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Bioremoval of Some Metals by Living Algae *Spirogyra sp.* and *Spirullina sp.* from aqueous solution

Mane, P. C.* and Bhosle, A. B.

School Of Earth Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, 431606, India

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ABSTRACT: Algae have been proven efficient biological vectors for heavy metal uptake. In order to further study their biosorption potential, two strains *Spirogyra sp*. and *Spirulina sp*. have been studied under different initial metal concentrations. In recent years, the biosorption processes have been studied extensively using microbial biomass as biosorbent for heavy metal ions removal. In these studies, metal ions removal abilities of various species of bacteria, algae, fungi and yeasts were investigated.The discharge of heavy metals into aquatic ecosystems has become a matter of concern over the last few decades. The biosorption of six metal ions from artificial wastewaters containing single metal ions was investigated in batch experiments.One strain of algae collected from National Chemical Laboratory (NCL), and one strain from natural area tested for Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Selenium (Se) and Zinc (Zn) removal from aqueous solution. The concentration of all the six metal ion species in the absorption medium was varied between 5 and 25 mg/ L. The residual concentration of metals from the absorption medium was determined by using UV spectrophotometer.From the tested algae after seven days of incubation period, the highest percent bioremoval by *Spirogyra sp*. for Cr (98.23%), Cu (89.6%), Fe (99.73%), Mn (99.6%), Se (98.16%) and Zn (81.53%) respectively. The same by *Spirulina sp*. for Cr (98.3%), Cu (81.2%), Fe (98.93%), Mn (99.73%), Se (98.83%) and Zn (79%) respectively at 5 mg/L initial metal concentration.

Key words: Algal biomass, Percent bioremoval, Heavy metal ions, Aqueous solution, UV spectrophotometer

INTRODUCTION

Heavy metals are one of the most important cause of pollution, both in water and in the soil, and increasing levels of heavy metals in the environment are causing mounting concern in public opinion. Because of their chemical characteristics, heavy metals cannot be biodegraded by micro-organisms into non-toxic, eventually assimilable or volatile compounds, as is frequently the case with organic pollutants, but they remain in the environment, changing from one chemical state to another, and eventually accumulating in the food chain. For this reason, chemical and physicochemical methods have traditionally been utilized to remove heavy metals from polluted water bodies but such methods have a number of disadvantages: they are not so efficient, in particular at low metal concentrations, and they are expensive (Volesky, 2001). Now a day, great interest has been given to a new technique, biosorption, which exploits the cell envelopes of microorganisms to remove metals from water solutions (Wilde *et al.*2006,). Biosorption is the process of adsorption by either living microbial biomass or dead microbial biomass and it offers some important advantages such as (i) low cost, (ii) high efficiency in removing metals even from very dilute solutions, (iii) the possibility of recovering the valuable metals adsorbed by the biosorbent, and (iv) a lower amount of metal containing biological sludges that have to be disposed of after treatment. Microbial cells can be viewed as natural ion-exchange material because they have many anionic groups on their cell surface (Kratochvil and Volesky, 1998) and this enables them to remove metal ions efficiently, mainly by means of an ion-exchange mechanism (Crist *et al.*, 1994; Schiewer and Volesky, 1996).

Several authors have been searching for alternative and better performing remediation strategies pertaining to toxic heavy metals, because conventional physico-chemical methods (e.g. precipitation and ion exchange) are not so effective; in addition, they are rather expensive (Bayramog and Arica, 2008; Doshi *et al.*, 2007), especially when the metal levels are of the ppm order of magnitude (Gupta and Rsatogi, 2008a; Zhang *et al.*, 1998). A more feasible approach depends on the metal binding and uptake capacities of living materials, which mainly include

*Corresponding author E-mail:pramodmane30@yahoo.co.in

microalgae (Fraile *et al.*, 2005; Leborans and Novillo, 1992; Rollemberg *et al.*, 1999; Solisio *et al.*, 2008). Use of microbial biomass to remove toxic heavy metals has become relatively popular, owing to its high adsorbing capacity and low cost. Additionally, metals removed by adsorption onto the cell surface, may be successfully recovered, after desorption brought about by chemical agents. (Costa and Franca, 1998) reported that a 10.0 g/l EDTA solution could totally recover the Cd previously removed by adsorption onto the cell walls of the microalga *Tetraselmis chuii*, whereas (Gupta and Rastogi, 2008b) obtained 85 and 80% recoveries of Cd ions from *Oedogonium sp.* biomass, when HCl or EDTA are used as desorbing agents, respectively.

Many investigations have been carried out for biosorption of heavy metals by the other important divisions of algae Green algae (*Chlorophyta*) and Red algae (*Rhodophyta*) (Handy, 2000). The effects of several factors such as *pH*, initial metal concentration and contact time were analyzed. Green algae *Cladophora fascicularis* was to be an effective and economical biosorbent material for the removal of heavy metal ions (Dend *et al.,* 2006).

In the present study, the biosorption of heavy metal ions such as Cr, Cu, Fe, Mn, Se and Zn by fresh water algae *Spirogyra sp.* and *Spirulina sp.* was investigated. The effect of initial metal concentration was studied.

MATERIALS & METHODS

The starting culture was obtained from the National Chemical Laboratory (NCL), Pune. *Spirulina sp.* in log phase used in the experiment was inoculated in the Spirulina medium and the *Spirogyra sp.* isolates were inoculated in modified Bold's basal medium, for mass multiplication. The medium was sterilized by autoclaving at 121^{o}C for 15 minutes. Medium was stored at 4^{o}C until inoculated. Culture was grown in liquid media in 2 L glass Erlenmeyer flasks and incubated at 25^{o}C in a growth chamber with a light and dark cycle of 8 h and 16 h and 3000 – 3500 lux, light intensity provided by cool white day light fluorescent tube lamps. Algal metal bioremoval was assayed by exposing the strains of *Spirulina sp.* and *Spirogyra sp.* to various metals concentrations, in triplicate. Defined aliquots of each metal stock solution were added to 90 mL of algal culture in a 250 mL conical flask separately, in order to obtain the desired final concentrations i.e. 5, 10, 15, 20 and 25 mg/L. The conical flasks were stoppered with a cotton wool bung and incubated for seven days. The total concentration of all the metals removed by micro algal cells was calculated as the difference between the initial and the remaining metal concentration in the supernatant. Replicated blank controls, containing culture medium plus metal at each concentration tested, were considered; all the metal concentration remained stable in those flasks for the time frame of each experiment, so no redox reaction or adsorption onto the vessel walls took place to any measurable extent.

The bioremoval efficiency (R) of the algae was calculated by the following formula.

$$R\,(\%) = \frac{(Ci - Ce)}{Ci} 100$$

Where, R = Bioremoval efficiency (%); C_i = initial concentration of metals in aqueous solution (mg/L); C_e = equilibrium concentration of metals in aqueous solution (mg/L).

After completion of incubation period, 10 mL sample was drawn from the flask, centrifuged at 5000 rpm for 15 minutes and then the supernatant was filtered through filter paper. The filtrate was analyzed for residual metal in the solution by UV Spectrophotometer. Chromium was analyzed by s-Diphenylcarbazide method, copper by Neocuproine, Iron by Thiocyanate, Manganese by Persulphate, selenium by 3-3' Diamino Benzidine and zinc by Dithiozone methods.

RESULTS & DISCUSSION

In the present study the bioremoval characteristics of the algal strain *Spirulina sp.* and *Spirogyra sp.* were examined with regard to as Cr, Cu, Fe, Mn, Se and Zn ions from aqueous solution.The residual concentrations of respective metals after seven days of incubation period with *Spirulina sp.* and *Spirogyra sp.* is given in Table 1 & 2 respectively. It was observed that the metal removal is highly dependent on metal concentration system. Cr, Fe, Mn and Se removal capacity by *Spirulina sp.* is high as compared to Cu and Zn at lower initial metal concentration (5 mg/L). The same in case of *Spirogyra sp.* revealed that with Cr, Fe, Mn and Se removal of Cu is also good as compared to its removal by *Spirulina sp.*

Within all the tested metals the bioremoval capacity for Cr, Cu, Fe, Mn, Se and Zn was highest [98.30%, 81.20%, 98.93%, 99.73%, 98.83% and 79.00%] respectively at initial concentration 5 mg/L after seven days of incubation period by *Spirulina sp.* as shown in Fig. 1.

The same by *Spirogyra sp.* for Cr, Cu, Fe, Mn, Se and Zn was highest [98.23%, 89.60%, 99.73%, 99.60%, 98.16% and 81.53%] respectively at initial concentration 5 mg/L after seven days of incubation period as shown in Fig. 2.

In order to simplify the ability of each alga to remove each heavy metals, we converted % removal

Table 1. Residual concentration of metals in the medium after seven days of incubation period with *Spirulina sp*

Metals	Metal Concentrations [mg/L]				
	5	10	15	20	25
Cr	0.085 ± 0.0030	2.120 ± 0.0655	4.843 ± 0.0251	12.12 ± 0.0450	19.38 ± 0.0264
Cu	0.940 ± 0.0300	2.253 ± 0.0208	4.846 ± 0.0251	7.746 ± 0.0251	14.06 ± 0.1216
Fe	0.053 ± 0.0057	0.616 ± 0.0152	1.766 ± 0.0251	4.650 ± 0.0264	8.870 ± 0.0300
Mn	0.013 ± 0.0057	0.583 ± 0.0351	2.426 ± 0.0251	6.876 ± 0.0321	10.97 ± 0.0208
Se	0.058 ± 0.0020	2.823 ± 0.0152	6.886 ± 0.0152	12.04 ± 0.0321	18.38 ± 0.0200
Zn	1.050 ± 0.0173	2.916 ± 0.0208	5.166 ± 0.1527	8.323 ± 0.0251	14.25 ± 0.0057

Table 2. Residual concentration of metals in the medium after seven days of incubation period with *Spirogyra sp*

Metals	Metal Concentrations [mg/L]				
	5	10	15	20	25
Cr	0.088 ± 0.0050	2.153 ± 0.0404	4.486 ± 0.0850	7.920 ± 0.0600	13.87 ± 0.0500
Cu	0.520 ± 0.0300	1.836 ± 0.0404	3.703 ± 0.0321	7.330 ± 0.3637	12.48 ± 0.0208
Fe	0.013 ± 0.0057	0.463 ± 0.0115	1.416 ± 0.0152	4.150 ± 0.0264	8.303 ± 0.0152
Mn	0.020 ± 0.0100	0.583 ± 0.0115	2.200 ± 0.0300	6.966 ± 0.0321	11.38 ± 0.0152
Se	0.091 ± 0.0020	2.966 ± 0.0251	6.933 ± 0.0208	12.30 ± 0.0200	19.41 ± 0.0251
Zn	0.923 ± 0.0251	2.313 ± 0.0115	5.130 ± 0.0264	8.423 ± 0.0404	14.21 ± 0.0152

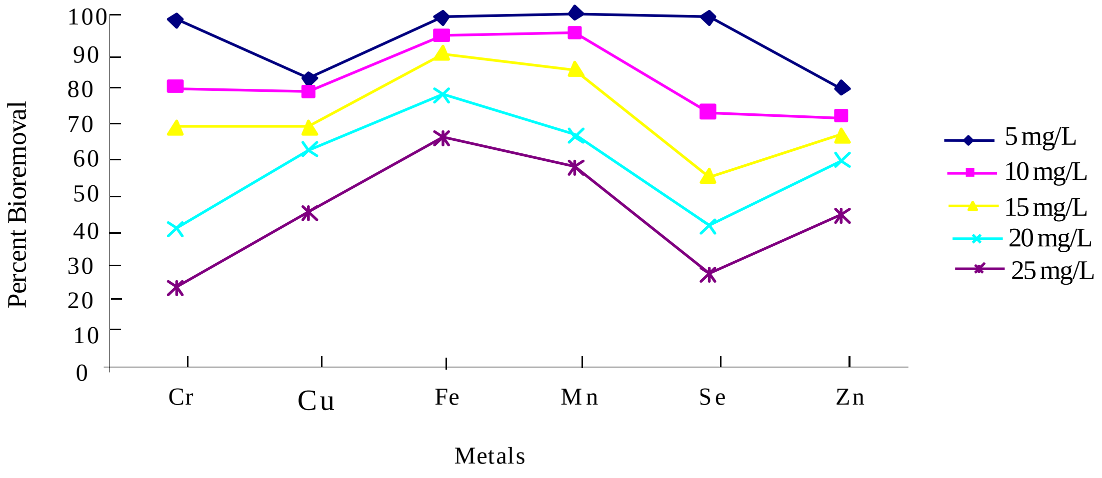

Fig. 1. Percent bioremoval of metals by *Spirulina sp.* from aqueous solution

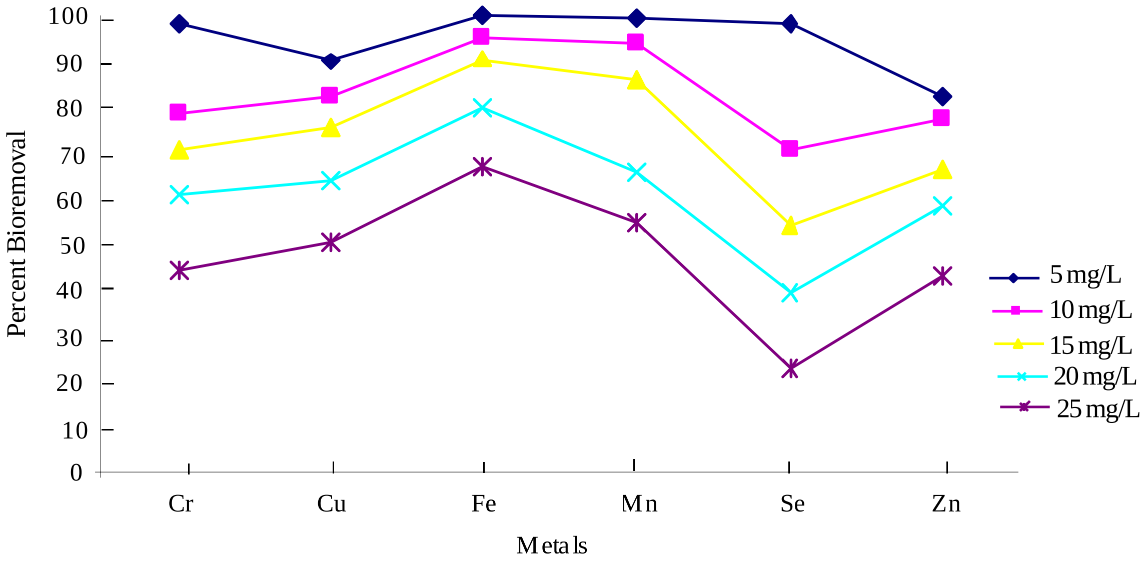

Fig. 2. Percent bioremoval of metals by *Spirogyra sp.* from aqueous solution

Table 3. Heavy metals removal (at 5 mg/L) in aqueous solution by *Spirogyara sp.* and *Spirullina sp*

Algae	Percent removal						Removal index					
	Cr	Cu	Fe	Mn	Se	Zn	Cr	Cu	Fe	Mn	Se	Zn
Spirogyara sp.	98.23	89.6	99.73	99.6	98.16	81.53	9.99	10	10	9.98	9.93	10
Spirullina sp.	98.3	81.2	98.93	99.73	98.83	79	10	9.065	9.91	10	10	9.68

into removal index by dividing % removal by the highest % removal and multiply the value obtained by 10. This would facilitate the comparison of each heavy metal removal ability by various microalgae. Table 3 was very helpful as it clearly denotes the effectiveness of each alga in removing the each metals viz. Cr, Cu, Fe, Mn, Se and Zn from aqueous solution.

In the present investigation, both the algal strains showed highest metal removal index to all the tested metals. In general heavy metals uptake by the cells of blue green algae by adsorption followed by metabolism-dependent intracellular cation intake as applicable to Zn, Cu, Cd and Zn, Cd, Al, Ni and Hg (Campbell and Smith, 1986; Johnson and Shubert, 1986; Les and Walker, 1984; Pettersson *et al.*, 1986; Shehata and Whitton, 1982; Singh and Yadava, 1985). In another studies, blue green algae had lower capacities than those found herein. For example, the blue green algae *Aphanothece halophytica* and *Spirulina platensis* could remove only 22% and 35% Pb, respectively, from battery factory effluent (Incharoensakdi and Kitjaharn, 1998; Kitjaharn and Incharoensakdi, 1992). In spite of this, we selected blue green algae to test for heavy metal adsorption because they have high growth rates and are easy to separate from solution by simple filtration.

Living algae biomass has been used for bioremediation processes of heavy metal-contaminated wastewaters, owing to its ability to remove such contaminants, either by adsorption onto the cell surface or by incorporation into the cells themselves. Two different biochemical paths can thus be followed: biosorption (or adsorption of metal ions onto the cell surface) and bioaccumulation (or absorption of metal into the cell) (Rangsayatorn *et al.*, 2002). The cell wall of microorganisms will play a crucial role as a defence mechanism—in that it is the first barrier to the uptake of toxic metals (O. zer *et al.*, 1999).

In this study, the amount of Cu removal was higher than Zn for both the algae this was explained as due to the high specificity that the active binding sites present on the biomass had for copper (Pradhan and Rai, 2000). Each of the metals showed different affinity toward algae. This could be due to the difference in cell wall composition and the intra group differences which cause significant differences in the type and amount of metal ion binding to them. The cell wall consists of variety of polysaccharides and proteins which offers a number of active sites capable of binding metal ions. In our study it was revealed that, in case of both the tested algae for all the metals as the initial metal concentration increases there is decrease in the metal removal capacity of the algae.

Literature reports that the accumulation of metal ions depends on external concentration of metal ions in the solution until their concentration leads to toxic effects and which leads to decreased performance of bioaccumulation (Yan and Pan, 2002). In the studied range of initial metal ions concentration this phenomenon was observed.

Our study indicates that both algae are able to remove the metals from aqueous solution at lower concentrations. At higher concentration there might be toxicity of metals which can reduce the biosorption capacity of the microorganisms. It has been described that microalgae can protect themselves against the toxicity caused by heavy metals using several mechanisms such as: exclusion mechanisms, adsorption to cell surface or intracellular accumulation some reported, in turn, that *Closterium lunula* cells did not undergo damage in the presence of 50 µg Cu/l, owing to their ability to exclude this metal (Abd-el-Monem *et al.*, 1998; Hassler *et al.*, 2005; Omar, 2002; Yu and Kaewsarn, 1999). Because of the lack of published data regarding metal removal by *D. pleiomorphus*, results reported in others studies, with similar microalgae were chosen for the sake of comparison. Some researchers stated that small amounts of cellular Zn were observed in *Chlorella kesslerii* cells, whereas some described that Chlorella sp. accumulated intracellular the highest proportion of Zn removed from the medium, in the range 0.1–2 mg/l; (Wong and Tan, 1998).

CONCLUSION

Biomass of both the algal strains of *Spirogyara sp.* and *Spirullina sp.* proves efficient towards removal of Cr, Cu, Fe, Mn, Se and Zn ions, to extents comparable to those reported for other algae, thus unfolding the potential of those strains for water and wastewater treatment processes.

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