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Research Paper

Effect of mercury on growth and pigment content of Cyanobacteria: *Westiellopsis prolifica* Janet

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***Abstract**: Today chemicals are widely used and may enter an aquatic ecosystem which causes an adverse effect on non-target organisms. These are particularly of concern due to increase in annual use of these chemicals worldwide. The present study was carried out to find out the effect of different concentration (2, 4, 6 and 8 ppm) of mercury (Hg) on the growth and photosynthetic pigments of a cynaobacterial species, Westiellopsis prolifica Janet. Experimental observations revealed a gradual growth reduction and the retardation of chlorophyll-a, b and c content with increase in concentrations of Hg+2 with the progressing time period progressed. Also, a fall in carotenoids and phycobillins content of W. prolifica was recorded at the end of incubation time. The results concluded that the Cyanobacterial species have been considered as candidates for use in bioremediation processes due to their potential of Bioaccumulation and Biosorption of heavy metals. But, proper studies are necessary for their practical use.*

Keywords: Cyanobacteria, Heavy metal, Mercury, *Westiellopsis prolifica*

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Introduction

The continuous discharge of heavy metals like, Hg, Cd, Cr, Cu, Ni, Sn, Pb, etc. into the aquatic environment, through industrial, domestic and agricultural activities has significantly contaminated our aquatic resources. Although many of the heavy metals are a trace requirement for most of the living organisms, a large dosage of them could adversely affect the enzymes and cellular membranes, disrupt biochemical pathways, etc and thereby render the organisms prone to death. Circulation of these harmful heavy metals within the food chain by transfer to higher trophic levels has begun to reveal a lot many inevitable consequences. These up comings have alerting us about the alarming rate at which the environment is being polluted.

Mercury is one of the most toxic pollutants in the environment. It is released into the environment from a variety of sources, both natural and anthropogenic [1]. It is impossible to separate out current levels of mercury in the environment as either anthropogenic or natural, but several experts have estimated that humans have doubled or tripled the amount of mercury released into the environment [2].

The quick paced mercury pollution has resulted in the need for quick, effective, economic and ecofriendly methods for their clean up. This can be accomplished by chemical, physical or biological methods. However, chemical and physical processes require a large amount of energy input and chemicals and thus are not cost effective [3]. Besides, it produce a considerable quantity of reactive chemical species as secondary wastes which would only contribute to further pollution. This leaves us with only one option- the biological method. Microbial biomass has been used for removal of heavy metals.

The use of microbial biomass is cost effective for industrial wastewater treatment[4]. Toxicological investigations was carried out by Singh and Singh, of the impact of inorganic mercury (Hg^{2+}) and methylmercury

(CH_3Hg^+) in terms of growth, NH_4^+ uptake and in vivo glutamine synthetase (transferase) activity in the diazotrophic cyanobacterium *Nostoc calcicola* Breb. Photoautotrophic growth of the cyanobacterium was extremely sensitive to mercury compounds, CH_3Hg^+ being 2.5 times more toxic than Hg^{2+} [5].

Effect of the carbamate insecticide, Sevin (50% w/v) was examined on survival, growth and nitrogen fixation of filamentous blue-green alga, *Westiellopsis prolifica* by Adhikary et al. Lower concentration of the insecticide (10µg/ml) increased survival, growth and nitrogen fixation while higher concentrations showed an inhibitory effect [6].

But, before applying microorganisms (or any other biological agent) as a means of bioremediation, the effect of the mercury on these agents, their response to presence of high doses of mercury, and at last, their capability to remediate contaminated sites have to be assessed, and if required, modified (by biotechnological tools), in order to obtain maximum remediation.

For this reason, in the present study, the effect of mercury, one of the many heavy metals posing a threat, is being evaluated on a cyanobacterium species namely, *Westeillopsis prolifica*, so that in future, based on further studies and standardisation, it can be used as a successful tool for bioremediation of heavy metal- polluted aquatic environments.

In order to determine the changes occuring in the cyanobacterial cell metabolites in response to mercury, following were the objectives which were framed in mind:
To determine effect of various doses of mercury on the pigment concentration. The pigments that were examined during the study are Chlorophyll- a, b and c, Phycobilins (Phycoerythrin, Phycocyanin and Allophycocyanin) and Carotenoids.

Materials and Methods

Sources of chemicals

The stock solutions and algal-medium were prepared using AR grade chemicals. All the solutions were prepared in sterilized double-distilled water.

Test organisms

Westiellopsis prolifica Janet, a nitrogen fixing, oxygenic,photoautrophic and heterocystous blue-green alga. It belongs to Hapalosiphonaceae family of Order Stigonematales.

Incubation and maintenance of culture for Cyanobacteria

Cyanobacterial cultures were obtained from National Facility for Blue-Green Algae, IARI, New Delhi, India.

Cultures were grown and maintained in BG11media [17.65 mM $NaNO_3$, 0.18 mM $K_2HPO_4.3H_2O$, 0.30 mM $MgSO_4.7H_2O$, 0.25 mM $CaCl_2.2H_2O$, 0.03 mM citric acid , 0.03 mM ferric ammonium citrate, 0.19 mM Na_2CO_3 , 0.003 mM EDTA (Na_2 salt), 0.05 mM H_3BO_3 , 9.15 mM $MnCL_2.4H_2O$, 0.77 mM $ZnSO_4.7H_2O$, 1.61 mM $Na_2MoO_4.2H_2O$, 0.37 mM $CuSO_4.5H_2O$, 0.17 mM $Co(NO_3)_2.6H_2O$] (Oberholster et al., 2005) at 25 ± 1°C under fluorescent illumination of 30 $\mu em^{-2}s^{-1}$ for 14:10 hours light: dark period. The medium was sterilized by autoclaving at 15 lbs for 20 minutes. The phosphate source ($K_2HPO_4.3H_2O$) was separately autoclaved and added to the medium immediately after autoclaving under aseptic conditions.

Heavy metal stock solutions

Mercury was used as the heavy metal in the form of mercuric chloride (Hg^{+2}). In order to determine the LC_{50} (in terms of chlorophyll content) of Hg^{+2} on *Westiellopsis prolifica*, the species was subjected to different concentrations of the heavy metal.

Stock solution of Hg^{+2} of concentration 1mg/ml was prepared. From the stock, BG11media of different concentrations were prepared viz. 1 ppm to 10 ppm, from which 20 ml was taken into previously autoclaved sugar tubes and 2 ml well mixed, homogenized algal culture was inoculated and incubated for five days.

Analytical methods:

a. Determination of survival and growth

Growth of culture was determined by directly taking absorbance at 650 nm in a spectrocolorimter (Bausch and Lomb spectronic 20 spectrocolorimter0 [7].

b.Determination of photosynthetic pigments

Westiellopsis prolifica J., was grown in different concentrations of heavy metal solution (mercury). Medium without mercury served as control. At the end of growth period estimation of pigments like chlorophyll a, chlorophyll-b, chlorophyll-c, carotenoids and phycobillins were estimated.

The following pigments were estimated to determine the effect of Hg^{+2}.

Chlorophyll-a, b, c and carotenoids

Estimation of chlorophyll- a, b, c was done using coefficients given by Jeffrey and Humphrey (1975) and carotenoids by Strickland et al. (1972).

Phycobilins

Phycobilin pigment contents viz. Phycocyanin (PC), Phycoerythrin (PE), and Allophycocyanin (APC) were estimated according to the method of Bennet and Bogorad (1973).

Results

Lethal concentration (LC50 (in terms of chlorophyll content))

The LC_{50} in terms of chlorophyll content of *W. prolifica* to Hg^{+2} was found out in order to decide the heavy metal treatments for estimating the response.

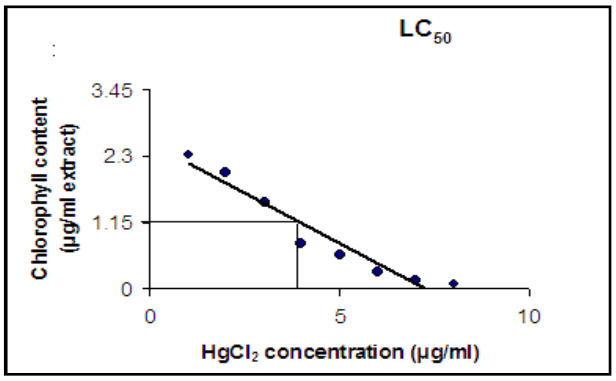

Figure 1: LC_{50} (in terms of chlorophyll content)

Figure 1 shows, that the LC_{50} (in terms of chlorophyll content) for Hg^{+2} was determined as 4 ppm. Thus, the concentration of heavy metal for different doses was decided as 2, 4 and 6 ppm for all the experiments

Growth

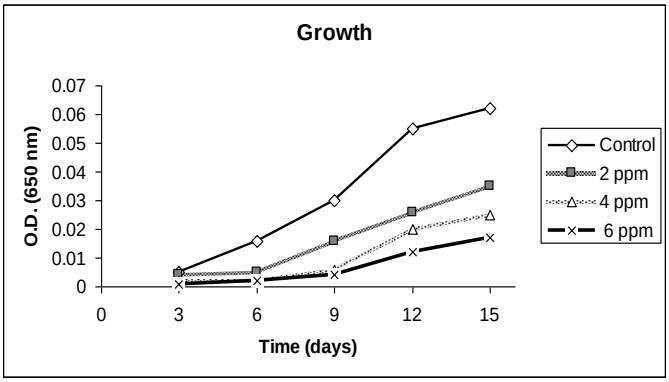

Figure 2: Growth pattern of *W. prolifica* as observed during an incubation period of 15 days with Hg^{+2}

Figure 2 shows that, the growth increases with an increase in time, despite the presence of the heavy metal. Although, the growth in treated *W. prolifica* is less when compared to the control. Also, it is evident that at higher concentrations of Hg^{+2}, the growth is less. The growth of *W. prolifica* varied from 0.005 to 0.062 (O.D. at 650 nm) in the medium. There was a gradual growth reduction of 44%, 60 % and 73% in 2ppm, 4ppm and 6ppm respectively, by the end of 15th day. The highest growth (0.035) was observed in 2 ppm treatment by the end of 15th day.

The chlorophyll-a content of *W. prolifica* varied from 0.12 to 1.44 µg ml^{-20} (Figure 3(a)). The retardation of chlorophyll-a content with increase in concentrations of Hg^{+2} as the time period progressed was observed. The rise of chlorophyll-a content by 0.019 to 0.571, 0.021 to 0.444 and 0.001 to 0.19 µg ml^{-20} was noticed at 2, 4 and 6 ppm doses, respectively.

A gradual fall by 60, 69 and 87 % was registered by the end of 15 days at 2, 4 and 6 ppm, respectively.

Pigments

(a) Chlorophyll-a

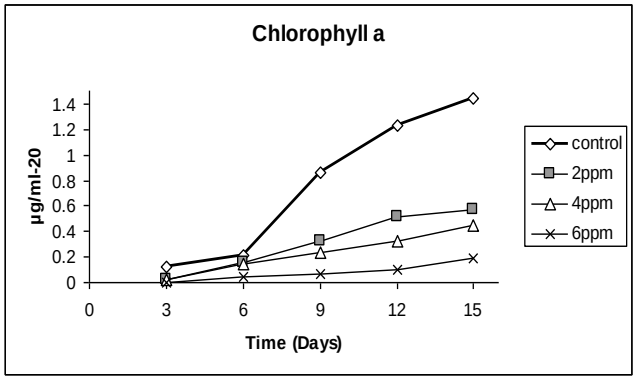

Figure 3(a): The reduction of Chlorophyll-a content, in response to addition of different concentrations of Hg^{+2}, during an incubation period of 15 days

(b) Chlorophyll-b

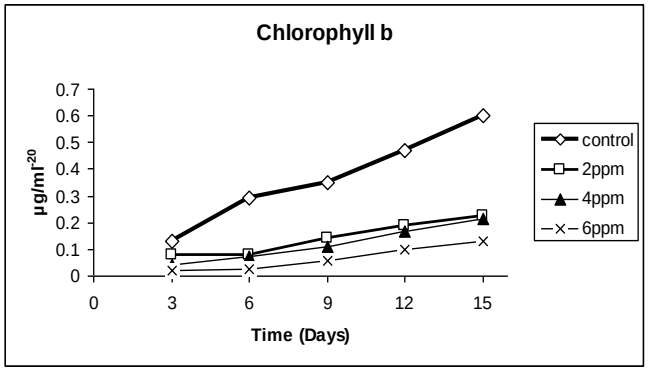

Figure 3(b): The reduction of Chlorophyll-b content, in response to addition of different concentrations of Hg^{+2}, during an incubation period of 15 days

The chlorophyll-b content of *W. prolifica* varied from 0.13 to 0.6 µg ml^{-20} (Fig. 3(b)). The retardation of chlorophyll-b content with increase in concentrations of Hg^{+2} as the time period progressed was observed. The rise of chlorophyll-b content by 0.076 to 0.223, 0.042 to 0.216 and 0.02 to 0.13 µg ml^{-20} was noticed at 2, 4 and 6 ppm doses, respectively. A gradual fall by 63, 64 and 78 % was registered by the end of 15 days at 2, 4 and 6 ppm , respectively.

(c) Chlorophyll-c

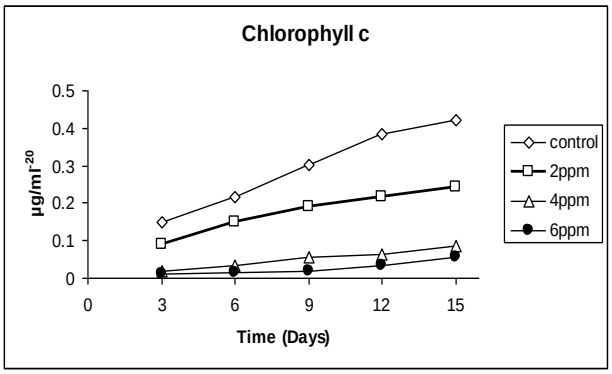

Figure 3(c): The reduction of Chlorophyll-c content, in response to addition of different concentrations of Hg^{+2}, during an incubation period of 15 days

The chlorophyll-c content of *W. prolifica* varied from 0.15 to 042 µg ml^{-20} (Fig. 3(c)). The retardation of chlorophyll-c content with increase in concentrations of Hg^{+2} as the time period progressed was observed. The rise of chlorophyll-c content by 0.09 to 0244, 0.02 to 0.084 and 0.01 to 0.057 µg ml^{-20} was noticed at 2, 4 and 6 ppm doses, respectively. A gradual fall by 42, 80 and 87 % was registered by the end of 15 days at 2, 4 and 6 ppm , respectively.

(d) Carotenoids

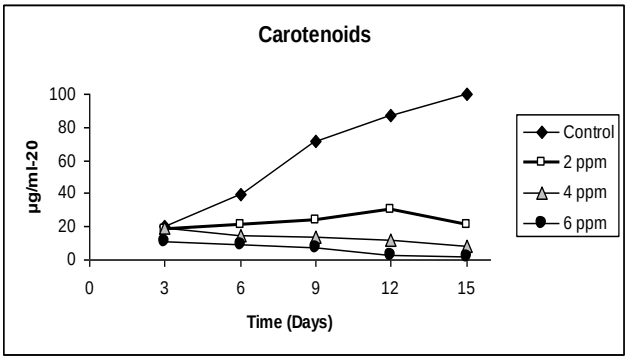

Figure 3 (d): The reduction of carotenoid content, in response to addition of different concentrations of Hg^{+2}, during an incubation period of 15 days

The effect on carotenoid content of *W. prolifica* to different concentrations of $Hg^{+2}$is shown in Fig.3(d). The carotenoid content ranged from 20µg ml^{-20} to 100 µg ml^{-20}. A fall of carotenoid content of *W. prolifica* by 79%, 92% and 98% was recorded by the end of 15th day at 2, 4 and 6 ppm, respectively. The highest amount of carotenoid content (21 µg $ml^{-20)}$ of the test organism was recorded at 2 ppm dose of Hg^{2+} by the end of 15th day.

(e) Phycobilins

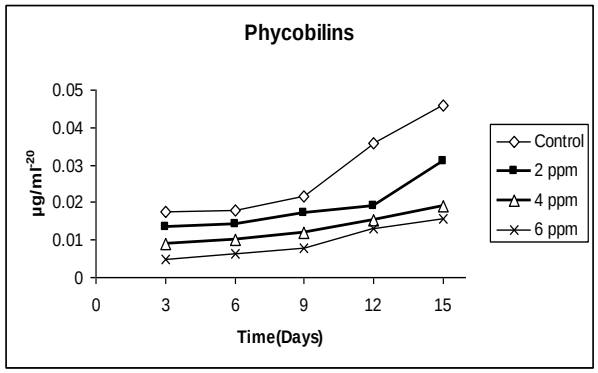

Figure 3(e): The reduction of phycobilin content, in response to addition of different concentrations of Hg^{+2}, during an incubation period of 15 days

The Phycobilin content of *W. prolifica* to different concentrations of is shown in Figure 3 (e). The Phycobilin content ranged from 0.018 µg ml^{-20} to 0.046 µg ml^{-20}. A fall of Phycobilin content of *W. prolifica* by 33%, 59% and 66% was recorded by the end of 15th day at 2, 4 and 6 ppm, respectively. The highest amount of Phycobilin content (0.031 µg $ml^{-20)}$ of the test organism was recorded at 2 ppm dose of Hg^{2+} by the end of 15th day.

4. Statistical analysis

Test of Significance (Student 't' test) of different variables of W. prolifica to different concentrations of Hg^{+2} was studied. The highest 't' value found was for Chlorophyll-a at 2 ppm (0.0249), and the lowest for chlorophyll-c at 6 ppm (0.000172).

A significant positive correlation (r = 0.883 to 0.986) was found between all the parameters i.e. Chlorophyll-a, chlorophyll-b, chlorophyll-c, Carotenoids and phycobillins treated with different concentrations (2, 4 and 6 ppm) of Hg^{+2}.

Table 1: Test of Significance (Student 't' test) of different variables of *Westeillopsis prolifica* to different concentrations of Hg^{+2}

Variables		Concentration (Hg^{+2})		
		2	4	6
chl a	t=	0.024959	0.025273	0.021775
p<		0.01	0.021	0.03
chl b	t=	0.006414	0.003677	0.003584
p<		0.052	0.097	0.099
chl c	t=	0.004701	0.001833	0.001726
p<		0.116	0.176	0.136
caro	t=	0.020769	0.019835	0.013061
p<		0.079	0.082	0.1
phyco	t=	0.019264	0.009382	0.003867
p<		0.102	0.086	0.04

p < = Level of significance, d.f. = 1

Table 2: Correlation matrix of different variables of *Westeillopsis prolifica* to different concentrations of Hg^{+2}

	1	2	3	4	5
1	1.000	0.943	0.986	0.987	0.919
2	1.000	1.000	0.977	0.965	0.929
3	1.000	1.000	1.000	0.993	0.918
4	1.000	1.000	1.000	1.000	0.883
5	1.000	1.000	1.000	1.000	1.000

Where: 1: Chlorophyll - a, 2: Chlorophyll-b,
3: Chlorophyll-c, 4: Carotenoids, 5: Phycobillins

Discussion

From the graph Figure 1, obtained for finding the LC_{50}, it was found out that the LC50 value of Hg^{+2} is 4 ppm for *Westeillopsis prolifica*, so the effect of various concentrations of mercury were done in a range of 2 to 6 ppm Hg^{+2}.

From the pattern observed in the Figure 2, it is evident that the growth of the W. prolifica, although increases in the presence of Hg^{+2}, it is less than that in control. Similar results were obtained by Mallick and Rai (1990) in their demonstration of effect of Copper as well as by Dubey and Rai (1990) in their studies involving Chromium and tin.

Chlorophyll a, b, and c content of *W. prolifica*, in response to 2, 4 and 6 ppm Hg^{+2} showed a decrease with the increasing concentration of Hg^{+2}(when compared to the control) as showed in Figure 3 (a), (b) and (c). This type of reduction in the chlorophyll content was also noticed by Surosz and Palinska (2005) while working with the different Heavy metals and their effect on Anabaena flos-aquae.

Carotenoid content of the treated *W. prolifica* with different concentrations of Hg^{+2} resulted in the decrease in the amount with both increase in the days as well as the dose of the Hg^{+2} as shown in fig 3(d), the reduction in the carotenoid content is also observed by Prasad et al. (2005) in their work based on the the effect of endosulfan (herbicide) on *Plectonema boryanum*.

Phycobilin content of the treated *W. prolifica* with different concentrations of Hg^{+2} resulted in the increase in the amount with both increase in the days as well as the dose of the Hg^{+2} as shown in figure 3(e), similar results were quoted in the work done by Padhy (1980) on Alloxan on *W. prolifica*.

The results obtained above, were analysed by using Students 't' test and Correlation coefficient (r). These statistical analyses were also performed by Rai et al. (1994) while working with Chlorella vulgaris sp. to see the effect of Cu and Ni on growth, mineral uptake, and photosynthesis and enzyme activities at different pH values.

Conclusion

The study aimed at evaluating the metabolic fate of Hg^{+2} on *Westiellopsis prolifica* have enlightened us about the important alterations taking place at cellular levels. Various parameters such as growth, Chlorophyll-a, b and c, carotenoids and phycobillins of the cyanobacterium were evaluated in response to treatments. Results revealed that with increasing concentrations of Hg^{+2}, the growth of *Westiellopsis prolifica* increased. On the other hand, other parameters like, Chlorophyll-a, b and c, phycobilins, carotenoids, showed a decline in their content with increasing Hg^{+2} concentration.

The statistical studies concluded that the correlation between carotenoid and chlorophyll-c reductase is highest whereas, carotenoids and phycobilins shows lowest correlation. The 't' value for chlorophyll-a (6 ppm) and chlorophyll-c (2 ppm) show the highest and the lowest values, respectively. Ultimately, it concludes with some important results about *Westiellopsis prolifica* that would in future help in determining the areas where it can be used efficiently.

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