INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

HEXAVALENT CHROMIUM TOXICITY TO CYANOBACTERIUM SPIRULINA PLATENSIS

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Article Received on: 30/09/14 Revised on: 11/11/14 Approved for publication: 22/11/14

DOI: 10.7897/2230-8407.0512184

ABSTRACT
The main industrial applications of heavy metals in leather and textile manufacturing, electro painting, corrosion inhibition, plating, glassware-cleaning solutions and the production of pigments has increased concentration of hexavalent chromium in the environment that lead to a serious problem to the ecosystem. Since, hexavalent chromium is found to be toxic in human beings. The present study has been designed to determine hexavalent chromium induced biomolecular changes in *Spirulina platensis*. *Spirulina platensis,* a filamentous *cyanobacterium* known as blue green algae of aquatic ecosystem has high economic importance due to its therapeutic potential. Blue green algae, *Spirulina platensis* was exposed to different concentration (0.01, 0.1, 1, 5, and 10 mg/l) of hexavalent chromium as potassium chromate and observed for different growth parameters for 9 days of incubation period. The concentration variation of carbohydrate, protein and chlorophyll- a pigment in *Spirulina platensis* was observed in the presence of chromium. The growth of *Spirulina platensis* was inhibited upon exposure of different concentrations of chromium. Maximum growth inhibition was observed in 10 mg/l of chromium treated cells as compared to control. The extent of toxicity increased with increasing concentration of chromium along with exposure time.
Keywords: *Cyanobacteria*, *Spirulina platensis*, Heavy metals, and Hexavalent chromium

INTRODUCTION

Environmental pollution due to heavy metals such as Pb, Cu, Fe, Zn, Hg, Co, Ni, and Cr, etc. is being widely spreaded because of anthropogenic activities. Although heavy metals are being discharged into rivers and sea from various industries in a limited quantity, yet these metals are taken up by the aquatic organisms since they have high solubility in the aquatic environment[1]. Aquatic organisms such as fish, present at top of the aquatic food chain are adversely affected. These metals transmit to human being through fish consumption since fish provides a good source of proteins, essential fatty acids, minerals and vitamins that are used to treat heart diseases and reduce blood cholesterol level[2]. Other than the fishes, Cyanobacteria which are one of the major micro flora of aquatic life, are also affected adversely. *Cyanobacteria*, also known as blue green algae are prokaryotic microorganisms that perform photosynthesis like higher plants by using photosynthetic pigment chlorophyll a to capture sunlight for energy. They are found almost at all biological niches including extreme condition. Therefore, they are exposed to various environmental stresses, whether tropospheric or aquatic. During evolution, these organisms have developed diverse strategies to maintain an equilibrated relation with heavy metal ions present and available in the surrounding medium[3]. Cells face two tasks, the first is to select those heavy metals essential for growth and exclude those that are not, and the second to keep essential ions at optimal intracellular concentrations[4]. The metal uptake process is considered as a two step process[5]. Firstly, they are adsorbed to the cell surface through interaction between different heavy metal ions and functional group such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thiol, etc. that are offered by the component of the cell wall like polysaccharides, proteins, lipids[6]. After that they get entry into the cell through penetration to the cell wall[7] and are localized into organelles. Heavy metals can be broadly divided into two groups. The first group consists of metals including As, Fe, Cr, Co, Cu, Ni, Se, Va and Zn, are essential as nutritional requirements at trace amount for many organisms but are toxic when present in greater amounts. The second group includes Pb, Hg, Cd, Ur, Ag and Be, all of them are highly poisonous even at very low concentration[8]. The eight most common pollutant heavy metals listed by the Environment Protection Agency (EPA) are: As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn[9]. Cr (VI) is considered as a micronutrient in trace amount but at higher concentration it become more toxic, carcinogenic and mutagenic. Hexavalent chromium is recognized by the International Agency for Research on Cancer and by the US Toxicology Program as a pulmonary carcinogen[10]. Among *Cyanobacteria* species, *Spirulina,* a filamentous *cyanobacterium* has gained considerable popularity in the human health food industry due to its content of a wide range of essential nutrients, such as provitamins, minerals, proteins and polyunsaturated fatty acids such as gamma-linolenic acid[11]. Due to high nutritional and economic value of *Spirulina platensis*, it has been receiving the interest of researcher to consider a good experimental model. In Indian context the discharge concentration of chromium should not exceed from 0.1 mg/l as per waste water discharge standard of Central Pollution Control Board[12]. Therefore, in the present study, the selected range of Cr (VI) concentration was 0.01 to 10 mg/l and *Spirulina platensis* was exposed to assess the toxic effect of this heavy metal by studying the biochemical changes such as carbohydrate, protein, and chlorophyll- a content.

MATERIAL AND METHODS

Culture and Maintenance of *Spirulina platensis*

Spirulina platensis was obtained from culture stock available in the department of Biotechnology, Madhav Institute of Technology and Science, Gwalior. *Spirulina platensis* was grown in the Zarrouk's medium (pH 9)[13] under aseptic

condition. The culture of *Spirulina platensis* was incubated and maintained at optimum temperature of 28 ± 2°C in a culture room illuminated with cool white fluorescent tubes providing a light intensity of 50 µmol/m^2sec^2 around the culture vessel following a 16:8-h light/dark regime. The culture was shaken three times a day manually.

Raising the test organism under heavy metal stress

To examine the effect of Chromium on exponentially growing *S. platensis*, cells were inoculated and immersed into chromate solutions (K_2CrO_4), each prepared in Zarrouk's medium to obtain the five final concentrations 0.01, 0.1, 1, 5, 10 mg/l. For the control experiment, cells of *S. platensis* were grown under identical culture conditions without metal solution. The culture was incubated and observed for 9 days. After incubation periods, different biochemical tests were performed on Cyanobacterial cells under control and stressed condition. All the experiments were conducted in triplicate.

Growth measurement

The progressive growth of *S. platensis* was observed alternatively over a period of 9 days under control and stressed conditions taking absorbance at 560 nm. For the estimation of growth of *S. platensis*, Chlorophyll-a, carbohydrate content and protein content were also measured alternatively under control and stressed condition. The specific growth rate, µ (h^{-1}) was computed by following formula.

$$\mu = \frac{ln(N2/N1)}{t}$$

Where, $t = T_2-T_1$; N_1 = Initial optical density/Protein concentration at time T_1, N_2 = final optical density/Protein concentration at time T_2

Chlorophyll-a estimation

The chlorophyll a content was estimated as per method described by MacKinney[14]. Sample of 3 ml algal suspension was centrifuged for 10 min at 5000 rpm. The resulting pellet was resuspended in equal volume (3 ml) of methanol (absolute) and homogenized. Samples were incubated in a water bath, at 70°C for 2 min. Then the samples were centrifuged (at same condition), and the clear supernatant was used for measurement. Optical density of the supernatant was measured at 665 nm against methanol as a blank.

Carbohydrate estimation

The carbohydrate content was estimated as per method described by Dubois *et al*[15]. 0.5 ml of an algal culture was taken into a thick walled test tube and 1.5 ml of distilled water was added to it. A reagent blank containing 2 ml distilled water and a set of glucose standards were prepared simultaneously. One ml of 5 % phenol was added to each tube. After through mixing, 5 ml of conc. of H_2SO_4 was added from a fast blowing auto pipette, directing the stream of acid to the agitated reaction mixture for fast mixing. These tubes were incubated at room temperature for 10 minutes for complete reaction, and here after, shaken and placed in a water bath at 30°C, for 20 min. The intensity of the characteristic straw color thus developed was determined by reading absorbance at 492 nm and the carbohydrate content was calculated from the glucose standard curve and expressed in µg/mL.

Estimation of protein content

The protein content was estimated as per method described by Lowry *et al.*[16] and Herbert *et al.*[17]. Exponentially growing harvested Cyanobacterial cells were homogenized in 0.1M potassium phosphate buffer (pH 7.8) and 1 mM EDTA (pH 7.8). The homogenate was centrifuged at 10, 000 rpm for 15 min at 4°C. 0.5 ml of the supernatant was taken in a test tube and 0.5 ml of 1N NaOH was added to it and then placed in boiling water bath for 5 minutes. After cooling in the cold water 4 ml of alkaline copper solution was added, allowed to react for 10 minutes followed by addition of 0.5 ml of 1N Folin reagent with thorough mixing. Blue color was developed after 30 min. The intensity of blue color was determined by reading absorbance at 650 nm and the amount of algal cell protein was calculated as µg/ml in culture with reference to a standard curve, obtained by using BSA. The same procedure is adopted in preparing standard curve using graded concentration of BSA.

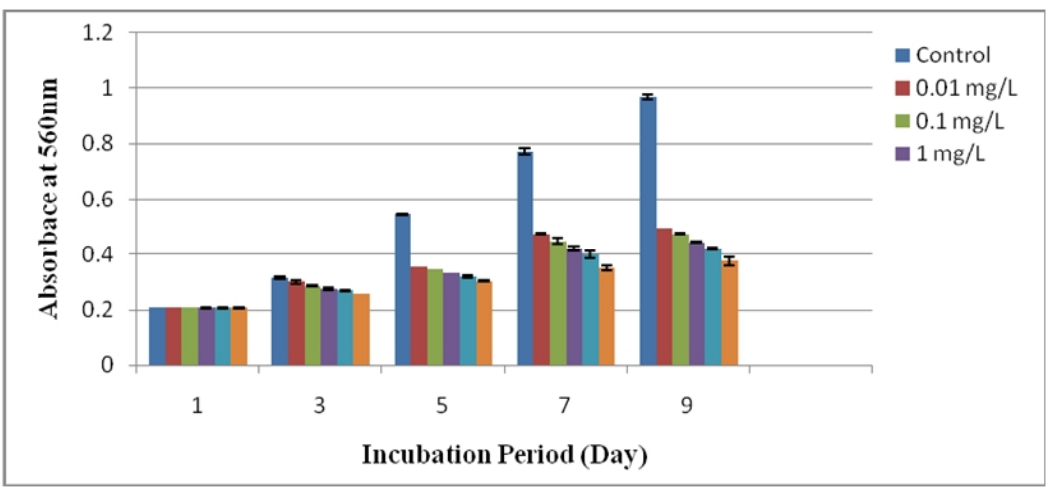

Figure 1: The effect of chromium on growth rate of *Spirulina platensis* upon exposure of different concentration of Chromium. Each value represents the mean of triplicate (± SEM)

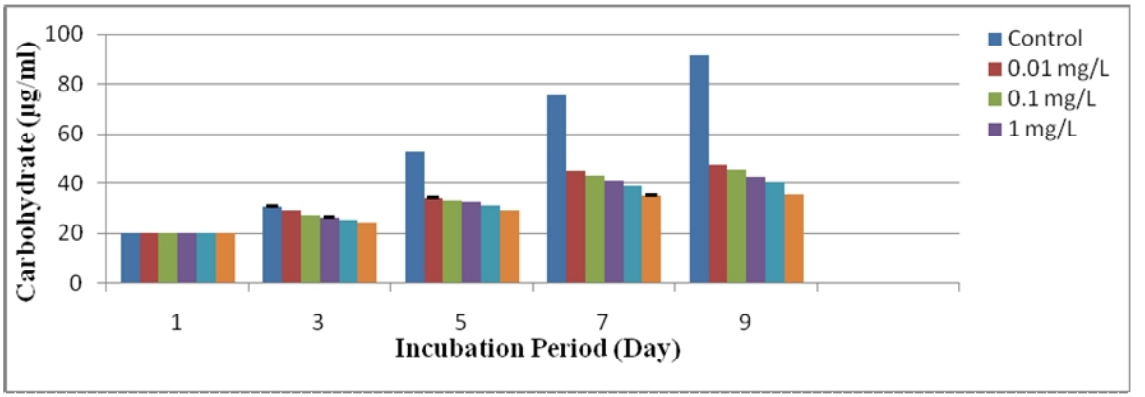

Figure 2: The effect of chromium on carbohydrate content of *Spirulina platensis* upon exposure of different concentration of Chromium. Each value represents the mean of triplicate (± SEM)

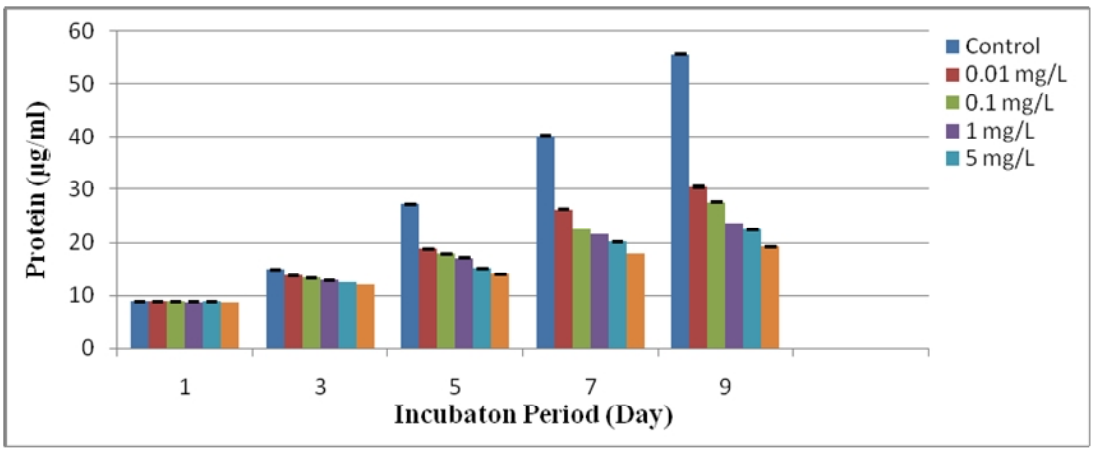

Figure 3: The effect of chromium on protein content of *Spirulina platensis* upon exposure of different concentration of Chromium. Each value represents the mean of triplicate (± SEM)

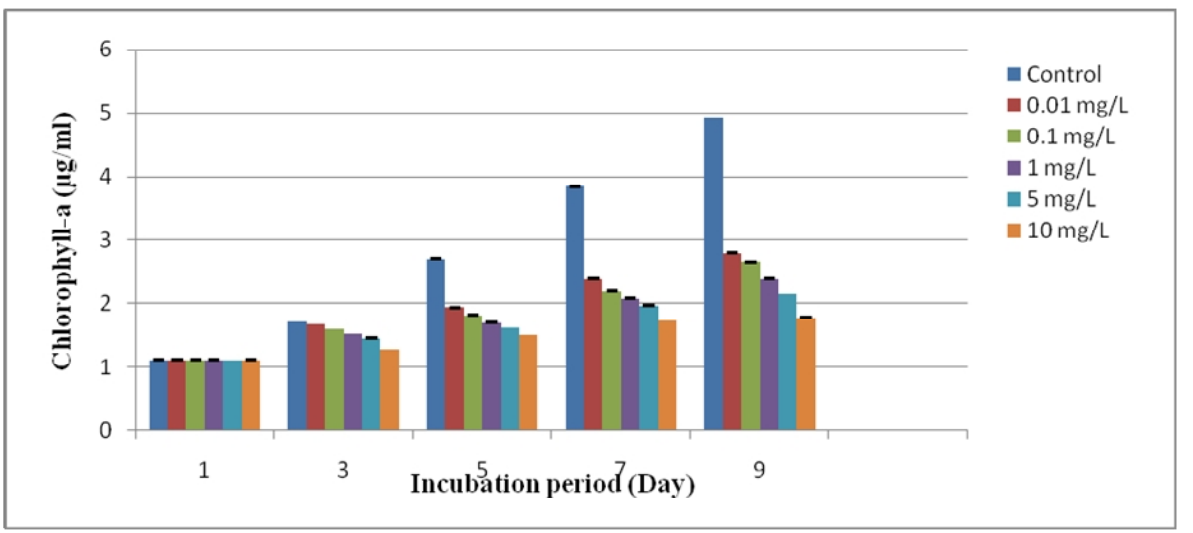

Figure 4: The effect of chromium on chlorophyll- a content of *Spirulina platensis* upon exposure of different concentration of Chromium. Each value represents the mean of triplicate (± SEM)

RESULT AND DISCUSSION

Growth is a good indicator to determine the effect of any toxic compound in susceptible microorganism since it reflects the metabolism of the cell. Therefore, carbohydrate, protein and chlorophyll-a content was observed in order to estimate the growth of *Spirulina platensis*. Toxic effect of Cr on *Spirulina platensis* was observed as concentration-duration dependent response curve. Figure 1 shows the growth behavior of control and treated *Spirulina platensis*. Exogenous addition of different concentration (0.01 to 10 mg/l) of Chromium showed toxicity to *Spirulina platensis*. The extent of toxicity increased with increasing concentration of Cr along with exposure time. A significantly maximum growth reduction by 62 % ($p = 0.05$) at higher concentration of Cr (10 mg/l) was observed as compared to the respective control at the end of incubation period.

At the 3rd day of incubation period, the decline in growth rate was less as compared to other incubation periods. It was followed by 5 %, 10 %, 13 %, 15 %, 18 % decline in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. At the 5th day of incubation period, the growth rate was decreased by 35 %, 37 %, 39 %, 42 %, 45 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. At the 7th day of incubation period, the growth rate was decreased by 39 %, 43 %, 46 %, 49 %, 55 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. At the 9th day of incubation period, a decline of 49.2 %, 51.2 %, 54.3 %, 57 %, 62 % in growth rate was observed in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. The findings were supported by Sultan *et al*.[18] who studied the biochemical changes in response to metal tolerance in *Anabaena doliolum* exposed to Cd and Cu. The growth inhibition may be due to the inhibition of photosynthesis, enzyme system, protein content, pigment degradation and nucleic acid synthesis. Environmental stress affects the functioning of PSII in *Spirulina* directly or indirectly causing growth inhibition[19]. Since *Spirulina* is a photosynthetic microorganism. As reported by Babu *et al*.[20] addition of heavy metals Cr and Ag, to intact cells of *Cyanobacterium Spirulina platensis* caused alteration in whole chain and PSII catalyzed electron transport activities. The reduction in growth could be due to the inhibition of normal cell divison by the metal as reported for *Chlorella vulgaris* exposed to Cu, Hg, and Cd [21]. The metal binds to the sulphydryl group present at cell surface is attributed to the reduction in the rate of cell division. Since sulphydryl groups are important for regulating the plant cell division[22,23].

Effect on carbohydrate content

The observed data demonstrates that the carbohydrate content was decreased with increasing concentration of Cr as well as incubation period. Figure 2 shows the carbohydrate content of control and treated *Spirulina platensis*. The carbohydrate content was increased up to 47.42, 45.67, 42.73, 40.42, 35.53 µg/ml in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control (91.47 µg/ml) at the end of incubation period. A significantly maximum decline was of 61.2 % ($p = 0.05$) at higher concentration of Cr (10 mg/l) as compared to the respective control.

At the 3rd day of incubation period, the carbohydrate content was less declined as compared to other incubation periods. It was followed by 5 %, 11 %, 14 %, 17 %, 20 % decline in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. At the 5th day of incubation period, the carbohydrate content was decreased by 35 %, 37 %, 38.5 %, 41 %, 45 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control. The carbohydrate content of *Spirulina* was decreased by 41 %, 43 %, 46 %, 48.2 %, 58.3 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control on 7th day. At the 9th day of incubation period, the carbohydrate content was decreased by 48.2 %, 50.1 %, 53.3 %, 56 %, 61.2 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control. The findings were supported by Sultan *et al*.[18] who studied the biochemical changes in response to metal tolerance in *Anabaena doliolum* exposed to Cd and Cu.

Effect on protein content

The observed data demonstrates that the protein content was decreased with increasing concentration of Cr as well as incubation period. Figure 3 shows the protein content of control and treated *Spirulina platensis*. The protein content was increased up to 30.45, 27.56, 23.61, 22.44, 19.22 µg/ml in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control (55.4 µg/ml) at the end of incubation period. A significantly maximum decline was of 65.3 % ($p = 0.05$) at higher concentration of Cr (10 mg/l) as compared to the respective control.

At the 3rd day of incubation period, the protein content was less influenced as compared to other incubation periods. It is followed by 7.1 %, 10.5 %, 13.4 %, 15.7 %, 18.7 % decline in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. At the 5th day of incubation period, the protein content was decreased by 30.8 %, 34.1 %, 37.1 %, 44.3 %, 48.6 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control. The protein content of *Spirulina* was decreased by 35 %, 44.2 %, 46.4 %, 50 %, 55.5 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control on 7th day. At the 9th day of incubation period, the protein content was decreased by 45.1 %, 50.3 %, 57.4 %, 59.5 %, 65.3 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control.

Effect on Chlorophyll-a content

The observed data demonstrates that the chlorophyll-a content was influenced in a dose and time dependent manner. Figure 4 shows the chlorophyll-a content of control and treated *Spirulina platensis*. The chlorophyll-a content was increased up to 2.8, 2.66, 2.34, 2.15, 1.78 µg/ml in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control (4.92 µg/ml) at the end of incubation period. A significantly maximum decline was of 63.9 % ($p = 0.05$) at higher concentration of Cr (10 mg/l) as compared to the respective control.

At the 3rd day of incubation period, the chlorophyll-a content was less influenced as compared to other incubation periods. It was followed by 2.2 %, 6.8 %, 11.4 %, 14.9 %, 25.7 % decline in 0.01, 0.1, 1, 5, 10 mg/l of Cr treated *Spirulina* respectively as compared to control. At the 5th day of incubation period, the chlorophyll-a content was decreased by 28.5 %, 32.8 %, 37.2 %, 40 %, 44.3 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control. The chlorophyll-a content of *Spirulina* was decreased by 38.1 %, 43 %, 46 %, 49 %, 54.9 % in 0.01, 0.1, 1, 5, 10 mg/l of Cr respectively as compared to control on 7th day. At the 9th day of incubation period, the chlorophyll-a content was decreased by 43.2 %, 46.1 %, 51.7 %, 56.4 %, 63.9 % in 0.01, 0.1, 1, 5,

10 mg/l of Cr respectively as compared to control. These findings were supported by previous study. *Cyanobacterium Spirulina sp*. was reported for decline in chlorophyll-a content treated with Cu, NaCl, and combination of both[24]. The decrease in chlorophyll-a pigment content which is necessary for photosynthesis, could be a reason of growth reduction. Sen *et al*.[25] suggest that 10 ppm Cr (VI) lowered the chlorophyll in *Pistia stratiotes* by decreasing the synthesis of chlorophyll as possible by decreasing chlorophyllase activity. The chlorophyll reduction is a marker for oxidative stress[26,27]. Impairment of the electron transport chain and replacement of Mg^{2+} ions associated with the tetrapyrole ring of chlorophyll molecules could be the primary reason of photosynthetic pigments destruction[28].

CONCLUSION

Environmental pollution of heavy metals is being widely spreaded due to anthropogenic activities. Heavy metals are transmitted to human along their food chain. It has brought a serious threat to the ecosystem. At lower concentration, heavy metals are necessary for proper metabolic function of an organism. But they become toxic at higher concentration and target the lipid which is a key biomolecules of the cell membrane and results in serious disorder to human. To treat the heavy metal pollution, Cyanobacterial cells are extremely used as they can accumulate a significant concentration of heavy metals. In the present study, the growth of *S. platensis* is adversely affected with increasing concentration of metals. The extent of toxicity was found to be increase with increasing concentration of metal. But the cyanobacterial cells could survive in the presence of metal. Therefore, *Spirulina platensis* can be used for the treatment of metal pollution present at lower concentration.

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Cite this article as:
Gupta Shilpi, Sharma Sunita, Singh Sweta. Hexavalent chromium toxicity to Cyanobacterium spirulina platensis. Int. Res. J. Pharm. 2014; 5(12):910-914
http://dx.doi.org/10.7897/2230-8407.0512184

Source of support: Nil, Conflict of interest: None Declared