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Bioremoval of Iron From Water Sources by Using One Species of Micro Algae (*Phormidium Tenue*)

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Abstract

Background: *The disturbance of aquatic ecosystems provoked by heavy metals pollution from industrial and domestic sources, has as consequence the loss of biological diversity, as well as increased bioaccumulation and magnification of toxicants in the food chain.* ***Aim:*** *the aim of the present study was to evaluate the efficiency of an isolated blue-green alga (cyanobacterium) Phormidium tenue in removal of iron (Fe^{3+}) from aqueous solutions.* ***Methods:*** *Experiment study using a wide concentrations range of metal solutions on the growth of the algal cultures was conducted. Chlorophyll (a), in addition to fresh weight of the isolated alga were estimated for various concentrations of the selected heavy metal (Fe^{3+}) throughout the experimental period.* ***Results:*** *The data show that the lower doses of the tested metal had stimulatory effect on biomass yield of Phormidium tenue, whereas the higher concentrations exhibited an inhibitory effects, depending on the metal concentration, exposure time, alga sensitivity,..etc. Phormidium tenue recorded 80% removal efficiency for Fe^{3+}. So, the studied alga offer a good system for bioaccumulation of the tested heavy metal. The morphological and anatomical changes in treated alga by heavy metal were studied by SEM & TM). These examinations showed some alterations in algal form and cellular components for tested alga.* ***Conclusion:*** *The results showed that Phormidium sp. can be used as a potential bioaccumulator for Fe^{3+} removal process .*

Keywords: Bioremoval ; Iron ; Phormidium tenue; Water

Introduction

Heavy metals are ubiquitous in nature in wide range of concentrations and mixtures. It occurs naturally in at least trace quantities, but their concentrations can be greatly increased by human activities[1, 2]. Many of the physical and chemical methods used for wastewater treatment can lead to contamination of water by chemicals and more expensive when dealing with water having a relative low metal content[3].

Heavy metals usually classified as the following categories: toxic metals, a precious metals and radionuclide[4,5]. Some of these metals have been to be essential for metabolism of plants and phytoplankton[6]. Some heavy metals are taken up by cation exchange and are concentrated within the cell[7]. Although, many heavy metals are essential for living beings such as iron, but at high concentrations they could be hazardous for organisms[8,9]. Toxic effects include ion displacement and /or substitution of essential ion from cellular sites and blocking of functional groups of important molecules[10], This results in denaturation and disruption of cell organelles and membrane integrity[11].

Removal and recovery of heavy metals from wastewater is important for the environmental protection and human

health. Physiochemical-based process, such as chemical precipitation, have been commonly employed for stripping toxic metals from wastewater and environmental pollution[4,12]. However, these methods have several disadvantages, such as incomplete metal removal and expensive equipment[4,12]. For these reason, bioaccumulation for the removal of heavy metal ion from aqueous solution have been launched[4,13]. Responses of algae cells to metal exposure are typically measured in terms of cell density, biomass, growth rate, chlorophyll content or absorbance[14]. Biosorption is the uptake of heavy metal ion and radionuclides from aqueous solution by biological materials. These biosorbents can decrease the concentration of heavy metal ions in solution from part/billion to part/trillion level[4]. In many sorption processes (as biosorption) several mechanisms often act in combination and it is difficult to distinguish between the single steps[15].

Bioaccumulation, involves two processes: the first is similar to biosorption, involving attachment of potentially toxic elements to the surface; and in the second step active transportation of metal ions into cells occurs[4]. Al-khiat[2] Identified bioaccumulation as the accumulation of a substance in the body of organisms without being metabolized and assimilated. This work suggests that the present biosorbents (***Phormidium tenue***) can be more useful for the removal of Iron from aquous solutions.

Aim of the study

The aim of this study was to evaluate the efficiency of micro-algal species (*Phormidium tenue*) in removing of Iron from aqueous culture and the effect of studied heavy metal on the morphological and ultrastructural changes of the treated microalgal cells.

Subject and Methods

Experimental study was done from March to the end of April 2018 The samples were collected from local areas (bany matar) in Sana'a, Yemen. The species (*Phormidium tenue*) was isolated and identified according to Prescott[16].

Culturing and isolation of algae: The isolation and culturing used the moist plate technique recommended by Jurgensen and Davey[17].

Media used for culturing microalgal taxa: Different media were used for isolation and cultivation of industrial wastewater algae (Modified Chu's medium, Bold's Basal medium, BG11 medium, Allen's medium, Z-medium.

Purification: Two method were applied (Dilution method , Plating out method) as described by Hilary and Erica[18].

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

Tested heavy metal: 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 previous salt in distilled water and kept in the refrigerator. Heavy metal 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 Phormidium tenue:- A preliminary experiment using a wide range of metal solutions, ferric citrate $Fe(C_6H_5O_7)$ was carried out to determine the suitable concentrations of this metal which could be tolerated

by the studied alga. Selection of these concentrations was based on the response of the studied alga 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 dist.water and algal cells with a known fresh weight 0.04 (mgL^{-1}) density (initial incoula) for ***Phormidium tenue***.

The culture media were aerated (to provide CO_2) 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 studied alga. The culture glasses for *Phormidium tenue* were incubated at 30 °C and continuous light at 3000 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 fresh weight for *Phormidium tenue* were measured.

In addition to, sample of 500 ml of studied algal species 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 desiccator for 30 min before digestion. Heavy metal removal ability was calculated from the equation $(C_i-C_f)/C_i \times 100$ (%) 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 preliminary 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 fresh weight for ***Phormidium tenue*** was 0.04 mgL^{-1} , using 250 ml conical flasks as culture vessels.

For *Phormidium tenue*

Pollutant	The used concentrations
Iron	1, 2, 4, 6, 8 and 10 mg/1000 ml ferric citrate

Determination of growth parameters during experimental period.

The growth of studied alga was determined by two methods

A) Chlorophyll content: Total chlorophyll content was determined according to the method described by Strickland and Persons[19]. 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). 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 Mackinney[20] and Arnon[21] which gave the specific absorption coefficient as follow:

$$\text{Chl (a)} = 11.93\ A_{664} - 1.93\ A_{647}.$$

B) Fresh weight:- Samples of 15 mls alga were harvested periodically at

the designated time periods (each 3 days) until the day 18, then filter paper drained to remove excess water and weighed.

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[22].

Preparing the studied algal cells for scanning and ultrastructure research:

After 15 days of experimental period ***Phormidium tenue*** was taken under certain concentration. *Phormidium tenue* harvested by filtration at concentration 4 mgL^{-1} of Fe^{+3}. Washing algal sample with dist. 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. Secondary 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 Electron Microscope (SEM) and Transmission Electron Microscope (TEM) (Jeol JSM-1011 electron microscope), according to Luong-Van and Hayward[23].

The data were analyzed used SPSS program. According to Trollope and Evans[24]. data were expressed as mean of three replicates ± SD.

Results

A fundamental goal in ecotoxicology is to predict ecological effects from xenobiotic stresses. Because ecosystem injury should be prevented where possible, scientists and regulators must refine their capacity to anticipate vulnerable components of ecosystems under stress as the potential outcomes. One strategy to reduce heavy metal solution is to use microorganisms. Microalgae, due to their ubiquitous occurrence in nature have been studied extensively in this regard. They can sequester heavy metal ions by adsorption and absorption as do by other microorganisms. The use of microalgae for metal removal has the potential to achieve greater performance at a lower cost than conventional wastewater treatment technologies. This is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metals from dilute aqueous waste.

In this study the microalgal strain (***Phrmidium tenue***) potentially suitable for Fe^{+3} removal in aqueous solution were selected. The researchers attained

our efforts to make a synthetic media contain high levels from iron. The iron concentrations used more than allowed in the International Environmental Low (4/1994). Preliminary tests were conducted by subjecting the recorded microalgal species to wide concentration ranges of Fe^{3+} solutions to determine the Range of algal resistance and also, to detect level of the tested metal to be studied.

Effect of the selected heavy metal on *Phormidium tenue*.

The effect of the selected heavy metal (Fe^{+3}) on the growth of ***Phormidium tenue*** throughout a period of 18 days on intervals of three days was estimated by chlorophyll (a) content and quantitative estimation of fresh weight.

Effect of Iron concentrations on *Phormidium tenue*:

Table 1: Effect of different Fe^{+3} concentrations (mgL^{-1}) on chlorophyll (a) content (μgL^{-1}) of *Phormidium tenue.*

Time /Days / Concentration (mgL^{-1})	Zero time	3	6	9	12	15	18
Control	0.85±0.01	0.943±0.07	1.28±0.01	1.47±0.01	1.48±0.01	1.62±0.01	1.323±0.59
1	0.85±0.01	0.95±0.01	1.67±0.01	1.8±0.01	2.15±0.01	3.32±0.01	1.43±0.01
2	0.85±0.01	0.99±0.01	1.96±0.01	2.18±0.01	2.28±0.01	3.33±0.01	1.49±0.01
4	0.85±0.01	1.84±0.01	3.12±0.01	3.19±0.01	4.35±0.01	6.88±0.01	3.88±0.01
6	0.85±0.01	0.99±0.01	2.56±0.01	3.04±0.01	3.75±0.01	3.95±0.01	1.49±0.01
8	0.85±0.01	0.99±0.01	2.33±0.01	2.37±0.01	2.4±0.1	3.57±0.01	1.11±0.01
10	0.85±0.01	0.99±0.01	1.54±0.01	1.56±0.01	2.05±0.01	2.21±0.01	0.7±0.1

(Data were expressed as mean of three replicates ± SD).

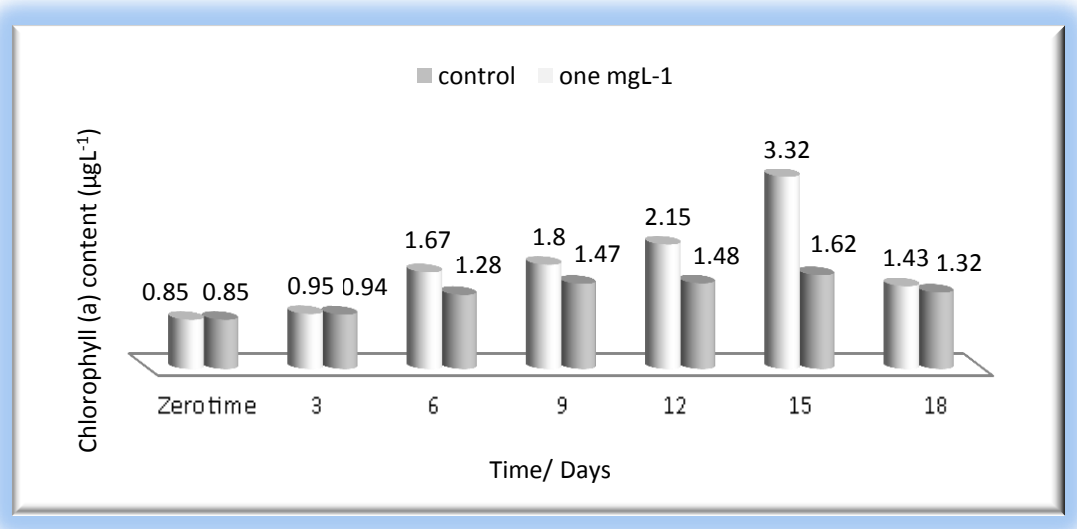

Figure 1: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Phormidium tenue*.(a)

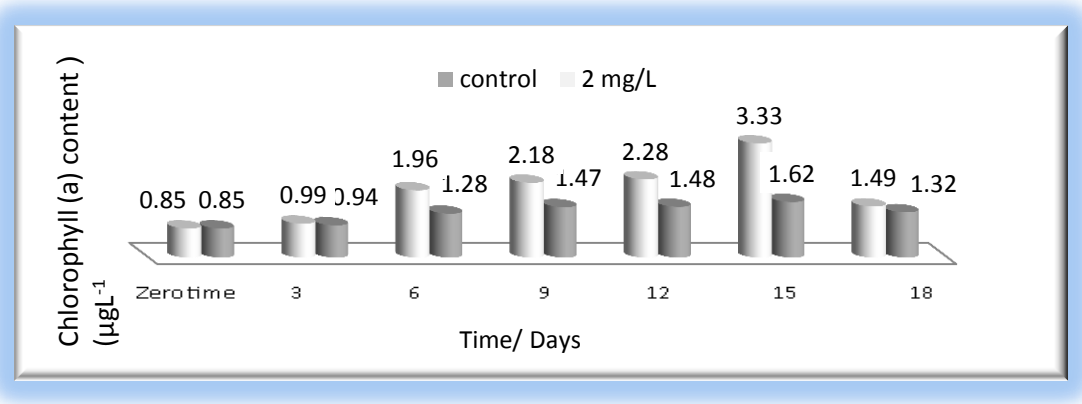

Figure 1: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Phormidium tenue*.(b)

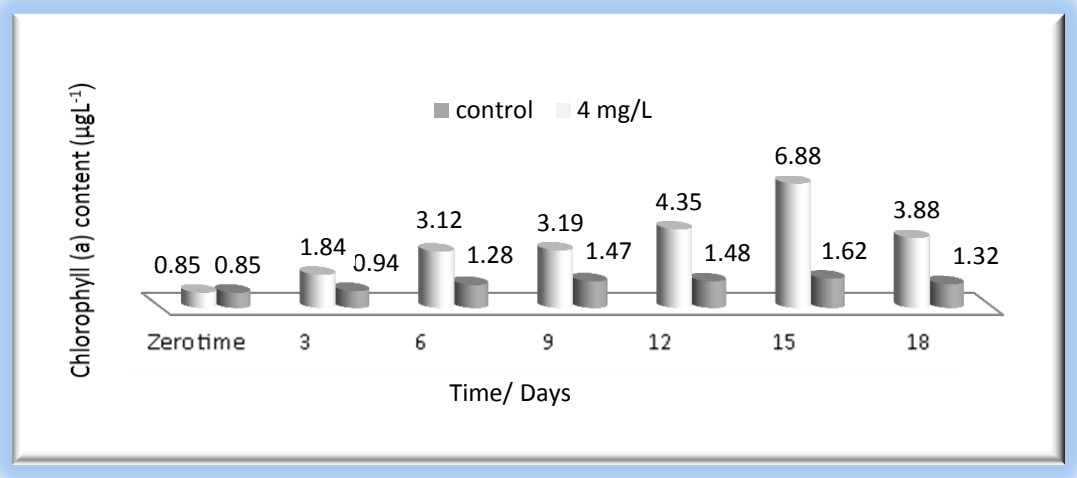

Figure 1: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Phormidium tenue*.(c)

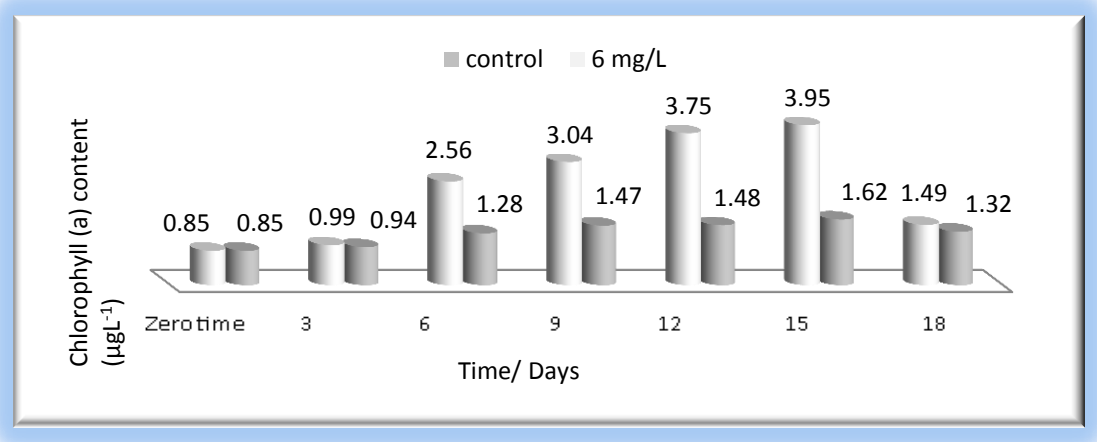

Figure 2: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Phormidium tenue*.(a)

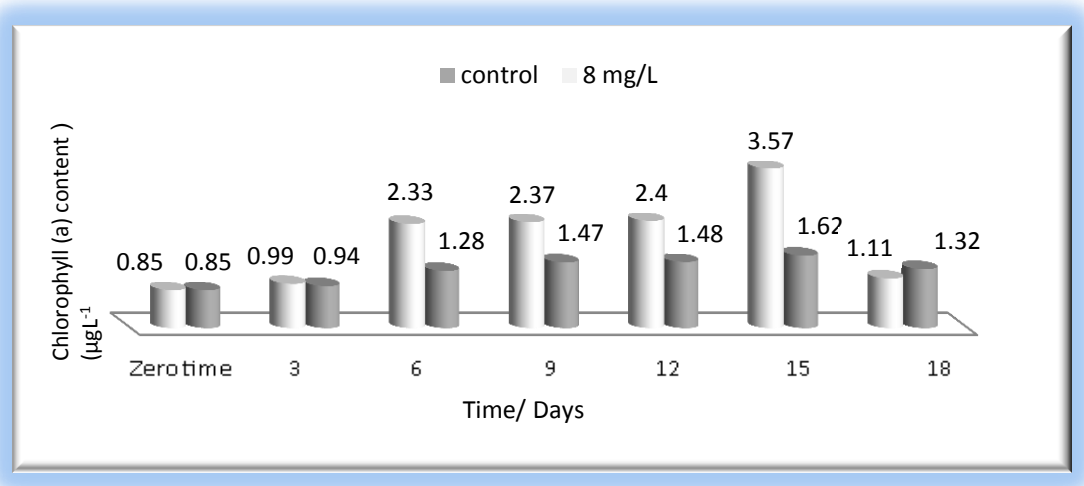

Figure 2: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Phormidium tenue*.(b)

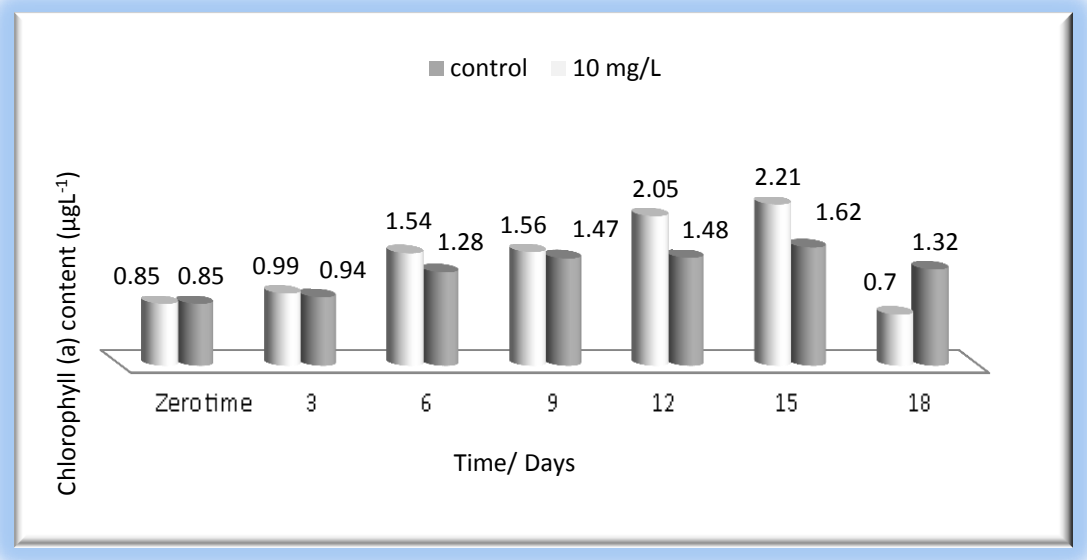

Figure 2: Effect of different Fe^{3+} concentrations on chlorophyll (a) content of *Phormidium tenue*.(c)

Table 1 and figures 1&2 Showed that most of the studied concentrations of Fe^{3+} metal (1, 2 , 4, 6, 8 and 10 mgL^{-1}) were exhibited gradual stimulation effect on chl (a) content of ***Phormidium tenue***. Furthermore, increasing of exposure time for 3 , 6 , 9 ,12, 15 & 18 days of ***Phormidium tenue*** under stress of 4 mgL^{-1} Fe^{3+} led to increase in chl (a) content to 1.84 ,3.12 , 3.19 , 4.35 , 6.88 and 3.88 μgL^{-1}, respectively comparing with control. So, the treated alga under this metal concentration (4 mgL^{-1} Fe^{3+}) was selected to be examined by the electron microscope.

Table 2: Effect of different Fe^{+3} concentrations (mgL^{-1}) on fresh weight (mgL^{-1}) of *Phormidium tenue.*

Time /Days / Concentration (mgL^{-1})	Zero time	3	6	9	12	15	18
Control	0.04±0.01	0.11±0.01	0.215±0.001	0.235±0.001	0.3±0.01	0.378±0.001	0.311±0.001
1	0.04±0.01	0.124±0.001	0.222±0.001	0.273±0.001	0.334±0.001	0.941±0.001	0.326±0.001
2	0.04±0.01	0.127±0.001	0.242±0.001	0.338±0.001	0.404±0.001	1.29±0.001	0.601±0.001
4	0.04±0.01	0.15±0.01	0.301±0.001	0.453±0.001	0.565±0.001	1.84±0.001	1.38±0.01
6	0.04±0.01	0.15±0.01	0.283±0.001	0.365±0.001	0.524±0.001	1.317±0.001	0.961±0.001
8	0.04±0.01	0.13±0.01	0.235±0.001	0.321±0.001	0.374±0.001	1.193±0.001	0.463±0.001
10	0.04±0.01	0.13±0.01	0.177±0.001	0.316±0.001	0.34±0.01	1.081±0.001	0.41±0.01

Data were expressed as mean of three replicates ± SD

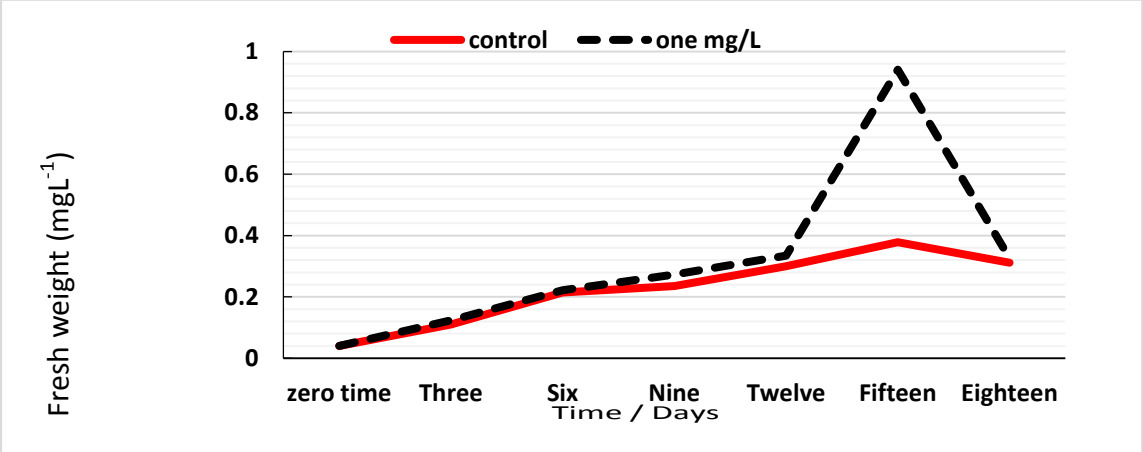

Figure 3: Effect of different Fe^{3+} concentrations on fresh weight of *Phormidium tenue*.(a)

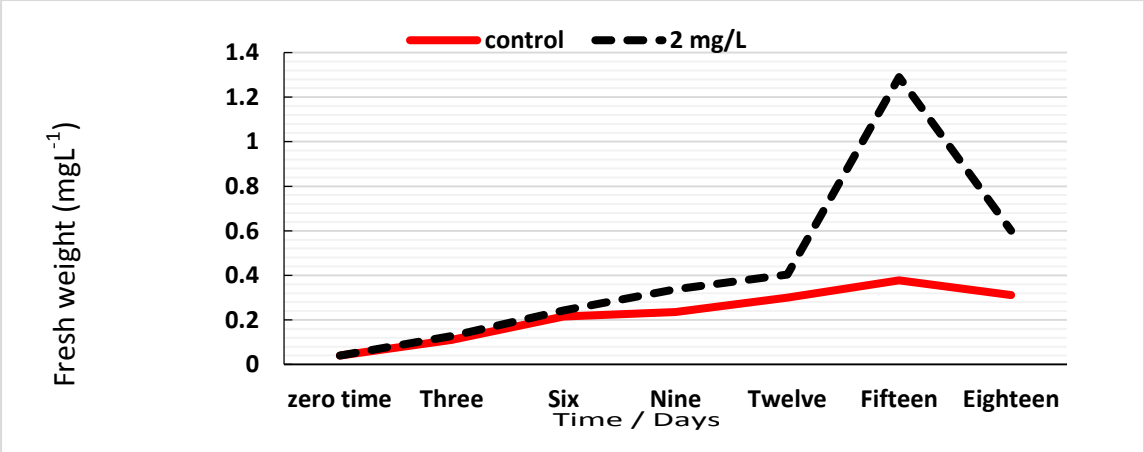

Figure 3: Effect of different Fe^{3+} concentrations on fresh weight of *Phormidium tenue*.(b)

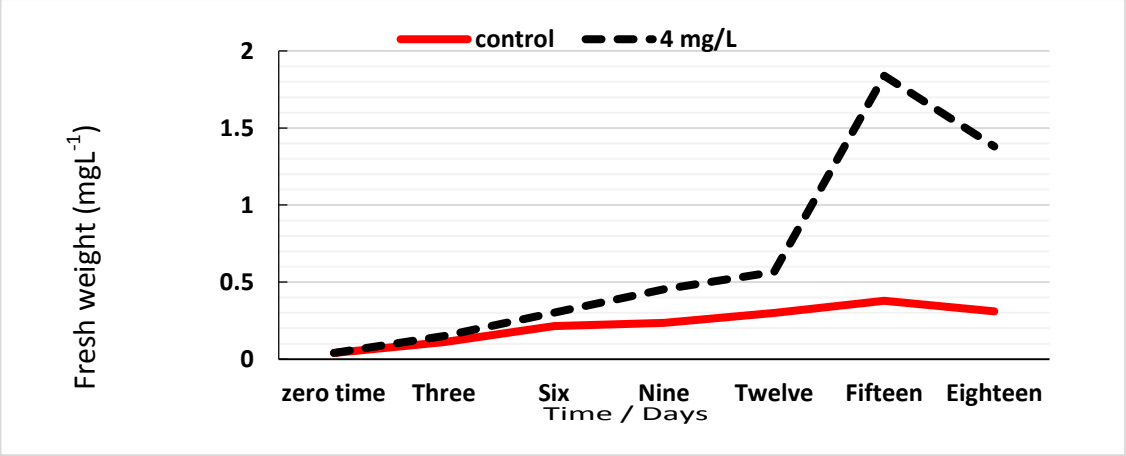

Figure 3: Effect of different Fe^{3+} concentrations on fresh weight of *Phormidium tenue*.(c)

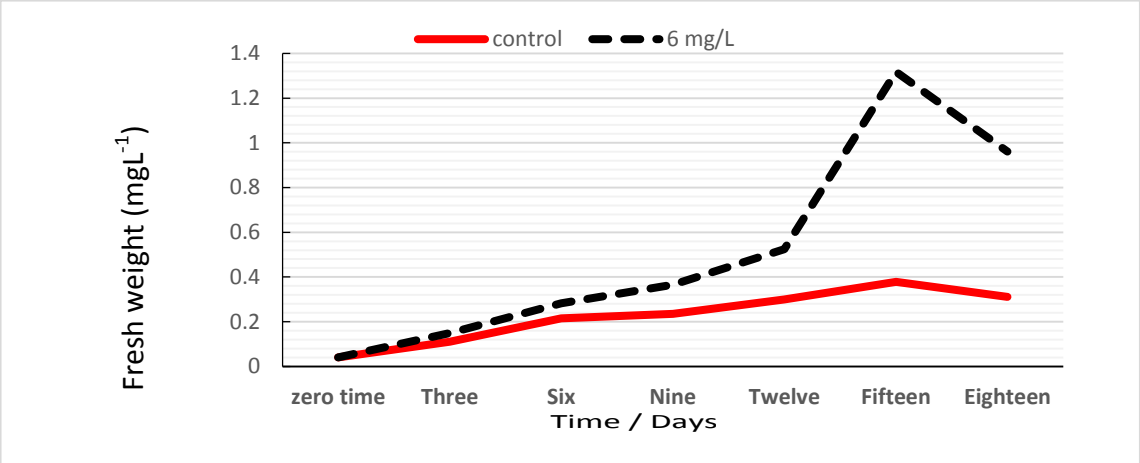

Figure 4: Effect of different Fe^{3+} concentrations on fresh weight of *Phormidium tenue*.(a)

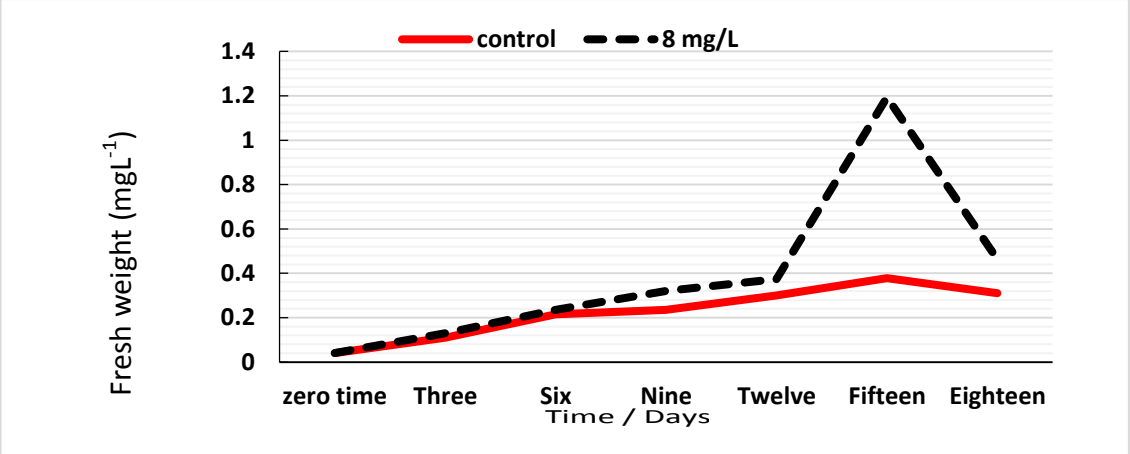

Figure 4: Effect of different Fe^{3+} concentrations on fresh weight of *Phormidium tenue*.(b)

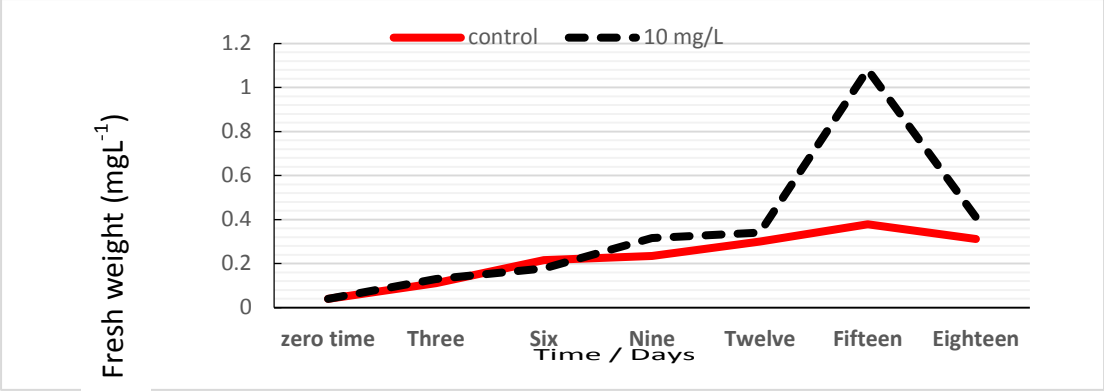

Figure 4: Effect of different Fe^{3+} concentrations on fresh weight of *Phormidium tenue*.(c)

The data in Table 2 and Figures 3 & 4 Revealed a noticeable and a gradual increase in fresh weight of *Phormidium tenue* (0.15 , 0.301 , 0.453 , 0.565 , 1.84 and 1.38 mgL^{-1}) with increasing growth period to 3, 6, 9 , 12 , 15 & 18 days, respectively at Fe^{3+} concentration 4 mgL^{-1}. The maximum fresh weight values of *Phormidium tenue* were obtained at 4 mgL^{-1} of Fe^{3+} concentration. The macro and micro-morphological changes in *Phormidium tenue* induced by the stimulatory concentration ($4mgL^{-1}$) of Fe^{3+} Through 15 days were illustrated in plates (2&3). Plate (1) shows a control cell of *Phormidium tenue* in a nutrient medium BGII For 15 days under constant conditions (3000 lux & 30 °C). The algal cell surface seems to be regular and healthy. The ultrastructural of *Phormidium* cell declare some cellular components as thylakoid membranes, vacuoles, glycogen granules and other cytoplasmic components surrounded by cell wall. Interesting, plates (2&3) declare the effect of Fe^{3+} concentration ($4mgL^{-1}$) on *Phormidium tenue* along 15 days, Fe^{3+} caused a fragmentation of algal filament into small fragments and disappearance of the apical round cells. Also, thickening of cell wall and increasing of crystalline inclusions inside algal cell which may be attributed to a defense mechanism against Fe^{3+} metal ions.

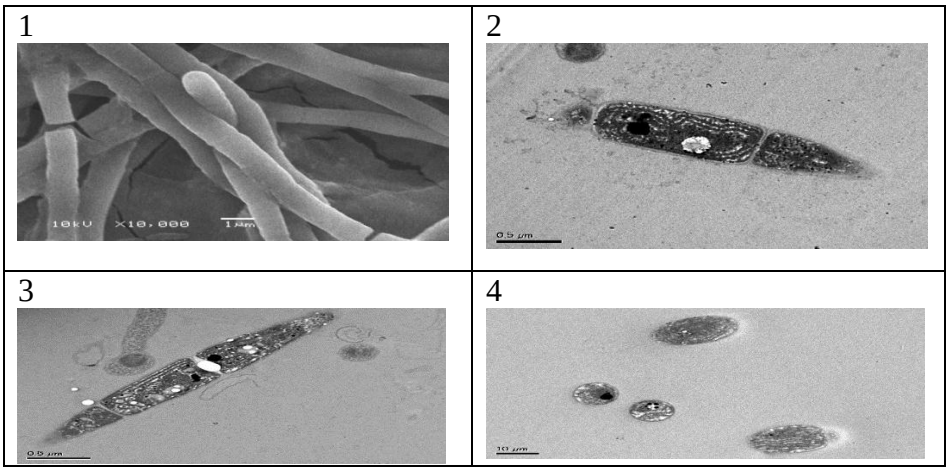

Plate (1): shows both SEM (1) and TEM (2,3&4) micrographs of ***Phormidium tenue*** without any treatment (control). *Phormidium* filaments grown in complete nutrient medium (BGII) For 15 days, under controlled conditions (3000 Lux & 30 C^{o}). Morphologically, the algal cell surfaces seems to be regular, healthy with a considerable length of filaments. The ultrastructural of ***Phormidium tenue*** cell shows some cellular components with distinguishable thylakoid membranes, glycogen granules, vacuole and surrounded by cell wall.

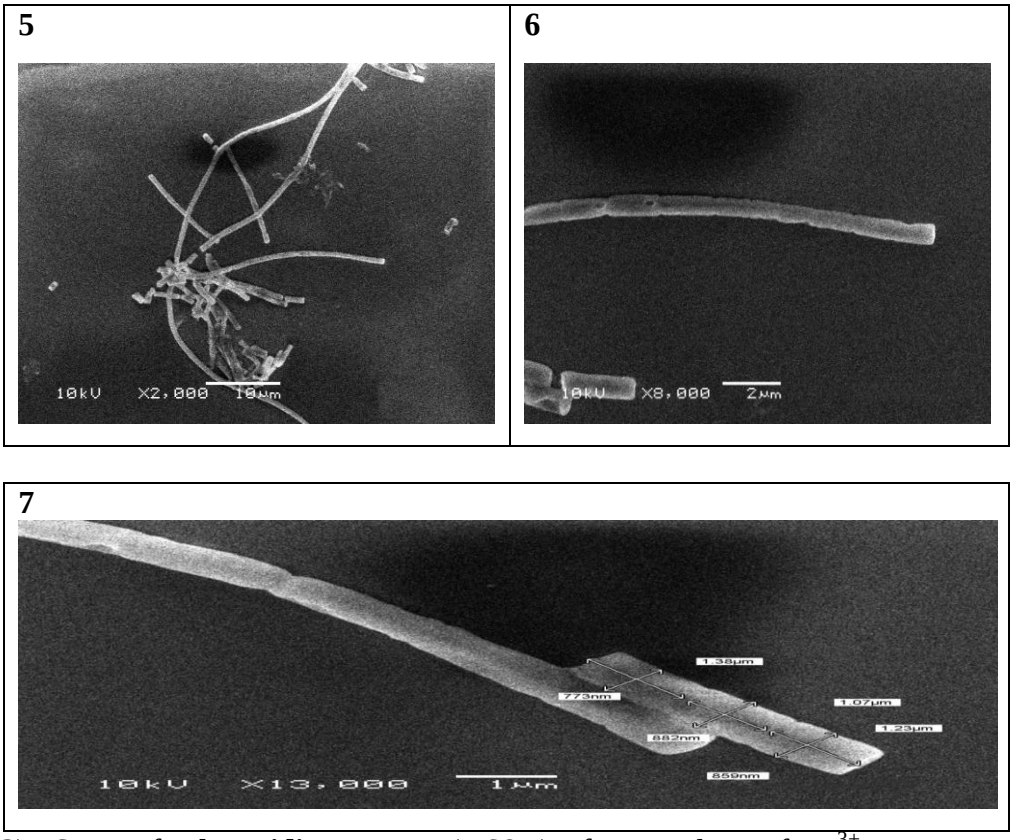

Plate (2): SEM of ***Phormidium tenue*** (5,6&7) after 15 days of Fe^{3+} treatment ($4mgL^{-1}$), shows more dwarf and breaking of *Phormidium* filaments into small pieces. So the round apex of the filament disappeared.

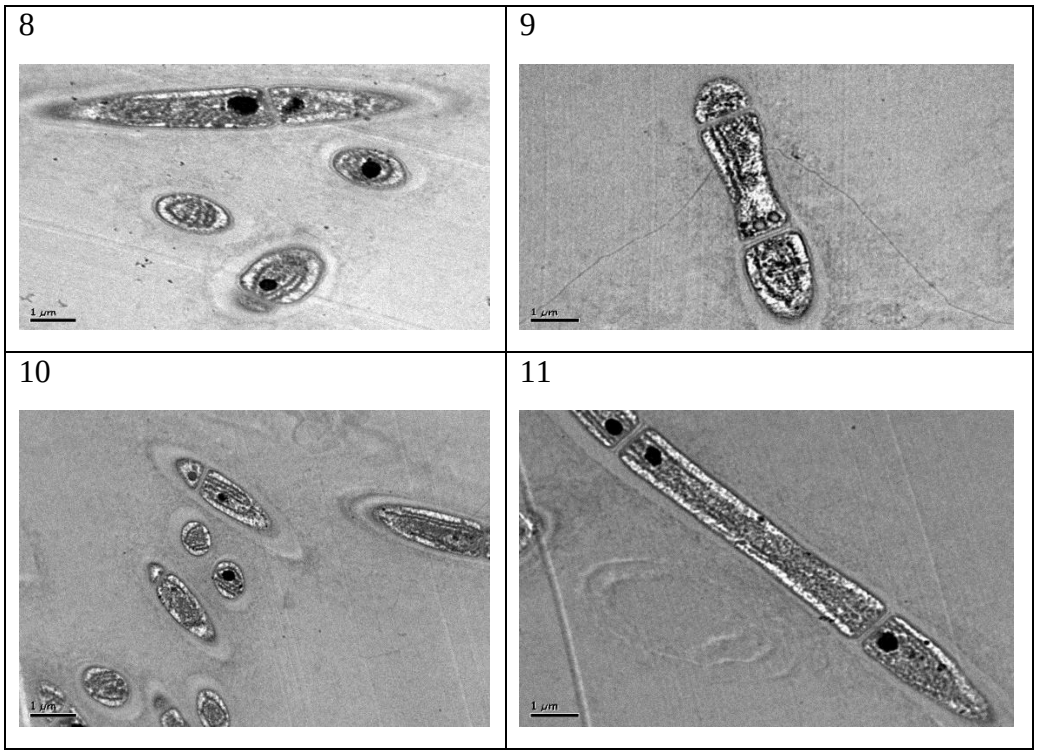

Plate (3): Pictures from 8-11 represent TEM of ***Phormidium tenue*** filament after 15 days of Fe^{3+} treatment ($4mgL^{-1}$), showing more thickening of cell wall, fragmentation of filament into small fragments, dilatation of the cell size and increasing of crystalline inclusions.

Discussion

Mechanisms of metal binding to algae

Bioaccumulation by living algae:

In order to differentiate between the biosorption of metals by dead algae biomass and heavy metal binding by live algae, in the case of biosorption by nonliving algae, the mechanisms can be thought of as occurring discretely at the cell wall while bioaccumulation normally implies intracellular binding by living organism. Even though both living and dead cells are capable of metal accumulation, there are differences in the mechanisms involved, depending on the extent of metabolic dependence in live algae. The parameters affecting performance of living biosorbents are as follows:

- Heavy metal concentrations.
- The physiological state of the organism.
- The age of the cells and density of the biomass.
- The availability of micronutrients during their growth.
- The environmental conditions during uptake (pH, temperature, light intensity etc.)

Accumulation of heavy metals by living algae has been shown to occur in two phases: (1) a rapid surface reaction followed by (2) a much slower metal uptake over a period of hours. An initial rapid uptake will correspond to extracellular adsorption and/or to passive intracellular uptake (metabolism- independent involving cell surface adsorption and simple diffusion into cell or intercellular spaces. A slower uptake will correspond to metabolism- dependent incorporation into the cell body or, in some cases, to a continuous or non-continuous excretion taking place in the macro-algae. In parallel experiments, 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 heavy metals there might be toxicity which can reduce the biosorption capacity of the microalgae. It has been described that microalgae can protect themselves against the toxicity of heavy metals using several mechanisms such as: exclusion mechanisms, adsorption to cell surface or intracellular accumulation[3,25].

According to Murphy[26], diagram (A) illustrates the routes of uptake for potentially toxic metal cations and possible tolerance mechanisms.

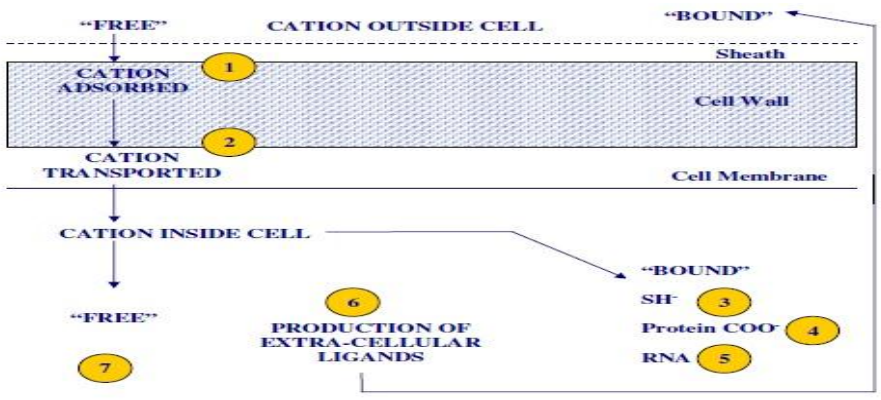

Diagram (A): Routes of uptake for potentially toxic cations and possible sites (1-7) of tolerance mechanisms.

by Goher et al[30] Petrou et al[8] and Behrenfeld and Milligan[31].

El-Sheekh et al[32] mentioned that heavy metals removal in wastewater mostly depending on the species of microalgae used. Moreover, it is also relying on the nature and charge of the cell wall polysaccharides of microalgae. Algae can remove metal ions efficiently, mainly by an ion-exchange mechanism, and the metal removal is highly dependent on metal concentration[3].

Bilal et al[4] and Kaplan[33] mentioned that The binding groups, for example RS^-, SH^-, $PO4^{3-}$, RNH^{2-} and RO^- exist outside and inside the cell wall. The biosorption mechanism of metal ions across the cell walls could be supported by cytosolic protein-mediated metal ions transfer**.** The cell wall is an initial hindrance to the biosorption of toxic elements. Most of the binding sites are due to polysaccharides and proteins[4,3]. Different algal strains have different cell wall structures and varying capacity of biosorption of toxic elements[4].

Our results in harmony with Onyeji and Aboje[34] they found that when the higher dose of adsorbent was used lead to increase the removal percentage similarly to pH. This was because more surfaces and functional groups were available on the adsorbent with which the metal could interact. Our study was agreement with Goher et al[30] they mentioned that The fast removal at the beginning may be attributed to a larger adsorbent surface area being available for the adsorption of the metal 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

Algae may produce extracellular compounds[7], compounds in/on cell wall[27] that can bind to certain metals rendering them non-toxic. Detoxification of metal ions at the cell surface is referred to as exclusion mechanism because the metal ions do not cross the cell membrane (Mechanism 1). Another possible exclusion metal is adsorption or detoxification of a metal ion by surface-living micro-organisms (Mechanism 2). If no exclusion mechanism is operating, a metal ion can enter the cytoplasm. Several detoxification mechanisms are then possible inside the cell. Mechanisms 3-5 refer to metal binding to-SH residues, protein carboxyl groups and RNA respectively. Mechanism 6 (production of extracellular legends in Diagram A) refers to the production of phytochelatins. Kumar and Gaur[28] showed showed that phytochelatins are cysteine-rich peptides that can be induced in higher plants when exposed to a range of heavy metals. Chakraborty et al[29] later extended these findings to the microalga *Chlorella fusca* and the marine macroalga *Sargassum muticum*. If neither exclusion nor inclusion mechanisms take place, the metal cation remains "free" within the cell and a toxic effect takes place (Mechanism 7).

The bioassay results as illustrated in Tables 1 and 2 showed clear differences in chl (a) and fresh weight of algal cells between control and treated ones when alga was exposed to different concentrations of Fe^{3+} metal. Previous results indicated a gradual stimulation in growth rate for the 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

concentrations. Iron deficiency leads to a shortage of chlorophyll production, although the pigments do not contain iron. 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 deficiency leads to a decrease in the activity of these enzymes and thus reduce the rate of chlorophyll a synthesis. Also, the cellular chl a content depends on the quantity of iron contributing in the photosynthetic electron transport chain, mainly at the PSI and PSII reaction centres. deficiency of synthesis at these photosynthetic reaction centres by iron availability will also decrease cell pigment[35,38]. Petrou et al[8] and Behrenfeld and Milligan[31] they found that iron lack causes a major reorganization of the thylakoid synthesis, This reorganization causes a disconnection between light-harvesting centers resulting in a decline in photosystem II efficiency, electron transport and carbon fixation under iron- lack conditions. Petrou et al[8] who found that Lowered electron transport rates can lead to reduced production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), energy equivalents that are required to uptake iron. Iron is essential in both photosystems (2–3 atoms for PSII; 12 atoms for PSI): the cytochrome b_6f complex (5 atoms), which contacts the two photosystems, and the ferredoxin molecule (2 atoms). Given the important requirement of iron within the photosynthetic electron transport chain, Fe-limitation strongly effects electron transport kinetics. Also, mentioned that, a lack of iron led to a reduction in iron uptake which led to changes in the Antarctic diatom Chaetoceros simplex, inducing high-affinity transport pathways to adsorption process and the metal ion absorption onto the adsorbent surface.

Our results indicate that low concentrations of iron cause a lack of growth rates (fresh weight and the chlorophyll (a) content) and this is because the iron is essintial component in the syntheses of some important metabolism enzymes which play important role in many of the functions of biochemical and physiological, whereas, iron affects the activity of nitrate reeducates and nitrogenase, and is directly involved in nitrate and nitrite reduction. The two most energy demanding systems in the cell, photosynthetic carbon reduction and nitrogen reduction, are both highly dependent on iron containing compounds. Nitrogen metabolism is closely connected with carbon fixation, as both processes compete for energy generated by the light reactions of the photosynthesis. Carbon metabolism is necessary to integrate nitrogen into protein. and these findings agrees with K¨utzing[35] and Wang et al[36].

Iron limitation generally causes decreased synthesis of chlorophyll pigments**.** The decrease of pigment synthesis leads to fewer photons captured, causing a severe decrease in the output of photosynthesis (the total carbon fixed) This explain why the carbohydrate accumulated in the *Microcystis aeruginosa* cell was limited by iron at low concentration[36.] K¨utzing[35] who found that a lower iron concentrations caused a marked reduction in the chlorophyll a content. And Sunda and Huntsman[37] noted falling growth rates with decreasing iron concentrations not only in the large oceanic diatoms *Thalassiosira pseudonana* and *Thalassiosira oceanica* but also in other phytoplankton species they examined. Similar trends examined by Davey and Geider[38], exhibited a decrease in cellular chl a at low iron

bioaccumulation, and this agree with Mane and Bhosle[3]. Our results showed 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[40] who reported that was because the use of all active sites leads to saturation of biomass, then causes an stability state of biosorption.

The initial metal ionic concentration affects biosorption. A high initial metallic concentration exhibits high biosorption capacity due to the availability of free active sites[41]. A similar trend was also reported by changing the adsorbent dose[42, 43].

Two stages are involved in the accumulation of toxic elements through microorganisms. During the first stage, fast inactive biosorption proceeds at the cell surface with no cellular metabolism involved, whereas active sorption occurs during the second stage, which involves the cell cytoplasm. The second stage is considered intracellular ion accumulation since it involves cell metabolism[4].

The efficiency of the investigated alga in heavy metal removal in aques solution:

The selected heavy metal resulting from the preliminary experiment as mentioned before in materials and method are Fe^{3+} for the blue green alga *Phormidium tenue*. In addition, determination of this metal in aqueous solution before and after treatment period, the assessment was extended also to determine the same metal within the studied algal tissue before and after treatment period, in the case of aqueous solution and algal tissue, the initial content of heavy metal were determined in one time, but the final content were determined after 15 days of the experimental period.

The amount of metal removed by the maximize iron uptake. K¨utzing[35] reported that, iron deficiency of the Baltic diatom *Cyclotella meneghiniana* lead to decreased in chlorophyll a, So Iron therefore be an important regulatory factor controlling the growth of diatom *C. meneghiniana*. Torres et al[7] reported that reduction of chlorophyll (a) content is a common symptom of heavy metals toxicity. Also, removal efficiency of Fe^{3+} by *Phormidium* may be attribute to gelatinous layer surrounded the cell wall, may be slow down the diffusion rate of the metal ions into chelating matrix[1]. The cellular structure in the experimental test have explained the sensitivity pattern in *Phormidium tenue*. These results were supported by morphological and ultrastructural examination.

Our results indicate that the optimum concentration of iron leads to the highest growth rate and highest production of pigments, when the concentration of iron is higher than the optimum concentration, it leads to the growth inhibition and decrease in the production of pigments. These results are in agreement with those obtained by Wang et al[36]. The lack of pigments is paralleled by a decrease in thylakoid membranes, these results also, agrees with Wang et al[36].

Furthermore, the stimulatory effect of iron recorded in this study with lower concentrations can be accounted for either as a result of algal requirement of this element in metabolic processes or explained by production of some organic compound which decreases metal toxicity[39]. If the initial metal concentration increases there is decrease in the metal removal capacity of the algae[3].

This study showed that, if the external concentration of metal ions in the solution was higher their leads to toxic effects and which leads to decreased performance of

towards metal ions and may offer a promising chelating agents for removal of heavy metals from wastewaters[47]. A wide difference in metal binding capacity of sheathed and non sheathed blue-green algal strain has demonstrated. A blue-green algal strain with thick gelatinous sheath showed higher metal binding capacity than strains devoid of or with a thin layer of sheath[48]. However, presence of gelatinous sheath around the blue-green algal cell may slowdown the diffusion rate of metal ions into the chelating matrix of cell wall[49,50]. In additionally Inthorn et al[51] who stated that blue-green algae can select to test for heavy metal adsorption because they have high growth rates and are easy to separate from solution by simple filtration.

Conclusion and recommendations

The results showed that ***Phormidium sp.*** can be used as a potential bioaccumulator for Fe^{3+} removal process . The importance of local isolates ***Phormidium tenue*** as biomaterial for heavy metal removal process from wastewater. The tested algal species can be selected for further study because they have high removal ability and easily separate from water. The use of blue green algae for heavy metal removal would be ecofriendly and cost effective in comparison to traditional methods.

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cells increased rapidly during the first time after the application time, and then steadily increased with days in used metal. In our study the removal percentage (Removal capacity: Removal efficiency) of iron (Fe^{3+}) by *Phormidium tenue* during the experimental period (15 days) was highest at 80%.

Ferreira et al[9] and Kumar and Gaur[44] They Mentioned that The removal efficiency decreased with increasing metal concentration, pointing out a passive adsorption process involving the active sites on the surface of the biomasses**.** The cell surface is the main site of metal binding in algae and the surface bound metals often far exceed the metal accumulated in the intracellular compartments. The cell surfaces have different functional groups, like, hydroxyl, phosphate, amino, aldehyde, sulfhydeyl, with various affinities for heavy metal binding[9,45]**.**

Ion exchange is the main mechanism of metal binding, and the carboxyl group plays a predominant role in metal binding. Furthermore the number and nature of functional groups involved in metal binding may also differ in various algal species. These differences obviously account for differential sorption of the tested metals by tested microalgae. Peptidoglycan, consisting of N-acetylglucosamine and 1, 4-N-acetylmuramic acids with peptide chain, is the major component of cell wall of blue-green algae, providing mostly carboxylic groups for metal binding[46]. It has been suggested that carboxyl groups on the blue-green algal cell wall are the dominant active sites for the binding of metal ions. Many strains of blue-green algae have an outer sheath made of polysaccharides. Due to the an ionic nature of blue-green algal gelatinous sheath usually show very high affinity

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