Environment Conservation Journal 17(3) 37-43, 2016
ISSN 0972-3099 (Print) 2278-5124 (Online)
Abstracted and Indexed

Growth and biochemical constituents of an indigenous cyanobacterium affected by heavy metal stress

Rajesh Dhankhar and Lalita Rana ✉

Received:21.07.2016 **Accepted: 15.09.2016**

Abstract

A cyanobacterium having high relative abundance in sewage irrigated soil was isolated and identified as *Lyngbya contorta*. The species was tested for tolerance towards heavy metals, Cu^{2+}, Zn^{2+}, Ni^{2+} and Cd^{2+} (0.5 to 10 mg/L) in single metal systems under controlled laboratory conditions. Our results show that the studied strain has a distinctive response towards each heavy metal, it was most affected by Ni^{2+} followed by Cu^{2+}, Zn^{2+}, Cd^{2+} ions. The studied strain showed better response as indicated by higher concentrations of sugar, proteins and photosynthetic pigments in aqueous Cd^{2+} solutions as compared to that at control. The incubation of cyanobacterial cells with lower concentrations of heavy metals (Cu^{2+}, Zn^{2+}, Ni^{2+}) enhanced the growth rate, soluble proteins and photosynthetic pigments, while elevated concentrations were observed to be inhibitory. The present study demonstrates the capability of isolated indigenous species to withstand heavy metal stress at low concentrations and can be utilized for bioremediation of contaminated lands.

Keywords: Heavy metals, indigenous, organic constituents, pigments, proteins

Introduction

Sewage irrigated agro-ecosystem represents one of the degraded environmental system. The main contributors are heavy metals, which can have toxic effects on many different types of organisms and affect biological processes at various levels of organizations (Chauvat and Chauvat, 2015). The toxicity of these metals is based on their chemical properties, which allow them to promote the production of reactive oxygen species (ROS); the inactivation of enzymes, basically by reaction with SH-groups; and/or the displacement of the normal metal cofactors of numerous metalloproteins(Imlay,2014). Several metals are essential for living organisms at very low concentrations, but at high concentrations most are toxic (Debelius et al., 2009). McGinna et al., 2012 found total depletion of Fe, Zn and Cd from the sewage wastewater by microalga, and lower, but substantial, uptake and/or adsorption of seven other elements. However, the metal uptake process is complex and dependent upon not only the specific surface properties of the organisms but also cell physiology (Goudey, 1987) and other abiotic factors.In most organisms, one-quarter to one-third of all proteins require metals (Waldron and Robinson, 2009). Cyanobacteria also have high requirements for metal ions. Copper and zinc, being cofactors of enzymes involved in a wide range of biochemical reactions (Bityutskii,1999; Udel'nova and Yagodin, 1993). Zinc is a component of all basic enzyme classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases; more than 300 proteins use zinc as a cofactor (Prasad, 1995). Among zinc containing enzymes of microorganisms are carbonic anhydrase, aldolase, RNA polymerase, protease, and others. Both these metals have adverse effects at high concentrations Copper is phytotoxic and has been used as an algicide to control algal blooms. Cadmium and nickel are amongst toxic metals. Cadmium is not known to have any biological functions. It inhibits most of the basic physiological processes like chlorophyll biosynthesis, photosynthetic carbon assimilation and growth (Fargasova, 1999). Nickel is known as a constituent of the active site of urease and nitrogenase in cyanobacterial cells (Morand et al., 1991). It has been reported that nickel ions are actively taken up by the cells through a high affinity transport system (Campbell and Smith, 1986), the cells grown without nickel has identical level of urease activity with the cells grown with nickel ion concentration.

Author's Address
Department of Environmental Science, MaharshiDayanand University,Rohtak, Harvana.
E-mail: lalita.777@gmail.com.

Cyanobacteria are known to inhabit heavily polluted environments (Dodor and Tabatabai, 2003), where they have a wide distribution and may be dominating microfloral populations in these systems. They are also significant contributors to global photosynthetic productivity(Castielli et al., 2009), thus making it relevant to study how environmental stresses can alter their physiological activities. Lopez et al., 2006 reported that spontaneous mutation rate in cyanobacteria exposed to stressed environments was two orders of magnitude higher than the mutation rates observed in other prokaryotes ($\approx 10^{-8}$), which assures the adaptation of large cyanobacterial population. Thus, this study aimed to investigate the responses of indigenous *Lyngbya contorta* isolated from the sewage waste water irrigated soil(stressed environment), towards various concentrations of heavy metals in order to explore their tolerance towards anthropogenic pollution.

Materials and Method

Isolation and cultivation of dominant indigenous cyanobacterial strains

For isolation purpose, the soil samples at the depth of 0-10cm were collected from sewage irrigated fields located along the sewage drain of Rohtak city, Haryana, India. Pure cultures of cyanobacterial strains were obtained by agar plate spreading and serial dilution techniques (Andersen and Kawachi, 2005) using BG-11 medium(Stanier et al., 1971). The identification was done with the help of keys given by Desikachary(1959). Cyanobacteria belonging to nine genera were isolated from sewage irrigated soil which included 13 strains of Lyngbya (Rana and Chhikara, 2011). These isolates were abundantly found showing the highest relative abundance of 100%. *Lyngbya contorta* was selected for the present study. Purified cyanobacterial strain was maintained at 28 ± 3 °C and illuminated under a 16:8 h light–dark cycle, using cool fluorescent tubes.

Metal treatments

Toxicity studies were performed separately for the four metals using 150 ml Erlenmeyer flasks. Copper sulphate ($CuSO_4$), Zinc sulphate ($ZnSO_4$), Nickel chloride ($NiCl_2$) and cadmium chloride ($CdCl_2$) were used as the sources of Cu^{2+}, Zn^{2+}, Ni^{2+} and Cd^{2+} respectively. Each flask was inoculated with 1 ml of exponentially growing, homogenized cyanobacterial suspension, which contained various initial metal concentrations (0, 0.5, 1, 2, 5 and 10 mg/L). Suspensions were continuously homogenized in a rotary shaker at 100 rpm and cultured for 12 days under the conditions as described above. Each experiment was conducted in triplicates and growth rate was determined on 4, 8 and 12th day whereas pigment concentration (chlorophyll) and organic constituents (sugars) were studied on 12th day.

Concentration	Sugars(µg/ml)	Proteins (µg/ml)	Total soluble proteins (µg/ml)	Chlorophyll(mg/l)
0 mg/l	85.16±0.11	32.6±0.03	65.7±0.54	6.2±0.21
0.5mg/l	56.9±0.94	27.3±0.63	67.7±0.17	5.1±0.27
1mg/l	60.2±1.74	27.6±0.38	84.3±0.18	7.7±0.64
2mg/l	63.5±0.63	29.5±0.26	85.8±0.44	7.8±0.66
5mg/l	66.7±0.16	32.4±0.30	119.3±0.18	7.5±0.43
10mg/l	107.4±0.35	26.1±0.33	108.5±0.22	4.3±0.23

Table 1. Effect of various cadmium concentrations on sugars, proteins, soluble proteins and chlorophyll content of isolated *Lyngbyacontorta*, on 12th day of observation(Values are arithmetic mean ± S.D. of three determinations)

Concentration	Sugars(µg/ml)	Proteins (µg/ml)	Total soluble proteins (µg/ml)	Chlorophyll(mg/l)
0 mg/l	85.16±0.11	32.6±0.03	65.7±0.54	6.2±0.21
0.5mg/l	44.15±0.38	13.4±0.33	27.6±0.08	5.1±0.23
1mg/l	54.9±0.12	17.7±0.35	60.7±0.75	5.4±0.19
2mg/l	61.7±0.23	18.4±0.21	64.8±0.12	4.6±0.16
5mg/l	58.7±0.18	14.6±0.27	61.2±0.22	4.2±0.093
10mg/l	53.8±0.16	11.8±0.37	56.8±0.28	3.5±0.025

Table 2. Effect of various nickel concentrations on sugars, proteins, soluble proteins and chlorophyll content of isolated *Lyngbyacontorta*, on 12th day of observation

Concentration	Sugars(µg/ml)	Proteins(µg/ml)	Total soluble proteins (µg/ml)	Chlorophyll(mg/l)
0 mg/l	85.16±0.11	32.6±0.036	65.7±0.54	6.2±0.21
0.5mg/l	44.45±0.06	22.2±0.29	49.4±0.20	5.8±0.07
1mg/l	27.41±0.02	28.5±0.2	72.2±0.37	7.1±0.09
2mg/l	28.3±0.09	25.7±0.29	54.8±0.38	6.2±0.08
5mg/l	30.17±0.38	19.1±0.24	49.2±0.24	4.1±0.2
10mg/l	36.32±0.39	13.9±0.09	43.8±0.43	3.7±0.1

Table 3. Effect of various copper concentrations on sugars, proteins, soluble proteins and chlorophyll content of isolated Lyngbyacontorta, on 12th day of observation

The growth rate of *Lyngbya contorta* was followed by measurements of absorbance at 750nm (Healy, 1985) using the equation: $\mu = ln\ (n_2/n_1)/t_2 - t_1$

where, µ is the growth rate and n_1, n_2 are absorbances of culture suspension at time intervals t_1 and t_2.Chlorophyll was estimated spectrophotometrically following hot extraction method using methanol and absorbance was read at 650 and 665 nm (Mackinney, 1941). The protein and soluble protein content were estimated according to the modified method of Lowry et al. (1951). Sugars were analysed following the method prescribed by Spiro (1966).

All the experiments were conducted in triplicates and variability was accounted for in statistical terms as standard error which is given as ±values in the tables. Variations in growth rate at various concentrations of heavy metals and with incubation time were tested using two way analysis of variance. One way ANOVA test was applied to confirm the significance of data for biochemical constituents with increasing concentrations of heavy metals.

Results and Discussion

The observed growth rate of *Lyngbya contorta* was found to depend on the type of metal and its concentration. For cadmium metal, the slowest growth rate was recorded on 4th day, thereafter, growth rate increased and was at maximum on 8th day(Fig. 1) Again, declined on 12th day except at 5 and 10mg/l concentration. The peak was obtained at 2mg/l concentration. The increased growth rate at higher concentration, corresponds to increase in cell size. It may be due to the fact that at high metal concentrations the existing damaged cells increase their size as a function of their individual complexity.

Concentration	Sugars(µg/ml)	Proteins (µg/ml)	Total soluble proteins (µg/ml)	Chlorophyll(mg/l)
0 mg/l	85.16±0.11	32.6±0.03	65.7±0.54	6.2±0.21
0.5mg/l	58.96±0.43	27±0.17	66.8±0.09	5.0±0.18
1mg/l	80.41±0.47	29.2±0.27	68±0.52	5.2±0.31
2mg/l	82.19±0.42	37.5±0.18	69.3±0.14	5.8±0.1
5mg/l	45.69±0.47	39.1±0.48	69.5±0.15	6.1±0.11
10mg/l	34.56±0.07	33.5±0.18	56.4±0.18	4.2±0.08

Table 4. Effect of various zinc concentrations on sugars, proteins, soluble proteins and chlorophyll content of isolated *Lyngbyacontorta*, on 12th day of observation

Similarly, for nickel metal, the maximum growth rate was recorded on 8th day(Fig. 2). As compared with the control, growth rate decreased gradually with increased metal concentrations. The highest growth rate was observed at 0.5mg/l. The 10mg/lt caused maximum growth reduction, which was visually apparent by clear and transparent cells. There was a tremendous increase in growth rate on 4th day in response to copper metal treatments (Fig. 3). However, with increase in incubation period and metal concentration(Zou et al.,(2015), growth rate showed a decline. Growth rate in zinc treated cultures (Fig. 4) showed the pattern similar to copper treated cultures. The maximum growth rate was recorded on 4th day at 2mg/lt. As compared to copper, zinc was less toxic as shown by the respective growth rates. This strain showed a decrease in growth rate with increase in incubation time for each concentration which could be due to inhibition of normal cell division by the metal, as it has been reported for Padina boegesenni (Mamboya et al., 1999) and for *Synechocystis aquatilis* (Shavyrina et al., 2001). The decrease in the rate of cell division caused by metals is primarily attributed to their binding to sulfhydryl groups which are important for regulating the plant cell division (Visviki and Rachlin, 1991). Two way analysis of variance revealed that the differences were not statistically significant ($P<0.05$) with different concentrations of all the four heavy metals, only incubation time had significant effect on growth rate of the studied species.Variations in chlorophyll, proteins, soluble proteins and sugar content of *Lyngbya contorta* in response to heavy metal treatments are presented in Tables 1-4.

Increasing concentration of copper and nickel upto 1mg/l increased chlorophyll content whereas cadmium and zinc treatments showed more favourable effect as chlorophyll content increased upto 2mg/l and 5mg/l respectively.Nickel and zinc both stimulated sugar content upto 61.7µg/ml and 82.19µg/ml at 2mg/L of metal concentration on 12th day of incubation. But further increase in metal decreased sugar concentration. For cadmium and copper, sugar content increased at all metal concentrations. Shashirekha et al., 2015, also demonstrated increased sugar content in *Lyngbya sp*. under heavy metal stress.

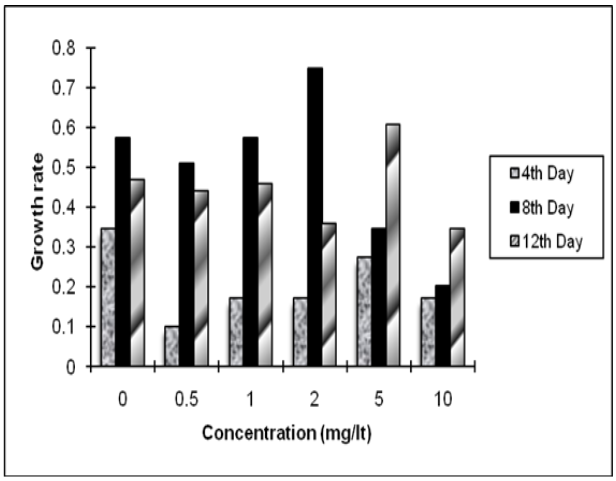

Figure. 1. Temporal variations in the growth rate of *Lyngbyacontorta*in response to different concentrations of cadmium metal.

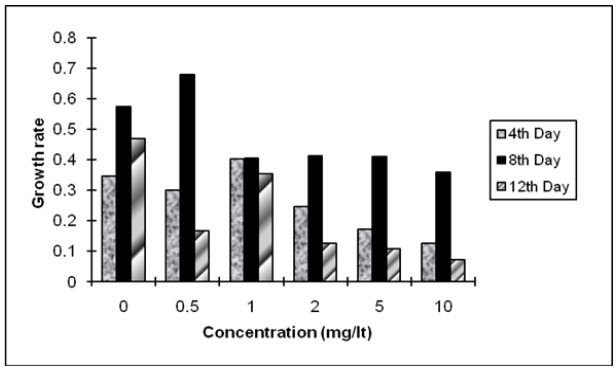

Figure 2. Temporal variations in the growth rate of *Lyngbyacontorta*in response to different concentrations of nickel metal

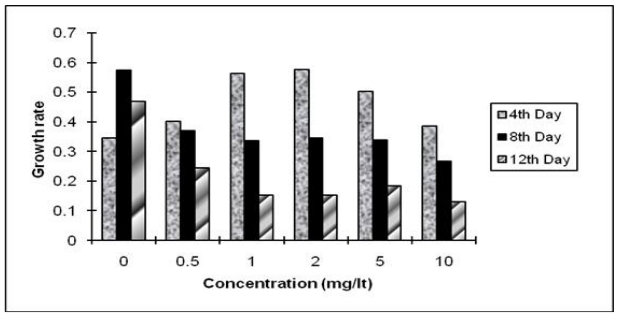

Figure 3. Temporal variations in the growth rate of *Lyngbyacontorta* in response to different concentrations of copper metal

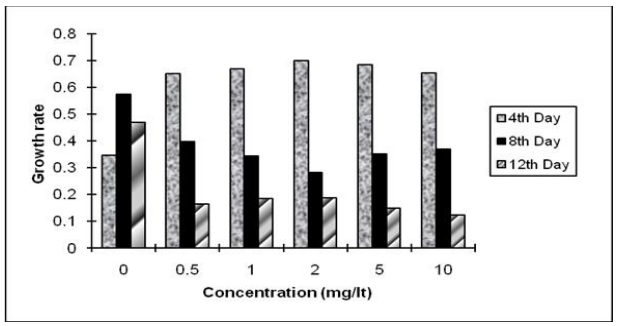

Figure 4. Temporal variations in the growth rate of *Lyngbya contorta* in response to different concentrations of zinc metal

The protein content in the studied cyanobacterium was sensitive to heavy metal treatments. Nickel and copper had more inhibitory effect on the protein and soluble protein content as compared to cadmium and zinc. Increasing concentration of copper up to 1 mg/L increased chlorophyll concentration but further increase in metal level decreases the chlorophyll concentration. This is supported by the finding of Bala and Thanasekaran (2011) and Mota *et al*., (2015), who reported a decrease in chlorophyll accumulation in the presence of copper at high concentrations. Copper may inhibit enzymes in the cytoplasm such as esterase and β-D-galactosidase (Franklin *et al*., 2001) and causes damage to the chloroplast lamellae, therefore, preventing the photosynthesis (Cid *et al*., 1995; Angeles *et al*., 1993). This strain was more sensitive to copper than zinc as indicated by lower concentrations of pigment, sugars and protein content in copper treated cultures. The strain was least tolerant to nickel metal, which exert its toxic effect by poisoning intracellular enzyme systems. In aged cultures of the *Lyngbya contorta*, pigment bleaching was observed which might be due to the due to the stress caused by higher levels of nickel leading to chlorophyll degradation and a retarded growth rate (Carrieri *et al*., 2008; Tam *et al*., 2001). The protein and sugar contents have also been found to be decreased at high nickel metal concentration, since extracellular binding contributes only slightly to Ni-resistance (Xiaolei-Jin and Nalewajko, 1996). Similar results have also been reported earlier (Rai *et al*., 1990). This strain showed increased protein and total soluble proteins, when the medium was spiked with increased concentration of cadmium. Babu *et al*., 2010, found that the absorption spectra of photosynthetic pigments in Spirulina platensis was decreased with increase in heavy metal concentrations. There are reports showing cadmium causing damage to various metabolic processes, pigment synthesis and chloroplast (Dubey, 1997;Lamaia *et al*., 2005; Machado *et al*.,2015), but the studied strain had developed tolerance towards cadmium and showed better performance in the presence of the metal.

The present strain was well acclimated to grow under a wide range of zinc concentrations. The exposure to zinc resulted in an increase in protein and soluble protein content upto 5mg/l, due to the synthesis of metal binding proteins, the metallothioneines, which have the capacity to bind metals (such as As, Cd, Cu, Hg and Zn) through the thiol group of its cysteine aminoacids (Shukla et al., 2009; Chauvat and Chauvat, 2015). In cyanobacteria, MTs were first identified in cells adapted to growth in elevated levels of Cd and Zn (Blindauer, 2011). Similarly, chlorophyll content also showed an increase with increase in

concentration of the zinc metal. However, both increase and decrease in chlorophyll have been reported earlier (Wong et al.,1994; Franklin et al., 2001). Decrease in growth rate was observed with increase in incubation time, which can be related to the extracellular zinc concentration (Jin et al., 2009). One possible mode of toxic action of zinc is at the cell membrane, where it may disrupt the uptake of calcium, which is necessary for Ca-ATPase in cell division (Stauber and Florence, 1990). *Lyngbya contorta* adaptation to zinc is evidently related to the formation of chelates with some cell secretions, which are loosely bound to the cell wall. It seems likely that the cell secreted chelates only during long term culturing in the presence of zinc. However, no significant difference was observed for all the organic constituents (one way analysis of variance) ($P<0.05$) with different concentrations of four heavy metals.

Conclusion

In conclusion, there was no observed inhibitory effect, when the studied species was exposed to increased concentrations of different heavy metals. It was found more sensitive towards nickel followed by copper, zinc and cadmium. This tolerance could have resulted from the natural selection in the sewage irrigated soil. Therefore, it may act as an ecological indicator and may facilitate the management of sewage irrigated soils.

References

Andersen, R.A., Kawachi, M., 2005. *Traditional microalgae isolation techniques*. In Andersen, R. A. (ed.), Algal Culturing Techniques. Elsevier Academic Press, Tokyo, 83–100.

Angeles, B. M., Cruz, C., Martins-Loucao, M. A. and Cerda, A., 1993. Nitrate reductase activity in wheat seedlings as affected by NO3-/NH4+ration and salinity, ***J. Plant Physiol.*** 142(5), 531-536.

Babu, N. G., Sharma, P. A., Attitalla, I. H., Murthy, S. D.S., 2010. Effect of Selected Heavy Metal Ions on the Photosynthetic Electron Transport and Energy Transfer in the Thylakoid Membrane of the Cyanobacterium, Spirulina platensis, ***Academic Journal of Plant Sciences***, 3(1), 46-49.

Bala, K., Thanasekaran, K., 2011. Metal tolerance of an indigenous cyanobacterial strain, Lyngbya contorta, ***International Biodeterioration & Biodegradation***. 65(8),1128–1132.

Bityutskiim, N. P., 1999. *Mikroelementy i rastenie (Micronutrients and Plant*), St. Petersburg S.-Pb. Gos. Univ.

Blindauer, C.A., 2011. Bacterial metallothioneins: Past, present, and questions for the future***. J. Biol. Inorg. Chem***. 16, 1011–1024.

Campbell, P. M., Smith, G. D., 1986. Transport and accumulation of nickel ions in the cyanobacterium Anabaena cylindrical. ***Arch. Biochem. Biophys***. 244, 470-477.

Carrieri, D., Gennady, A., Amaya, M., Garcia, C., Donald, A., Bryant, G., Charles Dismukes, 2008. Renewable hydrogen production by cyanobacteria: Nickel requirements for optimal hydrogenase activity. ***Int. J. Hyd. Ener***. 33(8), 2014-2022.

Chauvat, C., and Chauvat, F., 2015. Review Responses to Oxidative and Heavy Metal Stresses in Cyanobacteria: Recent Advances. ***Int. J. Mol. Sci***. 16, 871-886.

Cid, A., Herrero, C., Torres, E., Abalde, J., 1995. Copper toxicity on the marine microalga Phaeodactylum tricornutum: effects on photosynthesis and related parameters. ***Aqua. Toxicol***. 31(2), 165-174.

Debelius, B., Forja, J. M., Delvalls, A., Lubian, L. M., 2009. Toxicity and bioaccumulation of copper and lead in five marine microalgae. ***Ecotoxicol. Environ. Safety***. 72, 1503-1513.

Desikachary, T.V., 1959. *Cyanophyta, Indian Council of Agricultural Research*, New Delhi, India.

Dodor, D. E., Tabatabai, M. A., 2003. Effect of cropping systems on phosphatases in soils. ***J. Plant Nutr.Soil Sci***. 166, 7-13.

Dubey, R. S., 1997. *Photosynthesis in plants under stressful conditions*. - In: Pessarakli, M. (ed.):Handbook of Photosynthesis, M. Dekker, New York, 859-876.

Fargasova, A., 1999. The green alga Scenedesmus quadricauda– a subject for the study of inhibitory effects of Cd, Cu, Zn, Pb and Fe***. Biologia***. 54,393-398.

Franklin, N. M., Stauber, J. L., Lim, R. P., 2001. Development of flow cytometry-based algal bioassays for assessing toxicity of copper in natural waters Environ. ***Toxicol. Chem***. 20, 160-170.

Goudey, J. S., 1987. Modeling the inhibitory effects of metals on phytoplankton growth ***Aqua. Toxicol***. 10, 265-278.

Healy, F. P. 1985. Induction effects of light and nutrient limitation on the growth of Synchoccus linearis(Cyanophyceae). ***J. Phycol***. 21, 134-148.

Zoua, H.X., Pangb, Q. Y., Zhanb, A. Q., Linb, L.D., Lia, N., Yana, X. F., 2015. Excess copper induced proteomic changes in the marine brown algae Sargassum fusiforme. ***Ecotoxicology and Environmental Safety***. 111, 271–280.

Imlay, J.A., 2014. The Mismetallation of enzymes during oxidative stress. ***J. Biol. Chem***. 289, 28121–28128

Jin, Z., Liuyan, Y., Wen-Xiong, W., 2009. Cadmium and zinc uptake and toxicity in two strains of Microcystis aeruginosa predicted by metal free ion activity and intracellular concentration. ***Aqua.Toxicol***. 91(3), 212-220.

Lamaia, C., Kruatrachuea, M., Pokethitiyooka, P., Upathamb, E. S., Soonthornsarathoola, V., 2005. Toxicity and accumulation of lead and cadmium in the filamentous green alga Cladophora fracta: A laboratory study. ***Sci. Asia***. 31, 121-127.

Lowry, O. M., Rosenbrough, N. J., Farr, L. A., Randall, R. J., 1951. Protein measurements with the folin phenol reagent. ***J. biol. Chem***. 193, 265-275.

Machado, M. D., Lopes, A. R., Soares E.V., 2015. Responses of the alga *Pseudokirchneriella subcapitata* to long-term exposure to metal stress. ***J. Hazardous materials***. 296, 82-92.

Mackinney, G., 1941. Absorption of light by chlorophyll solutions. ***J. biol. Chem***. 140, 315-322.

Mamboya, F. A., Pratap, H. B., Mtolera, M., Bjork, M., 1999. *The effect of copper on daily growth rate and photosynthetic efficiency of the brown macroalgae Padina boegesenni*. :In Richmond MD, Francis J(eds) Proceedings of the conference on advances on marine sciences. Tanzania. 185-192.

McGinna, P. J., Dickinsona, K. E., Parka, K.C., Whitneya, C. G., MacQuarriea, S. P., Blackb, F. J., Frigonc, J., Guiotc, S. R., O'Learya, S. J. B., 2012. Assessment of the bioenergy and bioremediation potentials of the microalga Scenedesmus sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode. ***Algal Research.*** 1(2), 155-165.

Morand, L. Z., Cheng, R. H., Krogmann, D. W., Ho, K. K., 1991. *Soluble electron transfer catalysts of cyanobacteria*. In:D.A. Bryant(ed.), The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands. 381-407.

Mota, R., Pereira, S. B., Meazzini, M., Fernandes, R., Santos, A., Evans, C. A., Philippis, R.D., Wright, P. C., Tamagnini P., 2015. Effects of heavy metals on *Cyanothece* sp. CCY 0110 growth, extracellular polymeric substances (EPS) production, ultrastructure and protein profiles. ***Journal of proteomics***. 120, 75-94.

Prasad, A. S., 1995. Zinc: An Overview. ***Nutrition***. 11, 93-99.

Rai, L. C., Raizada, M., Mallick, N., Husaini, Y., Singh, A. K., Dubey, S. K., 1990. Effect of four heavy metals on the biology of Nostoc muscorum. ***Biol. Met***. 2(4), 229-234.

Rana, L., Chhikara, S., 2011. Cyanobacterial composition under sewage irrigated and tubewell irrigated agroecosystems in Rohtak city, Haryana, India. ***Environ. We Int. J. Sci. Tech.*** 6(1), 39-48.

Shashirekha, V., Sridharan, M.R., Swamy, M., 2015. Biochemical response of cyanobacterial species to trivalent chromium stress. ***Algal Research***. 12, 421–430

Shavyrina, O. B., Gapochka, L. D., Azovskii, A. I., 2001. Development of tolerance for copper in cyanobacteria repeatedly exposed to its toxic effect. ***Biol. Bull***. 28(2), 183-187.

Shukla, M. K., Tripathi, R. D., Sharma, N., Dwivedi, S., Mishra, S., Singh, S., Shukla, O. P., Rai, U. N., 2009. Responses of cyanobacterium Anabaena doliolum during nickel stress. ***J. Environ. Biol***. 30(5), 871-876.

Spiro, R. G., 1966. Analysis of sugars found in glycoproteins. ***Methods in Enzymol***. 8, 3-26.

Stanier, R. Y., Kunisawa, R., Mandal, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue green-algae(Order: Chrococcales). ***Bacteriol. Rev***. 35, 171-305.

Stauber, J. L., Florence, T. M., 1987. The mechanism of toxicity of ionic copper and copper complexes to algae. ***Mar. Biol***. 94, 511-519.

Tam, N. F. Y., Wong, J. P. K., Wong, Y. S., 2001. Repeated use of two Chlorella species, C. vulgaris and WW1 for cyclic nickel biosorption. ***Environ. Poll***. 114(1), 85-92.

Udel'nova, T. M., Yagodin, B. A., 1993. Zinc in the Life of Plant, Animal, and Human. Usp. Sovrem.***Biol***. 113, 176-189.

Visviki, I., Rachlin, J. W., 1991. The toxic actions and interactions of copper and cadmium to marine alga Dunaliella minuta in both acute and chronic exposure. ***Arch Environ Contam Toxicol.*** 2, 271-275.

Waldron, K.J., Robinson, N.J., 2009. How do bacterial cells ensure that metalloproteins get the correct metal? ***Nat. Rev. Microbiol***. 7, 25–35

Wong, S. L., Nakamoto, L., Wainwright, J. F., 1994. Identification of toxic metals in affected algal cells in assays of wastewaters**. *J. Appl. Phycol***. 6, 405-414.

Xiaolei-Jin, D. J. K., Nalewajko, C., 1996. Nickel uptake and release in nickel-resistant and –sensitive strains of Scenedesmus Acutus F. Alternans (Chlorophyceae). ***Environ. Exp. Bot***. 36(4), 401-411.