

# Biosorption of Selected Heavy Metals by Green Algae, *Spirogyra* Species and Its Potential as a Pollution Biomonitor

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## Abstract

In this paper, adsorption features of freshwater green algae as a function of contact time, initial pH and initial metal ion concentrations were studied using model solutions of metal ions with subsequent determination by flame atomic absorption spectrometry (FAAS). Green algae samples were employed as bioindicators of selected heavy metals in environmental water samples with determination using FAAS and ICP-OES (inductively-coupled plasma-optical emission spectrometry). The optimum pH for cadmium (Cd), chromium (Cr), copper (Cu) and lead (Pb) biosorption were found to be 5.5, 5.8, 5.9 and 5.0, respectively. The biosorption process follows second order kinetics and is well described by the Langmuir isotherm model. Adsorption capacities were found to be 22.52, 38.19, 35.59 and 94.34 mg/g for Cd, Cr, Cu and Pb, respectively, at contact times of 15-50 minutes and initial metal ion concentrations of 500-700 mg/L. Biomonitoring studies revealed great potential for freshwater green algae as a bioindicator with mean concentration factors in the range of 367-7154 for the selected elements, the uptake being mainly via a metabolically mediated pathway. This work has demonstrated the potential of freshwater green algae as both a biosorbent and a bioindicator for heavy metal pollution in water systems.

**Keywords:** Green algae, Biosorption, Biomonitoring, Toxic metals, FTIR, ICP-OES.

## 1: INTRODUCTION

Control of toxic metal pollution is becoming increasingly important as industrialization becomes the main economic activity of many nations. Heavy metals, including cadmium (Cd), chromium (Cr), copper (Cu) and lead (Pb) are among the most important of such pollutants because of their toxicity and non-biodegradability [Chang and Chang, 1997].

Heavy metals reach the environment mainly from industrial plating baths, acid mine-drainage and metal cleaning baths. In particular cadmium sources include manufacture of plastics, Ag-Cd batteries, paint and pigments, among others (Sarı and Tuzen, 2007). Pulp, paper, paper-board mills and the fertilizer industry are common potential sources of copper pollution. Lead comes into water through the combustion of fossil fuels and the smelting of sulphide ores.

The toxic effects of cadmium include renal dysfunction, hypertension, hepatic injury, lung damage and teratogenic effects (Hajjaligol *et al.*, 2006). Excessive intake of copper results in an accumulation in the liver and may also produce gastro-intestinal catarrh. It is also toxic to aquatic organisms even at very small concentrations in the natural waters (Gupta *et al.*, 2005). Chromium is more harmful as Cr(VI) which is an acute carcinogen and more mobile than Cr(III). Lead accumulates mainly in bones, brain, kidney and muscles and may cause many serious

disorders like anemia, kidney diseases, nervous disorders, mental retardation and sickness even death (Chen *et al.*, 2006).

Numerous processes have been used for the removal of heavy metal ions from aqueous solution. These include ion exchange, precipitation, reverse osmosis, phyto-extraction, ultra-filtration and electro-dialysis among others (Yu *et al.*, 1999). However, these methods are either inefficient or expensive in removing trace metal concentrations (Wilde and Benermann 1993, Kuyucak and Volesky, 1998).

Biosorption, which uses biological materials, is a relatively new technology for removing even trace concentrations of heavy metals from polluted water. Biosorbent materials such as naturally occurring green algae or other seaweeds are generally less costly than existing technologies (Holan *et al.*, 1993) and remove pollutants by physical sorption, complexation, chemisorption as well as metabolically mediated physico-chemical pathways of uptake (Fourest and Roux, 1992). In particular, algae have proved to possess high metal binding capacities (Schiewer and Volesky, 2000) due to the presence of polysaccharides, proteins or lipid on their cell wall surfaces. Various other microbial sources like moss, aquatic plants and leaf-based adsorbents have been used by various investigators (Chang *et al.*, 1997, Niu *et al.*, 1993, Itoh *et al.*, 1975). Freshwater green algae are particularly useful because of their wide distribution.

Biomonitoring is the science of inferring the ecological condition of an area by examining the organisms that live there. It is most often used to assess water quality of rivers, lakes, streams, and wetlands (Rosenberg and Resh, 1993). Biomonitoring involves the quantitative measurement of an organism's exposure to toxic substances in the environment by determining the substances or their metabolites in specified parts of the

organism. Biomonitoring measurements are quite health-relevant assessments of exposure because they indicate the amount of pollutant that actually gets into the organism from all environmental sources such as air, soil, water, dust and food.

In this work, biosorption studies were conducted on model solutions of Cd, Cr, Cu and Pb using green algae. The algae was then used for heavy metal biomonitoring studies around Thika and Juja. Fourier transform infrared spectroscopy (FTIR) was used to study functional groups responsible for biosorption in algae. Equilibrium and kinetics studies were conducted using batch biosorption experiments. pH, contact time and initial metal ion concentration were optimized. The adsorption capacities were evaluated from equilibrium adsorption isotherms. The results indicated that freshwater green algae are a suitable material for the development of high capacity biosorbent for the selected heavy metals. A good bioindicator should be sedentary, of suitable dimensions, easy to identify and collect, widely distributed, and be able to accumulate metals to a satisfactory degree (Conti *et al.*, 2002).

## 2: MATERIALS AND METHODS

### 2.1 Equipment and reagents

Flame Atomic Absorption Spectrophotometer (210VGP, UK) using air acetylene flame and hollow cathode lamps and a Perkin Elmer Optima 5300D ICP – OES spectrophotometer operating in axial mode were used for metal determination. pH measurements were done using a digital pH meter fitted with a temperature probe and a glass electrode (pH 211, HANNA Instruments) while filtration was done using a Millipore filter funnel equipped with a 0.45  $\mu\text{m}$  cellulose acetate filter membrane and attached to a vacuum pump. The algae spectra were generated from a Fourier Transform Infrared Spectrophotometer 8400CE (Shimadzu, Japan) fitted with a pellet cell. All chemicals used in this work, were of analytical grade and were used without further purification. All solutions were prepared using distilled water. Stock solutions were prepared by dissolving appropriate amounts of analytical grade salts in 250 mL distilled water, acidified with 5mL of concentrated nitric acid and made to a liter using distilled water. Working solutions were made by appropriate dilution of the stock solutions.

### 2.2 Sample collection

Fresh green algae was collected from a stationery fresh water pond in Juja, washed in tap water several times and rinsed with distilled water. The algae samples were sun-dried in the open for two days then oven-dried at 60  $^{\circ}\text{C}$  for eight hours. Finally it was ground, sieved to 0.5 mm particle size and stored in a plastic bottle at room temperature until use. A second batch of algae samples was collected from various water ponds along Thika road, Juja and around Thika town. Water samples were also collected from each site. The algae were treated as described above, while the water samples were acidified to a pH of 2 by addition of two drops of concentrated nitric acid. The samples were stored in a refrigerator at 4  $^{\circ}\text{C}$  in plastic bottles until use.

### 2.3 Optimization of pH

Batch biosorption experiments were conducted on model solutions of Cd, Cr, Cu and Pb. For each element, the stock solution was diluted to 200 mg/L using 0.1 molar acetate buffer and divided into two 50 mL batches. Both batches were adjusted to pH values of 2.0, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0 and 7.0 using concentrated sodium hydroxide (NaOH) and nitric acid ( $\text{HNO}_3$ ) solutions. The first batch was equilibrated with 0.20 g of crushed algae for two hours while the second batch (control) was allowed to stand for two hours without addition of any algae. Both solutions were filtered through a 0.45  $\mu\text{m}$  membrane and the filtrate analyzed for residual metal ion concentration by flame atomic absorption spectrophotometer (FAAS). All experiments were done in triplicate and the mean values reported. A plot of biosorption against pH was used to determine the optimum pH.

### 2.4 Optimization of contact time

The stock solutions of the selected metal ions were each diluted with acetate buffer solution to obtain 500 mL of 100 and 200 mg/L solutions of Cd, Cr, Cu and Pb. The pH of the solutions was adjusted to 5.5, 5.8, 5.9 and 5.0 for cadmium, chromium, copper and lead, respectively. Exactly 2.00 g of crushed algae was weighed out and added to 500 mL of each solution and equilibrated for 140 minutes with magnetic stirring at 300 rpm. 10 mL portions of this mixture were withdrawn at measured time intervals between 0 and 140 minutes. Each portion was immediately filtered through a 0.45  $\mu\text{m}$  membrane and the residual metal ion concentration determined by FAAS. All experiments were done in triplicate.

To determine the order of the reaction, the experimental data was fitted to both 1<sup>st</sup> and 2<sup>nd</sup> order rate equations. Linear correlation coefficient ( $R^2$ ) values were used to deduce the order of reaction.

### 2.5 Optimization of initial metal concentration

The initial concentration which gives rise to the highest metal uptake was determined. Initial concentrations ranging from 50 to 1000 mg/L and the respective optimum pH values were used for each metal. 50 mL of each

solution was equilibrated with 0.2 g of finely crushed algae for two hours with stirring at 300 rpm. The solution was filtered through a 0.45  $\mu\text{m}$  membrane and the residual metal ion concentration determined by FAAS. The data was fitted to the linearized Freundlich and Langmuir adsorption isotherms from which the adsorption capacity was calculated. A plot of metal uptake (mg/g) against initial metal ion concentration was done for all metals and used to determine the optimum initial metal concentration.

### 2.6 Biomonitoring studies

The metal concentrations in acid - leached algae, digested algae and in the water samples from which the algae was collected were determined. Leaching was done by shaking 1 g of ground algae sample with 20 mL of 0.1M hydrochloric acid for five minutes, filtering and rinsing the residue with enough distilled water to make 50 mL of filtrate while the digestion was done on dried algal biomass using a 3.1.1 mixture of concentrated perchloric, nitric and hydrochloric acids, respectively. The digested and acid - leached samples were filtered through a 0.45  $\mu\text{m}$  membrane filter and the filtrate made up to 50 mL before analysis by FAAS. Concentration factors were calculated from the total metal ion concentration in digested algae and that in the water samples.

## 3: RESULTS AND DISCUSSION

### 3.1 Functional groups in green algae

The functional groups responsible for heavy metal biosorption on green algae were investigated by FTIR analysis. The spectrum of free algae and algae loaded with lead metal is shown in Figure 1. The position of absorption bands and corresponding functional groups able to interact with metal ions are presented in Table 1.

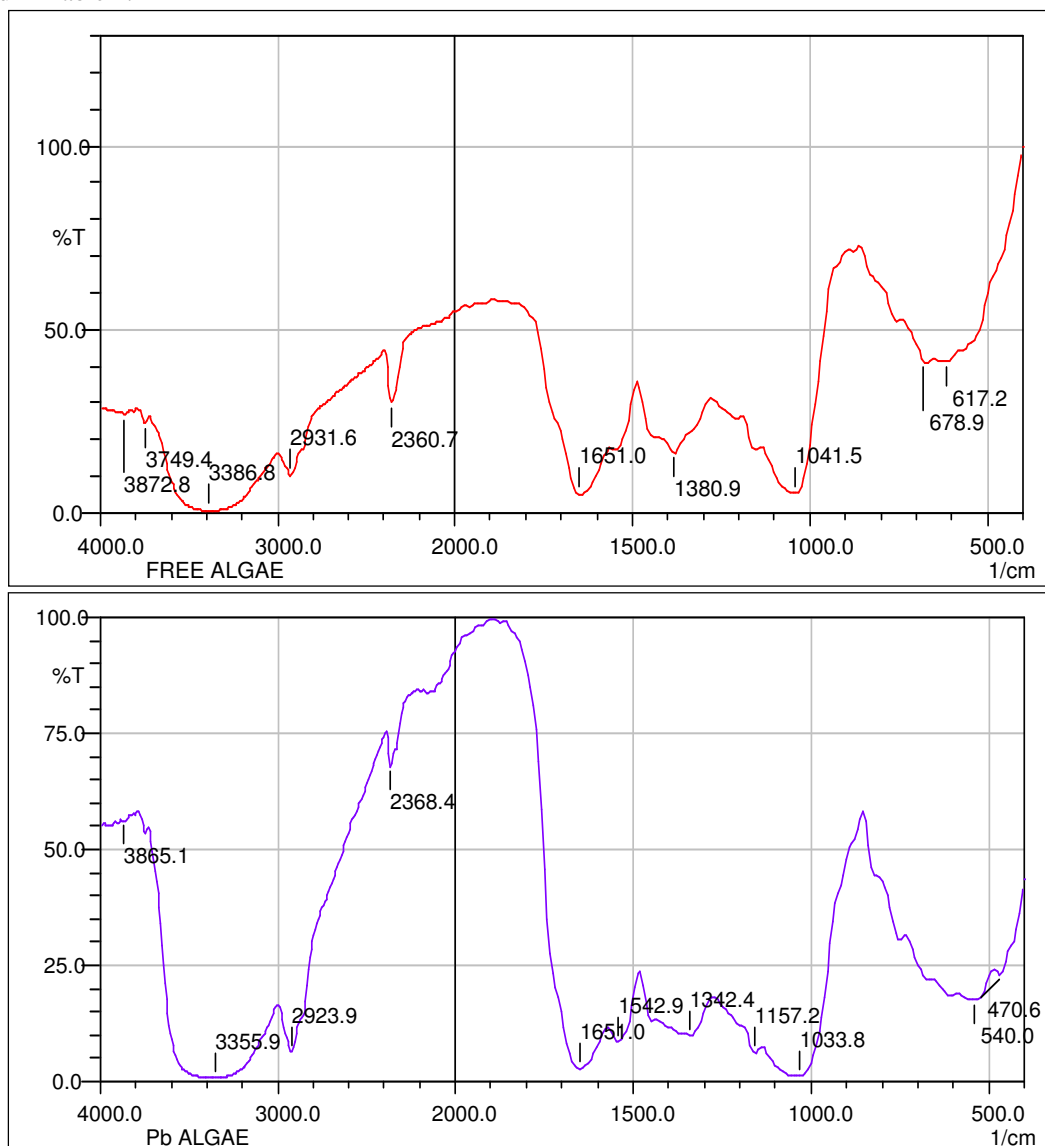


Figure 1. FTIR Spectra of free and lead loaded algae.

After adsorption of lead, slight changes were observed in the absorption peak frequencies between 2000 and 4000  $\text{cm}^{-1}$ . For instance, the peak at 2360.7  $\text{cm}^{-1}$  attributed to a cyanide group completely disappears. This was probably due to the coordination of the heavier metal atom to an active functional group resulting in a vibration frequency below the selected range. Other peaks (3386.8, 2931.6, 1380.9, 1041.5, 678.9  $\text{cm}^{-1}$ ) are also observed to shift to lower frequencies (3355.9, 2923.9, 1342.4, 1033.8, and 540.0  $\text{cm}^{-1}$  respectively) after loading the algae with lead. This was probably due to the attachment of the heavier metal atom to an active functional group resulting in lower vibration frequency. Similar trends are observed for all other selected metals.

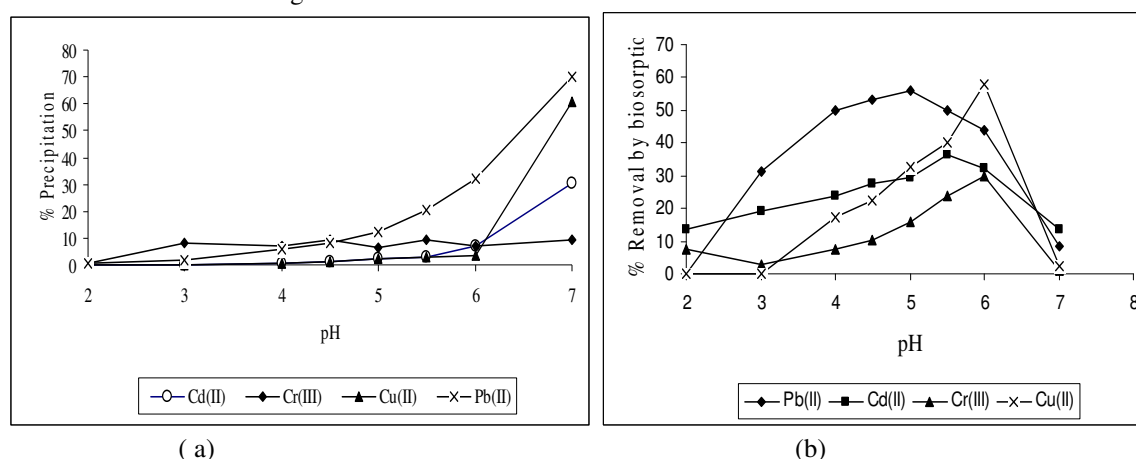
The peaks observed confirm the presence of carboxylic acids, hydroxyl ions and carbonyl groups among others on the algal surface as suggested in literature (Davis et al., 2003, Gupta and Rastogi, 2009).

**Table 1.** FTIR peaks and their possible assignments

Peak position before loading ( $\text{cm}^{-1}$ )	Peak position after loading ( $\text{cm}^{-1}$ )	Possible assignment
3872.6	3865.0	
3749.4	--	
3386.8	3355.9	O-H <sub>str</sub> , alcohols/phenols, N-H, amines
2931.6	2923.9	O-H <sub>str</sub> , carboxylic acids
	2368.4	
2360.7	--	-C≡N
1651.0	1651.0	C=N, C=O <sub>vib</sub> , C=C, N-H <sub>bend</sub>
	1542.9	
1380.9	1342.4	O-H <sub>bend</sub> , S=O <sub>str</sub> , CH <sub>3</sub> bend and ss, N-O
	1157.2	
1041.5	1033.8	C-N <sub>str</sub> , aliphatic amines
678.9	540.0	-C≡C-H: C-H <sub>bend</sub> alkynes
617.2	470.6	-C≡C-H: C-H <sub>bend</sub> alkynes, C-Br <sub>str</sub> , alkyl halides

### 3.2 Effect of pH

The residual metal ion concentrations after batch equilibration with algae at various pH values were determined. The metal removal is a combination of biosorption and other physical processes such as precipitation and complexation among and others. The percent removal of ions by biosorption alone was determined (figure 2b), while the percentage removal by other processes including complexation of metal ions with the acetate buffer are shown for each metal in Figure 2 a.



**Figure 2.** Percent metal uptake from solution (a) other processes and (b) biosorption

For all the metals considered, metal removal by all processes was low at low pH. For biosorption the removal rises to a peak between pH 5 and 6 then starts to decline. This is probably because at low pH there is high competition for adsorption sites between metal ions and protons. Furthermore, at low pH both the hydrogen ion and the metal ion concentration are high since the metals are not precipitated but are available in solution. This explains why both processes are ineffective at low pH values. Since algal biomass has a high content of carboxyl groups on its cell walls, biosorption process can be affected by changes in the solution pH (Matheickal and Yu, 1999). Change in pH affects both the nature of the functional groups as well as the metal chemistry. As the pH rises, the hydrogen ion concentration falls resulting in an increase in biosorption of heavy metals. The high pH also leads to precipitation of low solubility metal hydroxides as shown in Figure 2 a. Precipitation and

complexation interfere with the biosorption process because they immobilize the metal ions thus making them unavailable for biosorption. Precipitation is due to the formation of low solubility metal hydroxides (Chen *et al* 2006, Gupta and Rastogi, 2007, Gupta *et al.*, 2005), while complexation may occur between the acetate ions in the buffer and the metal ions in solution.

The optimum pH for biosorption is thus a compromise between interference from precipitation at high pH, competition with hydrogen ions for sorption sites at low pH and complexation. To obtain the optimum pH, a graph of percentage metal removal by biosorption against pH was plotted. This plot (figure 2 b), shows the difference between the total metal removal by biosorption and other processes (precipitation/complexation etc) less the metal removal by those other processes to obtain the removal by biosorption alone. The curves obtained for the selected metal ions are shown in Figure 2 b. From these curves the optimum pH values for the selected metals were found to be 5.5, 5.8, 5.9 and 5.0 for cadmium, chromium, copper and lead, respectively.

### 3.3.1 Effect of contact time

The minimum time required for quantitative uptake of metal ions from solution was determined. The contact time was obtained by plotting the mean percentage metal ion uptake against time as shown in Figure 3. For all metals considered, metal adsorption was very rapid and went to completion in less than an hour. Cadmium adsorption was the fastest with quantitative uptake being achieved in fifteen minutes while chromium and copper took forty minutes. For lead, quantitative uptake occurred in fifty minutes. Hence the contact times for the selected metals were found to be 15 minutes for cadmium, 40 minutes for both chromium and copper and 50 minutes for lead. The short contact times demonstrate the potential of algae as a suitable biosorbent for fast removal of heavy metals from contaminated waters.

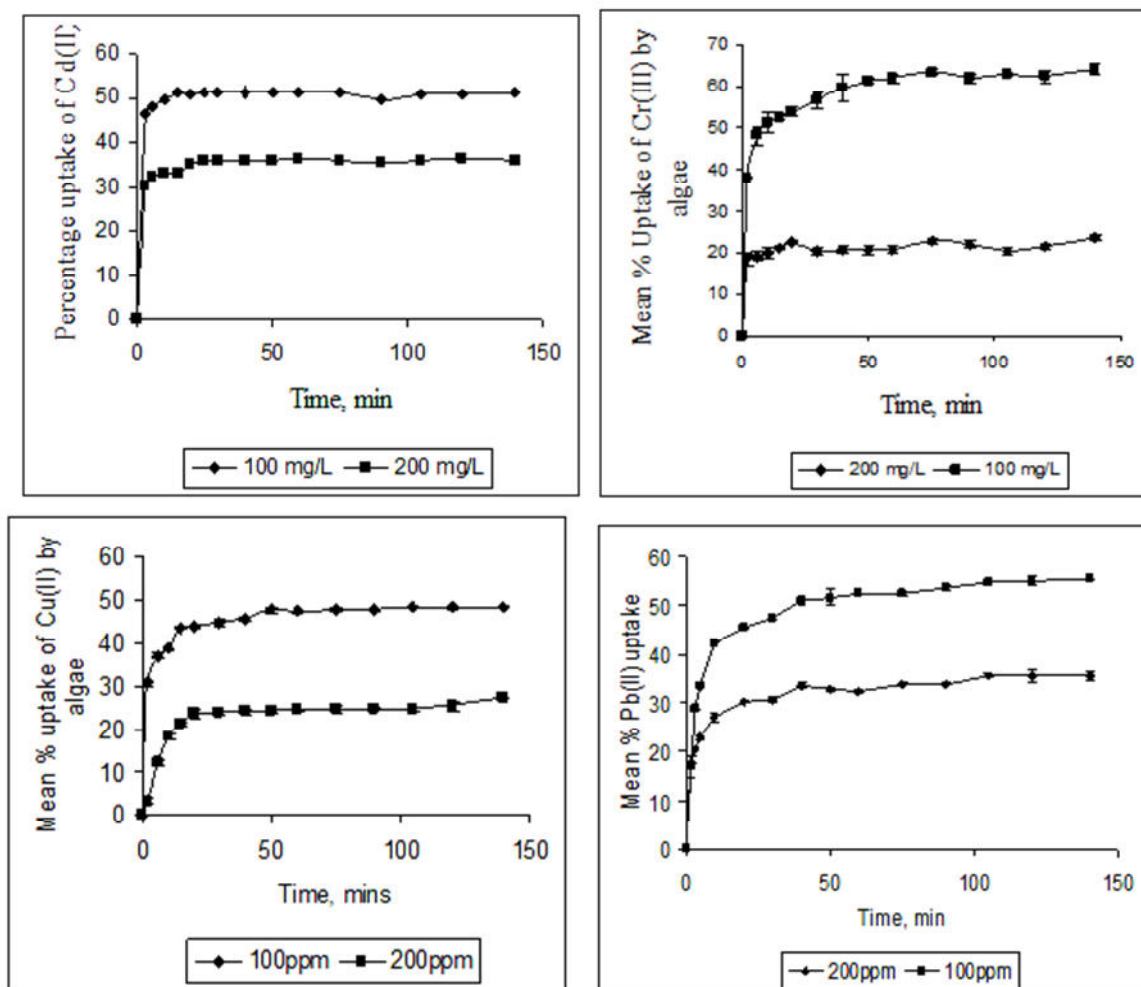


Figure 3. Percentage metal uptake as a function of time

### 3.4 Order of reaction

The first and second-order kinetics of biosorption processes are described by the rate equations (1) and (2) respectively, which upon integration yield solutions (3) and (4) as shown. Linear plots of  $\ln(q_e - qt)$  versus  $t$  and

$t/q_t$  versus  $t$  were used to test the data for first and second order kinetics. Linear correlation coefficients ( $R^2$  values) were used to deduce the order of reaction. From the sample plot for lead (Figure 4), the results fit better in to second order integrated rate equation for all metals studied. The kinetic parameters for all the metals considered are tabulated in table 2. This shows that the biosorption process is second order for all the selected heavy metals. These results agree with literature (Sari and Tuzen 2007, Patel and Suresh, 2008).

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (1)$$

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (2)$$

$$k_1 t = \ln q_e - (\ln q_e - q_t) \quad (3)$$

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \quad (4)$$

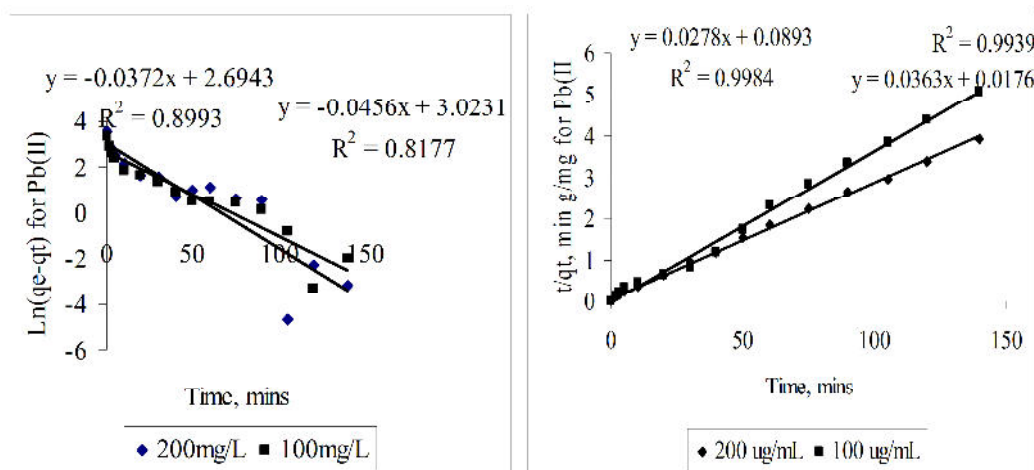


Figure 4. First and second order linearity plots for lead

**Table 2.** Kinetic parameters for Cd, Cr, Cu and lead

Metal	Initial metal concentration ( $\mu\text{g/mL}$ )	Calculated metal uptake $q_e$ in (mg/g) at equilibrium	R values for 1 <sup>st</sup> order linearity test	R values for 2 <sup>nd</sup> order linearity test
Cd(II)	200	3.64	0.338	1.000
	100	2.55	0.159	1.000
Cr(III)	200	8.06	0.598	0.999
	100	5.60	0.884	0.996
Cu(II)	200	26.39	0.719	0.998
	100	24.45	0.944	0.999
Pb(II)	200	35.97	0.904	0.999
	100	27.55	0.948	0.997

### 3.5. Adsorption capacity

Adsorption capacities were determined from the Langmuir adsorption isotherm. The Langmuir and Freundlich isotherms take the forms of equations (5) and (6) respectively where,  $q_e$  and  $q_{max}$  are the equilibrium and maximum metal uptake, respectively in mg per gram of biosorbent,  $C_e$  is the equilibrium metal ion concentration,  $b$ ,  $K_F$  and  $n$  are Langmuir and Freundlich constants, respectively. However, experimental results are best compared with the

Langmuir and Freundlich theories when the isotherms are in their linearized forms (equations (7) and (8)). Thus a linear plot was done for lead. Examination of the linear regression coefficients of the linearized isotherms revealed that the experimental data fitted better to the Langmuir isotherm, Figure 6. The data is reported in table

3.

**Table 3.** Equilibrium concentration  $C_e$  (mg/L) and metal uptake  $q_e$  (mg/g) data fitted to Langmuir and Freundlich isotherms

Metal ion	Cd (II)		Cr (III)		Cu (II)		Pb(II)	
$C_i$ ( $\mu\text{g/mL}$ )	$C_e$	$q_e$	$C_e$	$q_e$	$C_e$	$q_e$	$C_e$	$q_e$
50	14.25 $\pm 0.59$	8.94 $\pm 0.15$	4.21 $\pm 0.51$	5.72 $\pm 0.06$	12.12 $\pm 0.79$	9.47 $\pm 0.20$	30.33 $\pm 0.50$	34.83 $\pm 0.25$
150	87.43 $\pm 0.48$	15.64 $\pm 0.12$	30.75 $\pm 1.93$	14.91 $\pm 0.24$	84.20 $\pm 0.48$	16.45 $\pm 0.12$	83.17 $\pm 8.50$	58.42 $\pm 4.25$
300	213.95 $\pm 0.38$	21.51 $\pm 0.10$	104.18 $\pm 1.73$	24.48 $\pm 0.22$	208.56 $\pm 0.80$	22.86 $\pm 0.20$	146.67 $\pm 12.35$	76.67 $\pm 1.18$
500	410.85 $\pm 1.03$	22.29 $\pm 0.26$	257.63 $\pm 4.56$	30.30 $\pm 0.57$	388.99 $\pm 2.57$	27.75 $\pm 0.64$	338.17 $\pm 2.35$	80.92 $\pm 3.01$
600	517.85 $\pm 0.53$	20.54 $\pm 0.13$	349.20 $\pm 1.64$	31.35 $\pm 0.20$	485.54 $\pm 0.61$	28.62 $\pm 0.15$	426.00 $\pm 6.03$	87.00 $\pm 0.25$
700	616.73 $\pm 0.28$	20.82 $\pm 0.07$	424.07 $\pm 7.75$	34.49 $\pm 0.97$	574.70 $\pm 13.41$	31.32 $\pm 3.35$	537.61 $\pm 1.84$	81.19 $\pm 0.92$
850	760.21 $\pm 0.48$	22.45 $\pm 0.12$	559.71 $\pm 4.16$	36.28 $\pm 1.77$	721.58 $\pm 0.30$	32.11 $\pm 0.08$	631.50 $\pm 3.00$	84.25 $\pm 1.50$
1000	859.33 $\pm 0.59$	35.17 $\pm 0.15$	713.32 $\pm 5.70$	35.84 $\pm 0.72$	869.55 $\pm 4.56$	32.62 $\pm 1.15$	713.17 $\pm 11.18$	93.42 $\pm 5.59$

$$q_e = q_{\max} \left( \frac{bC_e}{1 + bC_e} \right) \quad (5)$$

$$q_e = k_F (C_e)^{1/n} \quad (6)$$

$$\frac{C_e}{q} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}} \quad (7)$$

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (8)$$

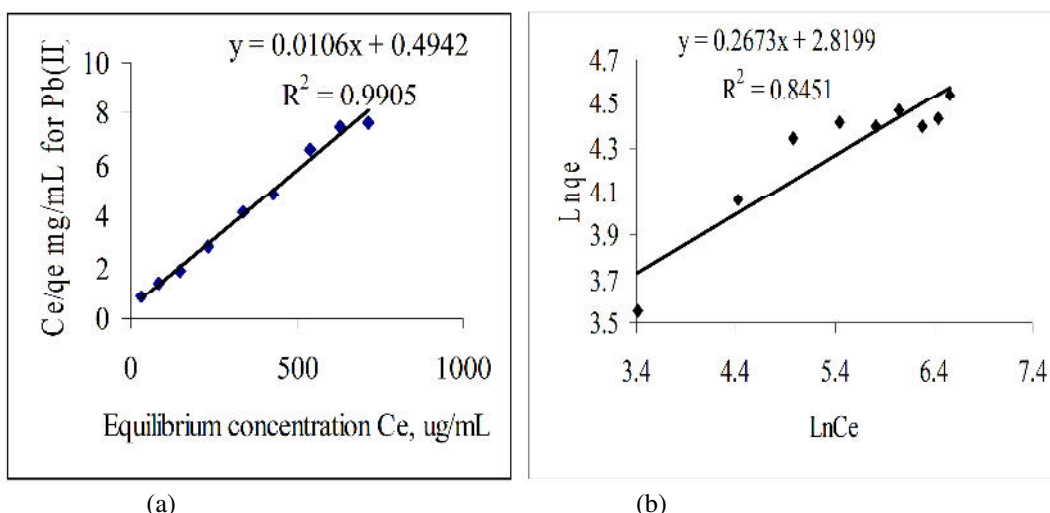


Figure 5. Data fitted to Langmuir (a) and Freundlich (b) isotherms respectively for Pb

The adsorption capacity,  $q_{max}$  was calculated from the linearized Langmuir isotherm and found to be 22.52, 38.19, 35.59 and 94.34 mg/g for cadmium, chromium, copper and lead, respectively. Table 4 gives a comparison of the adsorption capacity of algae with those of other biosorbents from literature. From the table it is clear that the adsorption capacity of freshwater green algae for the selected metals is high compared to other biosorbents. Green algae are therefore suitable for the biosorption of Cd, Cr, Cu and Pb from polluted water.

**Table 4.** Comparison of adsorption capacity of green algae for Cd, Cr, Cu and Pb ions with that of different biomasses

Biomass	Adsorption capacity (mg/g)				Reference
	Cd	Cr	Cu	Pb	
<i>Pinus sylvestris</i>	19.1	-	-	22.2	Rakhshae et al., 2006
<i>Caulerpa lentillifera</i>	4.7	-	-	28.7	Pavasant et al., 2006
<i>Mucor rouxii</i>	8.5	-	-	35.7	Yan et al., 2003
<i>Chlorella minutissima</i>	11.1	-	-	9.7	Roy et al., 1993
<i>P. palmate</i> (red algae)	-	-	6.7	-	Holan et al, 1993
<i>P.aerogenosa</i> (bacteria)	-	-	23.0	68.4	Chang et al., 1997
<i>C. cryptica</i> (diatom)	-	-	26.3	-	Gupta et al., 2001
Siliceous earth	-	-	0.1	-	Gupta et al., 2001
Alfalfa biomass	-	6.2	-	-	Yan et al., 2003
<i>Sargassum glaucescens</i> (brown algae)	-	6.1	-	-	Yang and Chen, 2008
<b>Green algae</b>	<b>22.5</b>	<b>38.2</b>	<b>35.6</b>	<b>94.3</b>	<b>This work</b>

The adsorption capacity calculated from the linearized Langmuir isotherm was compared with the experimentally observed values. The results are reported in table 5.

**Table 5:** Comparison of experimentally observed metal uptakes  $q_e$  (mg/g) against the Langmuir capacities for selected metals

Metal ion	Cd (II)	Cr (III)	Cu (II)	Pb (II)
Langmuir adsorption capacity (mg/g)	22.5	38.2	35.6	94.3
Experimentally observed adsorption capacity (mg/g)	22.29 ± 0.26	34.49 ± 0.97	31.32 ± 3.35	93.42 ± 5.59

The results show good agreement between the Langmuir values and the observed values. This further confirms that the Langmuir isotherm best describes the process.

$Pb^{2+}$  ions generally show high sorption capacity because they have a polarizable soft ion character. The other ions (Cd (II), Cr (II) and Cu (II)) are classified borderline metal ions. In soft ions covalent interactions as well as electrostatic attractions play significant roles in metal uptake while for the borderline to hard ions only electrostatic attraction is important (Can and Jianlong, 2007).



### 3.6 Effect of initial metal concentration

The trend in metal uptake  $q_e$  (mg/g) by green algae at various initial concentrations was determined. The uptake increased with increasing initial metal concentration to a limiting value at initial concentrations between 500 – 700 mg/L, Figure 6. At concentrations above 800 mg/L the observed uptake started to rise again perhaps as a result of the onset of precipitation. Thus the optimum initial metal ion concentrations range from 500 to 700 mg/L for all the metals considered.

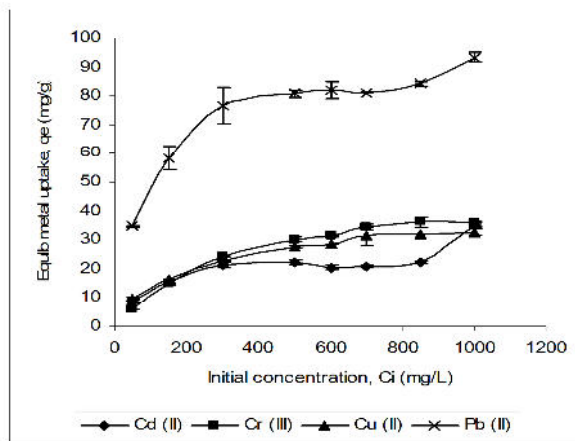


Figure 6. Metal uptake as a function of initial concentration

### 3.7 Concentrations in water, acid - leached and digested algae

The concentration of the heavy metals in environmental water samples and in algae collected from the water were determined. The summary of these concentrations is reported in Table 6.

**Table 6:** Average concentrations of the selected metals in environmental samples

Metal	Water	Algae	
	Total concentration ( $\mu\text{g/L}$ )	Leachable concentration ( $\mu\text{g/g}$ )	Total concentration ( $\mu\text{g/g}$ )
Cd	$1.82 \pm 0.11$	$0.01 \pm 0.09$	$2.30 \pm 0.09$
Cr	$64.33 \pm 0.35$	$4.43 \pm 1.86$	$12.17 \pm 0.20$
Cu	$17.14 \pm 0.15$	$8.23 \pm 0.59$	$25.61 \pm 0.74$
Pb	$12.08 \pm 1.80$	$0.19 \pm 0.01$	$60.50 \pm 1.57$

Concentration factors were calculated from the ratio of metal concentration in green algae to that in the parent water. The mean concentration factors were Cd (2547.01), Cr (367.02), Cu(1843.59) and Pb (7154.95). The results are reported graphically in Figure 10.

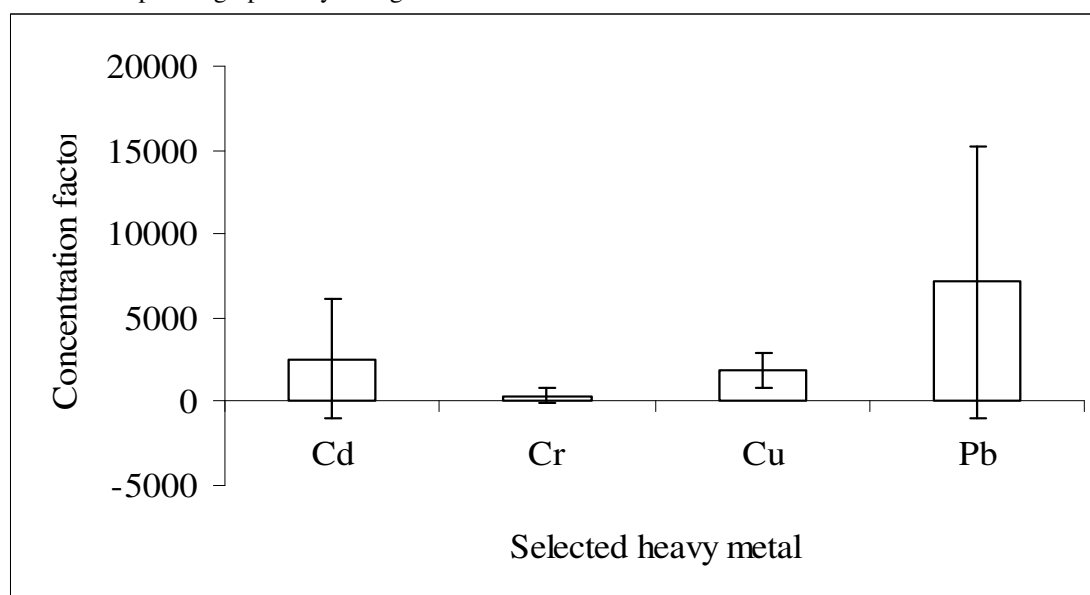


Figure 7. Average concentration factors of Cd, Cr, Cu and Pb by green algae

From the data, lead had the highest concentration factor and chromium the least. The trend is  $Pb > Cd > Cu > Cr$ , the mean concentration factor for each metal being Cr (367.02), Cu (1843.59), Cd (2547.01) and Pb (7154.95). These values could also possibly indicate the trend in the strengths of active metal transport by the algae. The high concentration factors confirm that algae is a good biosorbent and bioindicator for the metals studied.

#### 4: CONCLUSION

The biosorption and biomonitoring study conducted in this work provides significant information regarding the suitability of green algae as a biosorbent and a biomonitor for the selected heavy metals in solution. Adsorption parameters were determined. The best pH for adsorption of the selected metals was found to be 5.0, 5.5, 5.8 and 5.9 for lead, chromium, copper and cadmium, respectively and the times required for metal adsorption equilibrium to be established in model solutions were 15 minutes for cadmium, 40 minutes for both chromium and copper, and 50 minutes for lead. The adsorption process was found to be second order and the data fitted better to the Langmuir isotherm than the Freundlich. The adsorption capacities were found to be 22.52, 35.59, 38.19 and 94.34 mg/g for cadmium, copper, chromium and lead, respectively. The initial metal concentrations which resulted in highest metal adsorption onto green algae were between 500 - 700 mg/L for all the metals considered. The average concentrations of cadmium, lead, copper and chromium in water were  $1.82 \pm 0.11$ ,  $12.08 \pm 1.80$ ,  $17.14 \pm 0.15$  and  $64.33 \pm 0.35$  ng/mL, respectively while in the digested algae, cadmium, chromium, copper and lead concentrations were  $1.36 \pm 0.10$ ,  $12.42 \pm 1.74$ ,  $14.88 \pm 0.99$  and  $14.98 \pm 1.01$  µg/g, respectively. The adsorbed metal (leachable) fraction concentrations were found to be  $0.74 \pm 0.09$ ,  $4.95 \pm 1.86$ ,  $8.23 \pm 0.59$  and  $9.41 \pm 0.74$  µg/g, for cadmium, chromium, copper and lead, respectively.

There was correlation between the total and leachable metal concentrations, ( $R = 0.88, 0.55, 0.95$  and  $0.94$  for cadmium, chromium, copper and lead respectively). As expected, little correlation was found between the heavy metal concentration in algae and the parent water ( $R \leq 0.11$ ). This observation points towards an active transport assisted sorption process as opposed to diffusion mediated one. Furthermore average concentration factors in the range of 2547.01 for cadmium, 367.02 for chromium, 1843.59 for copper and 7154.95 for lead were observed.

From this work, green algae was found to be a suitable biosorbent for effectively removing heavy metals from polluted water. The algae is also suitable as a bioindicator because it is able to accumulate metals to a satisfactory degree. While the metal concentration in the water samples was negligible for all metals considered, the algae was much richer in heavy metal content. This is evidence for pre-concentration of heavy metals from water.

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#### Response to points raised by reviewers

- The removal of metal ions by other processes have been dealt with in section 3.2. Fig 2(a) shows metal ion removal without any algae while fig 2(b) shows the metal removal by green algae alone. Thus % removal by biosorption in figure 2(b) represents metal ion uptake by algae = uptake by algae and other processes – uptake by the other processes.
- FTIR spectrum of Cr loaded algae was replaced with a Pb loaded one.
- Most of the figures (linear Freundlich and Langmuir plots) have been replaced by table 3.
- Experimentally observed values of sorption capacities for the selected metals compared to those calculated from the Langmuir isotherm model have been given in table 5.
- Differences in sorption capacity of the selected metals have been discussed in section 3.5

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