, 3 and 4). The growth responses of *Aulosira fertilissima* in

Table 4. Effects of various concentrations of Sn^{2+} on Total amino acids (ppm)

Concentration (mg/l)	*Nostoc muscorum*	*Anabaena doliolum*	*Aulosira fertilissima*
Control	0.38	0.47	1.44
5	0.91	0.63	1.69
10	0.68	0.53	1.59
15	0.59	0.41	1.41
20	0.48	0.49	1.02
25	0.32	0.05	0.42
30	0.09	0.02	0.08
35	0.07	0.01	0.04
40	0.05	0.01	0.02
45	0.01	0.00	0.01
50	0.00	0.00	0.00
55	0.00	0.00	0.00
SEM^{+-}	0.0054	0.0045	0.0132
CD (5%)	0.0158	0.0131	0.0387
CD (1%)	0.0214	0.0178	0.0524
CV	3.14	3.56	3.57

ANOVA for Total amino acids (ppm) under the influence of Sn^{2+}

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	**SS**	**MSS**	**DF**	**SS**	**MSS**	**DF**	**SS**	**MSS**
Conc.	11	3.19	0.290217*	11	2.22	0.201419*	11	17.13	1.557175*
Error	24	0.00	0.000088	24	0.00	0.0000605	24	0.01	0.000527
Total	35			35			35		

(* Significant)

presence of different concentrations of Tin showed somewhat similar results. Total growth (Table.1), total proteins (Table.3) and total amino acids (Table.4) content increased upto 20-25 ppm showing high values compared to control (highest values at 5 ppm). Beyond that a rapid decrease was observed upto the last concentration tested. A decrease in heterocyst frequency observed in all the three algae upto 20-25 ppm in comparison to control (Table.5) and then it increases regularly upto the highest concentration studied which indicates heterocyst differentiation due to the higher concentration of heavy metal in the medium but it appears that the growth responses and metabolism of *A. fertilissima* are greatly affected by presence of Tin into the medium. Reduction in growth and content of macromolecules of all three test algae at increasing concentrations of Tin confirms its toxic characteristics. The authors' findings dealing with tin toxicity are in very good agreement with those of Dubey and Rai (1987) and Rai and Dubey (1989). PawlikSkowronska *et al.* (1997) observed that tin (II) salts inhibited the growth of planktonic cyanobacterium *Synechocystis aquatilis*. Toxicity increased with increasing tin concentrations, time of exposure and pH value of medium in the range 7.0-9.8. At lowest tin (II) concentration of 1ppm, there was a 36-40% decrease in growth and chlorophyll *a* content after 96 hrs at pH 9.8. Wong *et al.* (1982) observed that tin concentration of >5 ppm was toxic to cyanobacterium *Anabaena flos-aquae*. Also concentrations 12ppm and >50 ppm were toxic to green alga *Ankistrodesmus falcatus* and *Scenedesmus quadricauda* respectively. Data on analysis of variance of various growth parameters studied indicated highly significant values at 5 % or 1 % (Tables 1to 5).

Table. 5: Effects of various concentrations of Sn^{2+} on Heterocyst frequency (%)

Concentration (ppm)	*Nostoc muscorum*	*Anabaena doliolum*	*Aulosira fertilissima*
Control	4.95	4.63	3.64
5	3.36	4.18	3.40
10	3.93	4.68	3.43
15	4.07	4.95	3.36
20	4.30	5.16	3.92
25	4.81	4.97	4.01
30	5.02	5.36	4.29
35	5.09	5.87	4.05
40	5.63	5.92	4.12
45	5.89	5.88	4.22
50	6.82	6.07	3.96
55	6.83	6.01	4.17
SEM^{+-}	0.0668	0.0777	0.0667
CD (5%)	0.1949	0.2269	0.1946
CD (1%)	0.2642	0.3075	0.2637
CV	2.29	2.54	2.98

ANOVA for Heterocyst frequency (%) under the influence of Sn^{2+}

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	**SS**	**MSS**	**DF**	**SS**	**MSS**	**DF**	**SS**	**MSS**
Conc.	11	39.46	3.587287*	11	13.90	1.263631*	11	4.05	0.367784*
Error	24	0.32	0.013381	24	0.44	0.018134	24	0.32	0.013335
Total	35			35			35		

(* Significant)

Toxicity may be manifested either by disruption of the integrity of cell membranes or by inhibition of photosynthetic pigments and key enzymes of nitrogen metabolism, *viz.* nitrogenase, nitrate reductase and glutamine synthetase of cyanobacteria. Micronutrients may influence the bioavailability and uptake of heavy metals to the micro biota as aquatic environment is comprised of several toxic and non-toxic metallic ions and their inorganic/organic complexes. The exact mechanism has not yet been clearly explored as the toxicity of heavy metals is governed by several factors acting together at one time.

In the present investigation 5 ppm has been found to be the tolerable limit for test organisms. Thereafter all the four parameters showed a declining pattern but a gradual increase can be seen in heterocyst frequency at increasing Tin concentration. Also 20-30 ppm found to be toxic where a reduced growth in terms of total carbohydrate and protein was seen in comparison to control. Complete growth inhibition was seen at 50, 45 and 40 ppm in *N. muscorum*, *A. doliolum* and *A. fertilissima* respectively. So these studies demonstrated that *N. muscorum* can tolerate a high concentration of Tin as compared to other two algae and can be used as a purifying alga in tin polluted water bodies.

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