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Biosorption of Pb^{+2} by green marine Algae (Enteromorpha)

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ABSTRACT

Toxic lead has been found in many industrial and wastewater, and its removal becomes a challenging environmental protection aim. For this purpose, the biosorption of lead on the marine green algae Enteromorpha has been studied.

The amount of metal uptake increased steeply by increasing the weight of the biomass and reached equilibrium after 20.51mg/g(algae) at pH 7.0 in agitation experiment. In column experiment, the lead uptake capacity by Enteromorpha algae was found equal to 70 mg/g at optimum pH 3.0. The uptake process is relatively fast and equilibrium reached after 20 minutes of residence itme. The mechanism of equilibrium biosorption was described by Freundlich adsorption isotherm model. FTIR and thermal analysis of pure algae and algae material after lead uptake are compared.

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1. INTRODUCTION

Natural waters have been found contaminated with several heavy metals arising mostly from mining wastes and industrial discharges. The contamination of waste water and soil with heavy metal ions is a complex problem, since these metals are toxic in both their elemental and chemically combined forms **[3-4]**. From an environmental protection point of view, heavy metal ions should be removed at their source in order to avoid pollution of natural waters and subsequent metal accumulation in the food chain **[3]**.

Lead being one of the "big three" toxic metals, it is of profound concern as a toxic waste and contaminant of surface waters as it becomes concentrated throughout the food chain to humans **[1].** Lead damages different body organs (central and peripheral nervous systems and kidney). Lead also has a tratogenic effect, causing stillbirth in women and affecting the fetus **[2].**

Conventional methods for removal are chemical precipitation, chemical oxidation, chemical reduction, ion exchange, filtration, electrochemical treatment and evaporation. [**5-6**]. All these procedures have significant disadvantages, which are incomplete removal, high-energy requirements, and production of toxic sludge or waste products that also require disposal. These methods often are very expensive.

Alternative methods for heavy metal removal were developed in the last past decade, such as biosorption of heavy metal ions on biomass. Marine algae, a renewable natural biomass, has attracted the attention of many investigators because it was found in large quantities in the sea and was used as dead nonliving materials which has an ability to adsorb and remove heavy metals **[7-8]**.

In the literature, there are divergent mechanisms explaining the metal uptake by marine algae. A semi speculative model of the structure of the cell walls of the algae has been proposed recently. This could suggest that there are two common moieties to which the uptake ability of taxonomically different algal biomass was attributed: sulfated ester polysaccharides (fucodans,

carrageenans, galatansandxylans) and polyuronides (galactouronic, glucouronic, guluronic, and mannuronic acids). The carboxyl and sulfate groups are considered the bulk of metal uptake sorption **[14].** Also algae were found accumulating heavy metal in their habitat and are thus used as heavy metal pollution monitors in fresh and salty water such as river, sea and ocean in different regions of the world. They have been also used in on-site bioremediation of polluted natural water **[9-10]**.

In general the algae are very soft, with the tendency to disintegrate which prevents the follow- up experiments even in laboratory column **[15]**. The present work focused on the ability of green algae Enteromorpha to adsorb Pb^{2+} from aqueous medium at 25°C. The algae was collected from Riviera region near Manara in Lebanon. FTIR spectra and TG of Enteromorpha algae were detected before and after adsorption, the effect of mass, pH, concentration, and residence time were studied. Also column application for algae and algae after reflux were studied for maximum capacity per one gram. This was compared with the capacity of Sweden Wood powder.

2. THEORETHICAL

The Freundlich equation model was chosen for estimation of the maximum metal uptake. Whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied.

$$q = K_F (C)1/n$$

$$\text{Log } q = 1/n \text{ Log } C + \text{Log } K_F$$

Where "q" is the maximum metal uptake and "C" is the initial ion concentration in ppm, "K_F " and "n" are the Freundlich constants, the characteristics of the system. K_F and n are the indicator of adsorption capacity and adsorption intensity respectively **[21].**

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

2. EXPERIMENTAL

2.1. Chemicals. All reagents are from Fluka: disodium ethylenediamine tetra acetic acid (EDTA), Lead nitrate $Pb(NO_3)_2$, xylenol orange indicator, hexamine, nitric acid, sodium acetate and acetic acid for acetate buffer pH (2-8).

2.2. Algae collection. The raw algae Enteromorpha was harvested from the Lebanese coast at Manara (**Fig. 1**) washed with tap and deionized water in order to remove extra salts, sun dried, and grounded to particle size (0.5 mesh). Finally the fine powder is oven dried at 60 °C for 24 hours and ready to be used in metal uptake study [**11**].

Fig.1 Harvested of algae were done from Riviera shore near Manara(Beirut).

2.3. Equipments and Instruments. The potentiometric measurements were carried out using Denver instrument Model 225 pH – Ion selective electrode meter fitted with a combined glass electrode (reading to ± 0.01 pH unit). The reaction flask was kept constant at 25 ^{o}C (± 0.1 ^{o}C) by using a thermostat Model Heto HMT 200. The shaker (Wiggen Hauser OS-150, Germany) and centrifuge (Sigma 203) were used for agitation experiment. Infrared data were collected on a Schimadzu 8300 FTIR spectrophotometer using the KBr pellet method.

2.4. Metal analysis. Titration with EDTA (2.0 x10^{-4} M) was used in order to determine the concentration of Pb^{2+} (10-1000 ppm) using xylenol orange as indicator for color change from purple to yellow. Hexamine buffer was used to adjust the pH to 5.0-6.0.

2.5. Effect of mass of algae on metal uptake. The effect of mass of algae on metal uptake was studied in batch system. 25 mL solution of 300 ppm lead (at pH 4.00) in 50 mL Erlenmeyer flask was shaken with different masses of algae for 2 min at 200 r.p.m and left to stand for 24 hrs in water bath at 25 ^{o}C and then analyzed for the remaining lead [**12**].

2.6. pH effect on metal uptake. The mass of algae used in this experiment is the optimum mass 0.3 g done in batch system using 50-mL Erlenmeyer flasks with a reaction volume of 25 mL lead solution (300 mg/L) at 25^{o}C. The mixture was shaken for 2 min (at 200 r.p.m) and left to stand for 24 hrs, at different pH using sodium acetate buffer to cover the pH range 3.0 - 7.5 (3 , 3.6 , 4 , 5 , 6.08 , 7.08 , 7.50). In this experiment the pH value doesn't exceed 7.50 due to the precipitation of lead as lead hydroxide **[16].**

2.7. Effect of lead concentration on metal uptake. This experiment was done as above at only pH 3.0 (optimum value) using sodium acetate buffer but with different lead concentrations (10, 25, 50, 100, 150, 200, 250, 300, 400, 500 and1000 ppm).

2.8. Effect of residence time on metal uptake. This experiment was done at 25 ^{o}C with 25 mL lead (200 ppm) and 0.3 g algae in 50-mL Erlenmeyer flasks at pH 3.0 (using sodium acetate buffer) and shaked at 200 r.p.m for 2 minutes with variable waiting time (2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes) **[16].**

2.9. Procedure for column utilization (Breakthrough curve). The column used has a diameter of 2 cm and a length of 44 cm. It was packed uniformly with 1.0 g of green ***Enteromorpha*** algae powder before and after reflux. The lead solution (200 ppm) at pH 3.0 was drained through the column at a constant rate 2.5 mL/min. Aliquots of similar volume were collected repeatability from the column and analyzed for lead **[17].**

2.10. Swelling characteristics. The swelling characteristics (distention index, swelling ratio, and volume of absorbed solvent) were obtained from the weights and volumes of dry and swollen particles for algae alone, algae after reflux, algae mixed with silica and sawdust of sweden wood. The mass of each biomass was 1.440 g. Dry particles were swollen in cylinders with distilled or deionized water and degassed under lower pressure. The volume and weight were measured after two hours, from which the swelling characteristics have been calculated **[15]**:

1) *Distention index (DI) was calculated from the ratio:*

$$\mathbf{DI = V_s/W_d}$$

Where: $\mathbf{V_s}$:is the volume of the particles after swelling
$\mathbf{W_d}$:is the weight of the dry particles.

2) *The swelling ratio (Q):*

$$\mathbf{Q = W_s/W_d}$$

Where: Ws is the weight of swollen particles

3) *Volume of absorbed solvent(VAS):*

$$\mathbf{VAS = (W_s - W_d)/W_d}$$

3. RESULTS AND DISCUSSION

3.1 Infrared spectroscopy

FTIR spectra of Enteromorpha algae alone shows a strong stretch at 3395 cm^{-1} due to –NH of amino group, a strong stretch at 1646.5 cm^{-1} and a weaker ones at 1436 cm^{-1} due to carboxylate group, and strong bending vibration at 1105 and 1158 cm^{-1} due to C-O of ether and alcoholic group respectively, **Fig. 2 [18, 19].** FTIR spectra of Enteromorpha algae with adsorbed lead, also shows many peaks similar to free algae, **Fig. 2**. However the peak due to –NH strech has been shifted to lower wave number 3291 cm^{-1} indicating involvement of binding of lead to –NH group. The bands at 1651 cm^{-1} due to carboxylate shows

a little shift to higher wave number and a variation in the shape of the peak compared to free algae, also the peak at 1105 cm^{-1} disappears in the case of algae with biosorbed lead. Both indicate the involvement of carboxylate and ester groups in the binding with lead.

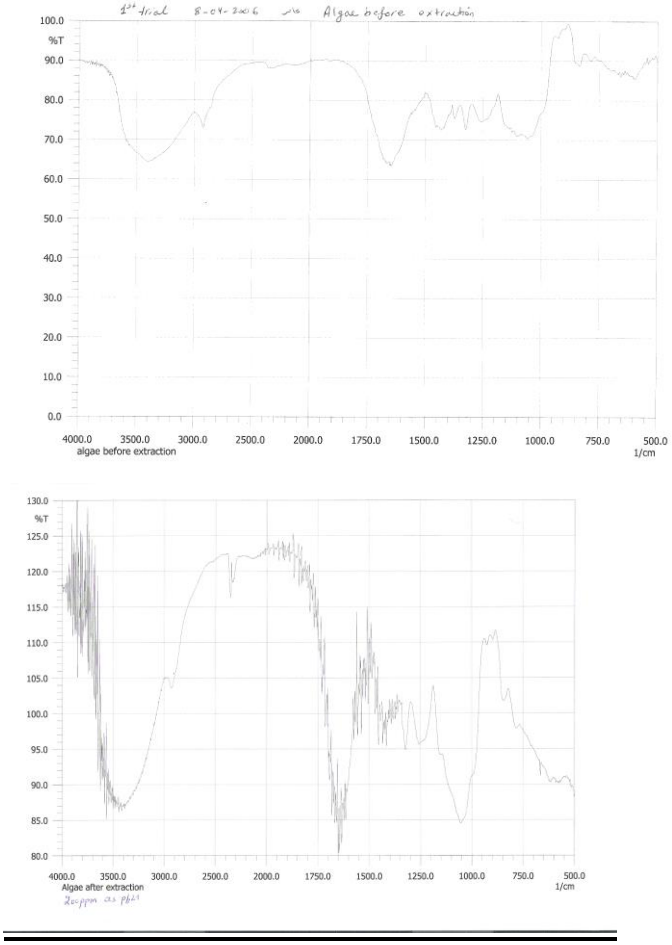

Fig. 2. FTIR spectra of Enteromorpha algae with or without adsorbed lead

3.2. Metal uptake

The effect of mass of algae on metal uptake was studied in batch system. There was a steep increase in the biosorption of mercury as the mass of algae increased from 0.05 g to 0.30 g. The optimum mass of algae 0.30 g corresponds to a maximum metal uptake of 82.14 % for a volume reaction of 25 mL lead (300 ppm), **Fig. 3**. With higher biomass level ranging from 0.3 to 0.35 g there were no significant increases in the metal uptake.

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

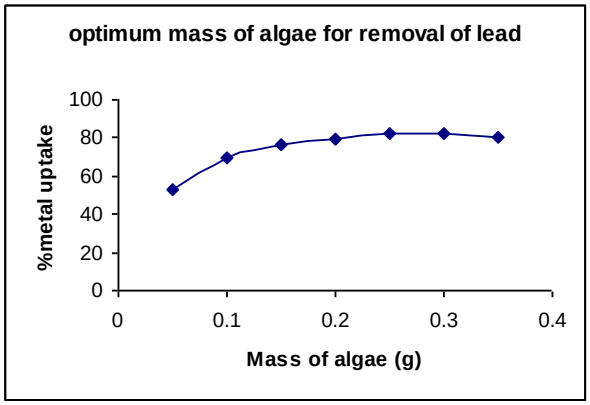

Fig 3. Effect of mass of Enteromorpha algae on metal uptake for a 25 mL of lead (300 ppm) solution.

The standard lead solution (300 ppm) was prepared in acetate buffer to cover a pH range 3.0-7.5. The data of metal uptake by 0.30 g algae in a 25 mL standard lead solution for different pH values are presented in **Fig. 4**. Lead shows maximum binding of biomass at an optimum pH 3.0 corresponding to a metal uptake of 86.62 % (21.55 mg/g) using sodium acetate buffer and there was no change in metal uptake for pH values between 3.6 and 7.5.

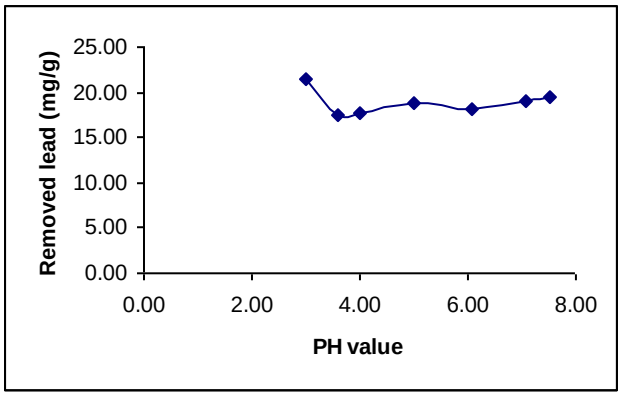

Fig 4. Effect of pH on metal uptake by 0.30 g algae in 25 ml lead solution (300 ppm) using acetate buffer.

The effect of lead concentration on metal uptake was done in batch system concentrations of 0.3 g at optimum pH 3.0 (using sodium acetate buffer) for a residence time of 24 hrs and different lead concentrations in the range (10 -

1000 ppm). There was a sharp increase in biosorption as the lead concentration increases from 10 to 150 ppm algae and reaches a maximum at 200 ppm lead with a metal up take of 95.50 % and a metal uptake capacity of 15.29 mg/g algae, **Fig. 5**. For lead solution greater than 200 ppm, the algae become saturated and no increase in % uptake was noticed.

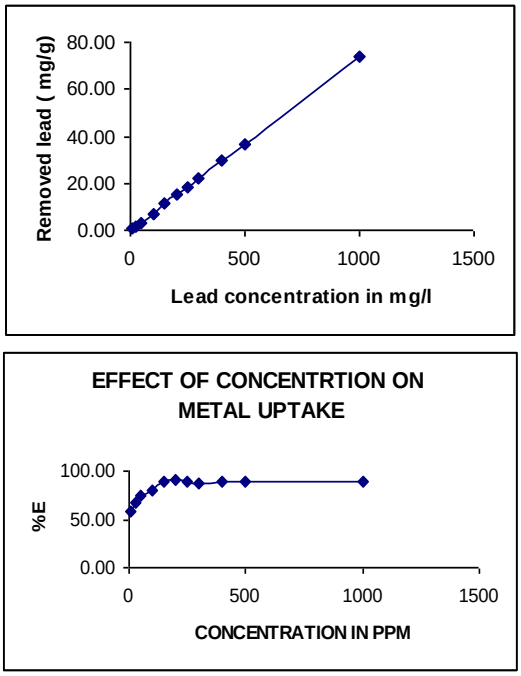

Fig 5. Effect of concentration on metal uptake by 0.30 g algae in 25 ml lead solution (PH = 3) using acetate buffer.

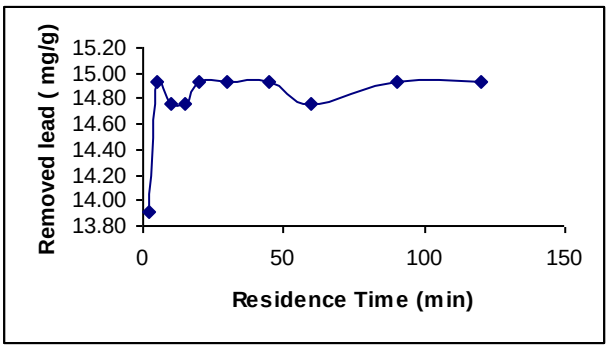

Fig 6. Effect of residence time on metal uptake

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

Effect of residence time on metal uptake was studied with 0.30 g algae in 25 mL lead solution 200 (ppm) at optimum pH 3.0 using sodium acetate buffer, and variable residence time (2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min) in which each flask was shaken at 200 r.p.m for 2 min, **Fig. 6**. This experiment shows that algae have bound most of the metal after 20 min, and that equilibrium was reached at maximum metal uptake of 88.94 % (capacity 14.49 mg/g). This result is in parallel with those previously obtained with different algae and fungi for heavy metal uptake **[17, 18].**

Breakthrough curve of lead was determined using column packed uniformly with 1.0 g of Enteromorpha algae powder at pH 3.0. Aliquots of 25 mL were collected from the column and analyzed for lead, **Fig. 7**. This experiment showed that 70.0 mg of lead was removed after passage of 525 mL solutions of lead (200 ppm), **Table 1**. This result is in parallel with those recently obtained with different microalgae Chalamydomonas Reinhardti for heavy metal uptake **[21].**

Table 1. Capacity of a column filled with 1 g algae using 200 ppm lead solution

Volume (mL)	Q mg/g	Volume (mL)	Q (mg/g)
25	5.0	300	55.13
50	10.0	325	59.2
75	14.69	350	62.75
100	19.28	375	65.68
125	23.87	400	67.57
150	28.35	425	68.94
175	32.83	450	69.28
200	37.31	475	69.51
225	41.79	500	69.74
250	46.27	525	70.00
275	50.75		

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

Also this experiment was repeated using Sweden powder wood with same conditions **Fig. 8**, showing a 9.65 mg lead uptake with total volume of 125 mL, **Table 2**.

Table 2. Capacity of a column filled with 1 g wood Pb solution

Volume mL	Q mg/g
0	0
25	4.07
50	7.01
75	7.89
100	8.77
125	9.65

While repetition using algae residue after reflux gave a 68.89 mg of lead uptake with a total of 625 mL, **Table 3**. Saturation of the column after passage of 25 mL aliquots of 200 ppm lead is shown in **Fig. 9**.

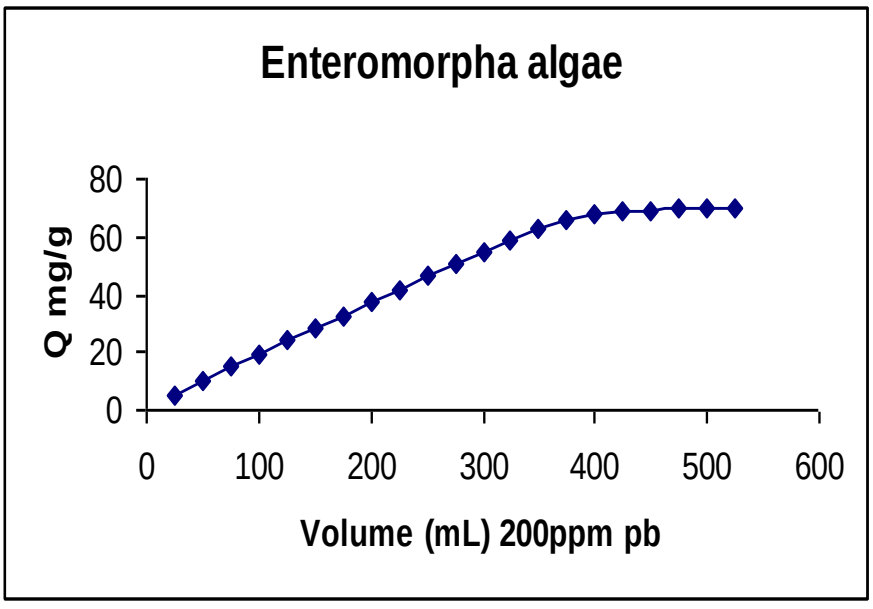

Fig. 7. Breakthrough curve: capacity of 1 gram of marine algae (Enteromorpha) at pH 3 and flow rate 2.5 mL/min with 200 ppm lead solution (partical size 0.5 mesh).

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

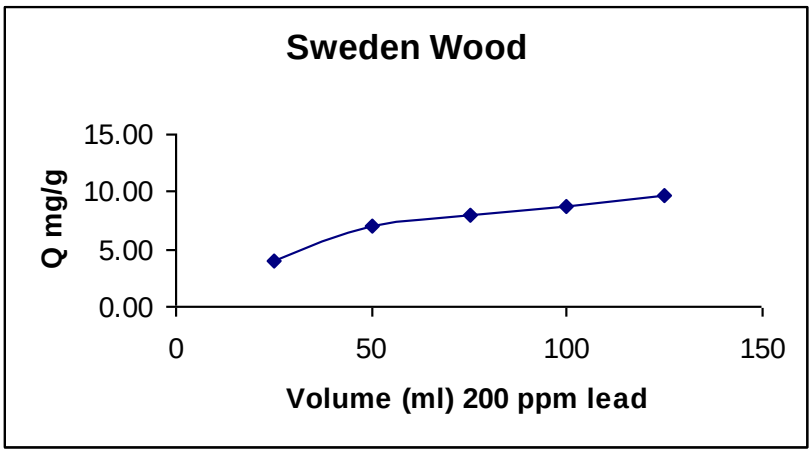

Fig. 8. Breakthrough curve: capacity of 1 gram of Sweden Wood powder at pH 3.0 and flow rate 2.5 mL/min with 200 (ppm) lead solution (partical size 0.5 mesh).

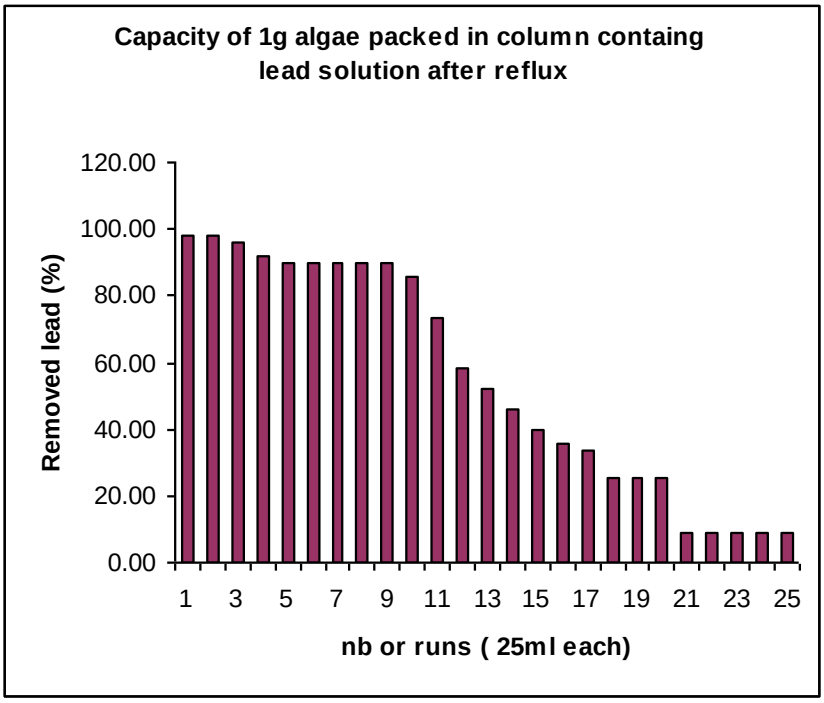

Fig. 9. Saturation of the column after passage of 25 aliquots of 25 mL, 200 ppm lead for a column filled with 1 g Enteromorpha algae residue after reflux.

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

Table 3. Capacity for a column filled with 1 g algae after reflux , for treatment of 200 ppm Pb solution.			
Volume (mL)	**Q (mg/g)**	**Volume (mL)**	**Q (mg/g)**
25	4.90	350	57.54
50	9.8	375	59.54
75	14.77	400	61.33
100	19.36	425	63.01
125	23.84	450	64.28
150	28.32	475	65.55
175	32.8	500	66.82
200	37.28	525	67.26
225	41.76	550	67.70
250	46.03	575	68.14
275	49.68	600	68.58
300	52.61	625	69.02
325	55.23		

The volume of swollen particles increased during the first 20 minutes then it remained constant during 2 hours. The values of DI, Q, and VAS of the biomass particles **Table.4**, are approximately the same except for algae mixed with silica and algae after reflux which indicates an improvement of stability and mechanical properties of the biomass **[15]**.

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

Table 4. Swelling parameters of the used biomass

Type of biomass	Particle size(mesh)	Ws	DI	Q	V_s	VAS
Algae alone (1.442 g)	0.5	10.41 g	6.934 cm^3/g	7.219	10 cm^3	6.219
Algae with reflux (1.442 g)	0.5	6.88 g	4.166 cm^3/g	4.775	7 cm^3	3.775
Algae mixed with silica (4.32 g)	0.5	11.54 g	2.43 cm^3/g	3.687	10.5 cm^3	1.671
Sweden wood (1.442 g)	0.5	8.92 g	6.935 cm^3/g	6.185	10 cm^3	5.185

3.3. Adsorption Isotherm Calculations

It was reported that Freundlich model was applicable in lead biosorption with Enteromorha algae [**17**].

Figure 10 shows the results of Freundlich adsorption isotherm model with a very satisfactory fit, $R^2 = 0.999$. The plot of (log C) versus (log q) was employed in order to determine the intercept value log K_F and the slope 1/n.

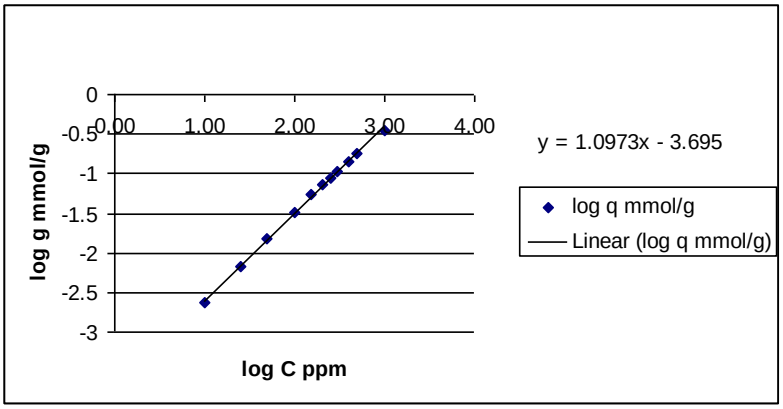

Fig. 10. Adsorption of lead onto Enteromorpha algae using Freundlich model.

The magnitude of Freundlich constants K_F and n reveals easy separation of Pb(II) ions from aqueous medium and indicates favorable adsorption, **Table 5**.

It is of importance to realize that values of 1/n greater than unity imply the formation of multilayer of metal on the surface of biomass.

Table 5. Freundlich model constants for adsorption of lead onto Enteromorpha algae.

Métal ion	***Freundlich constants***		
	K_F	***n***	R^2
Pb (II)	2.081×10^{-4}	0.9113	0.999

3.4. Thermal analysis

The results of thermo gravimetric analysis of free algae and algae with biosorbed mercury are shown in **Fig. 11 and 12** respectively. The algae materials were heated to 800 ^{0}C, where all relevant weight loss was complete. The % mass loss at each temperature for Enteromorpha algae are:

12.82 % (65^{o}C), 36.3% (217^{o}C), 21.58 % (448^{o}C), 25.8 % (717.2^{o}C)

while the % mass loss and temperature for algae with biosorbed lead are:

16.33 % (77.78^{o}C), 38.76 % (221^{o}C), 17.10 % (435^{o}C), 24.61 % (660^{o}C).

The two TGA curves reveal difference between loss in weight with temperatures for both materials, with greatest difference occurring at 220^{o}C where free algae shows a loss of 36.3 % while algae with biosorbed lead shows a loss of 38.7 %. The 2.46 % difference between the two could be attributed to the adsorbed amount of lead. Also, this process is exothermic in both cases.

H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*

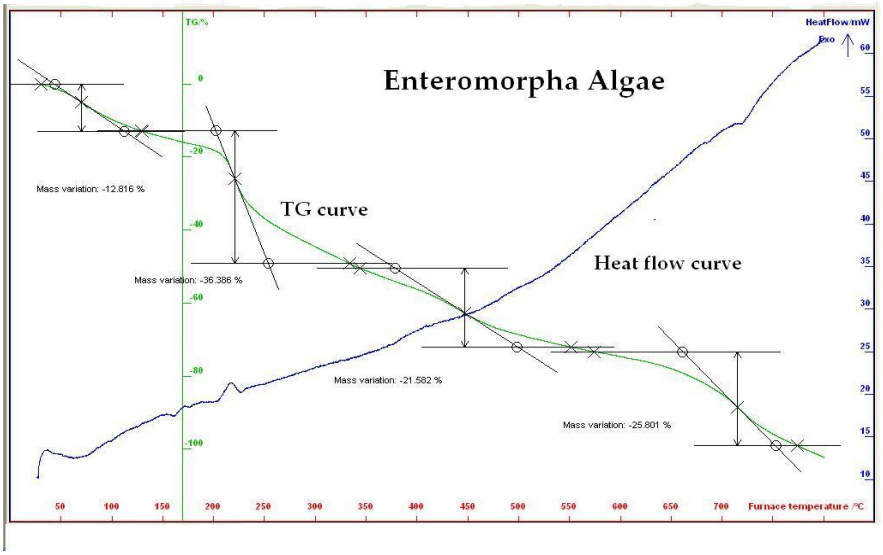

Fig. 11. Thermal analysis of Enteromorpha algae

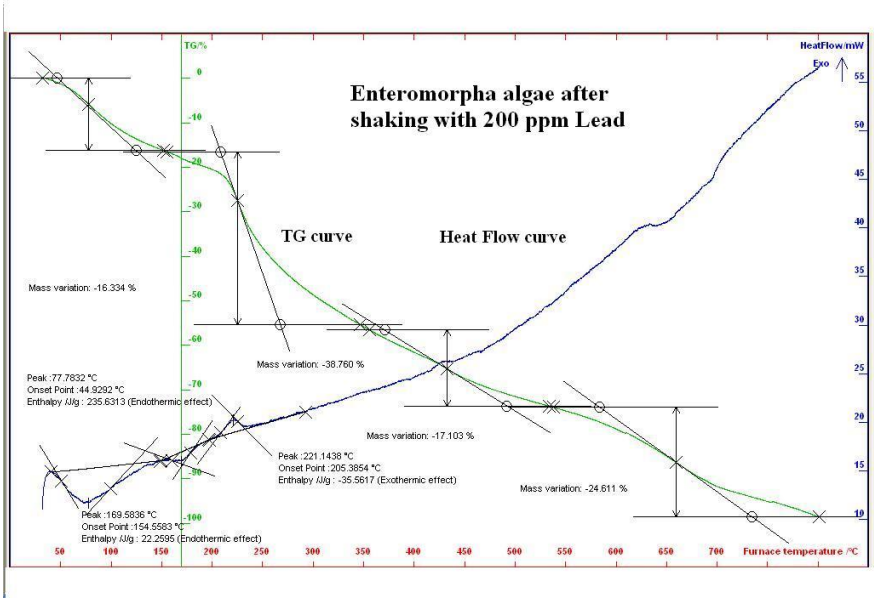

Fig. 12. Thermal analysis of Enteromorpha algae with adsorbed lead

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H. H. Hammud[1], E. M. E. Mansour[1], Ali El-Shaar[1],M. Akkileh[1], W. Mustapha[1], S. Shaalan[2]*