

BIOSORPTION OF IRON FROM AQUEOUS SOLUTION BY DRIED *SPIRULINA PLATENSIS* AND *CHLORELLA VULGARIS*

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Abstract

The present investigation shows that substantial amount of iron ions biosorption from aqueous solution onto the biomass of dried cells of blue green alga *Spirulina platensis* and green alga *Chlorella vulgaris* at different iron concentrations. The IR analysis for surface function group of algal biomass revealed the existence of amino, carboxyl, hydroxyl and carbonyl groups, which are responsible for the biosorption of iron. Also, the results of scanning electron microscope analysis suggested that the biomass of *S. platensis* and *C. vulgaris* are an extremely efficient biosorbent for the removal of iron from aqueous solutions.

Key Word: Biosorption; IR analysis; Scanning Electron Microscope analysis; *Spirulina platensis* and *Chlorella vulgaris*.

Introduction

The production of metals has rapidly increased since the industrial revolution. Some metals usually form compounds that can be toxic carcinogenic or mutagenic even in very low concentrations (**Picardo *et al.*, 2009**). Iron is commonly found in rocks and soil. Under proper conditions, iron will leach into the water resources from rock and soil formations. Exceeding iron concentration greater than 0.3 mg/L causes water staining that adversely affect plumbing fixtures, dishware, clothes and produce a yellow to reddish appearance in water. These levels may also impart taste and odor of drinking water. The US Environmental Protection Agency (USEPA) has established a secondary drinking water regulation of 0.3 mg/L for iron (**EPA, 2000; Ncibi *et al.*, 2007**).

Conventional techniques for metal removal from aqueous solutions include chemical precipitation, ion exchange, adsorption process, membrane process, crystallization and electrochemical treatment. Considering the cost, technical complexity and limitations of the conventional methods make the alternative biological methods more appealing (**Schiewer and Volesky, 1995**). Biological process for metal ions removal from aqueous solutions can be divided into three general categories: (a) sorption of metal ions onto the surfaces of a microorganism, (b) intracellular uptake of metal ions, and (c) chemical transformation of metal ions by microorganisms. The latter two processes require live organisms to proceed (**Holan and Volesky, 1994; Mamisahebei *et al.*,**

2007). Sorption of heavy metals by various biological materials has been proposed as an efficient and potentially cost effective tool for metal enriched industrial effluents. Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (Gadd, 1993).

Biosorption is a property of certain types of inactive, dead microbial biomass to bind and concentrate heavy metals from very dilute aqueous solutions (Gaur and Dhankhar, 2009). Capability of microorganisms to bind heavy metals in aqueous solutions has long been of scientific interest (Aksus and Gulen, 2002; Sen and Ghosh Dastidar, 2007).

Various biomasses such as bacteria (Iyeret *et al.*, 2005), yeast (Tunali *et al.*, 2006; Anayurt *et al.*, 2009) and algae (Gupta and Rastogi, 2008 a, b and 2009) were investigated as biosorbent for the removal of heavy metals. The most important criteria for developing algal biosorbents to decrease harmful metal contents in soil and water are the followings: ability for rapid adsorption of huge amounts of metal ions, availability in large quantities because of worldwide cultivation and relatively cheap production (Solisio *et al.*, 2006; Abu Al-Rub *et al.*, 2006). *Spirulina* and *Chlorella* cells were used by many researchers to test their ability to remove heavy metal ions from polluted water (Gokhale *et al.*, 2008; Çelekli *et al.*, 2010). The use of various conditions for the adsorption resulted in different adsorption properties. Mehta and Gaur (2001) compared the different adsorption features of living and dead biomass of *Chlorella vulgaris* cells and stated that the use of dead biomass is favorable because it tolerates high concentrations of toxic ions, a nutrient supply unnecessary and the culture conditions are not limiting. Blue-green algae including, *Spirulina* (*Arthrospira*), *Nostoc*, *Anabaena*, and *Synechococcus* were the typical examples that showed the potential as biosorbent for efficient removal of heavy metals from wastewaters (El-Sheekh *et al.*, 2005).

Cyanobacteria have some advantages over other microorganisms including their greater mucilage volume with high binding affinity, large surface area, and simple nutrient requirements (Roy *et al.*, 1993). Cyanobacteria are easily cultivated in a large scale in laboratory cultures providing a low cost biomass for the biosorption process. Several algae were tested for their ability to adsorb heavy metals.

The aim of the present work was to study the removal of iron from solutions by using dead *Spirulina platensis* and *Chlorella vulgaris* biomass.

Materials and methods

Algal samples

Spirulina platensis and *Chlorella vulgaris* dry material were obtained from Algae Unit at National Research Center.

Preparation of iron solution

Metal salt used in the preparation of the synthetic metal bearing solutions were FeSO₄ at different concentrations 100, 150, 200, 300 and 400ppm. Deionized water was used in all experiments.

Analytical methods

Determination of metals concentrations

The concentrations of metals in all samples were determined according to the (APHA, 2005) method using Atomic Absorption Spectrometer (Varian Spectra AA 220, USA) with graphite furnace accessory and equipped with deuterium arc background corrector. Precision of the metal measurement was determined by analyzing the metal concentration of all samples (at the Regional Center of Mycology and Biotechnology, Cairo, Egypt).

Batch biosorption experiments

General uptake procedure experiment was carried out in 250 ml conical flask containing 100ml of different concentrations of 100, 200, 300 and 400ppm of FeSO₄, 1g of biomass was introduced into conical flask. The flask was sealed with rubber cork. Then the flask was kept in rotary shaker with an agitation rate of 200rpm for 24h. Then it was filtered with the help of Whatmann no.1 filter paper. Filtered sample was analyzed for concentration of heavy metal in atomic absorption spectroscopy. To distinguish between possible metal precipitation and actual metal sorption, controls (blank) were used without biosorbent materials.

The percent biosorption of metal ion was calculated as follows:

$$\text{Biosorption (\%)} = \frac{C_i - C_f \times 100}{C_i}$$

C_i = initial concentration

C_f = final concentration

All the experiments were carried out in triplicate and the mean of the quantitative results were used for further calculations. For the calculation of mean value, the percent relative standard deviation for results was calculated and if the value of standard deviation for a sample was greater than 5%, the data were discarded.

Effect of pH on biosorption

The batch experiment was carried out by contacting 0.1 g of alga with 100 ml of 50 mg/L of metal solution in 250 ml stopper conical flask at different pH value, ranging from 2 to 8. The pH of the solutions was adjusted either by hydrochloric acid or sodium hydroxide. The mixture was shaken for 2 h at room temperature, filtered and the final pH for each sample was determined.

IR analysis

Infrared Spectroscopic (IR) spectra were taken to know the chemical bonding or molecular structure of organic or inorganic materials. Samples for IR analysis were prepared with the biomass loaded with and without metal solutions such as 100ppm and 400ppm for each species. Spectra were taken using IR-AVATAR 330 (Central Laboratory, Faculty of Science, Cairo University) in order to investigate the functional groups and the possible metal binding sites present in the *S. platensis* and *C. vulgaris* samples.

SEM Analysis

For Scanning Electron Microscopic the Samples coated by gold sputter coater (SPI- Module).The samples examined by scanning electron microscopy (JEOL-JSM-5500LV) by using high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt. Samples studied were prepared with biomass treated with and without metal solution. SEM analysis was used to study the morphological changes of *S. platensis* and *C. vulgaris*.

Results and Discussion

The effect of pH value on the biosorption of iron ions onto *S. platensis* and *C. vulgaris* biomass was evaluated and the results were presented in Table (1). It is clear that the maximum biosorption for iron reached 67.5 and 75% at pH 6 and 8, respectively for *S. platensis* while *C. vulgaris* recorded 52.5 and 54.5% at pH 4 and 6. On the other hand, *S. platensis* recorded 45.5 and 40% at pH 2 and 4, also *C. vulgaris* recorded 44.2 and 37.5% at pH 2 and 8. The current results indicated that the biosorption of iron was increased with increasing the pH value for two species. This is because, at lower pH, the concentration of positive charge (protons) increased on the sites of biomass surface, which restricted the approach of metal cations to the surface of biomass because of charge repulsion (Kaewsarn, 2002).

Table (1): Effect of pH on the biosorption of iron (at concentration 200 ppm) by *S. platensis* and *C. vulgaris* biomass.

pH	Final Iron Concentration (ppm)		Removal Efficiency (%)	
	<i>S. platensis</i>	<i>C. vulgaris</i>	<i>S. platensis</i>	<i>C. vulgaris</i>
2	109	111.5	45.5	44.2
4	120	95	40	52.5
6	65	91	67.5	54.5
8	50	125	75	37.5

As the pH increase, the proton concentration decreases and the biomass surface is more negatively charged. The biosorption of the positively charged metal ions increased till reaching their maximum biosorption around pH 6 and 8 for *S. platensis* and pH 4 and 6 for *C. vulgaris*. This could probably correlate to the different characteristics between the two species of algae iron affinity to the surface functional groups of the cell wall is higher *S. platensis* than *C. vulgaris* at high pH value affinity to the algal biomass, and their binding mechanism include a combination of ion exchange, chelation, and reduction reactions, accompanied by metallic iron precipitation on the cell wall matrix.

The data presented in Table (2) showed the effect of metal concentration on biosorption process. The concentration of iron was varied between 100 and 400 ppm at the optimum pH (6 for *C. vulgaris* and pH 8 for *S. platensis*). The results presented in Table (2) indicated that the biosorption of iron at the beginning was 71.6, 67.5, 51.5 and 24.67% respectively for *S. platensis*. On the other hand, *C. vulgaris* recorded 61.6, 54.5, 46.8 and 42.9% respectively. The biosorption was decreased with increasing the metal concentration. This behavior was attributed to the fact that, initially, all binding sites on the biomass surface were vacant resulting in high metal biosorption at the beginning. After that, with increasing metal concentration, the biosorption of metal was decreased because of a few active sites were available on the surface of the algal biomass.

Table (2): Effect of initial iron concentration on iron removal efficiency by *S. platensis* and *C. vulgaris* biomass

Initial Iron Concentration (ppm)	Final Iron Concentration (ppm)		Removal Efficiency (%)	
	<i>S. platensis</i>	<i>C. vulgaris</i>	<i>S. platensis</i>	<i>C. vulgaris</i>
100	28.4	38.4	71.6	61.6
200	65	91	67.5	54.5
300	154.6	159.5	51.5	46.8
400	301.7	228.6	24.67	42.9

The results illustrated in Fig. (1 A, B and C) showed the IR spectra of the unloaded biomass and iron-loaded biomass. These results represented the information about the functional groups (like sulphonic, carboxylic (fatty acids and amino acids), phosphate, amid and hydroxyl (polysaccharide)). **Campanella et al. (1998)** studied *Spirulina platensis* surface cell wall biomass and the possible interaction between metals and the functional groups. From these data, of figure (1A) it is clear that the strong and broad band's at 3961, 3263, 3205 and 3166cm⁻¹ might be related to the overlapping between NH and OH stretching vibration. Where the band at 3062 cm⁻¹ could be related to the CH stretch and the band 1900 cm⁻¹ could be the aliphatic group. Band at 1400cm⁻¹ related to the CH₃, also band at

1169 cm^{-1} could be assigned to asymmetric stretching vibration of CO. On the other hand, the intense and strong band at 617 cm^{-1} might be attributed to the stretching of CH group on the surface of the biomass (**Sari and Tuzen, 2008**). Meanwhile, some bands in the fingerprint region could be related to the phosphate groups. It could be observed that the bands at 3961, 3263, 3205, 3166, 3062, 1900, 1169 and 617 cm^{-1} were changed to 3946, 3278, 3220, 3067, 3024, 3004, 1627, 1415, 1384 and 613 cm^{-1} after loading of iron at 100 ppm concentration Fig.(1 B) and 3988, 3938, 3911, 3379, 3321, 3305, 3197, 1620, 1388, 1265, 1107 and 609 cm^{-1} after loading of iron at 400 ppm concentration Fig.(1 C). It was observed that the new bands at 466 cm^{-1} in Fig. (1 B) and bands at 597, 470, 432 cm^{-1} respectively in Fig. (1C) it could be presence of the metal (iron). The significant changes in the wave number of these peaks after loading of iron indicate that the functional groups (amido, hydroxyl and CO) were involved in the biosorption of iron on the surface of *S. platensis*.

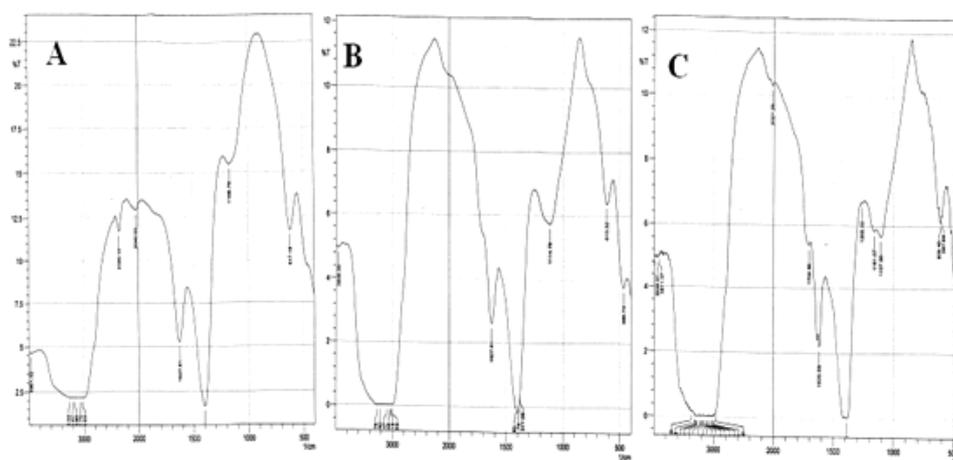


Figure (1): *Spirulina platensis*: A- Control; B- 100ppm iron concentration; C- 400ppm iron concentration

On the other hand, Figure (2 A) show that the strong and broad band's 3942, 3550, 3861, 3838, 3205 and 3093 cm^{-1} related to the OH group and band at 2345 cm^{-1} could be related to the CH stretch while band at 1631 cm^{-1} related to the CH_3 group and band at 1168 cm^{-1} it could be CN group finally band at 632 cm^{-1} might be attributed to the stretching of CH group on the surface of the biomass of *C. vulgaris*. New bands appear at 408 and 466 cm^{-1} after load by iron at 100 ppm Fig. (2 B) and at 424, 470 cm^{-1} at 400 ppm of iron Fig.(2 C).

FTIR analysis led **Ting et al., (1995)** to suggest the involvement of carboxyl groups in binding of Ag (III) on *Chlorella vulgaris*. Participation of carboxyl groups in adsorption of Cu, Cd and Pb on cyanobacterial cell wall has

also been demonstrated (Yee *et al.*, 2004). According to Greene *et al.* (1986) amino group plays an important role in binding of Ag to *C. vulgaris* as pretreatment of the alga with succinic anhydride that is known to react with amino groups making them unavailable for metal binding, resulted in a significant (50%) decrease in Ag binding.

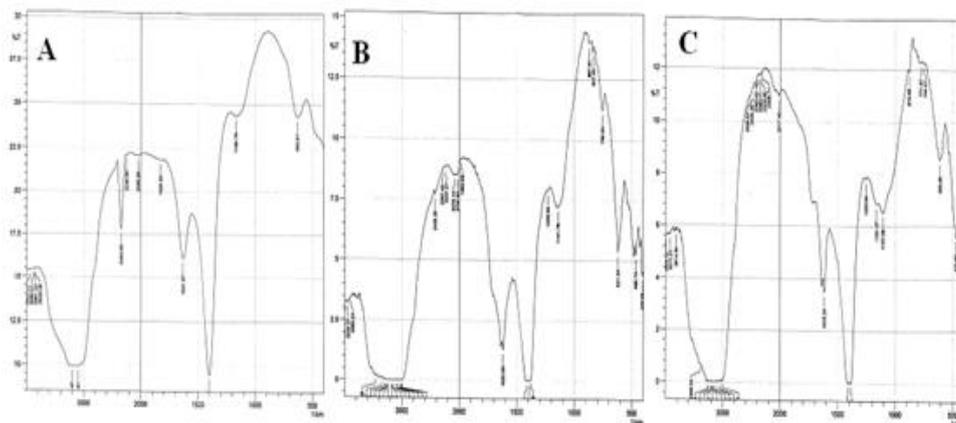


Figure (2): *Chlorella vulgaris*: A- Control; B- 100ppm iron concentration; C- 400ppm iron concentration

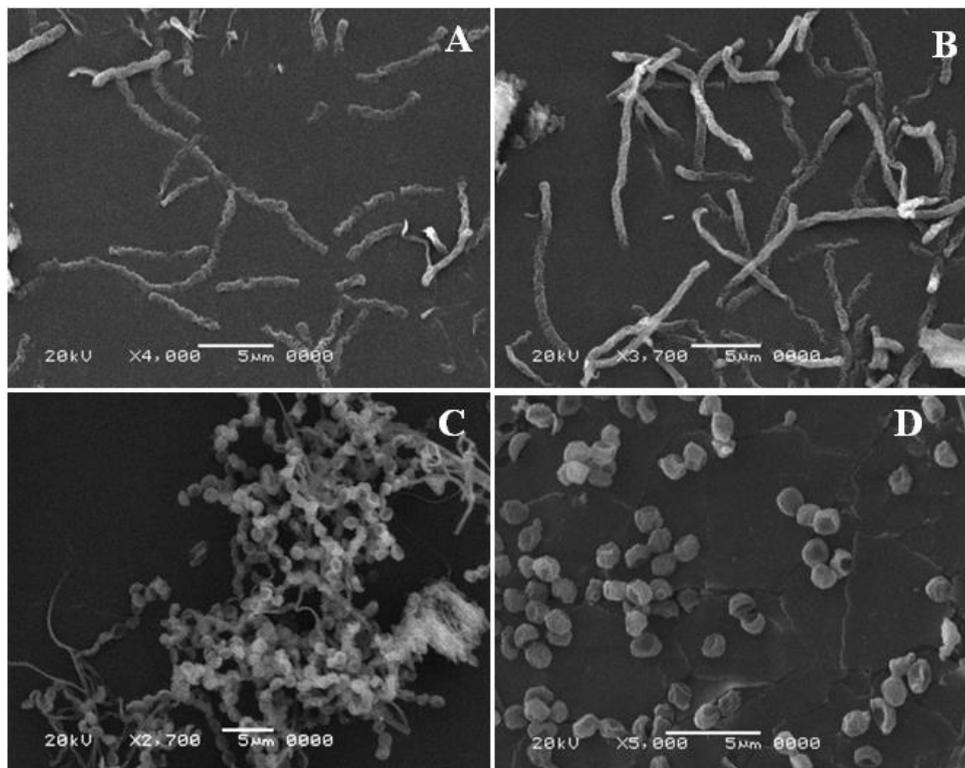
Based on IR spectra analysis, the most dominant role in the binding play the carboxyl groups from proteins and carboxylated polysaccharides Fang *et al.* (2011). These groups have the highest affinity for metal ions because they are deprotonated in the pH range of the highest adsorption. Other groups, such as phosphate, hydroxyl and amino groups present in the surface area of alga cells can play only a minor role. Gong *et al.* (2005) concluded that amino and hydroxyl groups play a predominant role at high pH in binding of Pb on *Spirulina maxima*.

It is well documented that the pH of the aqueous solution affects the metal solubility and the concentration of the counter ions on the functional group of the cell wall of the biosorbent. Consequently, the pH is considered as the most important parameter that could affect the biosorption of metal ions from solutions (King *et al.*, 2007; Gaur and Dhankhar, 2009).

Scanning electron microscope

SEM photographs of the samples are shown in Plate (1) *S. platensis* filaments some morphological changes when exposed to iron solution at low concentration showed electron dense metal are adsorbed on the cell wall (Plate 1B). While at high concentration (400ppm) there is the possibility of transport of these ions into transport cell membrane (Plate 1C). After sometime, *S. platensis* filament disintegration into separated cells as shown in Plate (1D).

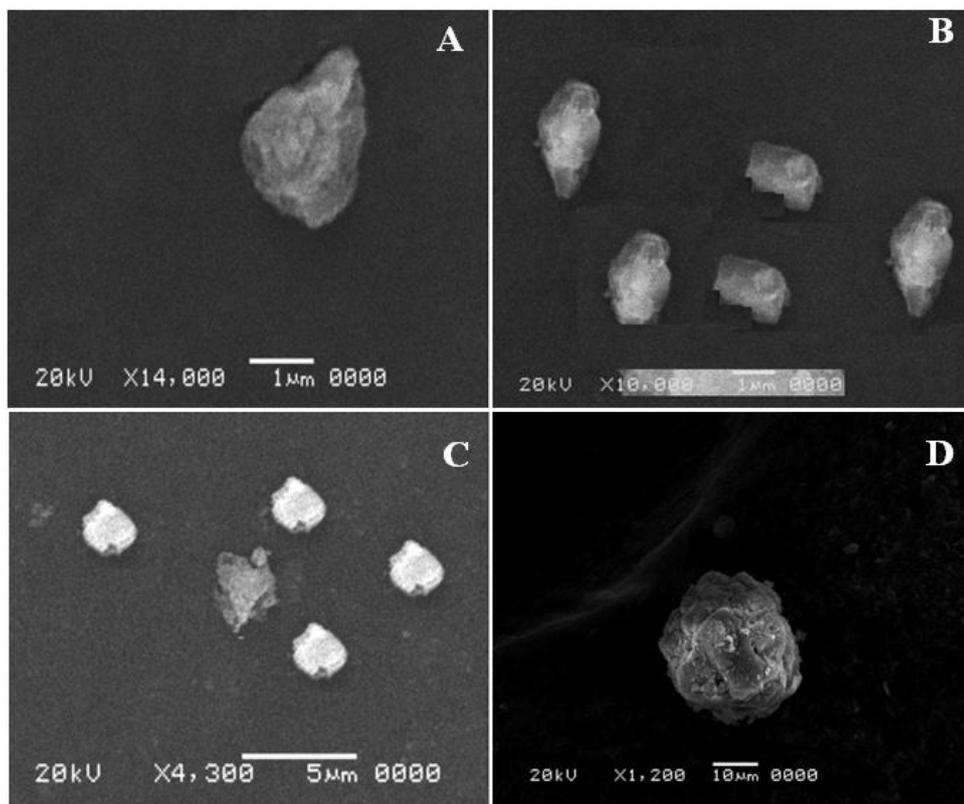
Plate (1)



***Spirulina platensis*: A- Control; B- 100ppm iron concentration; C and D- 400ppm iron concentration**

On the other hand SEM photographs of *Chlorella vulgaris* was shown in Plate (2). It revealed the change of surface morphology of biomass cell treated with metal ions at low concentration showing cells enlargement and surface modification of cell showed in Plate (2B). At high concentration crystallization was appear as precipitations on cell wall (Plate 2 C and D). **Tamilselvan *et al.* (2011)** reported the change in surface morphology of *Acanthopora spicifera* biomass treated with Cr metal ion, they found that the change in morphological characterization of seaweeds after interaction with chromium, size enlargement and surface modification. **Doshi *et al.* (2007)** demonstrated that the width of the filament of live *Spirulina* sp. varies from 2.00 to 5µm and expected the cell shrinks when it is dried, in dry species the surface of the biomass becomes rough after metal uptake. When treated with Cd⁺² metal ion there is possibility of transport of these ions through the cell membrane of biomass.

Plate 2



***Chlorella vulgaris*: A- Control; B- 100ppm iron concentration;
C and D- 400ppm iron concentration**

Conclusions

Our results demonstrated that the use of dead cells of *S. platensis* and *C. vulgaris* for biosorption of iron. The maximum biosorption capacities were 75% for *S. platensis* at pH 8 and 54.5% for *C. vulgaris* at pH 6. The IR indicated that the amino, carboxyl, hydroxyl and carbonyl groups on the surface of the biomass are responsible for biosorption of iron. This work will be helpful in developing technologies useable for bioremediation using *S. platensis* and *C. vulgaris*.

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**الامتصاص الحيوي للحديد من محلول مائي بواسطة خلايا جافه من طحلب
سبيرولينا بلاتنسيس و كلوريل فولجارييس
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تهدف الدراسة الحالية الى فحص قدرة الخلايا الجافه من طحالب (سبيرولينا بلاتنسيس و كلوريل فولجارييس) على امتصاص عنصر الحديد من محلول مائي . بعد تجارب المسح الاولية اوضحت النتائج تميز طحلب سبيرولينا على امتصاص اعلى نسبه من الحديد 75% عند الأس الهيدروجيني 8 بينما سجل طحلب الكلوريل اعلى معدل امتصاص عند الأس الهيدروجيني 6 بنسبه 54.5%. كما أثبتت نتائج الفحص للأشعة تحت الحمراء وجود مجموعه الكربوكسيل و الهيدروكسيل على جدار الخلية لكلا من الطحلبين وهو المسئول عن ادمصاص الحديد على الجدار مما يؤدي إلى عدم تراكم الحديد داخل الخلية. وقد تبين من الصور التي تم الحصول عليها باستخدام الميكروسكوب الألكتروني وجود بعض التغيرات في الشكل الظاهري لكلا الطحلبين و التي اكدت هذه التغيرات على التأثير الضار من زيادة تراكم عنصر الحديد.