Original Research *Al-Razi Univ J Med Sci 2019; 3 (2)*

RUJMS

Al-Razi University Journal of

Medical Sciences

Bioremoval of Iron From Water Sources by Using One Species of Micro Algae (*Chlorella Vulgaris*)

Soad H. A. Al-khiat[1*], Mahmoud M. Alazab[1], Gamal A. Q. Al-Mansori[2].

[1]*Department of biology, Faculty of Sciences, Sana'a University, Sana'a Yemen,* [2]*Department of Soil and water, Sana'a University, Sana'a Yemen.*

[*]***Corresponding author**: Dr. Soad Al-khiat; email; Omnoha2000@yahoo.com*

Abstract

***Background:** The disruption of aquatic ecosystems caused by heavy metals pollution from industrial and local sources leads to loss of biodiversity, as well as increased bioaccumulation and amplification of toxic substances in the food chain. **Aim**: The aim of this study was to assess the efficiency of an isolated green alga Chlorella vulgaris in the removal of iron (Fe^{3+}) from solutions. **Methods:** experimental study was performed using a wide range of metal solutions on the growth of the algal cultures. Chlorophyll (a), (b) and total cell counting of the isolated alga were estimated for different concentrations of iron (Fe^{3+}) during the experimental period. **Results:** The data indicate that the low doses of iron (Fe^{3+}) had stimulatory effect on the production of the Chlorella vulgaris, while higher concentrations showed an inhibitory effects, depending on the metal concentration, time of exposure and algal sensitivity. Chlorella vulgaris recorded a removal efficiency of 86.5% for Fe^{3+}. Therefore, the studied algae provide a good system for the bioaccumulation of the tested heavy metal (Fe^{3+}). The morphological and anatomical changes in Chlorella vulgaris by heavy metal (Fe^{3+}) were considered by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). These investigations exhibited some changes in algal form and cellular components for Chlorella vulgaris. **Conclusion**: We conclude that Chlorella sp. can be used as a bioaccumulator for Fe3+ removal process .*

Keywords: Bioremoval ; Iron ; Chlorella vulgaris; water.

Introduction

Heavy metals are one of the most dangerous water and soil pollutants which are non-degradable substances by microorganisms[1,2,3, 4]. Heavy metals are found in trace quantities in nature, but their concentration can be greatly increased by human activities[5]. Many of the physical and chemical methods used to treat wastewater can lead to chemical contamination of water and they are more expensive when handling water with a relative low metal content[1]. Heavy metals classified as: toxic metals, a precious metals and radionuclide[3,6]. Some of these metals have been to be essential for metabolism[7], but at low concentrations[8]. Heavy metals interfere with biological microbial processes[9]. Toxic effects include ion displacement and / or ion replacement from cellular sites and blocking of functional groups of important molecules[8], this effects in denaturation and disruption of cell components and cell membrane integrity. Also, these metals at high concentrations interact with enzyme active sites and nucleic acids[10]. Due to the searching for readily available and a low-cost materials for remove the heavy metals

from aqueous solution so the biological methods have been launched[3,11]. The ability of algae to live under various conditions makes algae the pioneers in colonizing new environments[11,12]. Wastewater contains high concentrations of nutrients, which are suitable for the growth of microalgae, so microalgae can be used in biological treatment[4]. Different species of algae exhibit different sorption properties[11]. ***Chlorella vulgaris*** is a unicellular green alga that can be found in fresh and marine water and can be used to remove heavy metals from wastewater[13]. Biosorption of heavy metals by algal cells is due to some benefits such as the presence of various functional groups on their extracellular polymer substances (EPS)[3,4,14].

The removal efficiency reduce with increasing metal concentration[15,16]. However, the effectiveness of metal removal using this procedure depends on microalgal species, microalgae concentrations and environmental factors[4]. Metabolism-dependent biosorption were termed bioaccumulation, and metabolism-independent biosorption were termed biosorption, Therefore the Biosorption is faster than bioaccumulation[3]. biosorption and bioaccumulation can be occurred in the living algae[1]. Biosorption is the uptake of toxic metal ion and radionuclides by biological substances. Biosorption can be based on the following mechanisms: ion exchange, physical adsorption, electrostatic attraction, chelation/ complexion and surface precipitation[3]. In various sorption processes, several mechanisms often act in combination[17].

The bioaccumulation is defined as the accumulation of a substance in the body of organisms without metabolization or assimilation[2,18]. Bioaccumulation, involves two processes: attachment of toxic elements to the surface; and transportation of metal ions into cells[3]. There are many variables can be effect on accumulation, such as pH and temperature[3]. This search proposes that the present biosorbent ***Chlorella vulgaris*** can be more useful for the removal of heavy metals such as Iron from aquous solutions. Phytoplankton need to iron in the metabolism[19]. Petrou et al[20], Kosakowska et al[21]and Wang et al[22] found that the iron deficiency lead to a lack of growth.

Aim of the study

The aim of this study was to assess the effectiveness of the micro-algal species (Chlorella vulgaris) in removing of Iron from aqueous solutions and the effect of iron (Fe^{3+}) on the morphological and ultrastructural changes of the Chlorella vugaris cells.

Subjects and Methods

Experimental study was done to assess the effectiveness of the micro-algal species (Chlorella vulgaris) in removing of Iron from aqueous solutions and the effect of iron (Fe^{3+}) on the morphological and ultrastructural changes of the Chlorella vugaris cells.

This study was done from May to the end of June 2018 The samples were collected from local areas bani matar in Sana'a, Yemen. *Chlorella vulgaris* was isolated and identified according to Prescott[23].

Culturing and isolation of algae: the culturing and isolation used the moist plate technique recommended by Jurgensen and Davey[24].

Media used for culturing *Chlorella vulgaris*: different media were used for cultivation and isolation of ***Chloralla vulgaris*** (BG11 medium, Z-medium, Bold's Basal medium, Allen's medium, Modified Chu's medium.

Purification: Two method were applied (Plating out method, Dilution method) as described by Hilary and Erica[25].

Algal growth conditions: light duration (12-24 hours), light intensities (1000-6000 Lux), The temperature (24-30 °C) and pH values (6-8).

Tested heavy metal: Is the ferric citrate $Fe(C_6H_5O_7)$

Preparation of tested heavy metal solution: The tested heavy metal was prepared as 1000 ppm stock solution of $Fe(C_6H_5O_7)$ in distilled water and kept in the refrigerator. Iron (Fe^{3+}) concentrations were calculated from the equation; $M_1 V_1 = M_2 V_2$ where M_1 was the stock solution concentration, M_2 the required concentration, V_1 the volume of the stock solution and V_2 the volume of the required concentration.

Uptake test of investigated heavy metal from culture media by *Chlorella vulgaris*:- a initial experiment using a wide range of metal concentrations, ferric citrate $Fe(C_6H_5O_7)$ was carried out to estimate the suitable concentrations of this metal which could be tolerated by ***Chlorella vulgaris***. Selection of these concentrations was based on the response of the ***Chlorella vulgaris*** to it, which had a slightly or marked effects on their growth and also to avoid the non-effective and directly lethal concentrations.

The actual experiment carried out by placing the appropriate volumes of selected concentrations of the studied metal into the culture media making up to 1000 mls with distribution water and algal cells with a cell count 20×10^5 (unit/ml) initial incoula for *Chlorella vulgaris*. The culture media were aerated through the cotton plugs. Three replicates for each concentration of the metals in addition to the control were prepared. Then the culture vessels were incubated under conditions required for the growth of *Chlorella vulgaris*. The culture glasses were incubated at 28 °C and continuous light at 2000 lux for 18 days. Flasks were shaken once per day to prevent clumping of algal cell. Every 3 days a known volume of treated cultures were taken then Chl(a) and (b) and cell counting for *Chlorella vulgaris* were measured.

In addition to, sample of 500 ml of *Chlorella vulgaris* was harvested at zero time and another sample was also harvested after the treatment. Then centrifuged (2500 rpm) for 15 min and the supernatant solution sampled for its heavy metal concentration by atomic absorption spectrophotometer. The algal residue was washed three times by distilled water then dried in an oven at 70 °C to obtain a constant weight. Samples were cooled in desiccators for 30 min before digestion. Heavy metal removal ability was calculated from the equation $(C_i\text{-}C_f)/C_i \times 100$ (%) where C_i was the initial concentration (mgL^{-1}) and C_f the equilibrium (final) heavy metal concentration (mgL^{-1}).

Tolerant test of heavy metal solution: A initial experiment using a wide concentration range of metal solutions on the growth of the algal cultures was conducted. The preliminary experiment carried out by placing each different concentration of heavy metal solutions into appropriate culture media making up to 100 ml with algal cells of known 20×10^5 Cell count (unit/ml) for ***Chlorella vulgaris*** using 250 ml conical flasks as culture vessels.

For *Chlorella vulgaris*

Pollutant	The used concentrations
Iron	0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/1000 ml ferric citrate

Determination of growth parameters during experimental period.

The growth of ***Chlorella vulgaris*** was determined by two methods:

A) **Chlorophyll content :**-Total chlorophyll content was determined according to the method described by Strickland and Persons[26]. A definite volume of well-shacked culture sample was filtered through glass fiber (Satorius, SM 13400). Then homogenized in 80% acetone and kept in freezer for about 24h, to ensure complete extraction. The extract was diluted to a definite volume (25 ml). After 10 min centrifugation (5000 rpm), one ml of the chlorophyll extract was used for the determination of chlorophyll (a) and (b). the extract was measured against a blank (80 % acetone) at wave-lengths 664 and 647 nm by spectrophotometer (Jenway 6300). According to the equation of [27]and[28] which gave the specific absorption coefficients as follows:

Chl (a) = 11.93 A_{664} – 1.93 A_{647}

Chl (b) = 20.36 A_{647} – 5.50 A_{664}

B) **Cell count:**- The laboratory technique of phytoplankton counting developed by Utermohl[29] and Utermohl[30] was applied for quantitative elaboration. The liquid algal culture was swirled to make a homogenous suspension and the containing was conducted using an improved Neubauer haemocytometer-

Digestion of microalgal cells:- Dried algal samples were digested with 1 ml of conc. HNO_3 in a dry thermo bath (Boekel series 02344, USA) until the solution was dry. After cooling, 1 ml of 30 % H_2O_2 was added. The sample was then further digested for 1 hour or until the solution had evaporated to dryness. This step was repeated 2 times until a white ash was obtained. Five ml of 0.5 N HNO_3 was added to the digested sample and heavy metal concentration was measured using an atomic absorption spectrophotometer as described above. All the experiments were performed in three replicates.

Heavy metal analysis: -Analysis of heavy metal (Fe^{+3}) was determined before and after the experimental study period as µg/L in liquid media and as µg/g fresh weight in algal cells using Perkin-Elmmer atomic absorption spectrophotometer model 2380 by the method described by Singh et al[31].

Preparing the studied algal cells for scanning and ultrastructure research:-

After 12 days of experimental period ***Chlorella vulgaris*** was taken under certain concentration. ***Chlorella vulgaris*** harvested at concentration 0.04 mgL^{-1} of Fe^{+3}, by centrifugation at 25000 r.p.m for 10 minutes at 4 °C. Washing algal sample with distribution water 3 times then, repeat the separation process for alga. Then algal cells became ready for preparation of scanning and transmission electron microscope as follows :

Fixation:- Primary fixative: by buffered Glutaraldehyde 2.5% over night in refrigerator wash by phosphate buffer pH=7.2.

Secondry fixative: by buffered Osmium Tetroxide 1% over night in refrigerator.

Dehydration:- Dehydration by series conc. of ethanol.

Embedding:- Embedding by resin mixture from SPI (SPI-Pon^{Tm} – Araldite$^{@}$ Epoxy Embedding Kit).

Cutting:- the block well cutting by (leica UC6 ultramicrotome) the section thickness is between 70-80 nm and it lode in cupper grid. Stained by aqua's urany1 acetate and lead citrate, examined under scanning

(SEM) and transmission electron microscope (TEM) (Jeol JSM-1011 electron microscope), according to Luong-Van and Hayward[32].

The data were analyzed used SPSS program. According to Torres et al[18]. data were expressed as mean of three replicates ± SD.

Results

The use of microorganisms is one of strategies to remove heavy metals from solutions. Microalgae have been studied extensively in this regard because of its spread in nature. They can remove heavy metal ions by absorption and adsorption as do by other microorganisms. microalgae has the potential to achieve greater performance at a lower cost for wastewater treatment than other conventional technologies. This corresponds to the recent trend for growing interest in biosorbent technology for removal of trace amounts of heavy metals from dilute solutions. In this study *Chlorella vulgaris* strain potentially suitable for Fe^{+3} removal in aqueous solution was selected, we attained to make media contain high concentrations of iron. The iron concentrations used more than allowed in the International Environmental Low (4/1994). Preliminary tests were conducted by subjecting the recorded *Chlorella vulgaris* to wide concentration ranges of Fe^{3+} solutions to detect level of the tested metal to be studied.

Effect of the selected heavy metal on *Chlorella vulgaris*.

The effect of the selected heavy metal (Fe^{+3}) on the growth of ***Chlorella vulgaris*** during the experimental period of 18 days on intervals of three days was measured by chlorophyll (a) and (b) content and cell counting.

Effect of Iron concentrations on *Chlorella vulgaris*:

Table 1: Effect of different Fe^{+3} concentrations (mgL^{-1}) on chlorophyll (a) content (μgL^{-1}) of *Chlorella vulgaris.* (Data were expressed as mean of three replicates±SD).

Time /Days / Concentration (mgL^{-1})	Zero time	3	6	9	12	15	18
Control	0.1±0.01	0.09±0.01	0.16±0.01	0.24±0.01	0.58±0.01	0.53±0.01	0.2±0.01
0.01	0.1±0.01	0.09±0.01	0.21±0.01	0.39±0.01	1.2±0.01	0.7±0.01	0.27±0.01
0.02	0.1±0.01	0.09±0.01	0.29±0.01	0.5±0.01	1.31±0.01	0.82±0.01	0.28±0.01
0.04	0.1±0.01	0.09±0.01	0.52±0.01	1.58±0.01	2.73±0.01	1.43±0.01	1.28±0.01
0.06	0.1±0.01	0.09±0.01	0.4±0.01	0.88±0.01	1.38±0.01	1.11±0.01	0.7±0.01
0.08	0.1±0.01	0.09±0.01	0.38±0.01	0.52±0.01	1.31±0.01	0.29±0.01	0.19±0.01
0.1	0.1±0.01	0.09±0.01	0.23±0.01	0.46±0.01	1.12±0.01	0.28±0.01	0.12±0.01

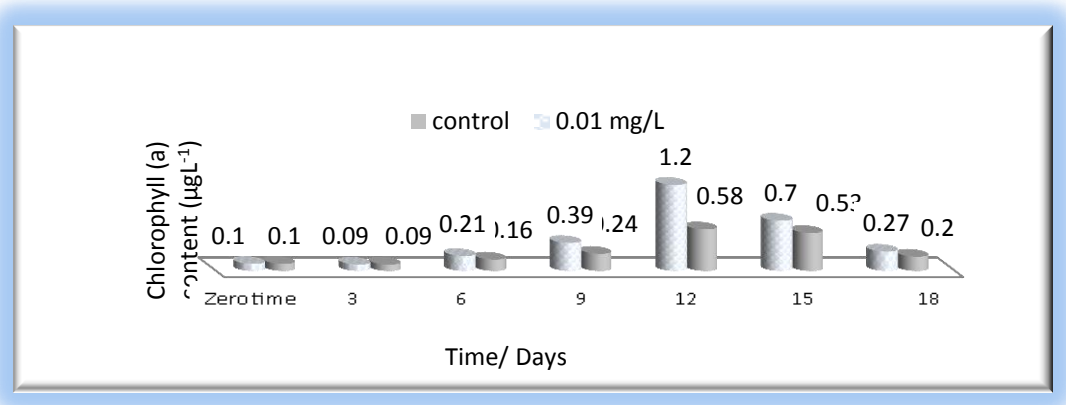

Figure1: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Chlorella vulgaris*.(a)

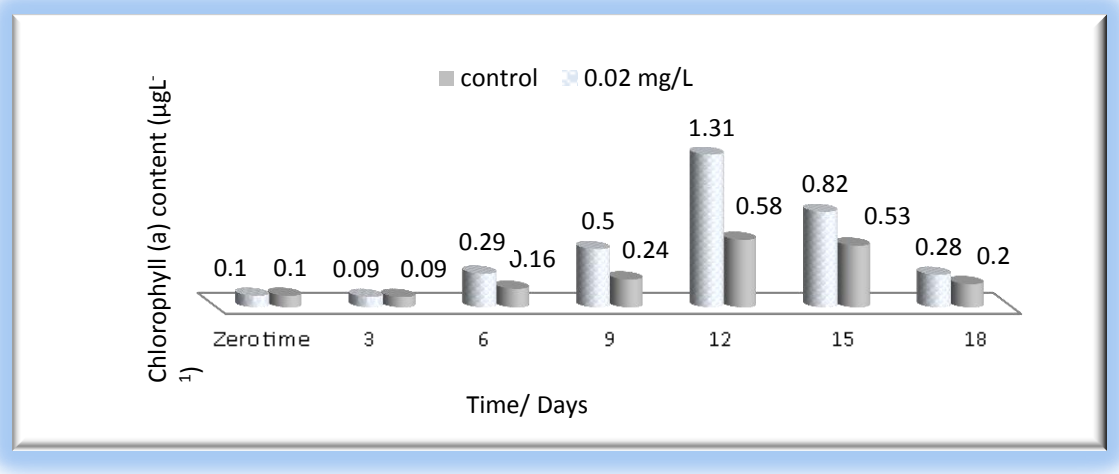

Figure1: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Chlorella vulgaris*.(b)

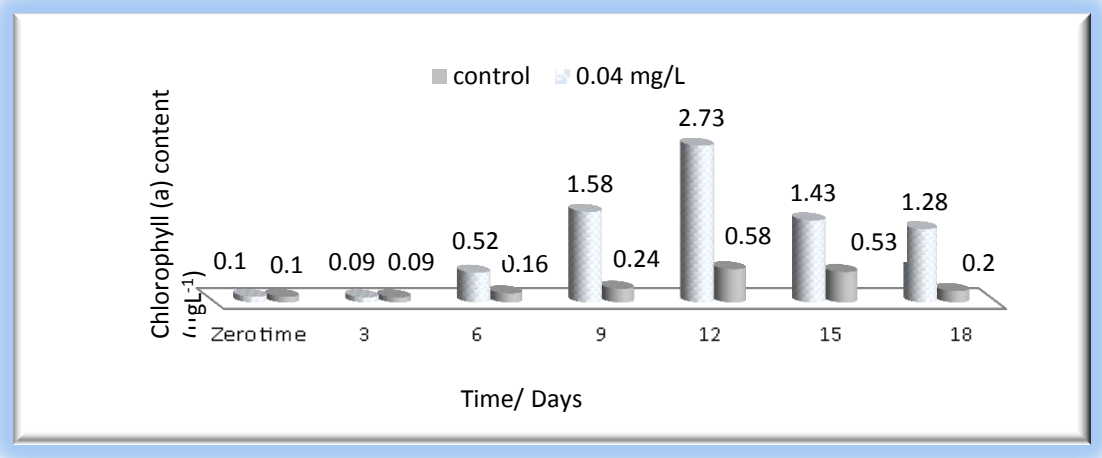

Figure1: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Chlorella vulgaris*.(c)

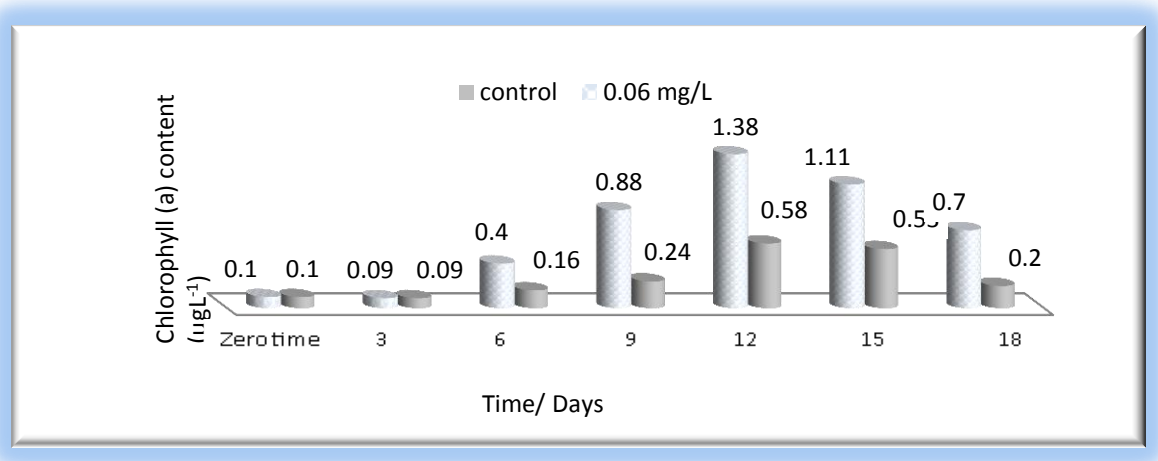

Figure 2: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Chlorella vulgaris*.(a)

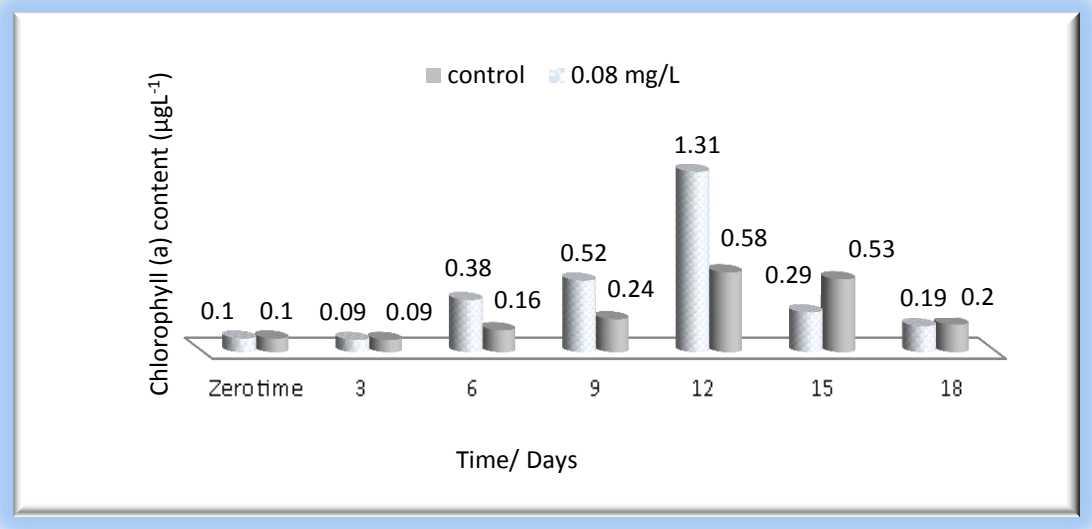

Figure 2: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Chlorella vulgaris*.(b)

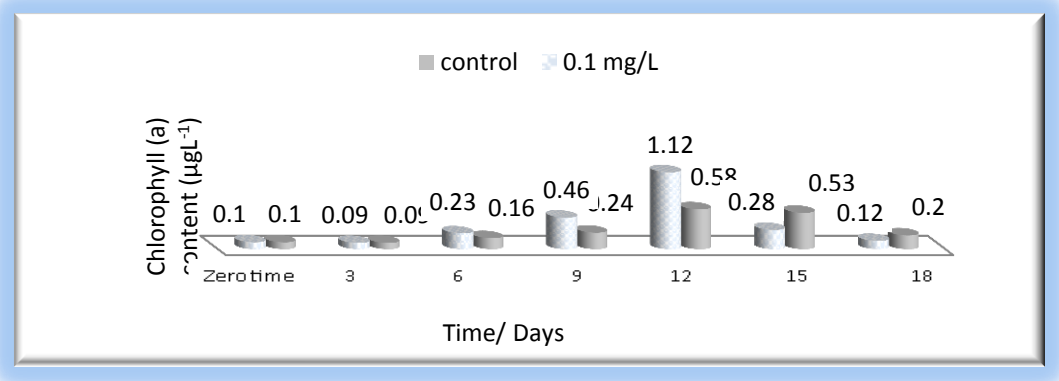

Figure 2: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Chlorella vulgaris*.(c)

Table 1 and Figures 1 & 2 illustrated the effect of different Fe^{3+} concentrations on chl(a) contents of ***Chorella vulgaris***. Data indicated that the highest values of chl(a) (2.73 μgL^{-1}) were recorded at 0.04 mgL^{-1} of Fe^{3+} concentration on day 12. Hence the treated alga under this metal concentration (0.04 mgL^{-1} Fe^{3+}) was selected to examine by electron microscope. Increasing in Fe^{3+} concentrations to 0.01 , 0.02 & 0.04 mgL^{-1} led to gradual stimulations in chl(a) contents to 1.2 , 1.31 & 2.73 μgL^{-1}, respectively along 12 days. Further increasing of Fe^{3+} concentrations to 0.06, 0.08 & 0.1 mgL^{-1} Led to dramatically inhibition of chl(a) contents to 1.38 , 1.31 & 1.12 μgL^{-1} , respectively compared with the highest values of chl(a) (2.73 μgL^{-1}) within 12 days, but all of them were more than control . A similar general pattern was observed for chl(b) calculation.

Table 2: Effect of different Fe^{+3} concentrations (mgL^{-1}) on chlorophyll (b) content (μgL^{-1}) of *Chlorella vulgaris.* (Data were expressed as mean of three replicates±SD).

Time /Days / **Concentration (mgL^{-1})**	**Zero time**	**3**	**6**	**9**	**12**	**15**	**18**
Control	0.08±0.01	0.09±0.01	0.15±0.01	0.46±0.01	0.5±0.01	0.33±0.01	0.09±0.01
0.01	0.08±0.01	0.09±0.01	0.17±0.01	0.57±0.01	0.7±0.01	0.35±0.01	0.13±0.01
0.02	0.08±0.01	0.09±0.01	0.19±0.01	0.6±0.01	0.81±0.01	0.39±0.01	0.15±0.01
0.04	0.08±0.01	0.19±0.01	0.36±0.01	0.76±0.01	0.85±0.01	0.76±0.01	0.3±0.01
0.06	0.08±0.01	0.1±0.01	0.18±0.01	0.36±0.01	0.81±0.01	0.48±0.01	0.13±0.01
0.08	0.08±0.01	0.09±0.01	0.15±0.01	0.24±0.01	0.62±0.01	0.25±0.01	0.05±0.01
0.1	0.08±0.01	0.09±0.01	0.06±0.01	0.07±0.01	0.09±0.01	0.07±0.01	0.03±0.01

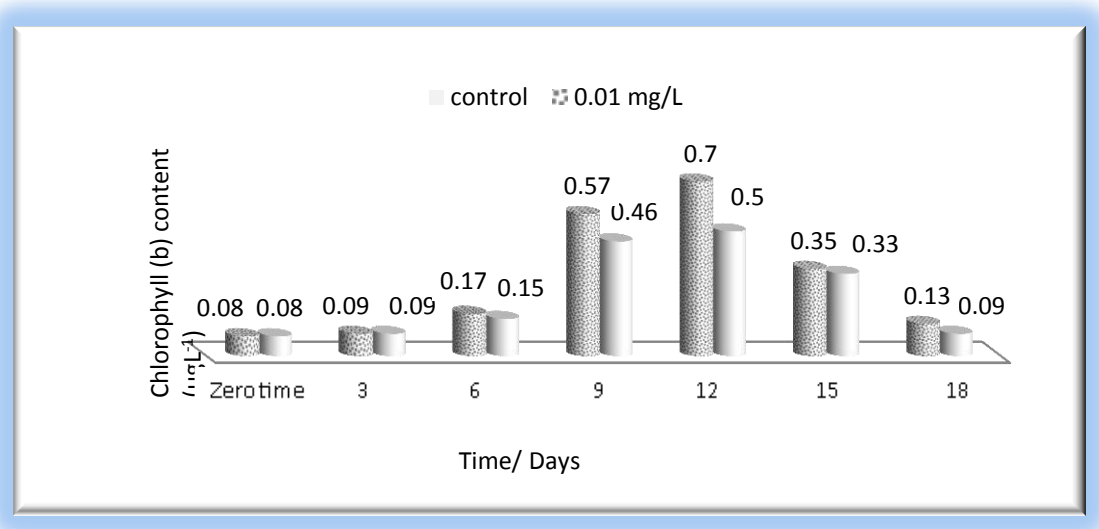

Figure 3: Effect of different Fe^{3+} concentrations on chlorophyll (b) content of *Chlorella vulgaris*.(a)

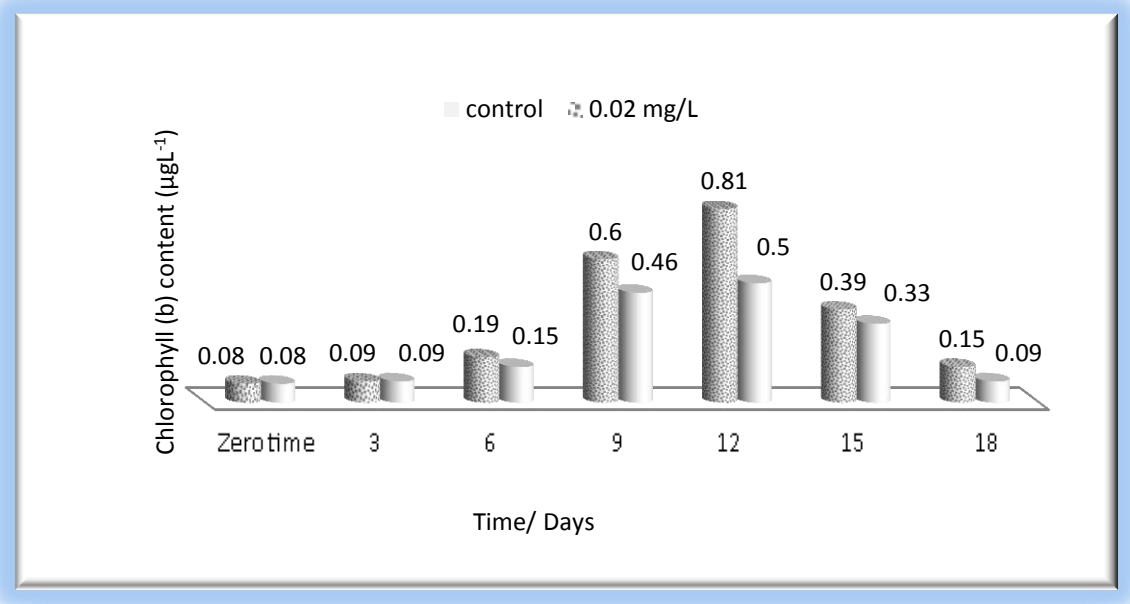

Figure 3: Effect of different Fe^{3+} concentrations on chlorophyll (b) content of *Chlorella vulgaris*.(b)

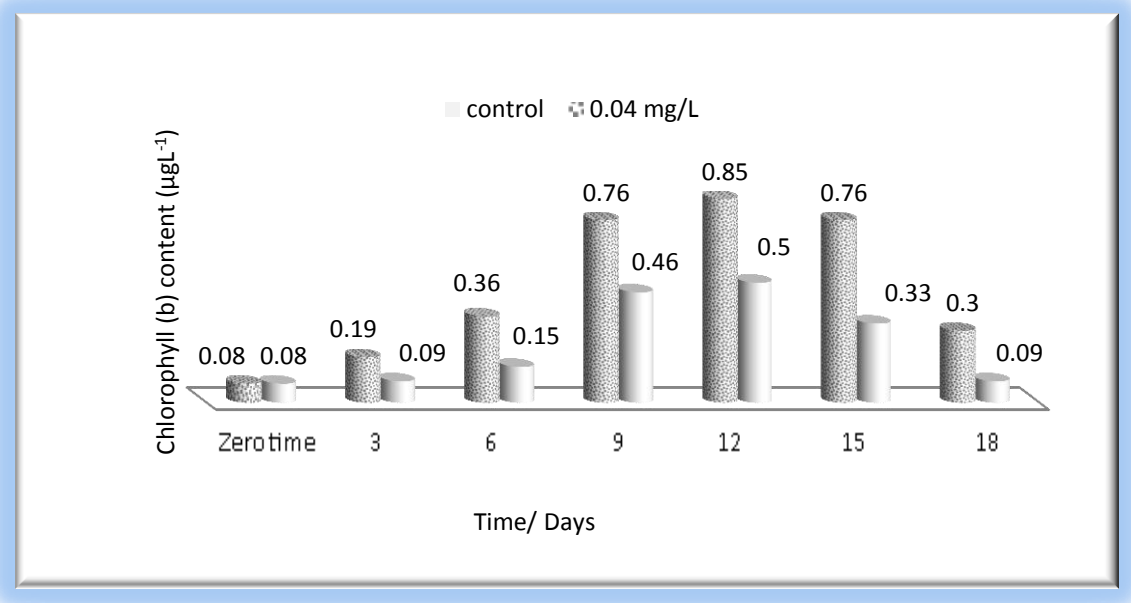

Figure 3: Effect of different Fe^{3+} concentrations on chlorophyll (b) content of *Chlorella vulgaris*.(c)

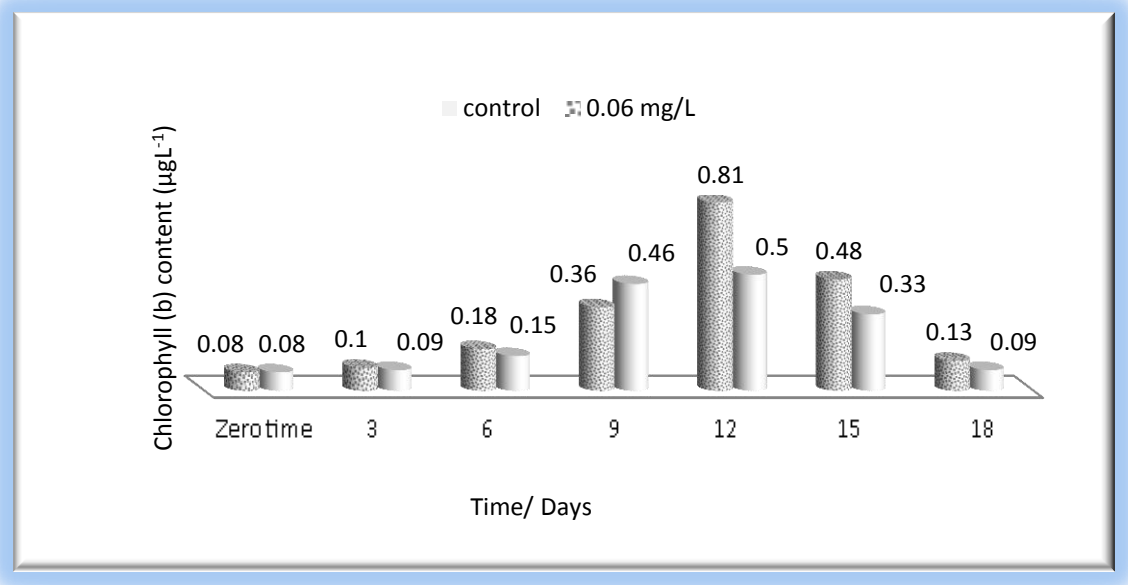

Figure 4: Effect of different Fe^{3+} concentrations on chlorophyll (b) content of *Chlorella vulgaris*.(a)

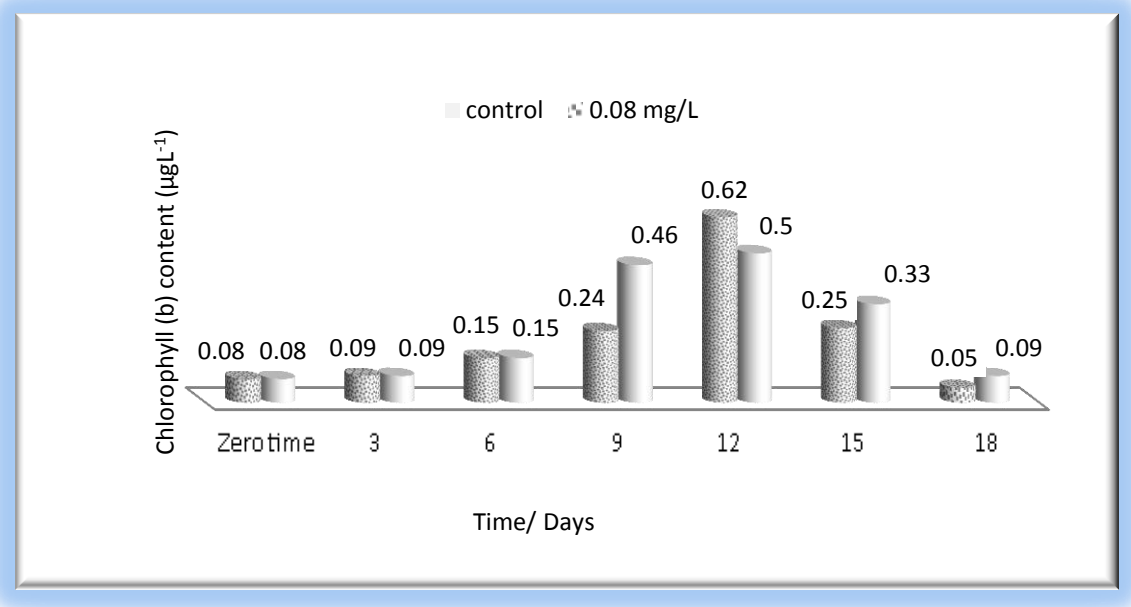

Figure 4: Effect of different Fe^{3+} concentrations on chlorophyll (b) content of *Chlorella vulgaris*.(b)

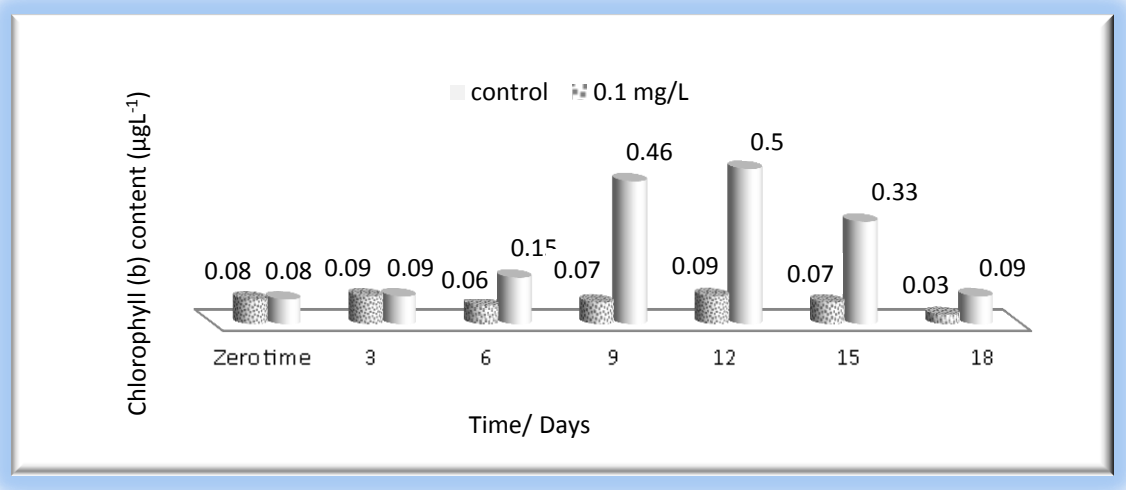

Figure 4: Effect of different Fe^{3+} concentrations on chlorophyll (b) content of *Chlorella vulgaris*.(c)

Table 2 and Figures 3 & 4 showed that low concentrations of Fe^{3+} (0.01 ,0.02 & 0.04 mgL^{-1}) induced chl(b) biosynthesis in ***Chlorella vulgaris*** to 0.7 , 0.81 & 0.85 μgL^{-1} within 12 days of experimental period respectively comparing with control. High concentrations of Fe^{3+} (0.06 ,0.08 & 0.1 mgL^{-1}) exhibited a deleterious effect on chl(b) synthesis which reached 0.81 , 0.62 & 0.09 , respectively relative to the highest values of chl(b) (0.85 μgL^{-1}) within 12 days, but all of them led to gradual stimulation in chl(b) content more than control, except the highest concentration of Fe^{3+} (0.1 mgL^{-1}) which recorded the lowest value of chl(b) (0.09 μgL^{-1}).

Table 3: Effect of different Fe^{+3} concentrations (mgL^{-1}) on total cell counting (unit/ml $\times 10^5$) of *Chlorella vulgaris.* (Data were expressed as mean of three replicates ± SD).

Time /Days / **Concentration (mgL^{-1})**	**Zero time**	**3**	**6**	**9**	**12**	**15**	**18**
Control	20±1	124±1	143±1	223±1	895±1	527±1	367±1
0.01	20±1	139±1	191±1	303±1	943±1	623±1	399±1
0.02	20±1	159±1	207±1	319±1	1007±1	671±1	463±1
0.04	20±1	159±1	223±1	351±1	1391±1	847±1	511±1
0.06	20±1	159±1	175±1	287±1	895±1	575±1	431±1
0.08	20±1	124±1	143±1	175±1	719±1	479±1	287±1
0.1	20±1	76±1	127±1	111±1	591±1	479±1	255±1

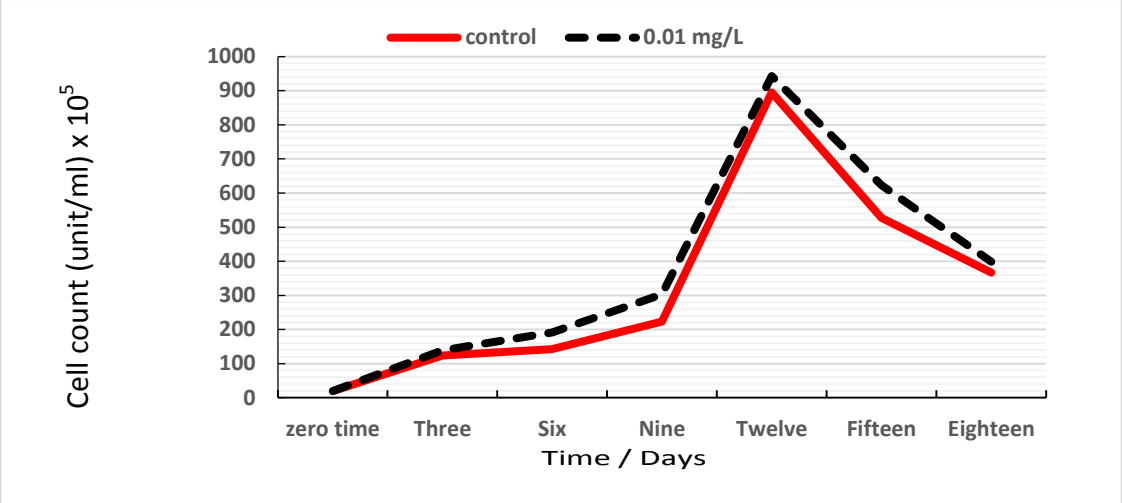

Figure 5: Effect of different Fe^{3+} concentrations on total cell count of *Chlorella vulgaris*.(a)

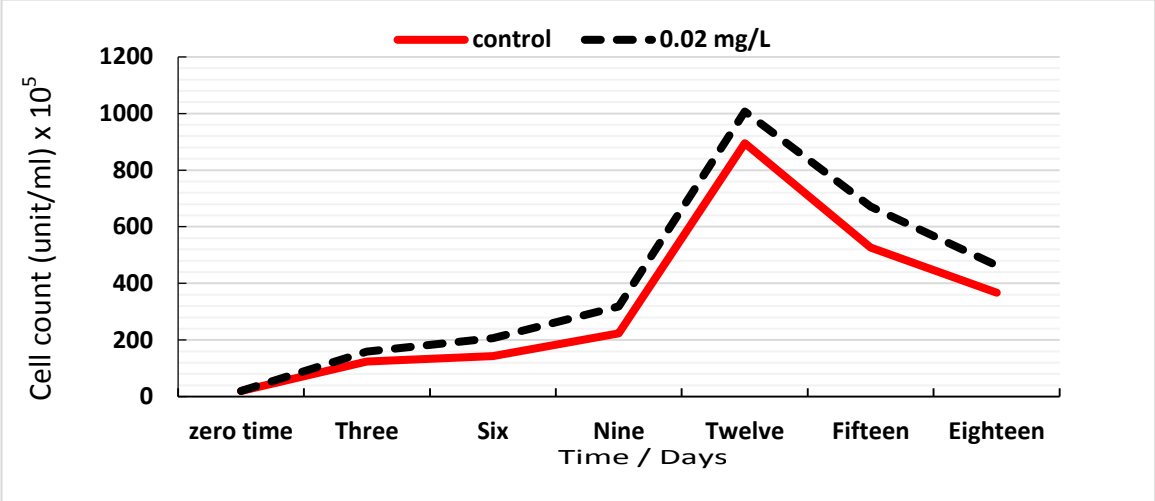

Figure 5: Effect of different Fe^{3+} concentrations on total cell count of *Chlorella vulgaris*.(b)

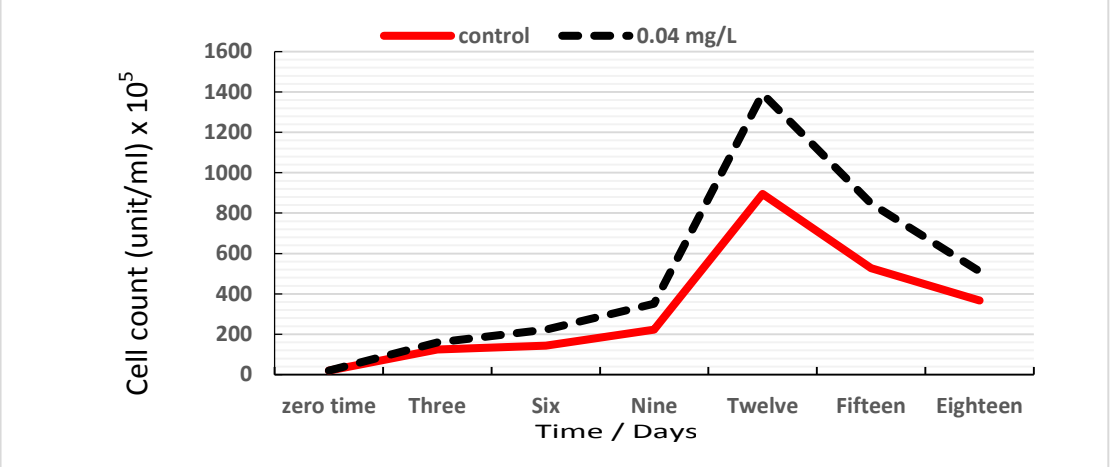

Figure 5: Effect of different Fe^{3+} concentrations on total cell count of *Chlorella vulgaris*.(c)

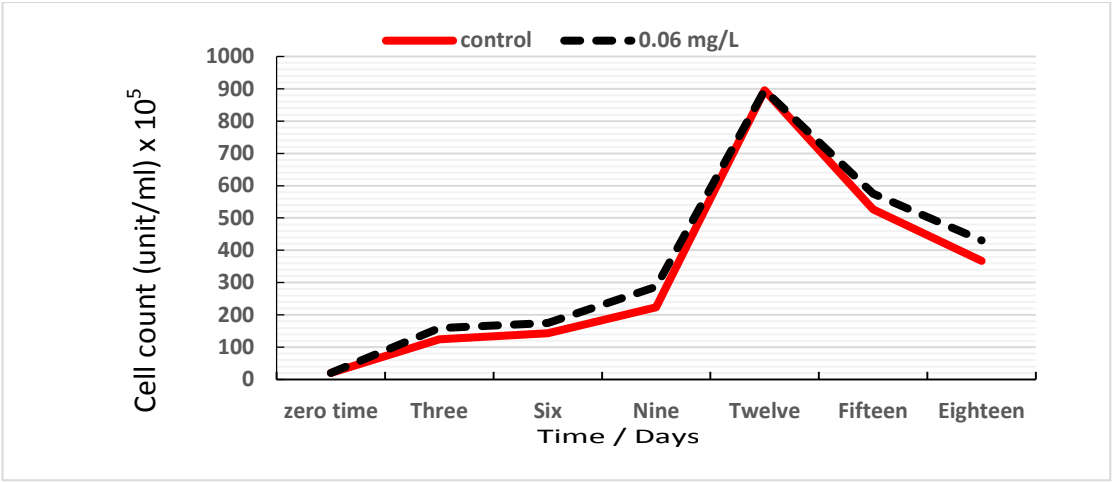

Figure 6: Effect of different Fe^{3+} concentrations on total cell count of *Chlorella vulgaris*.(a)

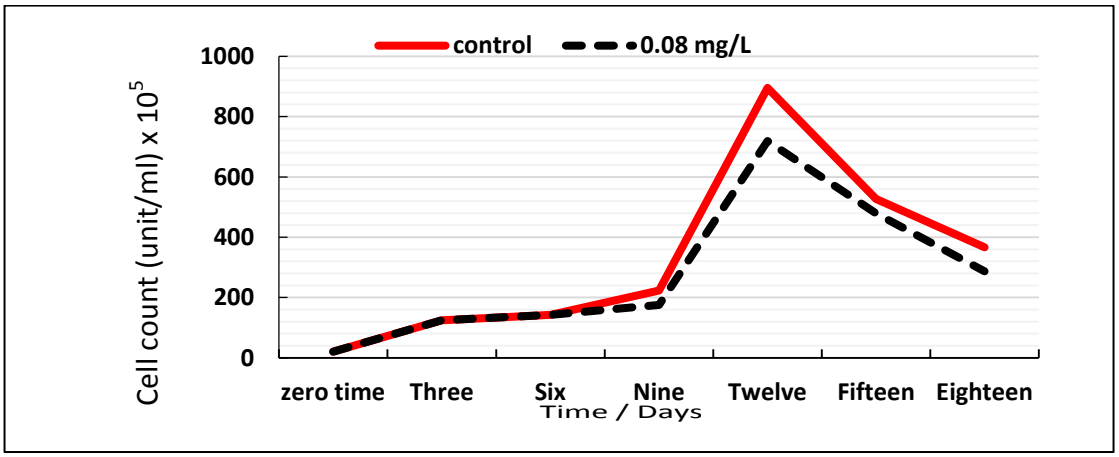

Figure 6: Effect of different Fe^{3+} concentrations on total cell count of *Chlorella vulgaris*.(b)

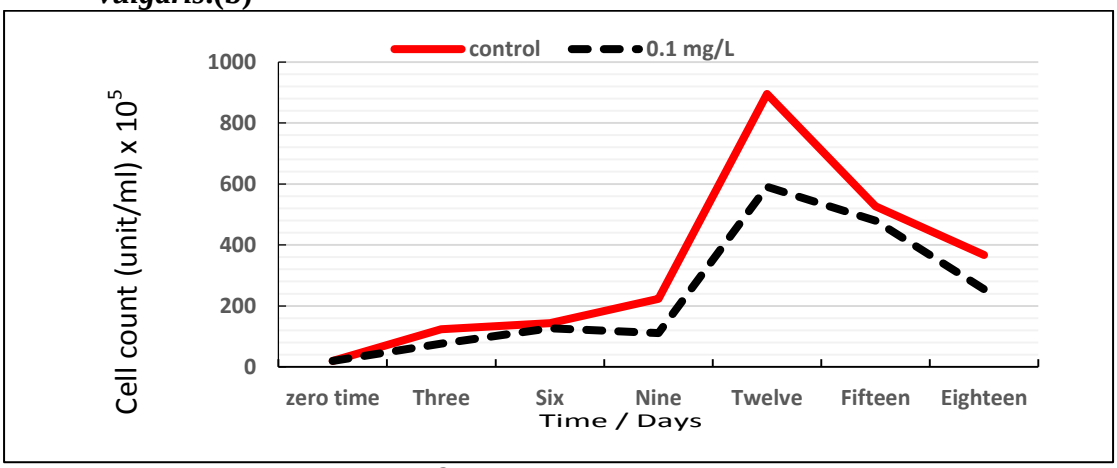

Figure 6: Effect of different Fe^{3+} concentrations on total cell count of *Chlorella vulgaris*.(c)

Accordingly, Table 3 and Figures 5 & 6 supported the previous results. The obtained data revealed that the total cell count of ***Chlorella vulgaris*** increased gradually as time of incubation lasted up to 12 days at low Fe^{3+} concentrations. It was reached 943

, 1007 & 1391× 10^5 unit/ml under Fe^{3+} concentrations of 0.01 , 0.02 & 0.04 mgL^{-1}, respectively. Increasing of the

Fe^{3+} concentrations to 0.06, 0.08 and 0.1 mgL^{-1} led to clear inhibition in *Chlorella* cells counting 895, 719 and 591 × 10^5 unit/ml, respectively comparing with the control.

The results were further supported by morphological (SEM) and ultrastructural examination (TEM) for ***Chlorella vulgaris*** which treated by 0.04 mgL^{-1} of Fe^3 for 12 days.

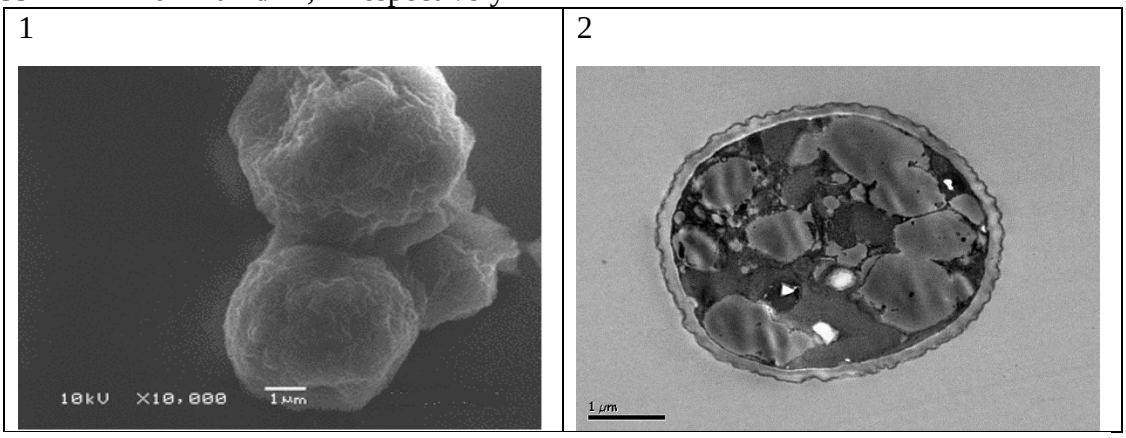

Plate (1): Pictures 1 (SEM) and 2 (TEM) of healthy ***Chlorella vulgaris*** cells grown in complete nutrient medium (BGII) under controlled conditions for 12 days (2000 Lux & 28 C^o) with a clear good appearance and regular cells as in picture (1). Accordingly, the ultrastructure of the same cell showing a regular and smooth cell wall, starch granules & cytoplasmic contents.

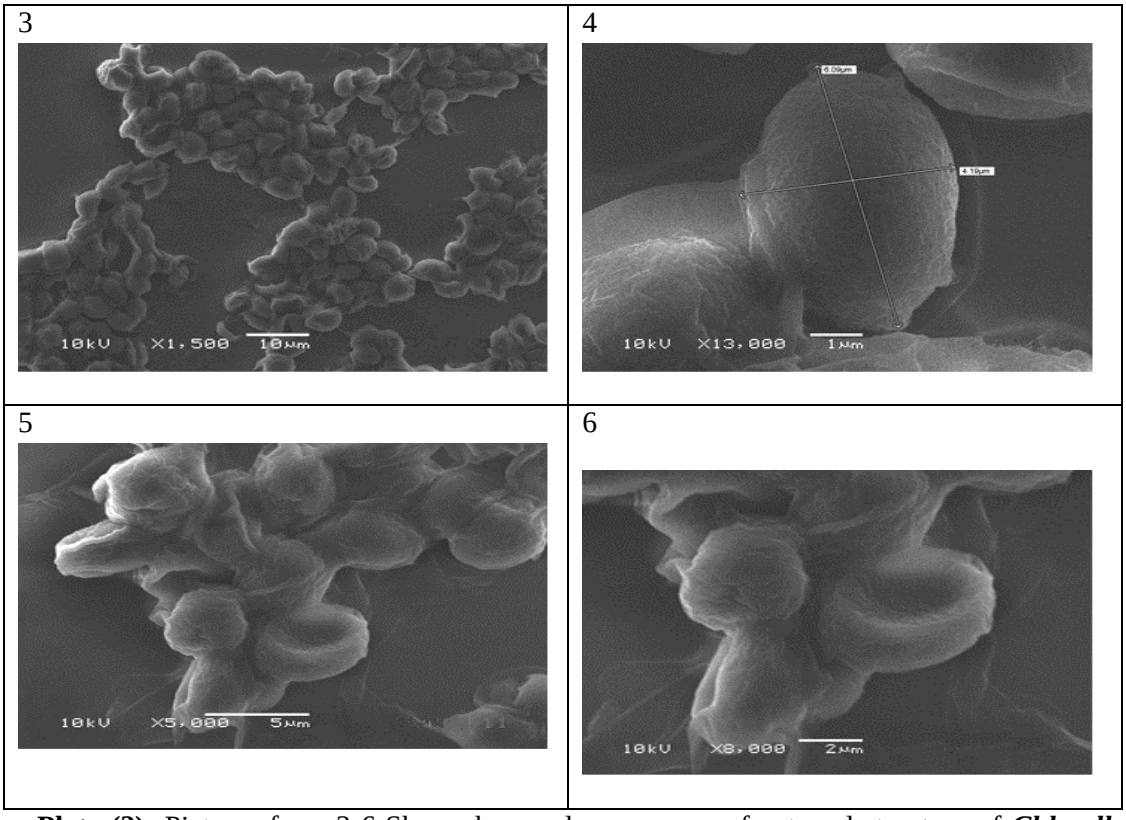

Plate (2): Pictures from 3-6 Show abnormal appearance of external structure of ***Chlorella vulgaris*** cells with 0.04 mgL^{-1} of Fe^{3+} for 12 days. A clear morphological change in the cell wall was observed.

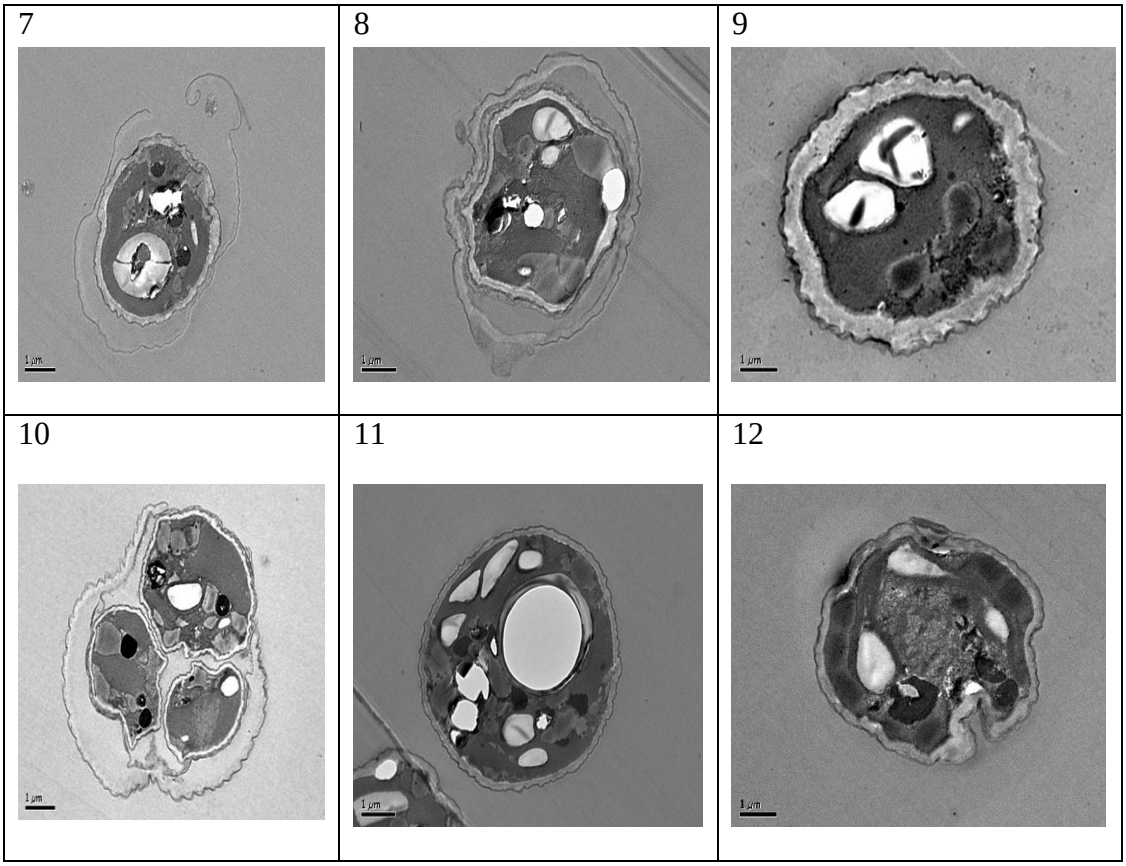

Plate (3): Pictures from 7-12 represent The ultrastructure examination (TEM) of treated ***Chlorella vulgaris*** cells by 0.04 mgL^{-1} Fe^{3+} for 12 days showing general increase in cell size, cell wall thickness and changes in shape. Also, the internal structure of the cell appears successively disorganized which led to clear separation in between the cell component and cell wall and aggregation of cell contents, formation of vacuoles and numerous granules. A clear dark patches scattered inside the cell.

Discussion

Mechanisms of metal binding to algae

In the case of biosorption by dead algae, the mechanisms can be thought of as occurring separately at the cell wall while bioaccumulation normally involves intracellular binding by living organism.

The factors affecting performance of living biosorbents are as follows:

- The physiological state of the organism.
- The availability of micronutrients during their growth.
- Heavy metal concentrations.
- The environmental conditions during uptake.
- The age of the cells and density of the biomass.

Accumulation of heavy metals by living algae has been shown to happen in two stages: a quick surface reaction followed by a much slower metal uptake over a period of hours. A quick uptake will correspond to extracellular adsorption. A slower uptake will correspond to metabolism- dependent incorporation into the cell body. Extracellular adsorption and intracellular uptake of metals may be analyzed separately by washing the algae with EDTA. The metal in the algae after the EDTA wash is defined as the intracellular metal while the metal in the extraction solution is defined as the adsorbed metal. At higher concentration of toxic metals

there might be harmfulness which can decrease the biosorption capacity. It has been described that microalgae can protect themselves against this effect of that metals by using various mechanisms such as: exclusion mechanisms, intracellular accumulation or adsorption to cell surface[1].

Algae may produce compounds in/on cell wall, and extracellular compounds, that can bind to some heavy metals and make them non-toxic[18,33]. Detoxification of heavy metal ions at the cell surface is referred to as exclusion mechanism. Another possible exclusion metal is adsorption or detoxification of a metal ion by surface-living micro-organisms, but If no exclusion mechanism is operating, a metal ion can enter the cytoplasm, and Several detoxification mechanisms are then possible inside the cell, such as Metal binding to-SH residues, protein carboxyl groups and RNA respectively. The last Mechanism is produce of extracellular legends which refers to the production of phytochelatins. If neither exclusion nor inclusion mechanisms take place, the metal cation remains "free" within the cell and a toxic effect takes place[34].

The bioassay results as illustrated in Tables 1 to 3 showed clear differences in chl (a) & (b), and cells counting of algal cells between control and treated ones when algae were exposed to different concentrations of Fe^{3+} metal. Previous results indicated a gradual stimulation in growth rate for tested alga at lower concentrations of Fe^{3+}. Whereas higher concentrations of Fe^{3+} caused a gradual reduction in growth rate. Regards to the stimulatory or inhibitory effect of Iron showed on this investigation, the present results are in agreement with those obtained by Goher et al[13] and Petrou et al[20]. Ghoniem et al[35] mentioned that ***Chlorella vulgaris*** cells have various functional groups according to their respective wave numbers. El-Sheekh et al[36] revealed that toxic metals removal is depending on the nature and charge of the cell wall polysaccharides and on the species of microalgae. Ahmad et al[37] and Goher et al[13] found that binding of heavy metals occurred on the surface functional groups of ***Chlorella vulgaris*** (–N-H groups, –OH groups, C-H alkane groups, –C=C groups and N-O groups). Bilal et al[3] and Kaplan[38] mentioned that The binding groups exist outside and inside the cell wall. The biosorption mechanism could be supported by cytosolic protein**.** The cell wall is an first hindrance to the biosorption of heavy metals. Most of the binding sites are due to polysaccharides and proteins. Different algal strains have varying capacity of biosorption of toxic elements due to different cell wall structures.

Our results in harmony with Onyeji and Aboje[39] who found that when the higher dose of adsorbent was used lead to increase the removal percentage. This was because more surfaces and functional groups were available on the adsorbent with which the metals could interact. Also, our results in agreement with Goher et al[13] who mentioned that The fast removal at the beginning may be attributed to a larger adsorbent surface area being available for the adsorption of the metals as well as a high number of available adsorptive sites. after that the removal decreased. This was probably caused by the decrease in the concentration gradient between the initial concentration and the equilibrium concentration of the solution with the progress of the adsorption process and the metal ion absorption onto the adsorbent surface. In addition to that, our results in harmony with Gani et al[4] who reported that the bio-removal of Fe by *Botryococcus* sp was increase with

increasing of concentration of cell/ml.

Our results indicate that a deficiency of iron cause a deficiency of growth rates (the chlorophyll (a) and chlorophyll (b) content and the total cell counting) and this is because the iron is vital component in the syntheses of some main metabolism enzymes which play vital role in numerous of the functions of biochemical and physiological, also the two most energy demanding systems in the cell, photosynthetic carbon reduction and nitrogen reduction, are both extremely dependent on iron containing compounds, and Nitrogen metabolism is strictly connected with carbon fixation, as both processes compete for energy produced by the light reactions of the photosynthesis, Whereas carbon metabolism is required to integrate nitrogen into protein, also iron limitation usually causes reduced synthesis of chlorophyll pigments, and The decline of pigment synthesis leads to fewer photons captured, causing a severe decline in the productivity of photosynthesis, and these results agrees with Wang et al[22], and also agree with Kutzing[19] who found that a lower iron concentrations produced a clear decline in the chlorophyll (a) content and the cell numbers. As Sunda and Huntsman[40] noted falling growth rates with decreasing iron concentrations in the large oceanic diatoms *Thalassiosira pseudonana* and *Thalassiosira oceanica* and other phytoplankton species they examined. in addition, Kudo et al[41] exhibited The reason is that iron has a direct or indirect role in the production of enzymes responsible for the synthesis of pigments. Iron also plays the role of a co-factor for some enzymes. Iron lack cause to a decrease in the activity of these enzymes and hence reduce the chlorophyll a synthesis. Also, the cellular chlorophyll a content depends on the amount of iron contributing in the photosynthetic electron transport chain, essentially at the PSI and PSII reaction centers. lack of synthesis at these photosynthetic reaction centers by iron availability will also reduction cell pigment[42]**.** Petrou et al[20] who found that Dropped electron transport rates can lead to reduced production of ATP and NADPH (the energy that are required to uptake iron). Iron is essential in photosystems (PSI; PSII); the cytochrome b_6f complex and the ferredoxin molecule. Fe-limitation strongly effects electron transport kinetics. In another study, K¨utzing[19] reported that, iron lack of the Baltic diatom *Cyclotella meneghiniana* lead to reduce in cell number and chlorophyll (a) content.

Our results indicate that the optimum concentration of iron leads to the maximum growth rate and maximum production of pigments, when the concentration of iron is greater than the optimum concentration, it leads to the growth inhibition and decline in the production of pigments. These results are in agreement with those obtained by Wang et al[22]. Furthermore, the catalytic effect of iron documented in this study with lower concentrations can be accounted for either as a result of algal requirement of this element in metabolism or explained by production of some organic compound which reduces metal toxicity[43]. This study exhibited that, if the outside concentration of metal ions was higher in the solution their leads to toxic effects and which leads to reduced bioaccumulation, and thus decline in the metal removal capacity of the algae, and this agree with Mane and Bhosle[1]. Also, Our results indicated that an increase in contact time increases biosorption up to the optimum contact time ; after that it becomes constant. these agree with Ibrahim et al[44] who reported that was

because the use of all active sites. The optimum time is differ for different types of biosorbents[45]. The initial metal concentration affects biosorption. A high initial metallic concentration exhibits high biosorption capacity due to the availability of free active sites[46].

Two stages are included in the accumulation of heavy metals in microorganisms. The first stage, fast passive biosorption process at the cell surface with no metabolism involved, whereas the second stage, active sorption process which involves the cell cytoplasm. The second process is considered ion accumulation and it involves cell metabolism[3].

Torres et al[18] reported that reduction of chlorophyll (a) content is a symptom of heavy metals toxicity. The cellular structure has explained the sensitivity of ***Chlorella vulgaris*** for metal toxicity. These results were supported by morphological and ultrastructural examination of studied alga. Also, our results are in conformity with Ahmad et al[37] who stated that Iron causes changes in the morphological structure observed by scanning electron microscope (SEM) after biosorption indicated the sorption of metal ions by pores present on the surface of the cell.

The efficiency of *Chlorella vulgaris* for heavy metal removal in aques solution:

The selected heavy metal resulting from the preliminary experiment as mentioned befor in materials and method are Fe^{3+} for the green alga ***Chlorella vulgaris***. In addition, determination of this metal in aqueous solution before (initial content) and after 12 days (final content) of the treatment period, the assessment was extended also to determine the same metal within the studied algal tissues before and after 12 days of the experimental period. The initial and final contents of heavy metal were determined in one time.

Heavy metal removal in aqueous solution by *Chlorella vulgaris*.

The amount of metal removed by the cells increased rapidly during the first time after the application time, and then steadily increased with days in used metal. The removal percentage of metal by ***Chlorella vulgaris*** during the experimental period (12 days) was 86.5%. While El-Sheekh et al[36] and Gao et al[47], found that ***Chlorella vulgaris*** was able to remove Fe concentration in sewage water and from domestic secondary effluent up to 100% after 10 days of treatment.

Our results in agreement with Ferreira et al[15] and Kumar and Gaur[16] who Mentioned that The removal efficiency reduced with increasing metal concentration, and they explained that because a passive adsorption process involving the active sites on the surface of the biomasses.

Adsorption of the studied metal, maybe it's because the nature of their cell wall. The cell wall is the main site of metal binding in algae and the surface bound metals often more than the metal accumulated in an intracellular parts. The cell surfaces have different functional groups, with various affinities for heavy metal binding[15]**.** Ion exchange is the main mechanism of metal binding, and the carboxyl group plays a main role in metal binding. Furthermore the number and kind of functional groups involved in metal binding may differ in various algal species. These differences explain the differential sorption of the tested metals by tested microalgae.

Conclusion and recommendations:

The inhibitory and stimulatory effects of the used heavy metal depend on metal concentration, algal efficiency & contact time. The results showed that *Chlorella sp*. can be used as a bioaccumulator for Fe^{3+} removal

process . The tested algal species can be selected for further study because they have high removal ability. The use of algae for the removal of pollutants is defined as phytoremediation. *Chlorella vulgaris* have rapid growth, and wide spread range and thus may be used as bioindicator. The use of green algae for removal of heavy metals is considered as an Environment friendly biotechnology.

References

1. Mane, P. C. and Bhosle, A. B. Bioremoval of Some Metals by Living Algae Spirogyra sp. and Spirullina sp. frvoom aqueous solution, J. Environ. Res. 2012, 6 (2):571-576.
2. Al-khiat, S.H.A. Bioremoval of heavy metals from water sources by using local microalgae, Botany and Microbiology Department, Faculty of Science, King Saud University, For Partial Fulfillment of the Ph.D Degree of Science in Microbiology (Algae), 2012.
3. Bilal, M., Rasheed, T., Hernández, J. E. S., Raza, A. Nabeel, F., Iqbal, H. M. N. Biosorption: An Interplay between Marine Algae and Potentially Toxic Elements—A Review, journal of Marine drugs. 2018, 16, 65.
4. Gani, P., Sunar, N.M., Matias-Peralta, H., latiff, A.A.A., Parjo, U.K. and Oyekanmi, A.A.R. Green Approach in the Bio-removal of Heavy Metals from wastewaters, Matec Web of Conferences 103, 06007. 2017.
5. Abdel-Raouf, N. and Ibraheem, I. B. M. Efficiency of Dunaliella sp. and Aphanocapsa elachista in removing of copper and nickel from culture media, Az. J. Microbiol. 2001 , 54,192-200.
6. Bulgariu, L. and Gavrilescu, M. Handbook of Marine Microalgae, Biotechnology Advances ,Chapter 30 - Bioremediation of Heavy Metals by Microalgae, Pages 457-469,Publisher, Elsevier. 2015.
7. Piotrowska-Niczyporuk, A., Bajguz, A., Zambrzycha, E. and Godlewska-Zytkiewicz, B. Phytohormones as regulators of heavy metal biosorption and toxicity in green alga Chlorella vulgaris (Chlorophyceae). J. Plant Physiology and Biochemistry. 2012, 52, 52-65.
8. Nies, D. H. Microbial heavy metal resistance. Appl. Microbiol. Biotechnol. 1999, 51:730-750.
9. Avery, S. V., Howlett, N. G. and Radice, S. Copper toxicity towards Saccharomyces cerevisiae: dependence of Plasma membrane fatty acid composition. Appl. Environ. Microbiol. 1996, 62:3960-3966.
10. Gadd, G. M. and White, C. Microbial treatment of metal pollution a working biotechnology. Tib Tech.1993. 11, 353-359.
11. Rajfur, M., Klos, A. and Waclawek, M. Sorption of copper (II) ions in the biomass of alga Spirogyra sp. Bioelectrochemistry, (Article in press). 2012.
12. Mehta, S. K. and Gaur, J. P. Use of algae for removing metal ions from wastewater: progress and prospects. Crit Rev Biotechnol. 2005, 25:113-152.
13. Goher M.E., Abd El-Monem A.M., Abdel-Satar A.M., Ali M.H., Hussian A.M., Napiórkowska-Krzebietke A.(2016). Biosorption of some toxic metals from aqueous solution using non-living algal cells of Chlorella vulgaris. J. Elementology. 21(3):703-714.
14. Li, W.W. and Yu, H.Q. Insight into the roles of microbial extracellular polymer substances in metal biosorption. J. bioresource Technology. 2014, 160:15-23.
15. Ferreira, L., S., Rodrigues, M., S., Carvalho, J., C., Lodi, A., Finocchio, E., perego, p. and converti, A. Adsorption of Ni^{2+}, Zn^{2+}, and Pb^{2+} on to dry biomass of Arthrospira (Spirulina) platensis and Chlorella vulgaris. 1-Single metal systems. 2011, 173, 826-333.
16. Kumar, D. and Gaur, J.P. Chemical reaction and particle diffusion-based kinetic modeling of metal biosorption by a phormidium sp. dominated

cyanobacterial mat. J. Bioresouce Technology. 2011, 102, 633-640.
17. Lacher, C. and Smith, R. W. Sorption of Hg (II) by Potamogeton natans dead biomass. Minerals Engineering. 2002, 15, 187-191.
18. Torres, M. A., Barros, M. P., Campos, S. C., Pinto, E., Rajamani, S., Sayre, R. and Colepicolo, P. Biochemical biomarkers in algae and marine pollution: A review. Ecotoxicology and Environmental Safety. 2008, 71, 1-15.
19. K¨utzing, Effect of iron limitation on cells of the diatom Cyclotella meneghiniana, OCEANOLOGIA. 2004, 46 (2), pp. 269–287.
20. Petrou, K., Trimborn, S., Rost, B., Ralph, P. J. and Hassler, C. S. The impact of iron limitation on the physiology of the Antarctic diatom Chaetoceros simplex ,Mar Biol. 2014, 161(4): 925–937.
21. Kosakowska, A., Lewandowska, J., Stoń, J., Burkiewicz, K. Qualitative and quantitative composition of pigments in Phaeodactylum tricornutum (Bacillariophyceae) stressed by iron, BioMetals. 2004. 17, 45–52.
22. Wang, C., Kong,H., Wang, X., Wu,H. D., Lin, Y., and He,S. B. Effects of Iron on Growth and Intracel lular Chemical Contents of Microcystis aeruginosa. Biomedical and environmental sciences journal. 2010. 23, 4852
23. Prescott, G . W. How to know the freshwater algae. 3rd Ed. Wm. C. Brown Company Publishers. USA. 1978.
24. Jurgensen, M. F. and Davey, C. B. Nitrogen fixating blue-green algae in acid forest and nursery soils. Can. J. of Microbiology. 1968, 14, 1179.
25. Hilary, B and Erica, S. Culturing algae. In: Natural environment research council, culture collection of algae and protozoa (CCAP), Freshwater biological Association, The Ferry House, Ambleside, Cumbria, United Kingdom. 18, 22. 1982
26. Strickland, J. D. H. and Persons, T. R. A practical handbook of seawater analysis. 2nd Eds. Bull. Fish. Res. Bd. Canada 167, 311.1972.
27. Mackinney, G. Absorption of light by chlorophyll solution. J. Biol. Chem. 1941, 140: 315.
28. Arnon, D. I. Copper enzyme in isolated chloroplasts. Plant Physiol. 1949, 24: 1-15.
29. Utermohl, H. Quantitative methods zur undersuchung des mannoplankton abderhalden's handbuch der biologischen, arbiets mothodern.1936. 2: 1879-1937.
30. Utermohl, H. Zur vervolkommnung der quanntitataven phytoplankton methodik. Limnol. 1958, 9: 1-38.
31. Singh, S. P., Verma, S. K., Singh, R. K. and Pandey, P. K. (1989). Copper uptake by free and immobilized cyanobacterium. FEMS Microbiological letters. 1989, 62, 336-348.
32. Luong-Van, J. T. and Hayward, E. Immobilized Tetraselmis sp. for ease of TEM processing and ultrastructure research. J. of Applied Phycology. 2007, 19(6): 685-688.
33. Goel, P. K. (2009). Water pollution causes, effects and control. Department of pollution studies Y. C. College of Science Vidyanagar, Karad -415124 Maharashtra.
34. Murphy, V. An investigation into the mechanisms of heavy metal binding by selected seaweed species. Ph.D, Waterford Institute of Technology. 2007.
35. Ghoniem M.M., El-Desoky H.S., El-Moselhy K.M., Amer A., Abo-El-Naga E.H., Mohamedein L.I. andAl-ProlA.E. (2014). Removal of cadmium from aqueous solution using green marine algae, Ulva lactuca. Egypt. J. Aquat. Res., 40(3): 235-242.
36. El-Sheekh, M.M. Farghl, A.A. Galal, H.R. and Bayoumi, H.S. Bioremediation of different types of polluted water using microalgae, Rend. Lincei. 2015, 22(2), 401 – 410.
37. Ahmad, A.; Bhat, A.H.; Buang, A. Biosorption of transition metals by freely suspended and Ca-alginate immobilised with Chlorella vulgaris:

Kinetic and equilibrium modeling. J. Clean. Prod. 2018,171, 1361–1375.
38. Kaplan, D. Absorption and adsorption of heavy metals by microalgae. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2nd ed.; John Wiley & Sons, Ltd.: New York, NY, USA,; Chapter 32, pp. 602–611. 2013.
39. Onyeji L.I., Aboje A.A. Removal of heavy metals from dye effluent using activated carbon produced from coconut shell. Int. J. Engin. Sci. Technol. (IJEST). 2011, 3(12): 8238-8246.
40. Sunda, W. G. and Huntsman, S.A. Iron uptake and growth limitation in oceanic and coastal phytoplankton, Mar. Chem. 1995, 50, 189–206.
41. Kudo, I., Miyamoto, M., Noiri, Y., Maita, Y. Combined effects of temperature and iron on the growth and physiology of the marine diatom Phaeodactylum tricornutum (Bacillariophyceae), J. Phycol. 2000, 36 (6), 1096–1102.
42. Davey, M., Geider, R. Impact of iron limitation on the photosynthetic apparatus of the diatom Chaetoceros muelleri (Bacillariophyceae), J. Phycol. 2001, 37 (6), 987–1000.
43. Yen-Chen, C., Weichang, H., Chunkao, P., Liag pan, J. and Shuchang, J. Biosorption of cadmium by CO_2-fixing microalga Scenedesmus obliquus CNW-N. J. Bioresource Technology. 2012, 105, 74-80.
44. Ibrahim, W. M.; Hassan, A. F.; Azab, Y. A. Biosorption of toxic heavy metals from aqueous solution by Ulva lactuca activated carbon. Egypt. J. Basic Appl. Sci. 2016, 3, 241–249.
45. Ibrahim, W. M. Biosorption of heavy metal ions from aqueous solution by red macroalgae. J. Hazard. Mater. 2011, 192, 1827–1835.
46. Vendruscolo, F.; da Rocha Ferreira, G.L.; Antoniosi Filho, N.R. Biosorption of hexavalent chromium by microorganisms. Int. Biodeter. Biodegr. 2017, 119, 87–95.
47. Gao, F. Li, C. Yang, Z. Zeng, G. Mu, J. Liu M. and Cui, W. Removal of nutrients, organic matter, and metal from domestic secondary effluent through microalgae cultivation in a membrane photobioreactor, J. Chem. Technol. Biotechnol., In Press. 2016.