

## ***Chlorella vulgaris* -A Potential Heavy Metal Bioremediator**

RENU<sup>1</sup>, SUNITA DUDI<sup>2</sup> AND S. SUNDARAMOORTHY<sup>3\*</sup>

Department of Botany, Centre of Advanced Study, Jai Narain Vyas University, Jodhpur 342001, India

Emails: <sup>1</sup> [dhenwalrenu@gmail.com](mailto:dhenwalrenu@gmail.com); <sup>2</sup> [suniita9dudi@gmail.com](mailto:suniita9dudi@gmail.com); <sup>3</sup> [jnvusundar@rediffmail.com](mailto:jnvusundar@rediffmail.com)

\* Corresponding author

### ABSTRACT

The capacity of the alga *Chlorella vulgaris* to remove Cadmium, Nickel and Zinc from waste waters and its kinetics were assessed in long-term experiments. Metal concentration was selected on the basis of individual metal tolerance limit of the alga. Results revealed that adsorption was comparatively more than absorption for all three heavy metals. Concentration (x) and adsorption (y) or absorption (y) followed parabolic path for Cd<sup>+2</sup> and Ni<sup>+2</sup>; whereas for Zn<sup>+2</sup> the relationship was linear (adsorption) and parabolic (absorption). *C. vulgaris* exhibits great efficiency to combat Cd<sup>+2</sup>, Ni<sup>+2</sup> and Zn<sup>+2</sup> stresses with the maximum accumulation factor of 4.2, 2.08 and 2.66, respectively. Metal tolerance efficiency and higher uptake rates make it a significant algal species for bioremediation purpose, more specifically for Cadmium.

Key Words: *Chlorella vulgaris*, Bioremediation, Cd<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup>.

### INTRODUCTION

Environmental contamination by heavy metals is a growing global problem, which is directly related to anthropogenic actions. For this motive, many techniques for environmental remediation of heavy metals are being studied (Ofer et al., 2003, Bayramoğlu et al. 2006, Rai 2008, 2010, Rawat et al. 2011). The presence of heavy metal ions such as lead, copper, cadmium, zinc and nickel as common contaminants in industrial wastewater leads to pollution of natural environment (Connell et al. 2008, Tsekova et al. 2010). Residual nutrients and heavy metal ions in domestic and agro-industrial wastewaters are also responsible for the pollution of rivers, lakes, and seas (de-Bashan and Bashan, 2010). Biosorption and accumulation of heavy metal ions in aquatic food chains can pass to humans causing major health problems (Chary et al. 2008).

Various methods are available for the removal and management of heavy metals, which involve technical inputs (Rajendran et al. 2003). Metal remediation strategies using microorganisms can minimize the bioavailability and bio toxicity of heavy metals (Lloyd et

al. 2001, Gadd 2000). Bioremediation has emerged as potential alternative tool to achieve the goal to invent a new separation method that reduces heavy metal concentration to environmentally acceptable levels at affordable cost (Gong et al. 2005).

The ability of algae to remove heavy metals from aqueous solution has been known from some decades (Harish et al. 2007). Algae possess the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water (Megharaja et al. 2003, Shamsuddoha et al. 2006). This study aims to determine the use of inexpensive natural material, algal cells of *Chlorella vulgaris* Beijerinck, a unicellular freshwater alga belongs to class Trebouxiophyceae, family Chlorellaceae.

### MATERIAL AND METHOD

#### **Experimental Condition**

The microorganism used in the present study *C. vulgaris*, was collected from Jojari river near Jodhpur. Pure

culture of algae was established by repeated isolation and selection of different colonies in Petri plates. These axenic cultures of algae were grown in growth room under continuous light, illuminated with cool fluorescent tubes at  $28\pm 2$  temperature. All the experiments were conducted in triplicate at same culture conditions. Bold Basal, BG-11, CHU-10, Hughes and Kertz & Mayer media were assessed and we found that BG-11 media supported better growth of *C. vulgaris*, and hence the same is used for all experiments.

### Adsorption / Adsorption Kinetics

One ml of algal cells with protein value  $100 \mu\text{g mL}^{-1}$  were withdrawn from exponentially growing homogenous culture of algae and inoculated in 100 mL freshly prepared BG-11 medium containing different heavy metal concentrations (Table 1).

Table 1. Treatment of different heavy metal ( $\text{mg l}^{-1}$ ) used for adsorption/adsorption kinetics

Metal ion	Concentrations used ( $\text{mg L}^{-1}$ )					
$\text{Cd}^{+2}$	0	4	8	12	16	20
$\text{Ni}^{+2}$	0	3	6	9	12	15
$\text{Zn}^{+2}$	0	4	6	8	10	12

After 15<sup>th</sup> and 30<sup>th</sup> day of inoculation, 10 mL of algal sample was harvested from homogenous culture, centrifuged (4000 G, 15 minutes) and supernatant media was separated. The algal sample in the sediment was mixed with 10 mL of EDTA ( $10 \mu\text{M}$ ) solution and gently shaken. Samples were once again centrifuged (4000G, 15 minutes). Supernatant EDTA was taken out for measuring the adsorbed ionic concentration. All three parts i.e. media, EDTA and algal pellets from each sample were dried, digested with double acid [ $\text{HNO}_3:\text{HClO}_4$ ] mixture [10:1, v/v] in boiling water bath for 1 h. After cooling sample were diluted to 25 mL with triple glass distilled water and analysed for heavy metal level by atomic absorption spectrometer (Spectrum SP-AA 5000). Accumulation factor was assessed as the ratio of concentration in the algae in relation to its surroundings. Observed variations of absorption or adsorption were assessed considering the initial concentration (Horizontal) and duration of growth (Vertical) as contributing

factors using strip-plot design (Gomez and Gomez). Cause effect relations were assessed (Snedecor and Cochran 1967).

## RESULTS

### Cadmium

*C. vulgaris* is not only a tolerant species but also found to be hyper-accumulator of metal  $\text{Cd}^{+2}$ . Adsorption was maximum ( $41.05 \mu\text{g mL}^{-1}$ ) at  $20 \text{ mg L}^{-1}$  during 30<sup>th</sup> day of experiment. Adsorption increased with the passing of time. Concentration in the media (X) was the only factor that contributed ( $p > 0.01$ ) for the observed variations. Concentration in the media (X) and adsorption (Y) were related parabolically for 15<sup>th</sup> and 30<sup>th</sup> day of growth ( $Y = -6.8267 + 6.8874X - 0.3631X^2$ ;  $R^2 = 0.99$ ;  $Y = -9.2625 + 8.824X - 0.0302X^2$ ;  $R^2 = 0.90$  respectively). Adsorption was maximum ( $22.933 \mu\text{g mL}^{-1}$ ) at  $8 \text{ mg L}^{-1}$  during 30<sup>th</sup> day of experiment. Adsorption increased with passing of days.  $\text{Cd}^{+2}$  concentration in media (X), days and their interactions contributed significantly ( $p > 0.01$ ) to the observed variations. Concentration in the media (X) and adsorption (Y) were related parabolically for 15<sup>th</sup> and 30<sup>th</sup> day of the growth ( $Y = 0.5917 + 0.3262X + 0.1857X^2$ ;  $R^2 = 0.71$ ;  $Y = -9.535 + 14.521X - 1.6762X^2$ ;  $R^2 = 0.71$  respectively; Figure 1). Maximum (4.2) AF value was observed on 30<sup>th</sup> day at  $4 \text{ mg L}^{-1}$  metal concentration; with increase in  $\text{Cd}^{+2}$  concentration in the media, AF value increased during the later stage of growth exhibiting the adaptive mechanism of tolerance (Figure 1).

### Nickel

*C. vulgaris* is not only a tolerant species but also found to be hyper-accumulator of  $\text{Ni}^{+2}$ . Adsorption was maximum ( $11.125 \mu\text{g mL}^{-1}$ ) at  $15 \text{ mg L}^{-1}$  during 30<sup>th</sup> day of experiment and it increased with passing of days (Figure 2). Concentration in the media (X) and adsorption (Y) were related significantly and followed a parabolic path for 15<sup>th</sup> and 30<sup>th</sup> day of growth ( $Y = -5.9283 + 5.5468X - 0.4732X^2$ ;  $R^2 = 0.93$ ;  $Y = -3.1375 + 3.645X - 0.1903X^2$ ;  $R^2 = 0.87$ , respectively).  $\text{Ni}^{+2}$  adsorption was maximum ( $10.90 \mu\text{g mL}^{-1}$ ) on 30<sup>th</sup> day at  $6 \text{ mg L}^{-1}$   $\text{Ni}^{+2}$  concentrations. Metal concentration in the media (X) and adsorption (Y) were related significantly and followed parabolic path for 15<sup>th</sup> and 30<sup>th</sup> day of growth ( $Y = -4.042 + 5.481X - 0.681X^2$ ;  $R^2 = 0.69$ ;  $Y = -4.2242 + 7.319X - 1.021X^2$ ;  $R^2 = 0.79$ , respectively). Maximum (2.08) AF value

was recorded on 30<sup>th</sup> day at 3 mg L<sup>-1</sup> metal concentration; with increase in Ni<sup>2+</sup> concentration in the media AF value increased during the later stage of growth exhibi-

ting the adaptive mechanism of tolerance for Ni<sup>2+</sup> similar to that of Cd<sup>2+</sup> (Figure 2).

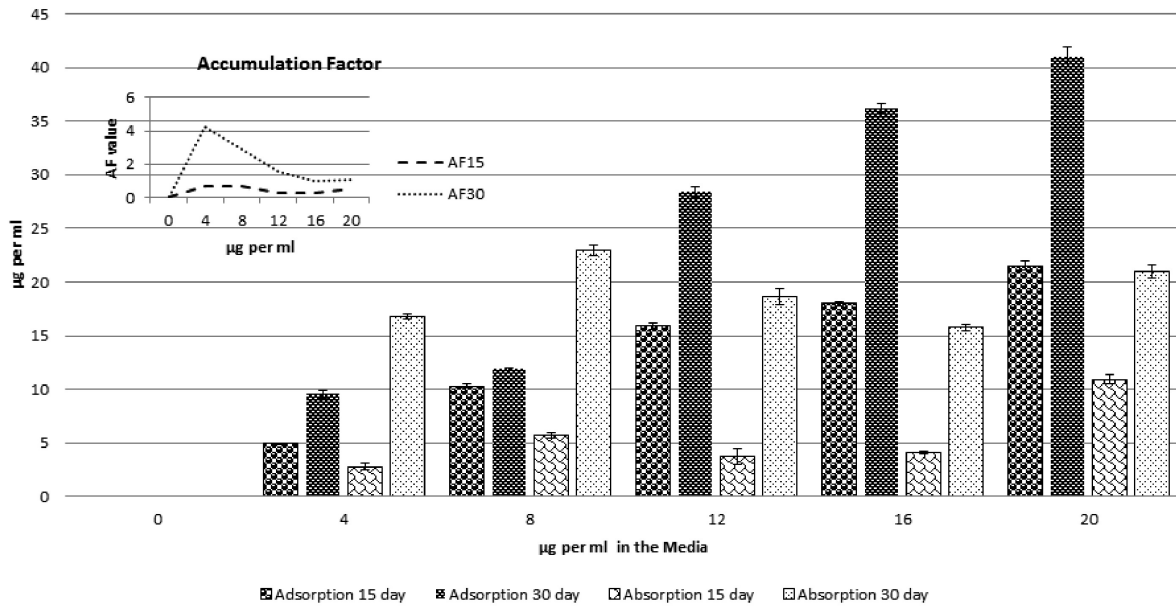


Figure 1. Adsorption/absorption kinetics of *Chlorella vulgaris* for Cadmium. Inset shows Accumulation Factor after 15 and 30 days.

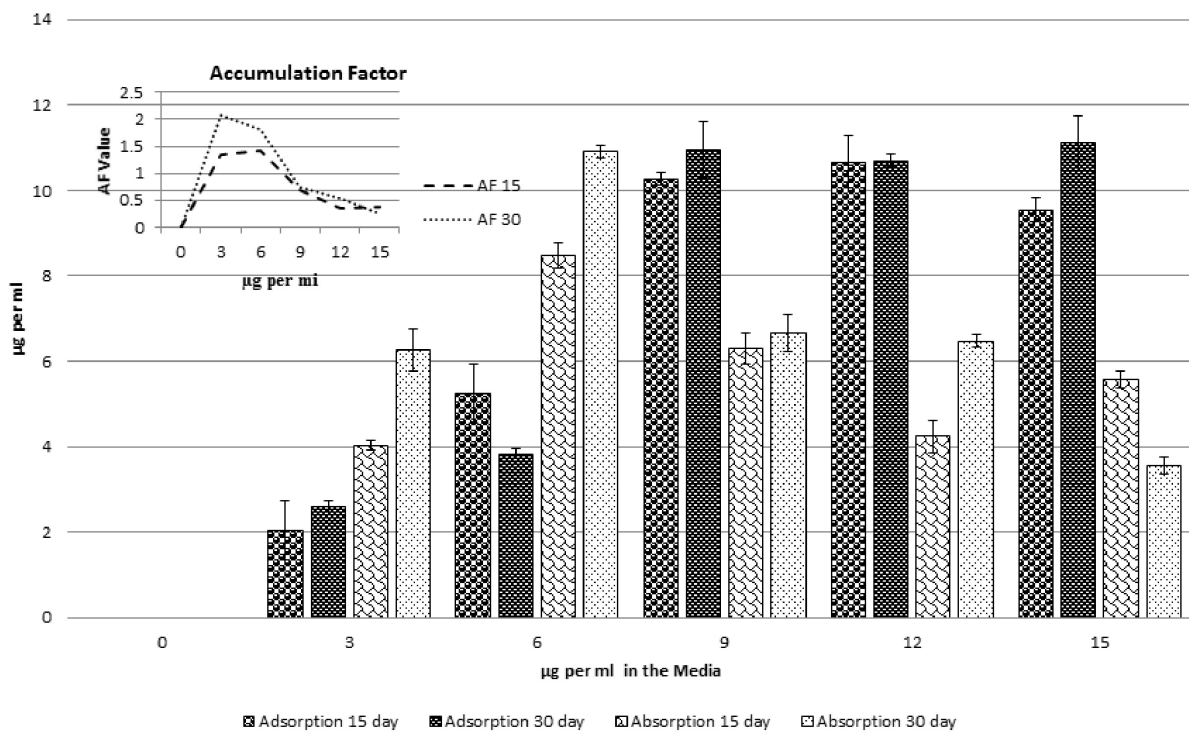


Figure 2. Adsorption/absorption kinetics of *Chlorella vulgaris* for Nickel. Inset shows Accumulation Factor after 15 and 30 days.

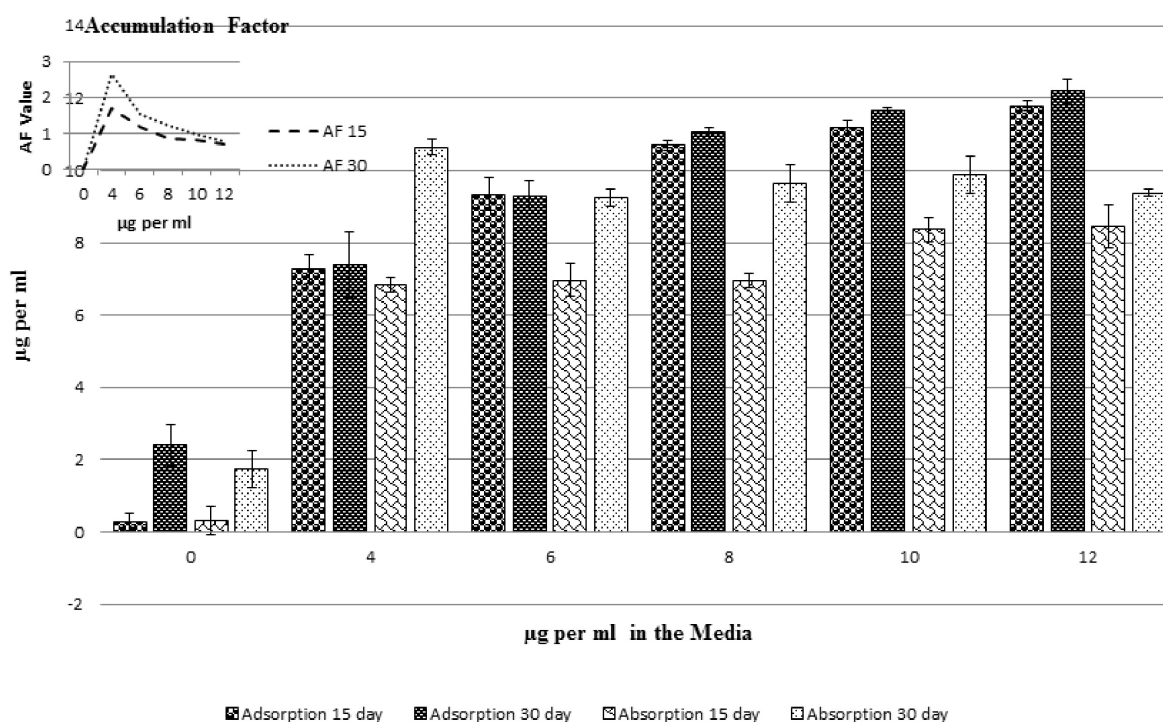


Figure 3. Adsorption/absorption kinetics of *Chlorella vulgaris* for Zinc. Inset shows Accumulation Factor after 15 and 30 days.

## Zinc

The algae was able to remove almost 93% of metal for initial concentrations 4 and 6 mg L<sup>-1</sup>, the removal remained at an average of 88% over 8,10 and 12 mg L<sup>-1</sup> concentration. Maximum (12.2 µg mL<sup>-1</sup>) adsorption was recorded on 30<sup>th</sup> day at 12 mg L<sup>-1</sup> concentration of metal. Analysis of variance revealed that metal concentration (X) was the only factor responsible for the observed variation and revealed linear relationship for 15<sup>th</sup> and 30<sup>th</sup> day of the growth ( $Y = -5.03 + 6.8139X - 0.6851X^2$ ;  $R^2 = 0.96$  and  $Y = -1.9217 + 5.2407X - 0.4893X^2$ ;  $R^2 = 0.98$  respectively). Maximum (10.64 µg mL<sup>-1</sup>) absorption was recorded at 4 mg L<sup>-1</sup> concentration and it decreased with increase in metal concentration. Zn<sup>+2</sup> concentration, days of growth and their interaction are contributing ( $p > 0.01$ ) factors for variations in absorption. Concentration in the media (X) and absorption (Y) revealed parabolic relationship for 15<sup>th</sup> and 30<sup>th</sup> day of growth ( $Y = -2.725 + 4.6762X - 0.4833X^2$ ;  $R^2 = 0.83$ ;  $Y = -1.9592 + 6.1019X - 0.7234X^2$ ;  $R^2 = 0.75$ , respectively). Maximum (2.66) AF was noted on 30<sup>th</sup> day at 4 mg L<sup>-1</sup> concentration. Similar to Cd<sup>2+</sup> and Ni<sup>2+</sup>, for Zn<sup>2+</sup> too this alga exhibited adaptive mechanism of tolerance (Figure 3).

## DISCUSSION

The efficiency of *C. vulgaris*, *Spirulina maxima*, and *Synechocystis* species for removal of Cu<sup>2+</sup> and Zn<sup>2+</sup> was assessed by Chan et al. (2014) who reported removal efficiency up to 81.7% for Cu<sup>2+</sup> and 94.1% for Zn<sup>2+</sup> when treated in ppb ranges, whereas in the present study the removal efficiencies were assessed in ppm ranges and were recorded to be comparatively higher. Şentürk and Yıldız (2016) assessed the biosorption of heavy metal in addition to macro elements and found 46% removal of Ni<sup>2+</sup> in 10ppm solution. In the present study the removal was 54% at 12 ppm, revealing the higher efficient strain of this species in the desert environment. Hyper-accumulation capacity (90%) of the algae *C. vulgaris* as compared to other studies for Cd<sup>2+</sup> may be the result of different environmental conditions as suggested by Monteiro et al. (2010). Increased absorption on initial stage followed by a decrease in Ni<sup>2+</sup> absorption was found in present study and similar results were also reported for this metal earlier (Sharma 2010). Higher Zn<sup>2+</sup> removal capacity (93%) of *Chlorella vulgaris* was also supported by the work done on *Chlorella marina* (97%; Kumar et al. 2013). Comparatively higher level of adsorption than absorption in present study and its

comparison with other studies suggests that different species may have different mechanisms to deal with excess amount of heavy metals. Some may respond by absorption (intra-cellular) strategy (Axtell et al. 2003) while other may respond with adsorption (extra-cellular; Anand et al. 2006).

*Chlorella vulgaris* collected from Indian desert revealed very high efficiency than reported earlier (Travieso et al. 1999, Inthorn et al. 2001, 2002, Dwivedi 2012). Earlier work done on unicellular Thar desert algae such as *Chlorococcum humicolum* (Harish et al. 2007) and *Treuboxia humicola* (Sharma 2010) revealed low efficiency of the algae i.e. only 60% of metal was removed from media, while *Chlorella vulgaris* is more efficient as it removed 90% of the metal from media. These results revealed that *Chlorella vulgaris* is not only a tolerant species but also a hyper-accumulator of the three selected metals ( $\text{Cd}^{+2}$ ,  $\text{Ni}^{+2}$  and  $\text{Zn}^{+2}$ ) and it can be used for bioremediation of even highly polluted or disturbed area.

#### ACKNOWLEDGEMENTS

We are grateful to the Professor and Head, Department of Botany Jai Narain Vyas University, Jodhpur, for providing laboratory facilities and Council of Scientific and Industrial Research (CSIR), New Delhi, for financial assistance in the form of fellowship (F.No: 09/098 (0104)/2011-EMR-1). Instrumentation facilities of UGC-CAS (University Grants Commission - Centre of Advanced Study) and DST-FIST (Department of Science & Technology - Fund for Improvement of S&T Infrastructure in Universities and Higher Educational Institutions) are gratefully acknowledged. Renu and Sunita Dudi carried out the experimental work and wrote the manuscript; Sundaramoorthy designed the experimental study, guided the research and critically reviewed the manuscript. Authors further declare no conflict of interest.

#### REFERENCES:

- Alison.C.; Salsali, H. and McBean E. 2014. Heavy metal removal (copper and zinc) in secondary effluents from waste water treatment plants by microalgae. *ACS Sustainable Chemistry and Engineering* 2:130-137.
- Bayramoğlu, G.; Tuzun, I.; Celik, G.; Yilmaz, M. and Arica, M.Y. 2006. Biosorption of mercury (II), cadmium (II) and lead (II) ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads. *International Journal of Mineral Processing* 81: 35-43.
- Chary, S.N.; Kamala, C.T. and Samuel Suman Raj, D. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety* 69: 513- 524.
- De-Bashan, L.E. and Bashan, Y. 2010. Immobilized microalgae for removing pollutant: review of practical aspects. *Bioresource Technology* 101: 611- 627.
- Dwivedi, S. 2012. Bioremediation of heavy metal by algae: current and future perspective. *Journal of Advanced Laboratory Research in Biology* 3: 195-199.
- Gadd, G.M. 2000. Bioremediation potential of metal mobilization and immobilization. *Current Opinion in Biotechnology* 11: 271.
- Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research*. John Wiley, New York, 680 pages.
- Gong, R.; Sun, Y.; Chen, J.; Liu, H. and Yang, C. 2005. Effect of chemical modification on dye adsorption capacity of peanut hull. *Dyes and Pigments* 67: 175-181.
- Harish; Sundaramoorthy, S.; Kumar, D. and Vijapurkar, S.G. 2007. A new Chlorophycean nickel hyperaccumulator. *Bioresource Technology* 99: 3930-3934.
- Inthorn, D. 2001. Removal of heavy metal by using microalgae. *Microorganisms in Environmental Biotechnology* 310:111-169.
- Inthorna, D.; Sidititona, N.; Silapanuntakula, S. and Incharoen-sakdib, A. 2002. Sorption of mercury, cadmium and lead by microalgae. *Science Asia* 28: 253-261.
- Kumar, M.; Sharma, M.P. and Dwivedi, G. 2013. Algae oil as future energy source in Indian perspective. *International Journal of Renewable Energy Research* 4: 913-921.
- Lloyd, J.R. and Lovley, D.R. 2001. Microbial detoxification of metals and radionuclides. *Current Opinion in Biotechnology* 12: 253.
- Megharaj, M.; Avudainayagam, S. and Naidu, R. 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. *Current Microbiology* 47:51-54.
- Monteiro, C.; Castro, P.L. and Malcata, F.X. 2010. Cadmium removal by two strains of *Desmodesmus pleiomorphus* cells. *Water Air Soil Pollution* 208: 17- 27.
- O'Connell, D.W.; Birkinshaw, C. and O'Dwyer, T.F. 2008. Heavy metal adsorbents prepared from the modification of cellulose: a review. *Bioresource Technology* 99: 6709-6724.
- Ofer, R.; Yerachmiel, A. and Shmuel, Y. 2003. Marine macroalgae as biosorbents for cadmium and nickel in water. *Water Environment Research* 75: 246-253.
- Ozaki, T.; Kimura, T.; Ohnuki, T.; Yoshida, Z. and Francis, A. 2003. Association mechanisms of Europium(III) and Curium(III) with *Chlorella vulgaris*. *Environmental Toxicology and Chemistry* 22: 2800-2805.
- Rai, P.K. 2008. Heavy-metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: An eco-sustainable approach. *International Journal of Phytoremediation* 10: 133-160.
- Rai, P.K. 2010. Phytoremediation of heavy metals in a tropical impoundment of industrial region. *Environmental Monitoring and Assessment* 165: 529-537.

- Rajendran, P.; Muthukrishnan, J. and Gunasekaran, P. 2003. Microbes in heavy metal remediation. *International Journal of Experimental Biology* 41: 935-944.
- Rawat, I.; Kumar, R.R.; Mutanda, T. and Bux, F. 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy* 88: 3411-3424.
- Senturk T. and Yildiz. S. 2016. Adsorbent effect of *Chlorella vulgaris* and *Scenedesmus* sp. (Chlorophyta) for the removal of some heavy metals and nutrients. *Turkish journal of Biochemistry* 41: 87-95.
- Shamsuddoha, A.S.M.; Bulbul, A. and Huq, S.M.I. 2006. Accumulation of arsenic in green algae and its subsequent transfer to the soil-plant system. *Bangladesh Journal of Microbiology* 22: 148-151.
- Sharma, D. 2010. Bioremediation of Cadmium, Copper, Nickel and Zinc. Ph.D. Thesis. Jai Narain Vyas University Jodhpur, 152 pages.
- Snedecor, G.W. and Cochran, W.G. 1967. *Statistical Methods*. Oxford & IBH Publishing, New Delhi. 593 pages.
- Travieso, L.; Cañizares, R.O.; Borja, R.; Benítez, F.; Domínguez, A.R. and Dupeyrón, R. 1999. Heavy metal removal by microalgae. *Bulletin of Environmental Contamination and Toxicology* 62:144-151
- Tsekova, K.; Todorova, D. and Ganeva, S. 2010. Removal of heavy metals from industrial waste water by free and immobilized cells of *Aspergillus niger*. *Biodeterioration and Biodegradation* 64: 447- 451.

*Received 13 December 2016*

*Accepted 16 March 2017*