



ISSN: 0975-833X

RESEARCH ARTICLE

BIOCHEMICAL RESPONSES OF CHROMIUM TO SOME FRESHWATER ALGAL SPECIES: A LABORATORY STUDY

Pramod Mane*, Deepali Kadam and Arjun Bhosle

School of Earth Sciences, Swami Ramanand Teerth Marathawada University, Nanded, 431606, (MH), India

ARTICLE INFO

Article History:

Received 25th December, 2012

Received in revised form

18th January, 2013

Accepted 22nd February, 2013

Published online 19th March, 2013

Key words:

Metal pollution,
Biochemical parameters,
Algae,
Inhibitory effects,
Stimulatory effects.

ABSTRACT

The disturbance of aquatic ecosystems due to metal pollution from various sources such as industrial and domestic, cause loss of biodiversity as well as increases the bioaccumulation and magnification of toxicants in the food chain. The objective of the present investigation was to evaluate the effect of chromium on several physiological activities of *Anabaena ambigua*, *Anabaena subcylindrica*, *Nostoc commune*, *Nostoc muscorum*, *Spirogyra sp.* and *Spirulina sp.* To carry out this research work, the algal strains were obtained from various sources. A standard initial inoculum of the isolated algal species was inoculated to culture flasks. The culture flasks were supplied with various concentrations (0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) of chromium. At the end of the incubation period 10 ml of sample was taken and centrifuged at 6000 rpm for 15 minutes and the pellets were used for measurement of the various experimental parameters. The results show that, the lower doses of chromium had stimulatory effects on total chlorophyll, total protein, total carbohydrate, total starch and total free amino acids of all the tested algal species. All the biochemical parameters of the tested algal strains were gradually decreased in a manner dependent on the metal concentration in the culture medium. The inhibitory and stimulatory effects of either of the used heavy metals depend on concentration. Different organisms, however, have different sensitivities to the same metal.

Copyright, IJCR, 2013, Academic Journals. All rights reserved.

INTRODUCTION

Water has the central role in mediating global-scale ecosystem processes, linking atmosphere, lithosphere and biosphere by moving substances between them and enabling chemical reactions to occur. Any physical, biological, or chemical change in water quality that adversely affects living organisms of makes water unsuitable for desired uses can be considered pollution. There are natural sources of water contamination, such as poison springs, oil seeps, and sedimentation from erosion, but here we will focus primarily on human-caused changes that affect water quality or usability. Water pollution occurs due to the presence of dissolved inorganic, organic materials and other substances (Mahajan, 2000). Over 40 elements in the environment are classified as metals. Macronutrients such as calcium, magnesium, iron, potassium and sodium are particularly important in sustaining life but may become toxic in excessive concentrations. Trace elements such as chromium, cobalt, copper, manganese, nickel, selenium and zinc are structurally part of important molecules and may serve as cofactors of enzymes in metabolic processes. Excessive concentrations of these elements are also toxic.

Metal contamination of the environment arises not only from natural sources, but from industrial activity (Sachan et. al. 2007). Combustion of fossil fuels releases about 20 toxicologically important metals into the environment including arsenic, beryllium, cadmium, lead and nickel. Industrial products and used industrial material may contain high concentrations of toxic metals. For example, mercury is used by the chlor-alkali industry to produce chlorine and caustic soda in the pulp and paper industry and in the production of battery cells, fluorescent bulbs, electrical switches, paints, agricultural products, dental preparations and pharmaceuticals. The pollution of aquatic Environments by metals is well documented worldwide. Metal

contamination in several rivers in Wales has been documented from the early 19th century, with some rivers having only invertebrate communities and showing no sign of fish life by the early 20th century (Mance, 1987). It is reported that, by international standards, about 70 percent of fresh water available in India is polluted. The sacred Ganga River is highly polluted in several regions and a major action plan to clean the river is in progress. In addition, the Central Pollution Control Board (CPCB) of India has identified several grossly polluted river stretches (Sastri, 1995). Algae are widely present in freshwater environments, such as lakes and rivers, where they are typically present as micro-organisms – visible only with the aid of a light microscope. Although relatively inconspicuous, they have a major importance in the freshwater environment, both in terms of fundamental ecology and in relation to human use of natural resources. Many thousands of algal species occur in nature. There few places on earth where algae of some kind cannot be found (Pelczar et al., 1986). Planktonic algae are considerably influenced by heavy metal pollution (Whitton, 1984), which can arise in a variety of ways – including sewage discharge (Seidl et al., 1998), resuspension of toxic sediments (Nayar et al., 2004) and industrial effluent discharge. (Cattaneo et al., 1998) studied the response of lake diatoms to heavy metal contamination, analyzing sediment cores in a northern Italian lake (Lake d'Orta) subject to industrial pollution. Some metals are mutagenic, which causes hereditary changes in the genome (Paul, 2009).

MATERIALS AND METHODS

Cultivation of algae

The starting culture of *Anabaena ambigua*, *Anabaena subcylindrica*, *Nostoc muscorum*, and *Spirulina sp.* was obtained from National Chemical Laboratory, Pune. The culture of *Nostoc commune* was brought from the School of Life Sciences, Swami Ramanand Teerth Marathawada University, Nanded while *Spirogyra sp.* was isolated

*Corresponding author: pramodmane30@yahoo.co.in

form the water body present in the campus of Swami Ramanand Teerth Marathawada University, Nanded. The strains of *Anabaena ambigua*, *Nostoc muscorum*, *Nostoc commune*, *Anabaena subcylindrica* were inoculated and grown in the Fog's medium at p^H 7.5 while the *Spirogyra sp.* was inoculated and grown in the modified Bold's basal medium and the *Spirulina sp.* was inoculated and grown in the Spirulina medium. All of these medium were sterilized by autoclaving at 121°C for 15 minutes. All of these medium were stored at 4°C until inoculated. Culture was grown in the respective liquid media in 2 liter glass Erlenmeyer flasks and incubated at 25°C in a growth chamber with a light and dark cycle of 8 hours and 16 hours and 3000 – 3500 lux, light intensity provided by cool white day light fluorescent tube lamps.

Experimental setup for toxicological study

Stock solution (1000 mg/L) of chromium was prepared in double distilled water. Three different sets of flasks (250 ml) along with one control were prepared each containing 100 ml of nutrient medium and sterilized by autoclaving at 121°C and 15 lb pressure for 15 minutes. Cooled at room temperature and metal solution was mixed aseptically in flasks for preparation of different concentrations. One ml of algae culture (one month old) was inoculated in each flask and incubated for 15 days under white fluorescent light of 2000 lux with 16/8 hours light and dark photoperiod at $28 \pm 2^\circ C$ in temperature controlled culture chamber.

Estimation of chlorophyll

Chlorophyll content was estimated by Arnon's method. 10 ml of sample was taken and centrifuged at 6000 rpm for 15 minutes. Supernatants were discarded, while pellets extracted with 5 ml of 80% aqueous acetone for at least 6 hours at 4°C temperature. The tubes were wrapped with aluminum foil and kept in dark. The samples were centrifuged again and the supernatants were used for measuring the optical density at 663 nm and 645 nm against 80% acetone as a blank by spectrophotometer. Total chlorophyll was calculated for each sample using the Arnon's formula (Arnon, 1949).

Estimation of protein

Protein contents were estimated by the Lowry method using Bovine Serum Albumin (BSA) as standard. The pellets remaining after chlorophyll extractions were dissolved in 0.1 N NaOH, centrifuged at 6000 rpm for 15 minutes. 0.2 ml of supernatant was mixed with 2.1 ml of working solution – I (1% $CuSO_4$ + 1% Na-K-Tartrate + 2% Na_2CO_3 in 0.1 N (NaOH)). After 10 minutes, 0.2 ml of 50% diluted Folin – Ciocalteu's reagent was added. Absorbance was recorded after 30 minutes at 750 nm by spectrophotometer against a blank having no protein (Lowry *et al.*, 1951).

Estimation of total carbohydrate

The total carbohydrate contents were estimated by the Anthrone reagent method. 10 ml samples were centrifuged at 6000 rpm for 15 minutes. Pellets were separated and extracted with 80% ethanol. On further centrifugation, the supernatants were used for total carbohydrate estimation. 0.5 ml of supernatants was added to 2.5 ml anthrone reagent in ice bath. Then the tubes were boiled in water bath at 100°C for 10 minutes. After cooling, the absorbance was recorded at 620 nm using spectrophotometer, against a blank having no carbohydrate (Dubois *et al.*, 1956).

Estimation of starch

The starch contents were estimated by the Anthrone reagent method. The pellets remained during total carbohydrate estimation, were used for starch estimation. The pellets were extracted with 52% perchloric acid for 30 minutes at 0°C centrifuged and supernatants were diluted upto 15 times. 1 ml of diluted sample was mixed with 2 ml cold anthrone reagent in ice bath. Boiled for 10 minutes at 100°C in water bath, cooled and recorded the absorbance at 630 nm by using

spectrophotometer against a blank having no starch. Calculated the starch content by multiplying with 0.9 to the values obtained from standard curve.

Estimation of free amino acids

The total free amino acids were estimated by the Ninhydrin method (Moore and Stein, 1948). 10 ml of samples taken and centrifuged at 6000 rpm for 15 minutes. Supernatants were discarded while pellets extracted with 5 ml of 80% ethanol. 0.1 ml of extract was mixed with 1 ml of ninhydrin solution and 0.9 ml of distilled water. The tubes were boiled in water bath for 20 minutes. 5 ml of diluent was mixed and after 15 minutes recorded the absorbance at 570 nm by spectrophotometer against a blank by taking 0.1 ml of 80% ethanol instead of the extract.

RESULTS AND DISCUSSION

Changes in the total chlorophyll contents of *Anabaena ambigua*, *Anabaena subcylindrica*, *Nostoc commune*, *Nostoc muscorum*, *Spirogyra sp.*, *Spirulina sp.*, in presence of different concentrations of chromium after fifteen days of incubation period were investigated.

The data given in Table 1 showed the effects of different chromium concentrations on total chlorophyll contents of algae. The data expresses that the total chlorophyll was inhibited 50% (IC_{50}) at chromium concentrations of 2.0 mg/L for *Anabaena ambigua*, *Anabaena subcylindrica*, *Nostoc commune*, *Nostoc muscorum*, and *Spirulina sp.* and 3.0 mg/L for *Spirogyra sp.* However, the chromium showed stimulatory effects on chlorophyll content of *Anabaena ambigua*, *Nostoc commune*, at 0.5 mg/L and *Nostoc muscorum*, *Spirogyra sp.* and *Spirulina sp.* at 0.1 mg/L of chromium concentrations. But at higher concentrations chromium showed inhibitory effects. Overall, data emphasize the stimulatory, inhibitory and toxic effect of chromium on chlorophyll content of the selected algal strains, which was also confirmed using different concentrations of metal separately. These findings were supported by several researchers. (Saxena, 2006), studied iron induced metabolic changes in the diazotrophic cyanobacterium *Anabaena PCC 7120*. The decline in chlorophyll content might be caused by a reduction in the synthesis of chlorophyll possibly by increasing chlorophyllase activity, by destruction in chloroplast membrane and due to deactivation of electron transport in photosystem I (Sen and Mondal, 1987). At higher concentration of heavy metals, chlorophyll synthesis will be inhibited as the heavy metals inhibit the enzymes that are responsible for chlorophyll synthesis (Prasad and Prasad, 1987).

Table 2 showed the effects of different chromium concentrations on total protein contents of algae. The data expresses that the total protein was inhibited 50% (IC_{50}) for *Anabaena ambigua* at 2.0 mg/L, *Anabaena subcylindrica* at 2.0 mg/L, *Nostoc commune* at 2.0 mg/L, *Nostoc muscorum* at 2.0 mg/L, *Spirogyra sp.* at 3.0 mg/L and *Spirulina sp.* at 2.0 mg/L of chromium concentrations. However, the chromium showed stimulatory effects on protein content of *Anabaena ambigua* upto 0.1 mg/L, *Anabaena subcylindrica* hasn't showed any stimulatory effects on chlorophyll at any concentration, *Nostoc commune* upto 0.1 mg/L, *Nostoc muscorum* upto 0.1 mg/L, *Spirogyra sp.* upto 0.1 mg/L and *Spirulina sp.* upto 0.1 mg/L. After fifteen days of incubation period, the protein content of all the algal strains was significantly decreased. The data given in table 2 clearly showed the effect of chromium on the protein content in six freshwater algal species, which decreased as increase in the dose of metal. Similar results were found by (Battah, 2010), during the study of effect of Zn and Cd on *Chroococcus mintus*. These findings also supported by (Vajpayee *et al.*, 2000) who observed that, at lower concentrations (0.01 to 0.1 ppm) of Ni increased the protein content in *P. stratiotes* leaves, which decreased at higher concentrations (1.0 and 10.0 ppm). They also reported that, such a reduction in the protein content could be attributed to effect on nitrate reductase activity. The stimulatory and inhibitory effects of metals on protein content at different metal concentrations also studied by (Odjegba and Fasidi, 2006)

Table 1: Effect of chromium concentration observed on chlorophyll content (mg/ml)

Conc. (mg/L)	Algae					
	<i>Anabaena ambigua</i>	<i>Anabaena subcylindrica</i>	<i>Nostoc commune</i>	<i>Nostoc muscorum</i>	<i>Spirogyra sp.</i>	<i>Spirulina sp.</i>
Control	0.0284±0.00020	0.0916±0.00020	0.7766±0.00024	0.1815±0.00016	1.7754±0.00169	0.3705±0.00016
0.1	0.0346±0.00021	0.0844±0.00020	0.8216±0.00021	0.1963±0.00012	1.9434±0.00016	0.4515±0.00021
0.5	0.0293±0.00020	0.0624±0.00028	0.8044±0.00020	0.1645±0.00029	1.7217±0.00012	0.3104±0.00016
1.0	0.0205±0.00021	0.0534±0.00020	0.6262±0.00012	0.1125±0.00016	1.6734±0.00012	0.2663±0.00017
2.0	0.0144±0.00029	0.0456±0.00021	0.3844±0.00024	0.0914±0.00016	1.1237±0.00016	0.1795±0.00016
3.0	0.0107±0.00016	0.0293±0.00020	0.1246±0.00012	0.0763±0.00020	0.8564±0.00020	0.1272±0.00012
4.0	0.0094±0.00029	0.0094±0.00021	0.0924±0.00016	0.0387±0.00012	0.4216±0.00017	0.0855±0.00021
5.0	0.0085±0.00012	0.0074±0.00021	0.0774±0.00024	0.0236±0.00017	0.1617±0.00017	0.0516±0.00020

Data are mean ± S.D. of three replicates per treatment.

Table 2: Effect of chromium concentrations on protein content of selected algae (mg/ml).

Conc. (mg/L)	Algae					
	<i>Anabaena ambigua</i>	<i>Anabaena subcylindrica</i>	<i>Nostoc commune</i>	<i>Nostoc muscorum</i>	<i>Spirogyra sp.</i>	<i>Spirulina sp.</i>
Control	0.4125±0.00020	0.0634±0.00028	0.9195±0.00016	0.4234±0.00024	0.6194±0.00010	1.1463±0.00017
0.1	0.4836±0.00024	0.0626±0.00020	0.9625±0.00032	0.4695±0.00029	0.6945±0.00024	1.1937±0.00012
0.5	0.4063±0.00021	0.0527±0.00017	0.7945±0.00020	0.3874±0.00020	0.5316±0.00020	0.8944±0.00016
1.0	0.3425±0.00029	0.0363±0.00020	0.5865±0.00024	0.2682±0.00012	0.4876±0.00029	0.6295±0.00028
2.0	0.2116±0.00024	0.0336±0.00014	0.4534±0.00029	0.2114±0.00029	0.3914±0.00028	0.5994±0.00020
3.0	0.0626±0.00021	0.0072±0.00017	0.1246±0.00010	0.0745±0.00034	0.3145±0.00020	0.1645±0.00024
4.0	0.0073±0.00024	0.0015±0.00016	0.0744±0.00029	0.0326±0.00020	0.1215±0.00033	0.0624±0.00024
5.0	0.0046±0.00017	0.0006±0.00020	0.0074±0.00160	0.0095±0.00016	0.0616±0.00017	0.0085±0.00026

Data are mean ± S.D. of three replicates per treatment.

Table 3: Effect of chromium concentrations on carbohydrate content of selected algae (mg/ml).

Conc. (mg/L)	Algae					
	<i>Anabaena ambigua</i>	<i>Anabaena subcylindrica</i>	<i>Nostoc commune</i>	<i>Nostoc muscorum</i>	<i>Spirogyra sp.</i>	<i>Spirulina sp.</i>
Control	0.0564±0.00024	0.0574±0.00024	0.2575±0.00024	0.0574±0.00028	0.1575±0.00024	0.0665±0.00016
0.1	0.0524±0.00033	0.0493±0.00020	0.2964±0.00028	0.0624±0.00024	0.2014±0.00028	0.0826±0.00029
0.5	0.0325±0.00016	0.0324±0.00028	0.2654±0.00033	0.0416±0.00024	0.1595±0.00028	0.0754±0.00029
1.0	0.0233±0.00020	0.0304±0.00024	0.1925±0.00024	0.0364±0.00033	0.0905±0.00024	0.0525±0.00020
2.0	0.0155±0.00024	0.0283±0.00020	0.1253±0.00024	0.0254±0.00028	0.0815±0.00024	0.0335±0.00034
3.0	0.0094±0.00034	0.0193±0.00020	0.0944±0.00028	0.0175±0.00028	0.0745±0.00033	0.0193±0.00020
4.0	0.0083±0.00012	0.0085±0.00028	0.0533±0.00030	0.0094±0.00024	0.0534±0.00020	0.0084±0.00024
5.0	0.0064±0.00028	0.0015±0.00020	0.0146±0.00029	0.0054±0.00024	0.0264±0.00028	0.0064±0.00028

Data are mean ± S.D. of three replicates per treatment.

Table 4: Effect of chromium concentrations on starch content of selected algae (mg/ml).

Conc. (mg/L)	Algae					
	<i>Anabaena ambigua</i>	<i>Anabaena subcylindrica</i>	<i>Nostoc commune</i>	<i>Nostoc muscorum</i>	<i>Spirogyra sp.</i>	<i>Spirulina sp.</i>
Control	0.3281±0.00455	0.0825±0.00033	1.3284±0.00028	0.3126±0.00020	1.6434±0.00020	0.6534±0.00028
0.1	0.3944±0.00024	0.0914±0.00020	1.3816±0.00024	0.3465±0.00024	1.7014±0.00020	0.8435±0.00028
0.5	0.3215±0.00024	0.0805±0.00020	0.9544±0.00024	0.2875±0.00016	1.5295±0.00033	0.6216±0.00020
1.0	0.1684±0.00034	0.0523±0.00024	0.7225±0.00020	0.2015±0.00029	0.9924±0.00028	0.5565±0.00028
2.0	0.1483±0.00024	0.0415±0.00021	0.6843±0.00024	0.1506±0.00024	0.8144±0.00020	0.3214±0.00024
3.0	0.0754±0.00024	0.0066±0.00024	0.2435±0.00028	0.0754±0.00020	0.5274±0.00034	0.0784±0.00024
4.0	0.0125±0.00028	0.0013±0.00020	0.0714±0.00034	0.0316±0.00029	0.0964±0.00024	0.0215±0.00020
5.0	0.0075±0.00016	0.0005±0.00029	0.0144±0.00020	0.0094±0.00024	0.0154±0.00024	0.0085±0.00016

Data are mean ± S.D. of three replicates per treatment.

Table 5: Effect of chromium concentrations on free amino acid content of selected algae (mg/ml).

Conc. (mg/L)	Algae					
	<i>Anabaena ambigua</i>	<i>Anabaena subcylindrica</i>	<i>Nostoc commune</i>	<i>Nostoc muscorum</i>	<i>Spirogyra sp.</i>	<i>Spirulina sp.</i>
Control	0.3605±0.00029	0.0695±0.00020	1.1226±0.00024	0.1496±0.15561	1.5426±0.00024	0.9845±0.00024
0.1	0.3945±0.00020	0.0694±0.00035	1.2926±0.00020	0.3975±0.00033	1.8274±0.00024	1.1097±0.00016
0.5	0.2924±0.00020	0.0514±0.00028	0.9946±0.00016	0.2464±0.00024	1.3166±0.00020	0.9334±0.00033
1.0	0.2145±0.00024	0.0494±0.00024	0.7724±0.00024	0.2016±0.00024	0.9690±0.03304	0.6545±0.00024
2.0	0.1804±0.00029	0.0344±0.00024	0.5765±0.00024	0.1796±0.00020	0.7934±0.00024	0.4924±0.00024
3.0	0.0764±0.00020	0.0084±0.00020	0.2114±0.00028	0.0854±0.00024	0.2316±0.00016	0.2165±0.00024
4.0	0.0224±0.00024	0.0025±0.00033	0.0846±0.00024	0.0215±0.00032	0.0695±0.00024	0.0695±0.00024
5.0	0.0064±0.00024	0.0015±0.00020	0.0565±0.00024	0.0045±0.00016	0.0266±0.00024	0.0155±0.00028

Data are mean ± S.D. of three replicates per treatment.

Table 3 showed the effects of different chromium concentrations on carbohydrate contents of algae. The data expresses that the carbohydrate was inhibited 50% (IC₅₀) for *Anabaena ambigua* at 1.0 mg/L, *Anabaena subcylindrica* at 2.0 mg/L, *Nostoc commune* at 2.0 mg/L, *Nostoc muscorum* at 2.0 mg/L, *Spirogyra sp.* at 3.0 mg/L and *Spirulina sp.* at 2.0 mg/L of chromium concentrations. However, the chromium showed stimulatory effects on carbohydrate content of *Anabaena ambigua* and *Anabaena subcylindrica* hasn't showed any stimulatory effects on carbohydrate when exposed to chromium, *Nostoc commune* upto 0.1 mg/L, *Nostoc muscorum* upto 0.1 mg/L, *Spirogyra sp.* upto 0.5 mg/L and *Spirulina sp.* upto 0.5 mg/L. The present results demonstrated a concentration dependent effect of chromium on carbohydrate formation, being stimulated by lower concentrations and inhibited at higher concentrations. Our study coincide with (Rolli *et al.*, 2010), who also reported the increase in the carbohydrate content of *Spirodela polyrhiza* at 0.1 ppm of Cd by 30.03% after 12 days of exposure. The above findings were also supported by (Chaudhary and Chandra 2005), who studied the toxicity of heavy metals with *Nostoc muscorum*, (Lim *et al.* 2006), also observed the effects of copper (I) oxide on biochemical compositions of two marine microalgae, *Tetraselmis suecica* and *Dunaliella tetriolecta*.

In Table 4 the effects of different chromium concentrations on starch contents of algae was showed. The data expresses that the starch was inhibited 50% (IC₅₀) for *Anabaena ambigua* at 1.0 mg/L, *Anabaena subcylindrica* at 2.0 mg/L, *Nostoc commune* at 2.0 mg/L, *Nostoc muscorum* at 2.0 mg/L, *Spirogyra sp.* at 2.0 mg/L and *Spirulina sp.* at 2.0 mg/L of chromium concentrations. However, the chromium showed stimulatory effects on starch content of *Anabaena ambigua* upto 0.1 mg/L, *Anabaena subcylindrica* 0.1 mg/L, *Nostoc commune* upto 0.1 mg/L, *Nostoc muscorum* upto 0.1 mg/L, *Spirogyra sp.* upto 0.1 mg/L and *Spirulina sp.* upto 0.1 mg/L. The increase in the starch content of all the six algal species at lower concentrations mainly at 0.1 mg/L after fifteen days of exposure period was determined. This was also mentioned by (Badr, 2004), who did a work on twenty day old *cucumber* plants to copper stress for five days, to observe the effect of copper on starch content. In this investigation they found that, the starch concentration measured in first and second leaves of the stressed plants increased significantly as compared to controls. After 0.1 mg/L concentration the starch content was decreased with increase in the metal concentrations.

The results given in the Table 5 showed the effects of different chromium concentrations on free amino acid contents of algae. The data expresses that the free amino acid was inhibited 50% (IC₅₀) for *Anabaena ambigua* at 2.0 mg/L, *Anabaena subcylindrica* at 2.0 mg/L, *Nostoc commune* at 2.0 mg/L, *Nostoc muscorum* at 2.0 mg/L, *Spirogyra sp.* at 2.0 mg/L and *Spirulina sp.* at 2.0 mg/L of chromium concentrations. However, the chromium showed stimulatory effects on free amino acid content of *Anabaena ambigua* upto 0.1 mg/L, *Anabaena subcylindrica* hasn't showed any stimulatory effects on free amino acid when exposed to chromium, *Nostoc commune* upto 0.1 mg/L, *Nostoc muscorum* upto 0.1 mg/L, *Spirogyra sp.* upto 0.1 mg/L and *Spirulina sp.* upto 0.1 mg/L. Total free amino acid at the increasing concentration of selenium decreased in all the six tested algal species. However, at lower concentration of selenium the total amino acid content was increased. These findings were supported by (Zsolt *et al.* 2006; Fathi *et al.* 2005).

Conclusion

The metals plays very important role in the growth and photosynthetic rate of the algae. But at higher concentrations of the metals the algae shows toxic effects. In the present study, we showed the toxic effects of the chromium at higher concentrations on some biochemical parameters of the algae. It is therefore clear that exposure of bio-organisms to metals can cause long-term and non-reversible effects. Because metals in the environment may have a profound impact on the physiology and general health of the exposed organism, this

present work will focus on the impact of well-known and frequently occurring metals on the metabolic parameters mainly the photosynthetic parameters of some selected algal species.

REFERENCES

- Arnon, D. I., (1949). Copper enzymes in isolated chloroplast: polyphenoloxidase in *Beta vulgaris*. *Plant physiol* 24, 1 – 5.
- Badr, A. S., Patricia, G., Florence, V. D., Marie, L. T., Daniel, E., Pierre, M. B. (2004). Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion contents. *Plant Science*. 166, 1213 – 1218.
- Battah, Mohamed G. (2010). Impact of different concentrations of zinc and cadmium chloride on microalgae *Chroococcus minutus*. *Australian J. of Basic and Applied Sciences*. 4 (12), 5739 – 5743.
- Cattaneo, A., Asioli, A., Comoli, P. (1998). Organisms response in a chronically polluted lake supports hypothesized link between stress and size. *Limnology and Oceanography*. 43, 1938 – 1943.
- Chaudhary, M. P., Chandra, R. (2005). Toxicity of heavy metals with *Nostoc muscorum* L. *J. of Environ. Biol.* 26 (1), 129 – 134.
- Dubois, M., Gills, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colourimetric method for determination of sugars and related substances. *Analyst. Chem.* 28, 350 – 356.
- Fathi A. A., Zaki F. T., Ibraheim H. A., (2005), Response of tolerant and wild types of *Chlorella vulgaris* to copper with special references to copper uptake system, *Protistology*, 4 (1), 73 – 78.
- Lim, C.Y., Yoo, Y.H., Sidharthan, M., Ma, C. W., Bang, I.C., Kim, J. M., Lee, K.S., Park, N.S., Shin, H.W. (2006). Effects of copper (I) oxide on growth and biochemical compositions of two marine microalgae. *J. of Environmental Biology*. 27(3): 461–466
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with the folin – phenol reagent. *J. Biol. Chem.* 193, 265 – 275.
- Mahajan, S. P. (2000). Pollution control in process industries. Tata McGraw – Hill Publ. New Delhi. 4.
- Mance, G. (1987). Pollution threat of heavy metals in aquatic environments, Elsevier Science Publishing Co., Inc., New York pp. 372.
- Moore, S., Stein, W.H. (1948). Photometric method for use in the chromatography of amino acids, In: *Methods in Enzymes* vol. III. Academic press, New York. 468.
- Nayar, S., Goh, B.P., Chou, L.M. (2004). Environmental impact of heavy metals from dredged and resuspended sediments on phytoplankton and bacteria assessed in situ mesocosms. *Ecotoxicology and Environmental Safety*. 59, 349 – 369.
- Odjeba, V.J., Fasidi, I. O. (2006). Effects of heavy metals on some proximate composition of *Eichhornia crassipes*. *J. Appl. Sci. Environ. Mgt.* 10(1), 83–87.
- Paul, A. (2009). Textbook of cell and molecular biology (2nd Ed.). - Books and Allied Publ., Kolkata. 1103.
- Pelczar, M. J., Chan, E. C. S., Krieg, Noel R. (1986). *Microbiology*, (5th Ed). - Tata McGraw – Hill Publ., New Delhi. 366 – 374.
- Prasad, D., Prasad, A. (1987). Altered- aminolaevulinic acid metabolism by lead and mercury in germinating seedlings of Bajra (*Pennisetum typhoides*). *J. Plant Physiol.* 127, 241 – 249.
- Rolli, N. M., Suvarnakhandi, S. S., Mulgund, G. S., Ratogeri, R. H., Taranath, T. C. (2010). Biochemical responses and accumulation of cadmium in *Spirodela polyrhiza*. *J. of Environmental Biology*. 31, 529 – 532.
- Sachan, S., Singh, S. K., Srivastav, P. C. (2007). Buildup of heavy metals in soil-water-plant continuum as influenced by irrigation with contaminated effluent. *Journal of Environ. Science and Engg.* 49(4), 293-296.
- Sastri, M. N., (1995). *Water* (1st Edⁿ). Himalaya publishing house, Girgaon, Mumbai – 400 004.
- Saxena, R. K. (2006). Iron induced metabolic changes in the diazotrophic cyanobacterium *Anabaena PCC 7120*. *Ind J. of Exptl Biol.* 44, 849 – 851.

- Seidl, M., Huang, V., Mouchel, J. M. (1998). Toxicity of combined sewer overflows on river phytoplankton. Environmental Pollution. 101. 107 – 116.
- Sen, A. K., Mondal, N. G. (1987). *Salvinia natan-* as the scavenger of Hg (II), *Water Air Soil Pollut.* 34, 439 – 446.
- Vajpayee, P., Tripathi, R. D., Rai, U. N., Ali, M. B., Singh, S. N. (2000). Chromium accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content of *Nymphaea alba*. *Chemosphere.* 41, 1075 – 1082.
- Whitton, B.A. (1984). *Algae as Ecological Indicators*. New York, Academic Press.
- Zsolt, H., Viktor, O., Arpad, B., Ilona, M., Laszlo, S., Gyula, L. (2006). Effect of chromium (VI) on growth, element and photosynthetic pigment composition of *Chlorella pyrenoidosa*. *Acta Biologica Szegediensis.* 50 (1-2), 19 – 23.
