

Original Research Article

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## Phycoremediation of Heavy Metals by *Chlorella pyrenoidosa* and *Spirogyra communis*

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### ABSTRACT

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In the present study, the phycoremediation capacities of live green algae, *Chlorella pyrenoidosa* and *Spirogyra communis* were evaluated for toxic heavy metals, Pb(II), Cu(II) and Cr(IV) from electroplating industrial effluent and synthetic solution. Both the algae proved efficient biological vectors for heavy metal uptake. Phycoremediation studies conducted on industrial effluent revealed that 100% Pb was removed from 20% effluent concentration in 20 days by both algae. Experimental results revealed that *Chlorella pyrenoidosa* and *Spirogyra communis* had maximum accumulation of Pb(II) followed by Cu(II) and Cr(IV) after 20 days of exposure. The growth performance of the two algae measured in terms of Chl a content was maximally reduced by Cr (>30 mg L<sup>-1</sup>) while it was enhanced by low levels of Pb and Cu (<50 mg L<sup>-1</sup>).

### Introduction

Environmental contamination by heavy metals is a serious problem due to their incremental accumulation in the food chain. Unlike most organic wastes and the microbial load in aquatic bodies, metal contaminants are not biodegradable, tending to accumulate in living organisms, thus becoming a permanent burden on ecosystems (Bayo, 2012). Most heavy metals are transition elements with incompletely filled d-orbitals. These d-orbitals provide heavy metal cations with the ability to form complex compounds. Trace amounts ( $\mu\text{g L}^{-1}$ ) of some metal ions such as

copper, zinc, cobalt, iron, nickel are required by living organisms as cofactors for the enzymatic activities. However, heavy metal ion concentrations at ppm ( $\text{mg L}^{-1}$ ) level are known to be toxic to the organisms because of irreversible inhibition of many enzymes by the heavy metal ions. The process of accumulation and adsorption of metals by algae involves adsorption onto the cell surface (wall, membrane or external polysaccharides) and binding to cytoplasmic ligands, phytochelatin and metallothioneins, and other intracellular molecules. The algal cell wall has many

functional groups, such as hydroxyl (OH), phosphoryl (PO<sub>3</sub>O<sub>2</sub>), amino (NH<sub>2</sub>), carboxyl (COOH), sulphhydryl (SH), etc., which confer negative charge to the cell surface (Gonzalez *et al.*, 2012; Bulgariu and Bulgariu, 2014). Since metal ions in water are generally in the cationic form, they are adsorbed onto the cell surface (Crist *et al.*, 1992; Xia and Liyuan, 2004; Romera *et al.*, 2007; Singh *et al.*, 2012). Algal cell walls are porous and allow the free passage of molecules and ions in aqueous solutions. The constituents of the algal cell wall provide an array of ligands with different functional groups capable of binding various heavy metals. These cells can be used live or dead (Zou *et al.*, 2014). They are generally rugged organisms with fast growth in simple medium, and the algal biomass produced can easily be processed into useful biosorbents (Tuzen and Sary, 2010).

The present study therefore aimed to compare the performance of *Chlorella pyrenoidosa* and *Spirogyra communis* in sequestering Pb(II), Cu(II) and Cr(IV) ions from industrial and aqueous solutions. The growth performance of the two algal species in terms of their Chl content after heavy metals accumulation was also examined. Both the algae were distinct from each other in their morphology, one is unicellular while other is multicellular. In literature no such study has been undertaken so far where a comparison is made between two morphologically distinct algae for bioremediation of heavy metals.

## **Materials and Methods**

### **Preparation of algal cultures**

*Chlorella pyrenoidosa* and *Spirogyra communis* were used as biosorbents for the bioremediation of heavy metals lead, chromium and copper from industrial

effluent as well as from aqueous solutions. *Spirogyra communis* was collected from the ponds located in Fisheries college, G.B. Pant University, Pantnagar while *Chlorella pyrenoidosa* (NCIM No.-2738) was obtained as pure culture from CSIR-National Chemical Laboratory, Pune-411008. Both the cultures were further maintained in Fog's medium. Slant cultures were prepared from the pure culture for further use. One loopful of algal biomass from best growth obtained above was inoculated in a sterile 15ml test tube with enriched medium (Fog's Medium). Total 20 numbers of 15ml test tubes were inoculated with isolated algae, maintained at 24°C±1°C and illuminated at 4000 lux light intensity with a light/dark cycle of 16/8-h for 10 days. After 10 days, inoculated algae from the test tubes were inoculated into 250ml Erlenmeyer flasks containing Fog's Medium for another 7 days. After seven days the medium inside the flask appear green, these were examined under microscope. At every 12 days new medium was prepared and the algal cells were inoculated to it to continue the algal cell generation. To avoid bacterial and fungal contamination appropriate amount of antifungal and antibacterial were added to the medium.

### **Preparation of heavy metal stock solutions**

Stock solutions of Pb(II), Cu(II) and Cr(IV) were prepared by dissolving their salts viz. lead nitrate Pb(NO<sub>3</sub>)<sub>2</sub>, copper sulphate CuSO<sub>4</sub> and potassium dichromate K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in distilled water. From this stock solution different concentration of heavy metals were prepared (30, 50, 100, 150 and 200 mg L<sup>-1</sup>).

### **Analysis of Electroplating effluent**

Electroplating effluent was analysed for various chemical and physical parameters

such as pH, BOD, COD, DO, TDS and Heavy metal estimation by standard prescribed methods in APHA (1995).

### **Analysis of algae**

#### **Measurement of Chlorophyll**

Chlorophyll a content in *S. communis* and *C. pyrenoidosa* was determined according to Vonshak (1997) where, 3g of fresh sample was ground in a mortar and pestle. Tissue was formed to a fine pulp with addition of 20 ml of 80% acetone. Centrifuge (5000 rpm for 5 min.) and the supernatant was transferred to a conical flask. This procedure was repeated until the residue was colourless. The mortar and pestle was washed thoroughly with 80% acetone and the clear washings was collected in the volumetric flask. The volume was made to 50 ml with 80% acetone. The absorbance of the solution was read at 645 and 663 nm against the solvent (80% acetone) blank using spectrophotometer, and then amount of Chl a ( $\text{mg g}^{-1}$ ) concentrations in the algal tissues were calculated according to the following equation:

$$\text{Chl a} = 12.7 (A_{663}) - 2.69(A_{645}) \times V/1000 \times W$$

Where,

A = absorbance at specific wavelength

V = final volume of chlorophyll extract in 80% acetone (ml)

W = fresh weight of the tissue extracted (g)

#### **Phycoremediation Experiments**

Algal metal bioremoval from industrial effluent was assayed by exposing *S. communis* (3 g FW) and *C. pyrenoidosa* (10

ml) to 100 ml waste water effluent (containing growth medium for algal biomass) in 250 mL conical flask separately. The experiments were performed in triplicates. The flasks were incubated for 10 and 20 days along with control for each day (without algae) at 20°C, under cool fluorescent lamps at maximum intensity of 3200 lux and periodicity of 12/12 hour Light/Dark. After 10 and 20 days the flasks were removed and analysed for heavy metal content using AAS. For aqueous solution the experiments were conducted with Pb, Cu and Cr at concentrations 10, 30, 50 and 70  $\text{mg L}^{-1}$  for each metal for 24 days, after which the growth performance of algae was measured in terms of Chl. content ( $\text{mg/g}$ ). The samples were digested using Nitric acid-perchloric acid method and were analysed for heavy metal concentration using AAS.

### **Results and Discussion**

#### **Characterization of biosorbents**

Qualitative studies regarding binding sites of heavy metals on the surface of biosorbents under investigation (*S. communis* and *C. pyrenoidosa*) were carried out by Fourier Transform Infra-Red (FTIR) Spectroscopy (Fig. 1 and 2). FTIR spectroscopy gives valuable information about the nature of the bonds present and allows identification of functional sites such as carboxyl, sulfonate, hydroxyl, and amino groups on the cell surface.

These groups have been proposed to be responsible for the biosorption of metals by algae. The region between 3200-3500  $\text{cm}^{-1}$  exhibits the stretching vibration of O-H and N-H which also confirms the presence of hydroxyl and amine functional groups in both algal structure. The region between 3000-2800  $\text{cm}^{-1}$  shows the C-H stretching

vibrations of sp<sup>3</sup> hybridized C in CH<sub>3</sub> and CH<sub>2</sub> functional groups. The peaks at 1652 cm<sup>-1</sup> (for *S. communis*), and 1649 cm<sup>-1</sup> (for *C. pyrenoidosa*) reveal the presence of carbonyl group. The presence of amide in the structure of each alga is confirmed by the peak at 1545 cm<sup>-1</sup> (for *S. communis*), and 1543 cm<sup>-1</sup> (for *C. pyrenoidosa*). The absorption peaks around 1240 cm<sup>-1</sup> and 1150 cm<sup>-1</sup> indicate the phosphate esters in *S. communis* and *C. pyrenoidosa*. The phosphate esters are the source of phospholipids. The absorption peaks at the respective frequencies of 1026 cm<sup>-1</sup> confirm the presence of sulfoxides in *S. communis* which are absent in *C. pyrenoidosa*. The observed frequencies in FTIR spectra of the algae used indicate the presence of amine (R-NH<sub>2</sub>), amide (R<sub>1</sub>(CO)NR<sub>2</sub>R<sub>3</sub>) (aminoacids, proteins, glycoproteins, etc.), carboxylic acids (fatty acids, lipopolysaccharides, etc.), sulfoxides (in case of *S. communis*) and phosphates

#### **Change in physicochemical parameters of electroplating effluent after treatment with *C. pyrenoidosa* and *S. communis***

The industrial effluents contain various toxic contaminants including heavy metals as cadmium, nickel, mercury, arsenic, copper etc. producing a significant toxic impact on aquatic environment (Singh *et al.*, 2006; Siddiqui and Sharma, 2009; Oyeku *et al.*, 2010 and Pandey *et al.*, 2010). Data represented in Table 1 and Fig. 3 clearly indicates the changes in physicochemical parameters and heavy metal concentration of electroplating effluent before and after treatment with algae *S. communis* and *C. pyrenoidosa*. After 20 days there was 100% removal of Pb by both algal biomass. 88.32% and 84.75% reduction in Cu and Cr content was observed by *C. pyrenoidosa* while a reduction of 94% and 60% was observed for Cu and Cr by *S. communis*

after 20 days of treatment (Fig. 4). Moreover, a significant change was observed in physicochemical parameters of electroplating effluent after 20 days of treatment with both algae under investigation. Study suggests that *C. pyrenoidosa* and *S. communis* shows promising approach towards the purification process of waste water at various parameters. Along with bringing the properties such as TDS, pH, BOD, COD etc. towards the desirable limit, both the algae has been found quite effective for the removal of heavy metals also. Among heavy metals, although a significant reduction was observed in all the three metals but the algae has been found to be especially effective in the reduction of Pb, followed by Cu and Cr.

#### **Effect of varied metal treatments on algae growth with respect to Chl "a" content**

The data represented in Fig. 5 shows chlorophyll "a" content of *S. communis* and *C. pyrenoidosa* and was found to be affected by different metal treatments. It can be seen from figures that the two algae tolerated the toxicity of Pb even at higher concentrations (10–50 mg/l) moreover the lower concentration of Pb (10 mg/l) induced a pronounced stimulation of chlorophyll "a" which was observed in both algae under investigation. On the other hand, Cr showed a strong inhibition of chlorophyll a biosynthesis even at the lower concentrations (10 mg/l) in both the algae and a complete destruction of the algal cell at concentration above 10 mg/l (Fig. 1 and 2). This means that the efficiency of the photosynthetic apparatus seemed to be less affected by Pb and severely altered by Cr. Cu toxicity was mostly intermediate (between that of Cr and Pb), it exhibited stimulatory effect to the algal growth (chlorophyll a) at lower concentrations (10–30 mg/ml) in case of *C. pyrenoidosa* while

in *S. communis* the enhancement effect was only restricted to concentration of 10 mg/l. The obtained results in this investigation concerning the tolerance of *S. communis* and *C. pyrenoidosa* to the tested heavy metal ions (Pb, Cu and Cr) were in agreement with the results reported by Foster, 1982 and Stokes, 1983 concerning the tolerance and resistance of green algal species to heavy metal ions (Cu, Cd, Pb and Zn). Increased chlorophyll ratios due to environmental stress have been reported in spinach leaves

(Delfine *et al.*, 1999; Monni *et al.*, 2001). The chlorophylls level in *Olisthodiscus luteus* decreased to 86.7% after 180 hr exposure to 0.5 mg/l Cr<sup>2+</sup>. In addition, high Cr<sup>2+</sup> concentrations reduced cell sizes and caused a decrease in growth rate (Fernandez-Leborans and Novillo, 1996). Nassiri *et al.*, (1997) found no growth inhibition at Cr<sup>2+</sup> concentrations < 1mg/l, but *Tetraselmis suecica* had 10, 30 and 50% growth inhibition, after 4 days in solutions contained 2, 5 and 10 mg/l Cr<sup>2+</sup> respectively.

**Table.1** Physicochemical parameters of electroplating effluent before and after treatment (20 days) with algae

Parameters	Values		
	Raw effluent	Treated with <i>C. pyrenoidosa</i>	Treated with <i>S. communis</i>
Colour	Dark brown	Lightened	Lightened
Odour	Acidic smell	Neutral smell	Neutral smell
pH	5.83	7.34	6.98
TDS (mg/L)	60	44	42
DO (mg/L)	7.73	6.32	6.12
BOD (mg/L)	148	54	48
COD (mg/L)	167	74	77
Zn (mg/L)	39	12	10
Cd (mg/L)	0.015	0.004	0.007

**Fig.1** FTIR spectra of *Chlorella pyrenoidosa* (a) Native (b) Pb loaded

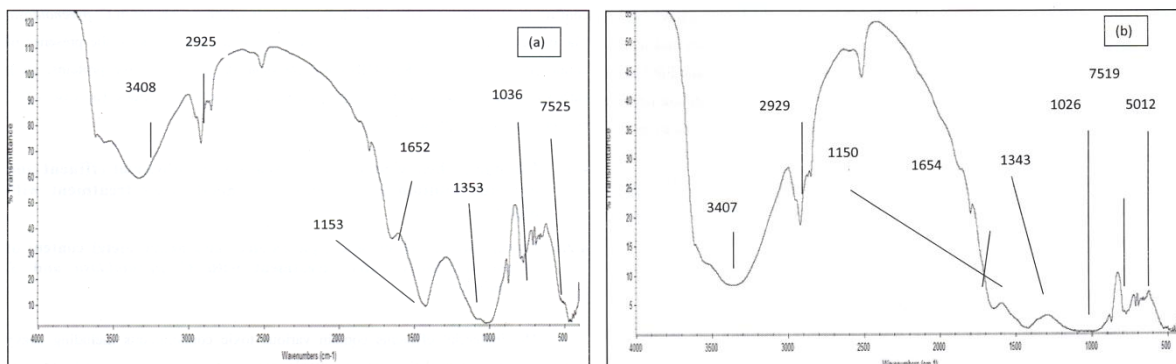


Fig.2 FTIR spectra of *S. communis* (a) Native (b) Pb loaded

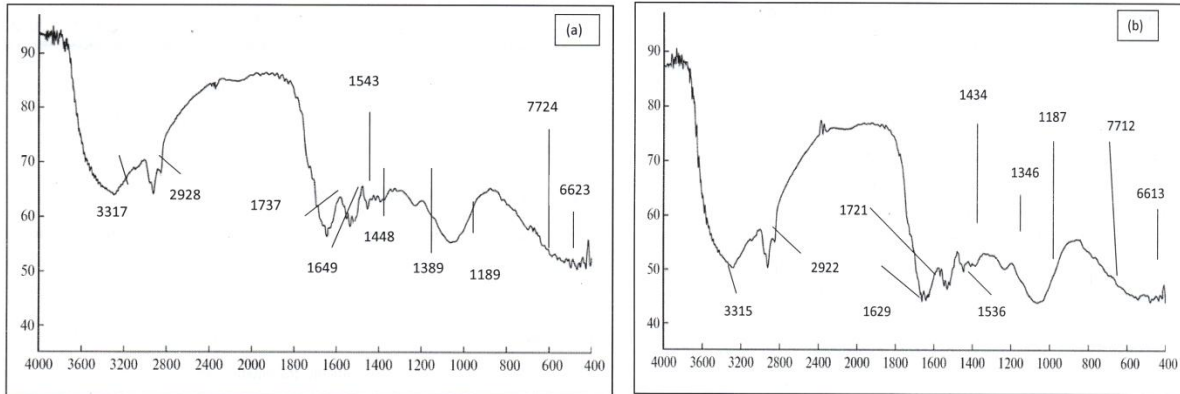
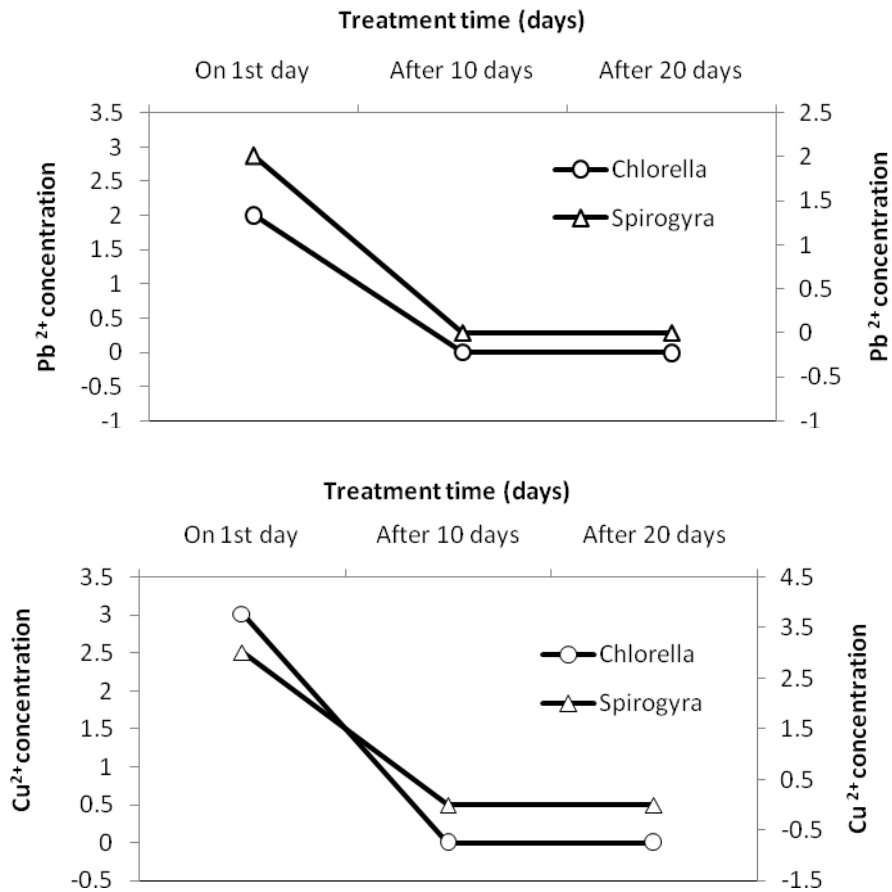
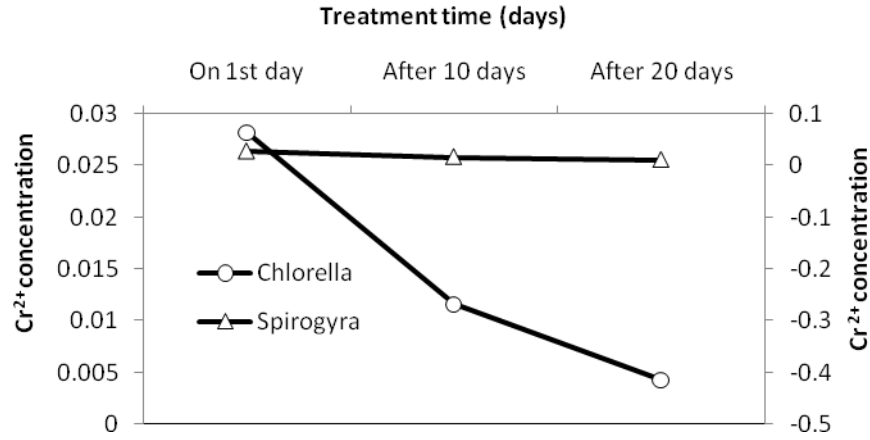
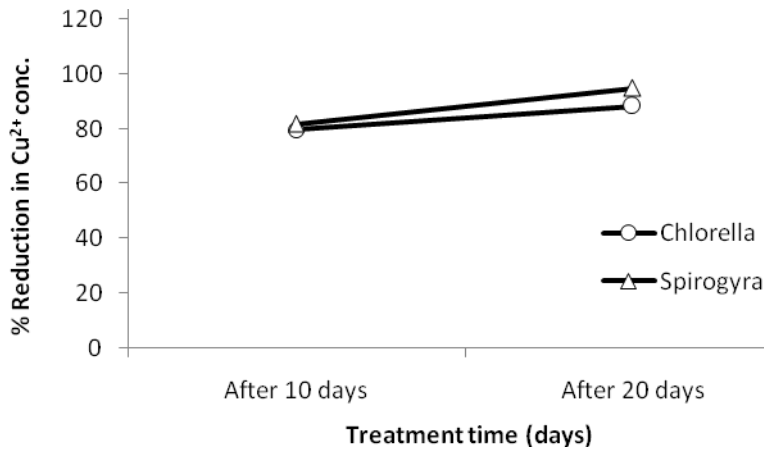
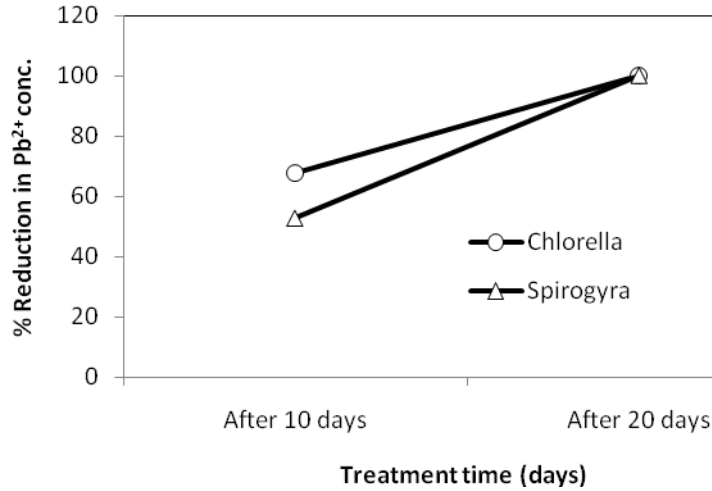


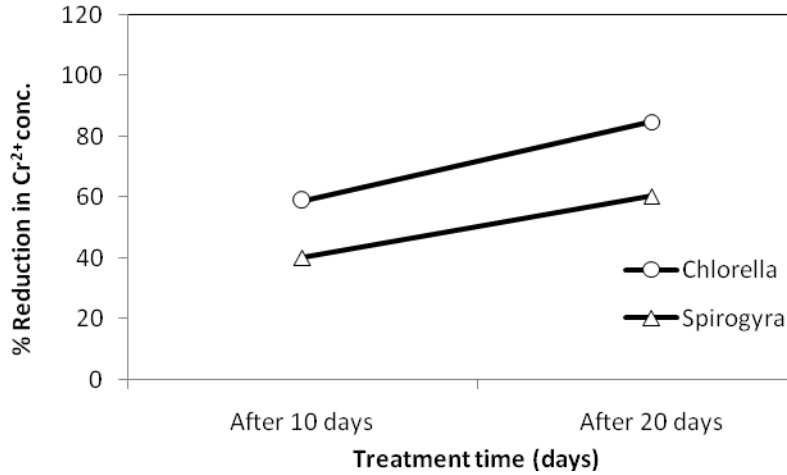
Fig.3 Change in concentration of heavy metals [(a) Pb, (b) Cu, (c) Cr] in electroplating industrial effluent after treatment with *C. pyrenoidosa* and *S. communis*



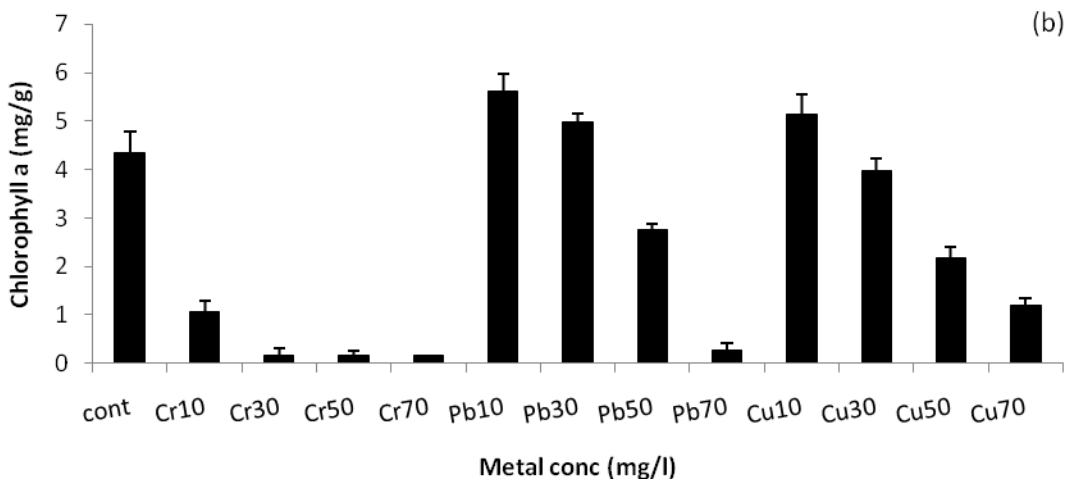
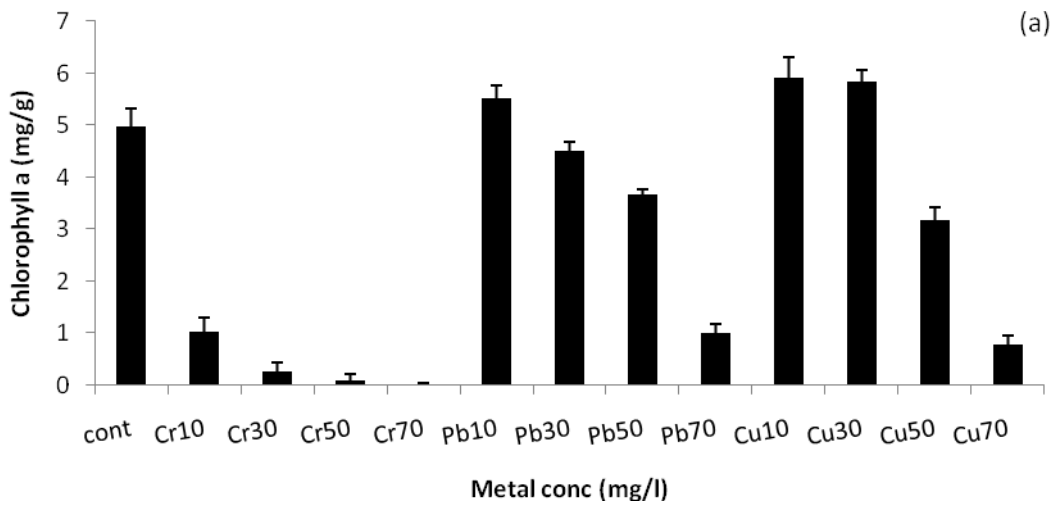


**Fig.4** Percent reduction in heavy metal concentration [(a) Pb, (b) Cu, (c) Cr] of electroplating industrial effluent after treatment with *C. pyrenoidosa* and *S. communis*





**Fig.5** Effect of different heavy metals concentration on Chlorophyll a content (mg g<sup>-1</sup>) of *S. communis* (a) and *C. pyrenoidosa* (b) after 24 days of exposure





Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium and chromium were reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid (ALA) synthesis (Stobart *et al.*, 1985). The inactivation of the enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content. The present results showed that lead, copper and chromium toxicity decreased the chlorophyll a content of the two algae under investigation. The highest reduction in chlorophyll a content was found in algae exposed to chromium, followed by copper and lead. A large reduction in chlorophyll content due to Cr toxicity can be explained on the basis of destruction of stomata and mesophyll cells, which decreases their efficiency of light utilization and electron transport rates involving PS I and PS II (Munne-Bosch and Alegre, 2003; Hernandez *et al.*, 2004).

In conclusion, in the present study, the bioaccumulation potential of two algal biomass *Spirogyra communis* and *Chlorella pyrenoidosa* have been assessed for the removal of Pb(II), Cu(II) and Cr(IV) from electroplating industrial effluent and aqueous solution. Experiments conducted on industrial effluent showed a significant decrease in physicochemical parameters and heavy metal content by both the algal biomass. The chlorophyll content of both algae was highly suppressed by high levels of Cr ( $>10 \text{ mg L}^{-1}$ ), while high concentration of Pb and Cu ( $>30 \text{ mg L}^{-1}$ ) has less inhibitory effect and low concentrations ( $<50 \text{ mg L}^{-1}$ ) has stimulatory effects. *S. communis* and *C. pyrenoidosa* both are efficient biosorbents for heavy metal removal from industrial wastewaters

containing low as well as high concentration of Pb, Cu and Cr. Their easy availability and cost effective nature make them most promising biosorbents over other conventional biosorbents. Bioaccumulation and biosorption of heavy metals utilizing *S. communis* and *C. pyrenoidosa* proved promising techniques to mitigate water pollution. The development of bioremediation processes using algal biomass requires further investigation

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