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COLLEGE OF ENGINEERING

USE OF ALGAE FOR REMOVING HEAVY METAL IONS FROM INDUSTRIAL WASTEWATER

BY

SAJEDA A. ALSAYDEH

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COMMITTEE PAGE

The members of the Committee approve the Thesis of

Sajeda Alsaydeh defended on 17/04/2019.

Fares AlMomani.

Thesis/Dissertation Supervisor

Majeda Khraisheh.

Committee Member

Mustafa Nasser

Committee Member

Ala'a Al-Muhtaseb

Committee Member

Approved:

Abdel Magid Hamouda , Dean, College of Engineering

ABSTRACT

ALSAYDEH, SAJEDA, A., Masters: June: 2019, Master of Science in Environmental Engineering

Title: Use of Algae for Removing Heavy Metal Ions From Industrial Wastewater

Supervisor of Thesis: Fares, AlMomani.

The presence of heavy metal ions in the environment has been a big challenge facing the world due to its harmful effect on the health of humans even at low concentrations. There are various treatment techniques that can be used to remove heavy metals from wastewater such as chemical, physical, and biological processes. However, the practical application of chemical and physical techniques is limited due to high cost, process complications and the need for well-trained personnel. Therefore, it is necessary to develop new, low cost, highly efficient and eco-friendly wastewater treatment methods that can be effectively eliminate and remove heavy metals from contaminated water solutions. Biological processes are considered the most economical alternative for the treatment of wastewater if compared with other processes in which its adsorbents are widely available. Thus, the main aim of this research work is to utilize microalgal species for removing heavy metal from industrial wastewater. The ability of non-living and living local algae strains to remove different heavy metal ions such as Nickel, Aluminum, and Copper from industrial wastewater samples was investigated. The secondary wastewater sample was modified in the laboratory by adding a specific concentrations of heavy metal ions to conduct the experiment. Batch experiments were conducted to find the optimal operating conditions and the most suitable material to remove Nickel, Aluminum, and Copper via adsorption. pH, initial metal concentration,

biomass dosage, and contact time were tested. Therefore, it was found that the optimum pH, biosorbent dosage, and the initial metal concentration are 5.5, 0.5 g/L, and 10 mg/L, respectively. The maximum removal% of heavy metals from industrial wastewater by non-living *spirulina sp.* biomass was found to be 57.38% for Nickel, 38.24% for Copper, and 93.46% for Aluminum within 4 hours. In order to fit the experimental data, Adsorption isotherms (Langmuir and Freundlich) have been used, then the process kinetics can be obtained. The results showed that the Langmuir isotherm was much better able to fit the linearized data points than the Freundlich isotherm with all metal; Nickel, Copper, and Aluminum based on the value of correlation coefficient (R^2). It was concluded that all metals have the same optimal operating conditions, although, all operating conditions showed positive results. Preliminary screening was conducted on 12 different living algae strains, in order so select the top 5 algae strains that abled to growth with 10 mg/L of the initial concentration of Aluminum ions. The results showed that the best growth of algae was with *Mychonastes, Chlorella, Chlorophyta, Desmodesmus,* and *Scenedesmus* that have the highest optical density @750nm. These algae species were used to conduct different experiment with different initial concentration of Aluminum 5, 10, and 15 mg/L. During these experiment OD@750 nm, pH values, and the percentage removal of Aluminum were reported. *Chlorophyta* showed the highest percentage removal of Aluminum at all the initial concentrations; 58%, 69%, and 45% at 5, 10, and 15 mg/L of Aluminum ions. However, in order to obtain new results on the other different parameters (i.e. agitation speed, temperature, adsorbent particle size, light), further investigations may be needed.

DEDICATION

To my role model in this life;

to my father and mother,

To my sisters, brothers, and friends,

To the one who is the only partner in each dream I had;

to my husband.

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CHAPTER 1: INTRODUCTION

This chapter shows the background of the research topic, the main problem, and the selection of the best solution.

1.1 General

During the 21st century, the demand for water is rapidly increasing as a result of population growth worldwide. In order to meet this growth, more amount of good quality water (i.e. water free of contaminants) is required for the health of different living organisms. Therefore, water pollution has become a global issue and caused great worldwide concern (Vijayaraghavan and Yun 2008, Dahiya, Tripathi and Hegde 2008). The sources of water pollution can be classified into two main categories which are direct and indirect sources of contaminant. The direct sources include the effluents from refineries, industries, and waste treatment plants in which the wastewater is emitted from these sources directly to the water supplies (i.e. lakes and oceans). However, the contaminants that enter water supplies from groundwater and soil systems, as well as from the atmosphere by rainwater are described as indirect sources of water pollution. Generally, the contaminants are divided into two main classes; organic and inorganic pollutants. However, organic wastewater contains volatile organic compounds, food processing wastes, and pesticides. Whereas, Inorganic wastewater includes fertilizers, heavy metals, and the acidity which is caused by industrial effluents (Rao et al. 2012). Among the inorganic pollutants, heavy metals are considered to be the major group of pollutants of concern. Recently, the presence of heavy metals in the systems of water supplies become a global issue faces the most developed and developing countries worldwide. Since these metals are extremely toxic, non-biodegradable, and when they enter the food chain they will have the ability to accumulate at low concentration in the living organisms in general and human bodies in specific. Under specific environmental conditions, heavy metals may be accumulated to toxic levels and cause many serious diseases such as kidney failures, nervous system damage, cancer, and it can be deadly at high concentrations (Visa 2016, Dieter 2011, Häyrynen et al. 2012, Al-Saydeh, El-Naas and Zaidi 2017). Therefore, the U.S. Environmental Protection Agency (USEPA), World Health Organization (WHO), and the other environmental protection agencies have listed the Maximum Contaminant Levels (MCLs) for each heavy metal in drinking water as well as industrial effluents (Vafakhah, Bahrololoom and Saeedikhani 2016). Nickel, Zinc, Silver, Aluminum, Lead, Chromium, Iron, Copper, Arsenic, Uranium, and Cadmium are the heaviest metals represented the major content of industrial wastewater (Jaishankar et al. 2014, Rajabi, Rezaie and Ghaedi 2015). However, the industrial effluents that contain high levels (exceed the regulation level) of these metals must be treated by any of the recent removal methods before discharging it to the aquatic systems.

1.2 Sources of Heavy Metals

Qatar is a peninsula located at 24-26°N latitude and 50°30'-51°31'E longitude on the midwestern coastline of the Arabian Gulf (see Figure 1). The long shoreline that lies in the north-south direction is considered to be an active area in which it has the most fishing activities and it contains several industries (Kureishy 1991). The coastal region of Qatar, especially in Doha Bay area, has witnessed fast development of different industries. Thus, the presence of heavy metals in the coastal environment is very expected and it can be combined within sediments together with clay, sulfides, oxides, and organic matters (Al-Naimi et al. 2015). The major industries that are located on the coast of Qatar are metals, paper, steel, textile, oilfield and refinery, soft drinks, petrochemical, fertilizers processes along with energy plants. However, each of these industries disposes of high levels of different metal ions to the environment, in which these metals can be aggregated and combined with other materials that lead to polluting the coastal water bodies. Therefore, the metal contaminated industrial wastewater is needed to be treated before discharging it into the environment, based on the limitations and regulations that are listed by the Ministry of Environment in Qatar.

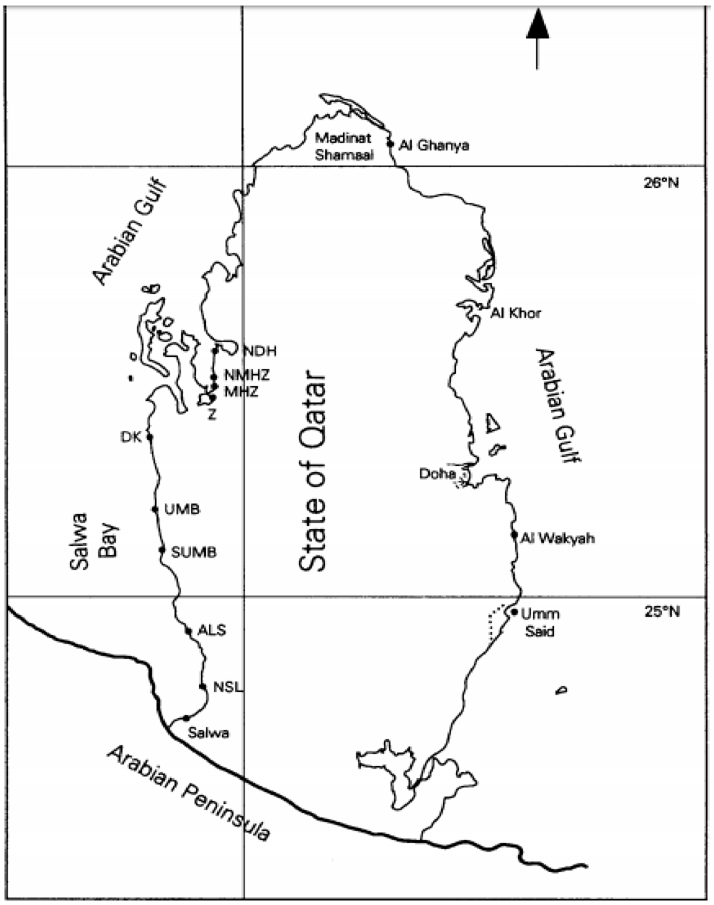

Figure 1: Map of Qatari Peninsula

1.3 Problem Statement

During the last years, many different treatment processes have been used for heavy metal ions removal from industrial wastewater. This includes chemical precipitation, ion exchange, and membrane filtration. However, these treatment processes have some limitations such as the production of a huge quantity of heavy metals rich sludge, high operation cost, and some processes inapplicable on large scales (Metcalf et al. 2003). On the other hand, adsorption as a removal process is widely used because of its low cost and it is applicable to large scale applications. Furthermore, the adsorption process by using low-cost adsorbents (i.e. fungi, yeast, bacteria, and algae) provides many advantages over the conventional treatment methods to heavy metals removal from industrial wastewater. For instance, it has a high removal efficiency with cost-effectiveness. Moreover, the use of bioprocesses technologies minimizes the production of chemical/biological sludge which makes this process environmentally friendly (Abbas et al. 2014). Algal cultures are considered to be the most suitable adsorbent which is more cost-effective and require fewer limitations such as high pressures and temperatures. Furthermore, it is widely available all over the Gulf of Qatar. Hence, there is growing attention in testing the ability of different local algae species for heavy metals removal from industrial wastewater. Therefore, finding suitable operating conditions is very important to address the concerns of metal-contaminated wastewater.

1.4 Thesis Objectives

In order to achieve and fulfill the problem statement, the following objectives will be conducted to remove Nickel, Copper, and Aluminum from industrial wastewater using non-living and living algae cells.

- Test the performance of non-living and living local algae species for removal of Copper, Nickel, and Aluminum from industrial wastewater samples.
- Optimize the adsorption parameters by changing the local algae biomass dosage, pH value of the sample, initial metal ions concentration, and contact time for Copper, Nickel, and Aluminum removal by non-living *Spirulina sp.* biomass from industrial wastewater samples.
- Find the maximum capacity of adsorption and the kinetics using different adsorption isotherms such as Langmuir and Freundlich isotherm models, and kinetics models which are pseudo-first-order, pseud-second-order, Elovich's, and intraparticle models.
- Perform preliminary screening for 12 different living local algae strains and select the best 5 based on their growth in 10 mg/L of Aluminum ions.
- Test the performance of best 5 living local algae species for removal of Aluminum ions from industrial wastewater samples at different initial Aluminum concentrations 5, 10, and 15 mg/L.
- Study the effect of pH value of the sample for Aluminum removal by best 5 living algae species from industrial wastewater samples at different initial Aluminum concentrations 5, 10, and 15 mg/L.

CHAPTER 2: LITERATURE REVIEW

The chapter discusses the literature review of different studies that are published by other researches. It consists of general information about heavy metals such as the negative effects, the sources of each metal, and the acceptance level of these metals in drinking water and industrial water. The chapter summarizes the advantages of using a biological treatment process and low-cost adsorbents in terms of time, efficiency, and cost. Furthermore, the several factors that affect the algal culture during heavy metals removal are mentioned in this chapter.

2.1 Heavy Metals

Generally, Heavy metals have a high density that is relatively bigger compared to the water density by five times and it is poisonous or toxic at low concentrations. However, the heavy metals are considered highly toxic because they cannot be destroyed or degraded and easy to accumulate in the living organisms. Of these metals, Lead (Pb), Mercury (Hg), Cadmium (Cd), Copper (Cu), Chromium (Cr), Nickel (Ni), and Aluminum (Al) are the heaviest metals found in wastewater. These metals can be found naturally in the earth in which a small quantity of some heavy metals is necessary to maintain the metabolism in the human bodies such as zinc and copper (Chowdhury and Chandra 1986). However, recently, they become concentrated in aqueous streams, soil, and subsequently into the food chain due to the different human's activities. However, because of their high level of toxicity, it is very important to treat the industrial wastewater and costumer wastes before discharge it to the environment (Luch 2012).

2.1.1 Copper

Copper is the most type of heavy metals that are available in the aquatic environment, in which it is widely used metal in the industrial processes. Copper is an essential material for human bodies and living organisms at certain levels but it can be toxic if it exceeds these levels (Yu et al. 2000). For example, reactive free oxygen species can be generated if copper was presented in the blood system, as well as, it may damage the lipids, protein, and DNA (Brewer 2010). Moreover, regarding the aquatic environment, excess levels of copper can damage the marine ecosystem and affect the life of fishes by damaging the liver, gills, kidneys, and the nervous system. However, the toxicity of copper depends on other parameters such as pH, alkalinity, hardness and organic compounds that are presented in the water (Vaishya and Prasad 1991, Hodges 1977). The main industrial sources of copper-contaminated wastewater are metal finishing, electroplating, petroleum, and fertilizer industries (Shell 1981). The United State Environmental Protection Agency (USEPA) reported the permissible limit of copper ions in industrial wastewater which is 1.3 mg/L in which it was stated by World Health Organization (WHO) that the copper concentration should not exceed 2 mg/L in drinking water (El-Ashtoukhy, Amin and Abdelwahab 2008).

2.1.2 Nickel

Nickel is widely used in different industrial activities, such as electroplating, mineral processing, batteries manufacturing, mining, metal finishing, production of paints and batteries. Nickel ions exist in high concentration in industrial wastewater which can cause several bad effects in humans, such as lungs and kidneys damages, nausea, vomiting, diarrhea, and pulmonary fibrosis (Aksu and Dönmez 2006, Moore and

Ramamoorthy 1984). According to the World Health Organization (WHO) guidelines, the maximum admissible concentration of nickel ions in drinking water is only 0.07 mg/L (Edition 2011).

2.1.3 Aluminum

Aluminum is considered as a reactive metal which usually bends to other elements or components. The dissolve of aluminum in water is affected by the different conditions of the water. The main factor that determines the solubility of aluminum in water is pH values. Hence, aluminum is known to be highly dissolved in water at basic and acidic conditions. Otherwise, the solubility of this metal will be very low in water at the natural conditions (Al-Muhtaseb, El-Naas and Abdallah 2008). The adverse effects of aluminum are more chronic than acute which means that these effects will accrue over the long term and then it will be noticeable in the human bodies. Therefore, once aluminum metal enters the bloodstream, it will act as a neurotoxic component. Bone mineralization disorders and encephalopathy are the main adverse effects that can be as a result of long term exposure of human to aluminum-contaminated wastewater (Golub 2005). Aluminum contamination exists in the environment from different industrial sources such as metal plating, metal smelters, and mining operations (Goher et al. 2015). The maximum allowable aluminum concentration extent in drinking water is set to be 0.2 mg/L as reported by the U.S. Environmental Protection Agency (USEPA) (2016).

2.2 Heavy Metals Removal Methods

During the last decades, different methods for heavy metals removal from industrial wastewater have been studied. These removal methods can be classified into physical, chemical, and biological removal method which include chemical precipitation, cementation, membrane filtration, and adsorption. Physico-chemical methods are the most suitable treatment methods can be used for heavy metals removal from industrial wastewater for many reasons which are described below.

2.2.1 Chemical Precipitation

The chemical precipitation process is considered as one of the conventional processes and the most widely used to remove heavy metals from industrial wastewater. The concept of the chemical precipitation process is to change the heavy metal ions in wastewater into solid particles which can be described with the following equation (Wang et al. 2005):

$$M^{2+} + 2(OH)^{-} \leftrightarrow M(OH)_2 \downarrow$$

In which the dissolved heavy metal ion, the precipitant, and the insoluble metal hydroxide are represented by M^{2+}, OH^{-}, and $M(OH)_2$, respectively. The precipitant agent plays the main role in this process. However, limestone and lime are considered to be the most common precipitant agents used because they are widely available with low-cost in most of the countries (Mirbagheri and Hosseini 2005, Aziz, Adlan and Ariffin 2008). The removal of heavy metals by using lime precipitant is an effective treatment of inorganic effluent since it can remove up to 1000 mg/L. of heavy metals concentrations. In addition, the lime precipitation process has other advantages such as the simplicity of the process, safe operation conditions, low-cost equipment needed. On the other hand, the chemical precipitation method needs a huge amount of chemicals that are used to reduce the heavy metals to an acceptable level which leads to produce a large amount of sludge that needs to be treated before discharge.

2.2.2 Membrane Filtration

During the last years, the membrane filtration method has received good attention for an inorganic effluent treatment (i.e. heavy metals). Ultrafiltration, nanofiltration, and reverse osmosis are considered to be the most types of membrane that can be used to remove heavy metals from industrial wastewater. These types are classified based on the size of the particles that will be removed (Vigneswaran et al. 2005). However, the using of membrane filtration method has many advantages such as no phase change, energy saving, cost effective, high efficiency in separation, easy to scale up, and environmentally safe (Déon et al. 2013, Escobar and Van der Bruggen 2011, Zhu et al. 2014). Membrane fouling, integrity failure, and faulty installation and/or maintenance are the main disadvantages of using membrane filtration process for heavy metals removal from industrial wastewater (Nakatsuka, Nakate and Miyano 1996, Childress et al. 2005).

2.2.3 Adsorption

The “adsorption” word describes the mass transfer process that occurs at any two phases, such as gas-liquid, liquid-solid, liquid-liquid, and gas-solid interfaces. In the case of the liquid-solid interface, which is the interface of interest in wastewater treatment, the solute is shifted from the liquid phase to the solid phase surface, where it will be bounded with physical or/and chemical interactions (Kurniawan 2003). Depending on the type of interactions involved, adsorption can be classified to the reversible adsorption process which is physisorption (physical adsorption) and chemisorption (chemical adsorption) that is considered to irreversible adsorption process (Rouquerol et al. 2013). Compared with the other removal methods, adsorption process is the most common method used and considered the most suitable process to remove the heavy metal ions from the industrial wastewater for many reasons. For instance, the simplicity of its design and the cost-effectiveness (Yadanaparthi, Graybill and von Wandruszka 2009, Kwon et al. 2010, Ramakrishna and Susmita 2012). Many different low-cost adsorbents have been developed to utilize the process in heavy metal ions removal from industrial wastewater (Maleki et al. 2016). The different low-cost adsorbents can be derived from the natural material, biological wastes, and modified biopolymers. (Barakat 2011).

Generally, the advantages and disadvantages of these treatment methods are summarized in Table 1 (Dahiya et al. 2008, Al-Saydeh et al. 2017).

Table 1: Advantages and Disadvantages of Different Treatment Method

Removal method	Advantages	Disadvantages	References
Chemical precipitation	• Cost-effective • Simple design • Non-metal selective	• Limited to a certain concentration of metal • Resulting sludges • Slow metal precipitation	(Chen et al. 2009, Kousi et al. , Ku and Jung 2001)
Membrane Filtration	• Small space requirement • Low operating pressure • Quality of effluent for recycling the water • High selective separation	• High pressure and membrane scaling • High operation cost • Low flowrate	(Petrov and Nenov 2004, Chen and Chen 2003, Gautama et al. 2014)
Electro-chemical methods	• High selective separation • Metal recovery • Eco-friendly in nature	• High operation cost • Very expensive at higher concentration of metal • High energy consumption • pH-sensitive	(Oztekin and Altin 2016, Kurniawan et al. 2006)
Photo-catalysis	• Removal of organic and metals contaminates together. • Produced less toxic by-products	• The duration time is long • It has limited applications	(Wang et al. 2017, Gautama et al. 2014)

2.3 Biological Treatment Process

2.3.1 Definition and properties

The biological treatment method is considered to be the eco-friendly solution to treat the industrial wastewater from heavy metals compared to the physic-chemical methods. However, the chemical method is the only method to remove some of the toxic inorganic compounds that cannot be removed by any of biological or physical methods (Gunatilake 2015). The biological treatment process is a description of the non-directed physicochemical interaction which happen between heavy metals and microbial cells in an aqueous solution (Shumate SE and S tranberg 1985). In other words, this process is based on the ability of biological species (living and non-living species) to adsorb the heavy metals ions that are dissolved in industrial wastewater onto the cell surface. In contrast, the bioaccumulation is an active process in which heavy metals removal requires the metabolic activity of a living organism. However, the biosorption process is based on the use of non-living biomass but the bioaccumulation process can be defined as the uptake of heavy metal ions by using the living microorganisms (i.e. algae, bacteria, and fungi). In general, the biological process involves two phases which are the solid phase (sorbent i.e. biological material) and the liquid phase (solvent i.e. industrial wastewater) that is containing the dissolved species (sorbate i.e. metal ions) to be adsorbed (Alluri et al. 2007, Kotrba 2011).

2.3.2 Advantages and disadvantages

The biological treatment methods provide several advantages over the conventional treatment methods for heavy metals removal from industrial wastewater. For instance, it can be carried out in situ at the sources of metal-contaminated wastewater. Further, it has a high removal efficiency with cost-effectiveness. Moreover, the using of bioprocesses technologies minimizes the production of chemical/biological sludge which makes this process Environmentally friendly. Hence, this removal method has proven to be more economical, versatile, effective, and environmentally friendly when compared with other conventional methods (Abbas et al. 2014). However, the use of living organisms for heavy metals removal and recovery has some certain disadvantages, such as the difficulty in controlling and maintaining the growth of microorganism due to the normality of wastewater that contains a high concentration of toxic metals and widely fluctuation of the pH conditions. A further limitation of using this process is that the recovery of heavy metal ions is not applicable while maintaining the ions viability, and the reason is that the adjustment of pH value or the addition of some specific complexing agents which can be toxic, is necessary to remove bound metals from the biomass. Due to these limitations, huge interests have been focused on the use of non-living biomass as adsorbents. (Cheremisinoff 2001, Kotrba 2011)

2.3.3 Mechanisms of heavy metals removal by biological treatment process

The mechanisms that are involved in the removal of heavy metal by biological treatment can be classified based on the cell metabolism. The first mechanism is biosorption which is considered to be a metabolism non-dependent process and the second mechanism that is metabolism dependent is found to be as a bioaccumulation process. The word "biosorption" describes the physio-chemical properties of non-living or inactive biomass that give them the ability to bend and remove the nondegradable pollutants (i.e. heavy metals) from aqueous solutions. Biosorption is considered to be a fast and low-cost treatment process with high efficiency of the heavy metals removal using the microorganism's biomass as well as this technique provides the possibility of metals recovery thus allowing the environmentally acceptable disposal of the heavy metals (Rao et al. 2010). Comparing with the other conventional methods, biosorption process has several advantages such as its low operation cost, high efficiency in removing heavy metals at low concentrations, and no nutrient requirements (Sheng, Ting and Chen 2007). The biosorption removal process is very rapid and it can occur immediately when the cell contacts with the heavy metal ions (Aksu 1998). The second metabolism, bioaccumulation, is slower process compared to the biosorption process, it is related to some types of metabolic activity and the metal uptake by this mechanism requires energy before it can be transported inside the cell. This slow phase of metal uptake can be due to a number of processes which include covalent bonding, redox reactions, surface precipitation, crystallization on the surface of the cell, and the diffusion into the cell interior and the metal ions will be bonded to proteins and other intracellular components (Aksu 1998) The relative importance of the two stages may vary with algal species and metal ions. Many research studies have proved that the non-living or inactive biomass can be more effective in removing the heavy metal ions from wastewater compared to the living or active cells. Furthermore, the inactive biomass can be easier to use in heavy metals removal for many reasons, include no requirement for food or any essential elements for biological growth, and it can be available as a waste or by-product material. During the last decades, many efforts have been done into identifying the available living and non-living biomass that have the effective properties to remove heavy metals from wastewater. These sorbents typically include algae species (Figueira et al. 2000, Davis, Volesky and Mucci 2003).

2.3.4 Algae as sorbent

During the last two decades, many different biological adsorbents have been extensively studied such as fungi, bacteria, algae, and yeast. Among all the studied microorganisms, algae gained huge attention, because the marine algae are considered to be as a rich source in the aquatic environment (i.e. sea and oceans), relatively cheap material, and they have the ability to adsorb and accumulate high concentration of heavy metals (Wilde and Benemann 1993). In general, "Algae" word is used as a classification of a large group of organisms that are referred to as aquatic plants which are mostly photosynthetic and oxygenic autotrophs (Hoek, Mann and Jahns 1995). Algae species can be used in industrial wastewater treatment processes in which it has mainly been to extract and remove heavy metal ions from wastewaters. There are many features that make the algae an effective adsorbent to extract the heavy metal ions from contaminated wastewater. One of these features is the ability to bind metal ions on the algae surface. to this point, several research works have shown that the algal cell wall contains a Phosphate, Carboxyl, and other functional groups that will create a negative net charge on the cell wall (Rai and Gaur 2012). Many studies proved that both non-living and living algae have the ability to remove heavy metal ions from contaminated wastewaters in which both are able to absorb metals available in their surroundings. However, Living micro-algae cells are considered to be the most efficient for wastewater treatment processes due to its ability to remove more metal ions using two mechanisms together which are biosorption and bioaccumulation as well as it has the ability to retain the extracted metals for a longer period of time (Hu 2006).

2.3.5 Batch Experiment

Testing the ability of living and non-living algae to remove heavy metal ions via the adsorption process can be achieved by using several modes such as continuous and batch modes of operation. The batch mode in laboratory scale is more preferable compared to the other modes in order to analyze how the adsorbate and adsorbent perform under various operating conditions such as initial concentration of heavy metals, pH levels, the dosage of adsorbent material, contact time, and temperature.

2.3.6 Factors influencing batch biosorption

2.3.6.1 Effect of pH

Many studies showed that the affinity of the cations (heavy metals ions) to join anions which are the functional groups that present in the surface of algal species is a function of pH of the solution. At low values of pH, the biosorption capacity becomes lower and increases when the pH value increased until it reached the optimum pH. However, at pH higher than the optimum value, the metal ions begin to precipitate due to the formation of M(OH) (Joo, Hassan and Oh 2010). Therefore, in order to maximize heavy metals removal by algal species, huge efforts have been done to find the optimum pH. For instance, (Yu and Kaewsarn 1999) studied the effect of pH value on copper ions removal by *Durvillaea potatorum*. They found that a very little of copper ions have been adsorbed at pH below 2, but it was increasing when pH increased. Therefore, the maximum copper ions uptake was found at pH between 3 and 4. Many different researchers proved that the increase in heavy metals uptake can be reached by increasing the pH value. For example, (Zhou, Huang and Lin 1998) found that the removal of copper and cadmium ions by *Sargassum kjellmanianum* and *Laminaria japonica* can be done at pH between 4 and 5, but the optimum was found to be 6.7.

2.3.6.2 Effect of temperature

Another important factor that can affect the heavy metal ions removal is temperature of the algal system. Heavy metal ions removal can be enhanced by increasing the temperature which leads to increase the activity of the surface and the kinetic energy of the heavy metal ions, but sometimes it may damage physical structure of biosorbent (Park, Yun and Park 2010). The biosorption process at a temperature within a range 20-35 ^{o}C may remain inefficient in which increase the temperature of the system to reach 50 ^{o}C will increase the metals uptake in some cases (Ahalya, Ramachandra and Kanamadi 2003, Goyal, Jain and Banerjee 2003). On the other hand, White *et al.* showed that the maximum biosorption capacity for nickel and palladium ions by using *S. cerevisiae* was reached at 25 ^{o}C and it decreases when the temperature was increased up to 40 ◦C (White, Sayer and Gadd 1997).

2.3.6.3 Effect of the initial pollutant concentration

As mentioned in the previous studies, the increase in the initial pollutant concentration leads to an increase in the quantity of adsorbed heavy metal ions per unit weight of adsorbent, but it decreases the removal efficiency (Park et al. 2010). The initial concentration is a very important factor in the algal systems in order to provide a driving force that helps in overcome all mass transfer resistances of heavy metal ions between the solid and liquid phases (Zouboulis, Matis and Hancock 1997). It was proved that the maximum removal percentage of heavy metal ions can be reached at low initial metal concentration. Hence, the heavy metal ions uptake increases when the initial concentration increases, at a specific concentration of biomass (Abbas et al. 2014).

2.3.6.4 Effect of biosorbents dosage

The biosorption process is strongly dependent on the dosage of a biosorbent. Some research studies proved that when the biosorbent dosages decrease, the metal uptake will increase which may occur due to the complex interaction of different factors. However, in general, increasing the concentration of biomass leads to increase the amount of biosorbed metal ions. The reason is that the number of bending sites on the surface of the biosorbent will increase. (Ahmady-Asbchin, Safari and Tabaraki 2015).

2.3.7 Factors influencing batch bioaccumulation

2.3.7.1 Effect of light

The light intensity and its availability are a very important factor that can affect the failure or success of algae cultures, in which the light can be the main source of energy for the phototrophic algae. If the light intensity is very low, the net growth of algal biomass will be zero and this point called compensation point as mentioned by (Lee 1997, Wang, Liu and Liu 2015, Sorokin and Krauss 1958). (Richmond 2000, Goldman 1979) studied the effect of increasing the light intensity on the growth of algal cultures and the results showed that when the light intensity increases, the photosynthesis will increase until reaching the saturation point, in which the growth rate will be the maximum rate. However, increasing the light intensity to exceed the saturation point will not increase the growth rate but it can lead to the photo-oxidation, which will decrease the rate of photosynthesis by damaging the light receptors of the algae (Richmond 2004, Richmond, Cheng-Wu and Zarmi 2003).

2.3.7.2 Effect of temperature

The temperature parameter is considered to be the second most important limiting factor that is affecting the cultures of algae. Tillett *et al.* (Tillett 1988, Sheehan et al. 1998, Pulz 2001) mentioned that the algal productivity will increase with increasing the operating temperature up to an optimum temperature, in which the algal respiration will be increased. The optimum temperature is often within a range of 28-35˚C for most of the algae as reported by (Soeder et al. 1985). However, many different algae species can easily acclimate at temperatures up to 15°C that is lower than their optimum temperature, but if the operating temperature exceeded the optimum temperature by only 2°C to 4°C, major biomass will be damaged as mentioned by (Moheimani and Borowitzka 2007, Nirbhay and Dolly 2011).

2.3.7.3 Effect of pH

The accumulation process of heavy metals is highly dependent on pH. Typically, the cell wall of the algae is considered to be anion which means that it possesses a negative charge, however, the metal ions carries a net positive charge. Thus, in acidic solution, the metal ions will compete with hydrogen ions in the binding on the algal wall. Wherefore, higher pH (maximum 8, otherwise the metals will precipitate) is preferable in the algal cultures for heavy metals removal (Fraile et al. 2005, Romera et al. 2007). (Kong et al. 2010) mentioned that the optimal pH of many algae species is around 7.5 and it can be adjusted by adding 0.2 M NaOH to the algal systems. Furthermore, (Weissman, Goebel and Benemann 1988) proved that the productivity of the algal culture decreases when the pH value exceeds 8. They studied the two species of algae which are *Chlorella sp.* and *Chaetoceros sp.* in which the productivity of selected species was reduced by 22% when the value of pH increased from 8 to 9. However, the proved that the ability of some algae species to grow under pH value that exceeds 8. For instance, *Ankistrodesmus sp.* and *Amphora sp.* couldn't be inhibited at pH 10 and 9, respectively.

2.3.7.4 Effect of CO_2 & O_2 availability

The availability of atmospheric CO_2 plays an important role in algae growth, in which the carbon is a main component in the algae and it is occupied around 45–50% of algal biomass (Doucha, Straka and Lívanský 2005). If the concentration of O_2 exceeded the saturation level in algal cultures, the chlorophyll reaction will be damaged which is inhibiting photosynthesis, thus reducing productivity (Ugwu, Aoyagi and Uchiyama 2007). Microalgae can grow within a large range of CO_2 concentration. For example, many species reach the maximum growth rates when the CO_2 concertation <10% (Maeda et al. 1995, Hirata et al. 1996, Nakano et al. , Hanagata et al. 1992), in which *Cyanidium caldanum* can grow in 100% of CO_2 (Seckbach, Gross and Nathan 1971, Graham and Wilcox 2000).

2.4 Modeling

2.4.1 Adsorption Isotherms

The adsorption process is a description of the removal of an adsorbate from a solution and collecting it at adsorbent surface until the amount of the adsorbate on the surface becomes in equilibrium with that in the solution. This process is usually studied via graphs that are known by adsorption isotherm. Therefore, the maximum adsorption capacity can be obtained from the same graphs. In order to represent the various types of adsorption isotherms, many empirical and theoretical models have been studied and developed. However, Langmuir and Freundlich are considered to be the most common models used to describe solid-liquid adsorption systems in wastewater treatment applications (Sheela et al. 2012, Kumar and Chawla 2014, Karabacakoğlu et al. 2008).

2.4.1.1 Langmuir adsorption isotherm

The Langmuir adsorption isotherm describes the equilibrium conditions for the adsorption process in different systems. Therefore, it is applicable to the homogeneous sorption in which the sorbate molecule has been uniformly banded on the surface of the adsorbent. In other words, this type of sorption has equal activation energy for each sorbate molecule. The linearized form of Langmuir adsorption isotherm is described by the following equation (Kumar and Chawla 2014, Karabacakoğlu et al. 2008):

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m} \qquad Equ. (1)$$

Where,

q_m (mg/g) and K_L (L/mg) are constant.

q_e: the equilibrium adsorbed solute amount per unit weight of adsorbent (mg/g of solid).

C_e: the equilibrium solute concentration in the solution (mg/L).

2.4.1.2 Freundlich adsorption isotherm

The Freundlich adsorption isotherm is the most important adsorption model to describe the heterogenous sorption process for a single solute system. The model has found broad acceptance because due to its accuracy and widely applicable. The linearized form of Freundlich adsorption isotherm is described by the following equation (Karabacakoğlu et al. 2008, Kumar and Chawla 2014):

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \qquad Equ.(2)$$

Where,

K_F (mg/g)(L/mg): Freundlich constants of the adsorbent related to adsorption capacity.

1/n: the empirical parameter that is related to the adsorption intensity.

q_e: the equilibrium adsorbed solute amount per unit weight of adsorbent (mg/g of solid).

C_e: the equilibrium solute concentration in the solution (mg/L).

2.4.2 Adsorption Kinetics

Adsorption kinetics describes the pathways of the adsorption reactions and the time needed to reach the equilibrium, in which the chemical equilibrium doesn't give any information about pathways and rates of the reaction (Kumar et al. 2010). The adsorption kinetics can be described by the different diffusions that occur through the adsorbent cell that can either be pore or film diffusion and sometimes a combination of both of them which depends on the system hydrodynamics (El-Naas, Al-Zuhair and Alhaija 2010b). In order to study the controlling mechanism of the adsorption processes and to determine the adsorption time needed to achieve equilibrium status, different kinetics models will be applied at different experimental conditions (Ocampo-Perez et al. 2011). These kinetics models include pseudo-first-order, pseudo-second-order, Elovich's, and intraparticle diffusion models which will be analyzed to model the kinetics of copper, aluminum, and nickel adsorption onto different algal species (El-Naas, Al-Zuhair and Alhaija 2010a). These models are described below.

2.4.2.1 Pseudo-first-order model

The pseudo-first-order model is considered to be the most commonly used to describe the adsorption reaction (Qi et al. 2017). The pseudo-first-order rate expression can be represented by the differential law (Bensacia et al. 2014):

$$\frac{dq_t}{dt} = k(q_e - q_t) \qquad Equ. (3)$$

Where,

K (1/min): the rate constant of pseudo-first-order.

q_e (mg/g): the adsorbed amount at equilibrium.

q (mg/g): the adsorbed amount at time t (min).

The linearized form of this model can be achieved by integrating for the boundary conditions t = 0 to t = t and q_t = 0 to q_t = q_t, the linear expression is given below:

$$\ln(q_e - q) = \ln(q_e) - \frac{kt}{2.303} \qquad Equ.(4)$$

2.4.2.2 Pseudo-second-order model

The pseudo-second-order kinetic model assumes that the rate of occupation of surface sites is directly proportional to the square of the unoccupied sites number (Qi et al. 2017). In this model, the surface adsorption is considered to be the rate-limiting step which involves chemisorption, in which the solute (solid phase) will be removed from the solution (liquid phase) by physicochemical interactions between the two phases (Ho 2003). The pseudo-second-order chemisorption equation can be expressed as (Ho and McKay 1999):

$$\frac{d_{qt}}{d_t} = K_2(q_e - q_t)^2 \qquad Equ.(5)$$

The linearized form of this model is represented as:

$$\frac{t}{q} = \frac{1}{K_2 q_t^2} + \frac{1}{q_e}t \qquad Equ.(6)$$

Where,

K_2 (g/mg/min): the rate constant of pseudo-second-order.

2.4.2.3 Elovich's model

In order to describe the adsorption of pollutants from aqueous solutions, Elovich's model has been successfully used during the last years. The kinetic model of Elovich's can be given by the following equation (Hariz and Monser 2014):

$$\frac{d_{qt}}{d_t} = a\exp(-bq_t) \qquad Equ.(7)$$

The linearized form of this model is represented by the following equation:

$$q = \frac{1}{b}\ln(ab) + \frac{1}{b}\ln(t) \qquad Equ.(8)$$

Where,

a (mg/g/min): the initial adsorption rate (mg/g/min)

b (g/mg): a parameter related to the extent of surface coverage.

2.4.2.4 Intraparticle diffusion model

The intraparticle diffusion model has been used to describe the adsorption processes in which the adsorption rate depends on the speed of the solute at which it diffuses towards the adsorbent, this model is presented by the following equation (Krishna 1993):

$$q = K_d\sqrt{t} + \boldsymbol{\theta} \qquad Equ.(9)$$

Where,

k_d (mg/g/min$^{1/2}$): the rate constant of the intraparticle transport

θ (mg/g): a constant related to the boundary layer thickness, in which bigger values of θ indicate that the boundary layer effect is high.

CHAPTER 3: MATERIALS AND METHODS

This chapter mentions the methodology that is followed to achieve the specific objectives of the research. This chapter contains the procedures, methods, assumptions, apparatus, equipment, and other information that was used to conduct the experiment.

3.1 Materials

3.1.1 Chemicals

For the preparation of the industrial wastewater samples, the heavy metal ions (Cu^{2+}, Ni^{2+}, Al^{3+}) were added artificially in the laboratory. the standard solutions 1000 ppm of Copper, Aluminum, and Nickel were purchased from Fisher Scientific and used as purchased without any further treatment. All other samples were prepared from these standards (see Figure 2). Secondary wastewater was collected from Doha West Sewage Treatment Plant and took from secondary clarifier which comes after the biological treatment. Before starting the experiment, each wastewater sample was analyzed and found that it has only three metal ions dissolved with the specific concentrations.

During the experiment period, approximately 10 mL of growth medium was added to each sample of living algal species. This was done in order to maintain the growth of the species in each flask. The growth medium consists of: $NaHCO_3$, EDTA, $FeSO_4.7H_2O$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, KCL, Na_2SO_4, K_2HPO_4, $NaNO_3$ (see Figure 3).

Figure 2: Standard solutions 1000 ppm of Copper, Aluminum, and Nickel.

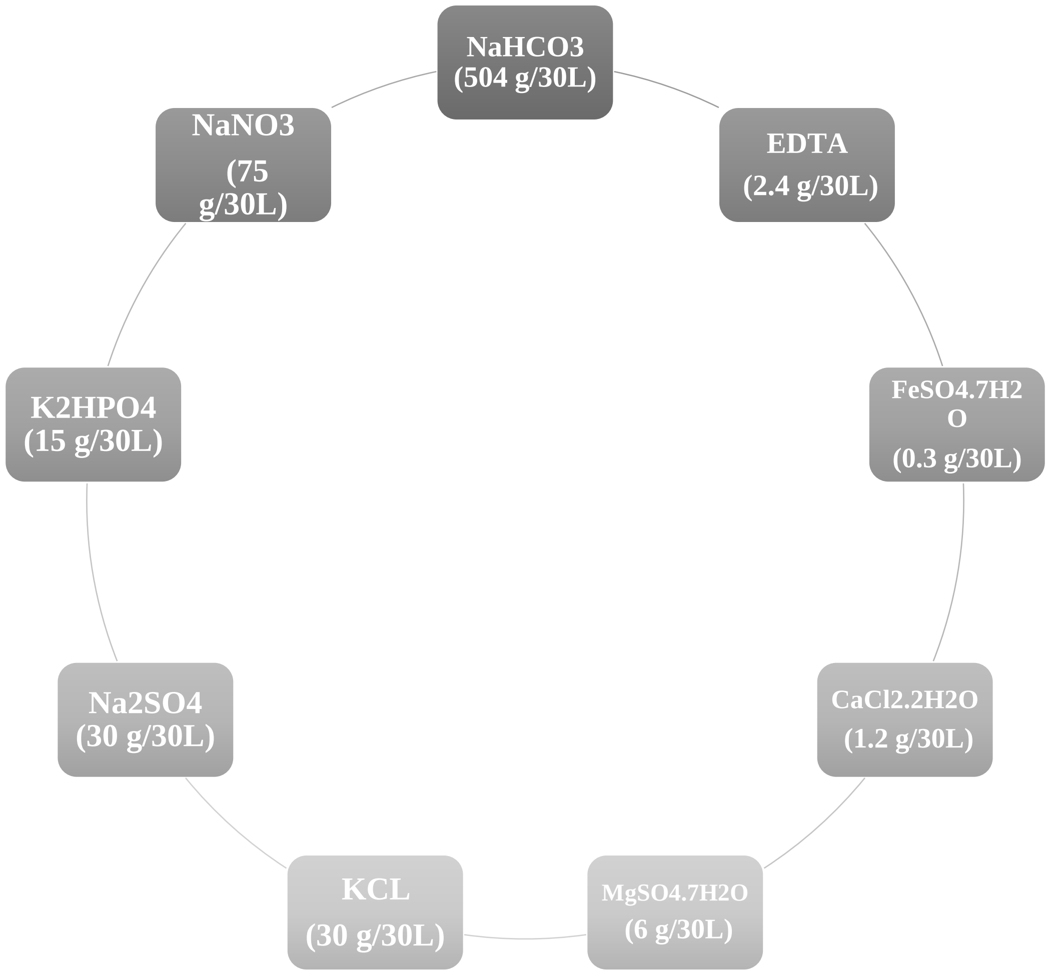

Figure 3: The content of the growth medium.

3.1.2 Adsorbent

3.1.2.1 Microalgae strains

The living algae strains used in this project were supplied by the Center for Sustainable Development at Qatar University at Qatar University. However, the non-living algal strain (*Spirulina sp.*) was supplied from the pilot plant that is in the chemical engineering department in Qatar University. The algae species that were used during this research are listed below (more details about algae strain are mentioned in the appendix):

1. *Monoraphidium sp.*
2. *Mychonastes sp.*
3. *Chlorella sp.*
4. *Oorococcun sp.*
5. *Neochloris sp.*
6. *Chlorophyta sp.*
7. *Desmodesmus sp.*
8. *Scenedesmus sp.*
9. *Dictyosphaerium sp.*
10. *Protosiphon sp.*
11. *Chlorococcum sp.*
12. *Chlamydocapsa sp.*

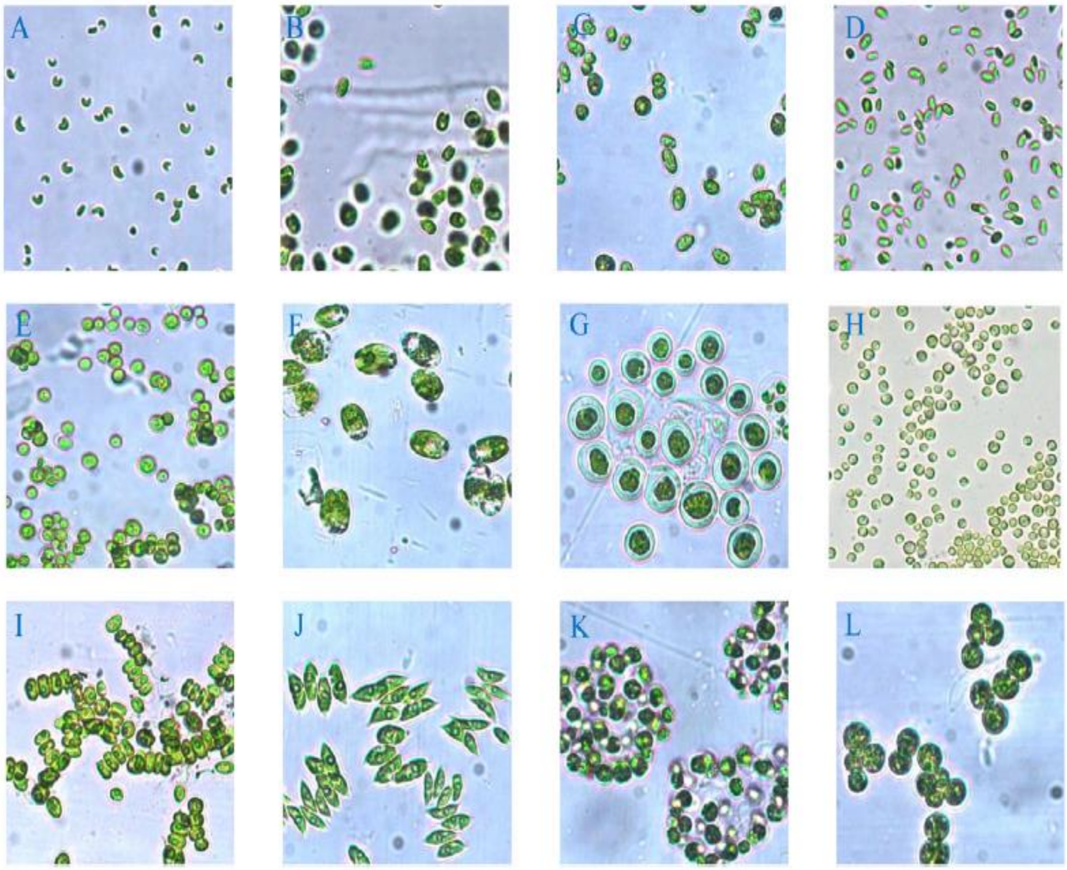

Figure 4: Microscopic algae strains via light microscopy, 40× magnification (A: *Monoraphidium*, B: *Chlamydomonas*, C: *Mychonastes*, D: *Oorococcun*, E: *Chlorophyta*, F: *Chlorella*, G: *Chlorococcum*, H: *Neochloris*, I: *Desmodesmus*, J: *Scenedesmus*, K: *Dictyosphaerium*, and L: *Protosiphon*) (Saadaoui et al. 2016).

3.1.3 Algae photo-bio-reactor

The photo-bio-reactors can be classified into open or close system that enables phototrophic microorganisms (i.e. algae) to be cultivated outside their environment. In this experiment, a closed photo-bio-reactor system has been used in which it provides a good environment for microalgae where all the parameters can be easily controlled and effectively measured. This photo-bio-reactor was prepared by adding 12/12 light/dark, 400 uM m^{-1} s^{-1}, and air bubbling by pumping CO_2 stream, as shown in Fig. 5.

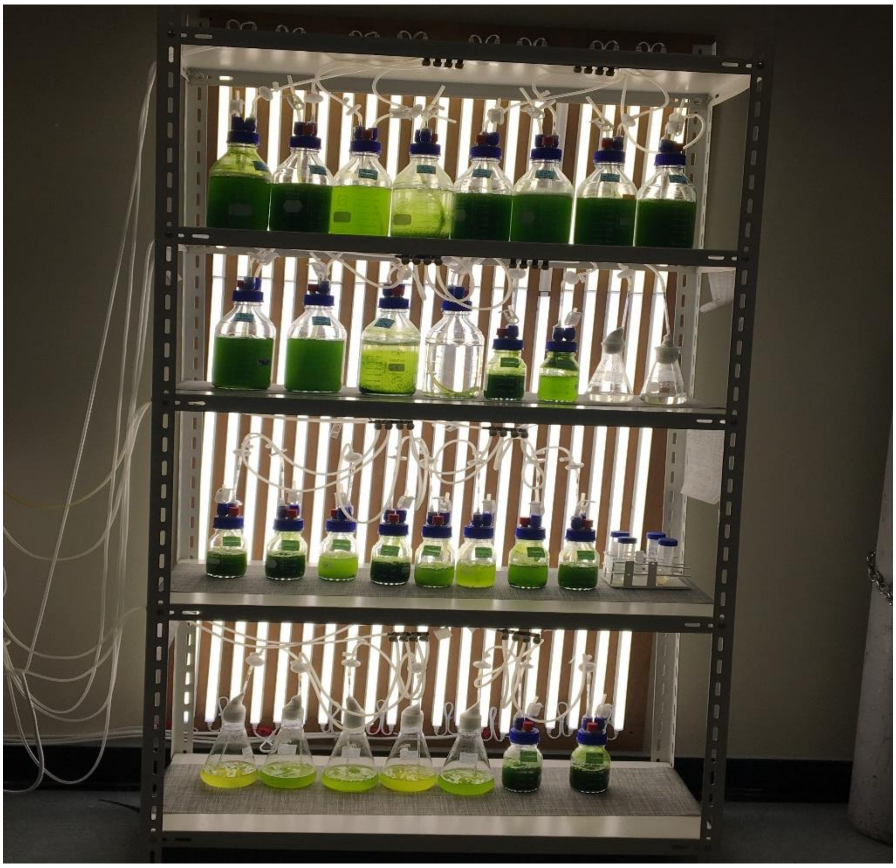

Figure 5: Algae photo-bio-reactor

3.1.4 Equipment

3.1.4.1 Inductively Coupled Plasma (ICP-ES)

The Inductively Coupled Plasma/Optical Emission Spectrometry (ICP/OES) is considered to be the most popular and powerful analytical tool to determine a trace of metals and some nonmetals in a wide variety of samples such as solids, powders, suspensions, and liquids. The ICP/OES device consists of two parts which are the inductively coupled plasma and the optical emission spectrometry. The main components of this equipment are sample introduction system (nebulizer), ICP torch, transfer optic, high-frequency generator, and computer interface. The sample solution will be introduced into a nebulizer in which the sample's portion will turn into droplets and the remaining will be sent to the drain. These droplets will go to the inductively coupled plasma (ICP) which sustains a temperature between 70000 to 10000 K, so the droplets of the sample will be vaporized quickly. The device which produces the IC plasma is commonly referred to as the ICP torch which is consisting of three concentric tubes made of quartz or some other suitable material. It consists of three Argon flows depending on the manufacturer:

1- Nebulizer gas flow and sample Aerosol (inner Argon flow).
2- Auxiliary gas, lifts the plasma above the injector tube, used when measuring organics.
3- Plasma gas which sets the plasma conditions, such as excitation temperature.

3.1.4.2 Conductivity and pH meter

All the samples were tested by the conductivity and pH meter (Orion™ Versa Star Pro™ Conductivity and pH Benchtop Meter, ThermoFisher Scientific, USA). The meter can operate at ambient temperature within a range between 5 °C and 45 °C. however, the measuring range of conductivity meter is between 0.00 μS/cm to 3000 mS/cm with accuracy of 0.5% of reading ± 0.01 μS. Also. the accuracy of pH reading is ± 0.002 or ± 0.2 mV.

3.1.4.3 UV-spectrophotometer

UV-Vis spectrophotometry DR 5000™ is used to determine the nutrients concentration and it provides a large range of water analysis tests with more than 240 pre-programmed tests. This method offers an automatic method detection with "Test N Tube ™ "regents that may save time and reduces the potential errors. It operates at wavelength within a range between 190 to 1110 nm with an uncertainty of ±1. Therefore, it capable to work at ambient temperature which is between 10 °C and 40° C.

3.2 Experimental Procedures

3.2.1 Preparation of non-living algal biomass and biosorption experiment

The algae specie that is used in biosorption experiment is *Spirulina sp*. The *Spirulina sp.* was living in the growth medium for one month to get the proper amount of biomass. A sample has been taken from the living *Spirulina sp.* and the centrifuge has been used at 5000 rpm for 15-20 minutes to separate the algae biomass from the wastewater samples. Then, in order to remove the extra materials and common ions (i.e. Ca^{2+} and Na^{+}) that present in the solution, the algae biomass was washed with deionized water. After that, the washed biomass was dried at 50°C for 24 h before it is used and stored in a dry cabinet and crushed. The industrial wastewater samples were prepared by using the standard of heavy metal ions ion as shown in *Figure 6*, the stock solution was prepared for 5, 10, and 15 ppm of copper, Nickle, and Aluminum. A known quantity of biomass powder was in contact with a known concentration of the stock solution in which a three different biomass dosage were tested which are 0.5, 1.5, and 2 g (dry weight)/L. Then, the solution (adsorbent and adsorbate) was put in 100 mL flasks and agitated at 150 rpm in Laboratory orbital shaker (IKA , KS 501). The adsorbate was separated from the adsorbent by using the centrifugation (Hermle Z 206 A) at 5000 rpm for 30 min. Then it was filtrated using 0.45 μm membrane filters. Finally, the supernatant liquid was analyzed for metal ions by using ICP (see Figure 7).

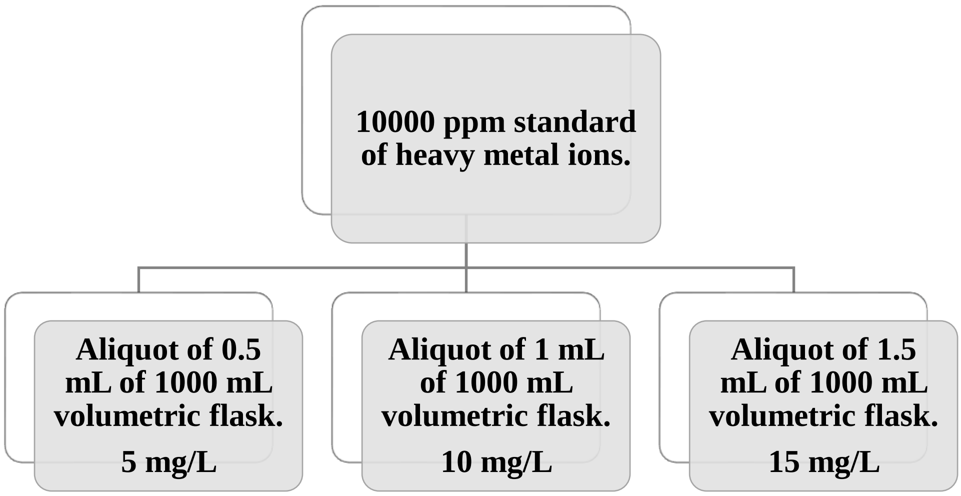

Figure 6: Preparation of stock solutions

Figure 7: Steps of non-living *Spirulina sp.* biomass experiment for Copper, Aluminum, Nickel removal.

3.2.2 Biosorption studies

Many Batch experiments were conducted to optimize the effect of different parameters such as the initial concentration of metal, contact time, biomass dosage, and pH on the removal of heavy metal ions by biomass. Each parameter was studied independently, by fixing the other parameters to be constant. However, the adsorption capacity and the percentage of removal were calculated by following equations (C. R. Holford 1997):

$$Q = \frac{(C_i - C_F) \times V}{W_g} \qquad Equ. (10)$$

$$\%Removal = \frac{(C_i - C_F)}{C_i} \times 100 \qquad Equ. (11)$$

Where C_i (mg/L) is the initial metal concentration in the wastewater samples, C_f (mg/L) is the final metal concentration in the wastewater samples, V (L) is the volume of wastewater for each sample. W_g (g) is the mass of algae species. All the batch tests were conducted twice, and the average values are reported in this report.

3.2.2.1 Effect of biomass dose

The impact of biomass dosage on the removal of heavy metal ions was studied by using different biomass concentrations; 0.5, 1.5, and 2 g/L at the initial metal concentration of 10 mg/L.

3.2.2.2 Effect of contact time

The impact of contact time on the removal of heavy metal ions was observed at different periods of times; 5, 10, 20, 30, 40, 60, 90, and 120 min at the initial metal concentration of 10 mg/L.

3.2.2.3 Effect of pH

In order to determine the effect of pH value on the removal of heavy metal ions by algae biomass, the experiment was conducted at different pH values; 5.5, 7, and 8.5 at the initial metal concentration of 10 mg/L. The adjusted pH samples were prepared by adding 1 N HCl or 1 N NaOH before mixing the algae biomass. Therefore, pH values were measured by using pH meter.

3.2.2.4 Effect of the initial metal concentration

The effect of the initial metal concentration on the removal of heavy metal ions by algae biomass was studied at different initial concentrations; 5, 10, and 15 mg/L. These samples were prepared by using the standard solutions 1000 ppm of Copper, Aluminum, and Nickel.

3.2.3 Preparation of living algal biomass and bioaccumulation experiment

For the screening part, the microalgae species were initially cultivated on solid nutrient-rich medium in sterile Petri dishes under low light for one week. 12 microalgae strains were tested by 10 mg/L of Al^{3+}-rich wastewater samples and the growth of these species was observed for 7 days by different stages. The first stage was the cultivation of microalgae isolates using Al^{3+} -rich wastewater in the scale of 10 mL using illuminated shaker under 12/12 light/dark, 100 uM m^{-1} s^{-1} and agitation of 150 rpm (initial OD @750nm = 0.1). In the second stage, the samples were scaled up to 100 mL under 12/12 light/dark, 400 uM m^{-1} s^{-1} and add air bubbling by adding CO_2 stream (initial OD @750nm = 0.1). The following equation will be used to increase the volume from 10 mL to 100 mL at the specific OD value (LeVan, Carta and Yon 1997).

$$OD_i V_i = OD_f V_f \qquad Equ. (12)$$

OD_i: The initial OD that is used for the lower scale.

V_i (mL): The amount of sample that will be taken from the lower scale (unknown).

OD_f: The initial OD that is used for the higher scale.

V_f (mL): The final volume of higher scale (unknown).

By comparing the optical density values by the end of day 7 under scale 100 mL, top 5 of algae species were selected to conduct the rest of the experiment. After the selection of 5 algae species, the samples scaled up to 200 mL (initial OD @750nm = 0.2) which was the third stage. In the last stage, the 5 algae species scaled up to 500 mL with an initial OD @750nm = 0.2 to check the ability of these species to grow under a large scale with initial Al^{3+} concentration of 10 mg/L. Finally, the top 5 algae strains were cultured separately in 700 mL bottles with Al^{3+}-rich industrial wastewater samples with different concentrations of Al^{3+} where the different kinetics (i.e. pH, conductivity, and dissolved oxygen) were recorded and compared.

3.3 Heavy Metal Analysis

After adsorption, the algae species were filtered from the solution that contain the remaining amount of heavy metal ions through 0.45 µm membrane filters. Finally, the final concentrations of metal were detected by using ICP-OES OES (Thermo Scientific

- iCAP 6500 - ICP-OES CID Spectrometer. The samples were analyzed in twice and the relative standard deviation was lower than 4%.

CHAPTER 4: RESULTS AND DISCUSSION

This chapter explains the results which are obtained during the experiments in both lab and pilot scales. The chapter contains the discussion of the obtained results and presents the optimal operating conditions which are essential to reach the most efficient adsorption process. The experimental data of the adsorption were analyzed by Langmuir and Freundlich isotherm models. In addition, the experimental data was fitted with different adsorption kinetics such as pseudo-first-order, pseud-second-order, Elovich's, and intraparticle models. Error bars represent an experimental error of 5% of data obtained from two repeated experiment for each run.

4.1 Non-living Algae Experiment

4.1.1 The effect of contact time on heavy metals removal

Results presented in *Figure 8* indicate that the removal percentage of copper, nickel, and aluminum by using *Spirulina sp.* biomass increased when the contact time increase up to 90 min and it remains stable until 120 min at the maximum removal% was recorded. It was noticed that the contact time has a direct effect on the removal of heavy metal ions by non-living algae species from industrial wastewater samples. It was found that the maximum removal% values of Cu^{2+}, Ni^{2+}, and Al^{3+} were almost 41%, 25%, and 45%, respectively within the first 90 min of contact time. In addition, it was clear to see that the level of removal% of Cu^{2+}, Ni^{2+}, and Al^{3+} remained in a range of 41-43%, 25-26%, and 45-47%, respectively during the period between 90 to 120 min, which indicate the saturation point for heavy metal removal by *Spirulina sp.*. However, it becomes very important to find the optimum contact time in order to achieve the maximal biosorption of heavy metals by algal biomass. As shown in *Figure 8*, the ability of *Spirulina sp.* biomass to remove the ions of heavy metal (Cu^{2+}, Ni^{2+}, Al^{3+}) from wastewater in short periods of contact time has been proved. Many different researches proved that the biosorption process becomes slower in the later stage compared to the initial stage due to the large number of unmanned sites on the surface were available to remove the heavy metal traces compared to the unmanned sites in the later stages (Prathima, Rao and Mahalakshmi 2017, Klimmek et al. 2001, Zinicovscaia et al. 2016).

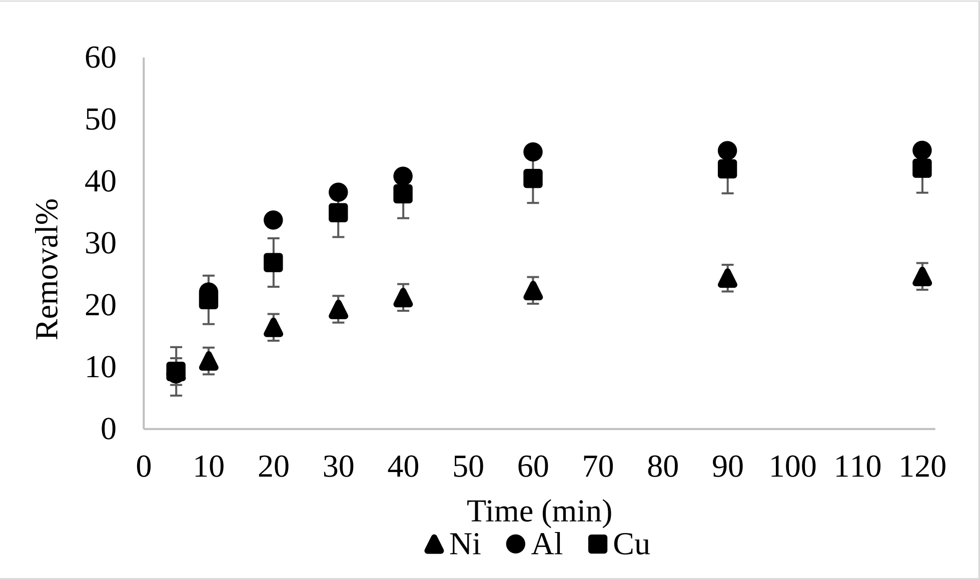

Figure 8: Effect of contact time on the removal% of Cu^{2+}, Ni^{2+}, Al^{3+} by non-living *Spirulina sp.* biomass (1 g/L, 26 °C, pH 7.0, and C_o= 10 mg/L).

4.1.2 The effect of algae dosage on heavy metals removal

The effect of *Spirulina sp.* cell biomass on heavy metals biosorption process is represented in *Figure 9*. Results show that when the algal biomass dosage increases within a range of 0.5-2 g/L, the percentage removal of Cu^{2+}, Ni^{2+}, and Al^{3+} decreased. The biomass concentration of 0.5 g/L has shown maximum percentage removal of 38.14% (7.63 mg/g), 57.38% (11.48 mg/g), and 93.46% (18.69 mg/g) of Cu^{2+}, Ni^{2+}, and Al^{3+} from the wastewater sample, thus 0.5 g/L considered to be the optimum biomass dosage under current conditions. Decreasing the metal percentage removal while the algal biomass dosage increases can be explained by the formation of the aggregates of biomass species at higher algal doses that leads to decrease the surface area of the biomass during the biosorption process. Thus, the adsorption sites will remain unsaturated during the biosorption process and that is because of the reducing in adsorptive capacity utilization thus decrease the efficiency of the process (Karthikeyan, Balasubramanian and Iyer 2007, Fourest and Roux 1992). (Markou et al. 2015) proved that the removal of Ni^{2+} and Cu^{2+} decreases which increase the biomass dosage within a range of 0.1-1.0 g/L. Therefore, the optimum biomass dosage was reported to be 0.1 g/L at an initial metal concentration of 100 mg/L with metal uptake of 90 and 80 mg/g for Cu^{2+} and Ni^{2+}, respectively.

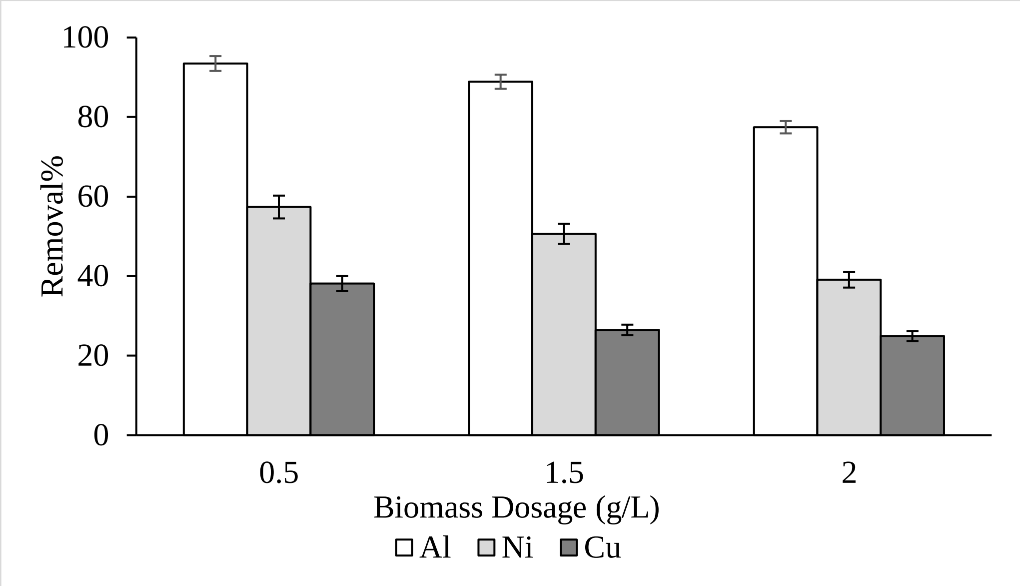

Figure 9: Effect of biomass dosage on the removal% of Cu^{2+}, Ni^{2+}, Al^{3+} by non-living *Spirulina sp.* biomass (240 min contact time, 26 °C, pH 7.0, and C_o= 10 mg/L).

4.1.3 The effect of pH on heavy metals removal

The value of pH is considered to be one of the most important factors that affect the biosorption process of heavy metals ions since it is directly related to the dissociation degree of the functional groups that are located on sorbent's surface. As shown in *Figure 10,* the biosorption of Cu^{2+}, Ni^{2+}, and Al^{3+}, by *Spirulina sp.* was studied as a function of pH. Results showed that under these conditions (1 g/L, 240 min contact time, 26 °C, and 10 mg/L of the initial concentration of heavy metal), heavy metals uptake slightly decreases with an increase in pH from 5.5 to 8.5. Although, the optimal pH value was found to be 5.5 to reach the maximal metal biosorption values which were 28.14% (2.81 mg/g), 42.38% (4.24 mg/g), and 93.46% (9.35 mg/g) for Cu^{2+}, Ni^{2+}, and Al, respectively. Generally, the metal biosorption rate decreases with an increase in the alkalinity mainly at pH > 6.0-7.0. Furthermore, an increase in pH value means that the number of protons will be lower which leads to decrease in the competition between heavy metal ions and the proton (Vannela and Verma 2006, Kaewsarn 2002). (Çelekli, Yavuzatmaca and Bozkurt 2010) reported that the zero point charge pH_{zpc} of *Spirulina sp.* was found to be 8.5 in which the electrostatic repulsion between adsorbent molecular will be the minimum. Thus, in this case, the surface of the algal biomass gets positively charged in which the pH ≤ 8.5. Therefore, they mentioned that the maximum Cu^{2+} uptake was 30 mg/g at pH 5 with an initial concentration of 100 mg/L. On the other hand, this test recorded a higher removal% compared to the test that has been done by (Zinicovscaia et al. 2018). They tested the ability of *Spirulina sp.* to remove Al^{3+} ions from industrial effluents. Under pH = 6, the maximum percentage Al^{3+} removal was found to be 60%, while the maximum removal% of Al^{3+} reached in this test was 93% at pH within a range between 5.5-7. (Jiang et al. 2018) mentioned that at pH = 5, the adsorption capacity of Cu^{2+} was 35 mg/g at the initial Cu^{2+} concentration of 33 mg/g which is much higher compared to 2.81 mg/g at the same pH value during this test. They did the test at 25˚C for 24h instead of 240 minutes with biomass dosage of 0.5 g/L.

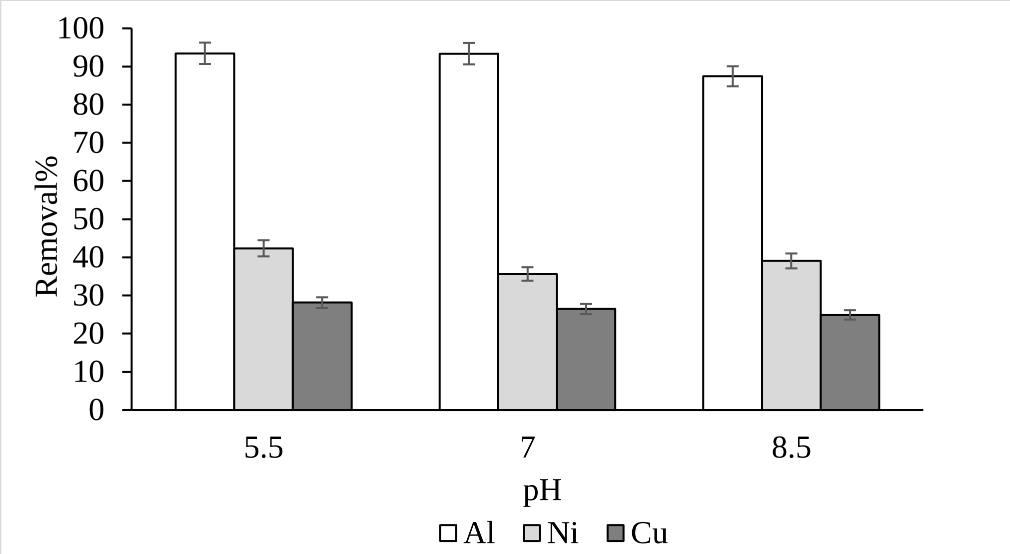

Figure 10: Effect of pH on the removal% of Cu^{2+}, Ni^{2+}, Al^{3+} by non-living *Spirulina sp.* biomass (1 g/L, 240 min contact time, 26 °C, and C_o= 10 mg/L).

4.1.4 The effect of the initial metal concentration on heavy metals removal

Results presented in *Figure 11* proved the effect of the initial metal concentration (mg/L) on the biosorption process by *Spirulina sp.* biomass. As shown in *Figure 11*, the initial metal concentration plays the main role in the biosorption process. In the present study, the increase in the initial concentration of metals (Cu^{2+}, Ni^{2+}, Al^{3+}) within a range from 5 to 15 mg/L leads to slightly decrease in the removal of all metals examined. Among this range, the removal of Cu^{2+}, Ni^{2+}, and Al^{3+} decreased from 32.18-20.83%, 47.18-37.85%, 93.60-89.8%, respectively. Therefore, this decrease in the percent removal of heavy metal indicated that all the bending sites on the surface of algal biomass became saturated because of the metal ions and establishment of equilibrium between the biosorbent and adsorbate (Kumar and Oommen 2012, Pugazhendhi et al. 2018, Kariuki, Kiptoo and Onyancha 2017). (E and P 2017) showed the relation between the initial Cu^{2+} concentration and removal% by *Spirulina sp.* biomass. It has been proved that when the initial Cu^{2+} concentration increases within a range of 50-300 mg/L, the percent of Cu^{2+} removal reduced with 20%.

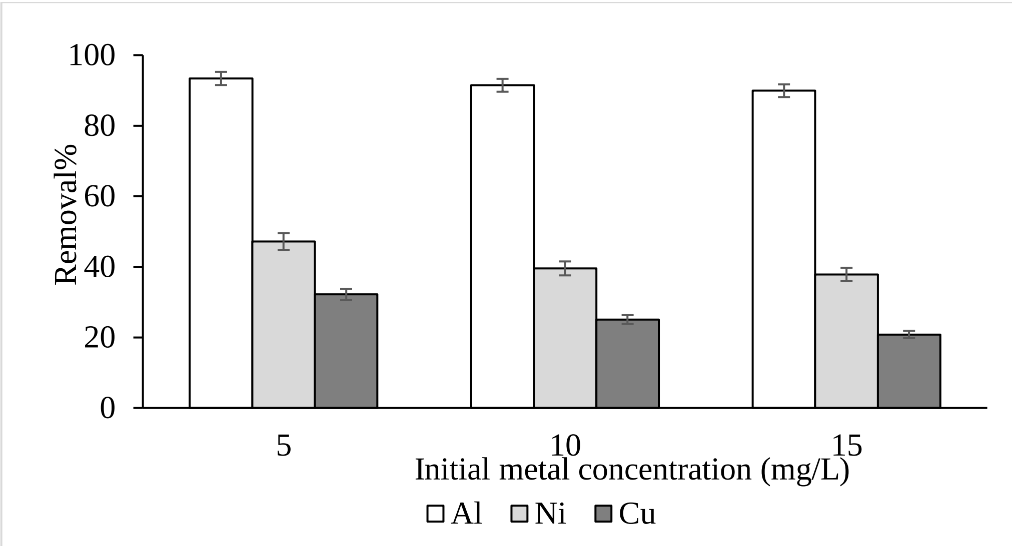

Figure 11: Effect of the initial metal concentration on the removal% of Cu^{2+}, Ni^{2+}, Al^{3+} by non-living *Spirulina sp.* biomass (1 g/L, 240 min contact time, 26 °C, and pH 7.0)

4.2 Modeling

4.2.1 Adsorption Isotherm

The experimental data of the adsorption of Cu^{2+}, Ni^{2+}, and Al^{3+} onto *Spirulina sp.* from industrial wastewater were analyzed by Langmuir and Freundlich isotherm models. Adsorption isotherms were obtained at the initial metal concentration of 5, 10, and 15 mg/L. In all cases, the metal uptake was found to increase from 2.22-7.81 mg/g, 1.42-2.27 mg/g, and 2.14-3.09 mg/g with the increase the concentration of Al^{3+}, Cu^{2+}, and

Ni^{2+}, respectively. The isotherm parameters and correlation coefficient (R^2) are given in Table 2 and the experimental data of two isotherms for Al^{3+}, Cu^{2+}, and $Ni^{2+}$are presented in *Figure. 11 and 12*. The parameters were found from the linear plot of the isotherm. The biosorption of Al^{3+}, Cu^{2+}, and Ni^{2+}on *Spirulina sp.* could be described by the Langmuir model in which the values of R^2 were higher compared to the Freundlich model, 0.9903 for Al^{3+}, 0.9853 for Cu^{2+}, and 0.9847 for Ni^{2+}which indicate that the monolayer adsorption is prevalent. Therefore, as shown in *Figure 12 and 13*, the maximum monolayer capacity values that were given by the Langmuir model, are 1.108 mg/g for Al^{3+}, 0.839 mg/g for Cu^{2+}, and 0.864 mg/g for Ni^{2+}.

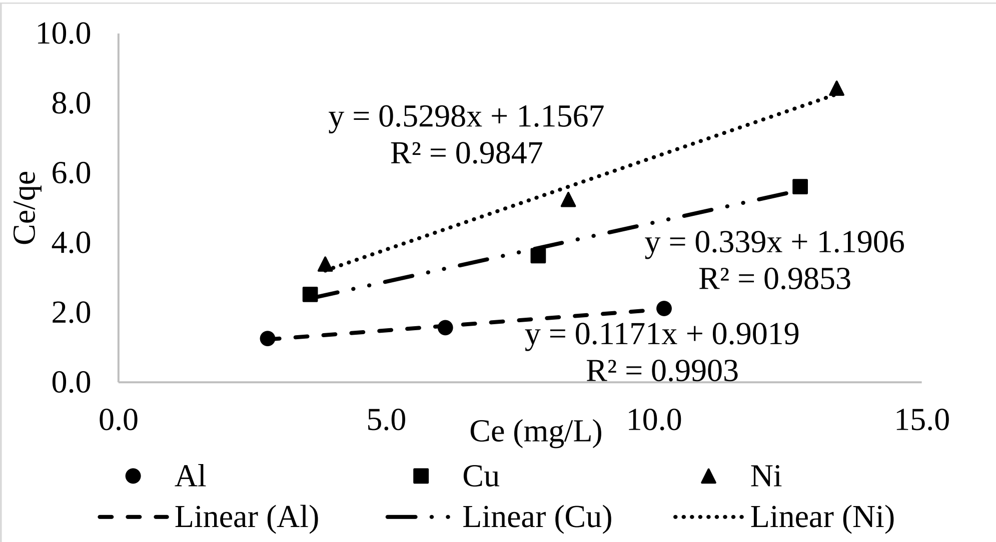

Figure 12: Langmuir equilibrium isotherm data for Al^{3+}, Cu^{2+}, Ni^{2+} at 25˚C, Biomass Dosage 1 g/L, and pH 7.0.

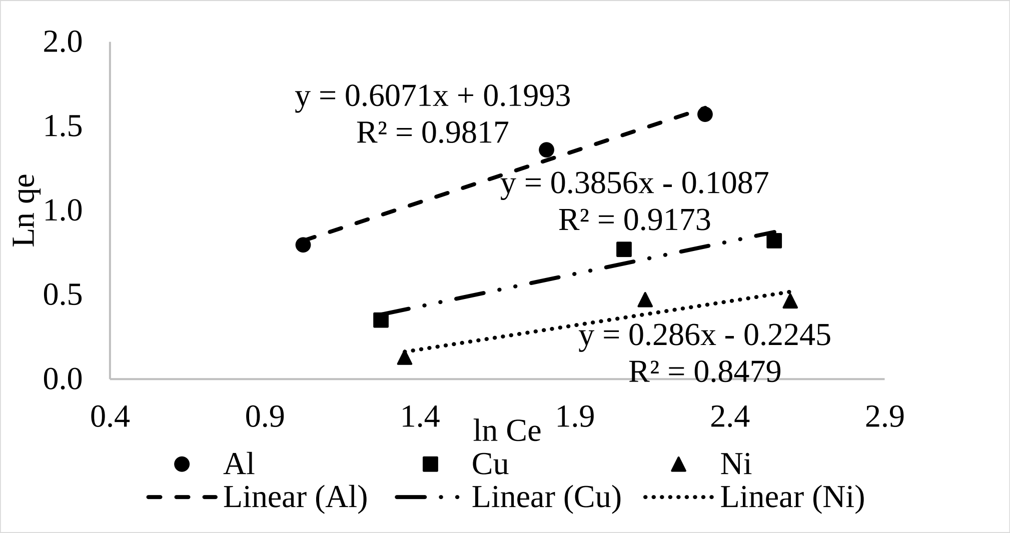

Figure 13: Freundlich equilibrium isotherm data for Al^{3+}, Cu^{2+}, Ni^{2+} at 25˚C, Biomass Dosage 1 g/L, and pH 7.0.

Table 2: Isotherm Parameters for Langmuir and Freundlich Models.

	Langmuir Model		Freundlich Model	
Aluminum	Q_m (mg/g)	1.11	K_f (mg/g) (L/mg)	1.22
	k_L (L/mg)	7.70	N	1.65
	R^2	0.99	R^2	0.98
Copper	Q_m (mg/g)	0.84	K_f (mg/g) (L/mg)	0.89
	k_L(L/mg)	3.51	N	2.59
	R^2	0.99	R^2	0.91
Nickel	Q_m (mg/g)	0.86	K_f (mg/g) (L/mg)	0.79
	k_L (L/mg)	2.18	n	3.49
	R^2	0.98	R^2	0.85

4.2.2 Adsorption Kinetics

The adsorption rate of Cu^{2+}, Ni^{2+}, and Al^{3+} from industrial wastewater solution by *Spirulina sp.* was determined with the initial metal concentration of 10 mg/L and biomass dosage of 0.001 g/L. The experimental data were fitted into four kinetic models which are pseudo-first-order, pseudo-second-order, Elovich and intraparticle diffusion. The constant parameters for all models are listed in Table 3. The correlation coefficient (R^2) was used to compare between these models and to select the best model for each metal of Cu^{2+}, Ni^{2+}, Al^{3+}. According to the linearized pseudo-first-order equation, *Figure 14* represents a plot of ln(qe-qt) as a function of time. By using the slope and intercept of this figure, the rate constant k_1 and equilibrium capacity q_e were calculated. The values of R^2 were 0.9025, 0.8664, and 0.8717 for Al^{3+}, Ni^{2+}, and Cu^{2+}, respectively. These low values indicate that the adsorption process of the three metals does not follow the pseudo-first-order model. Therefore, the magnitude of R^2 of this model was found to be the lowest values among all model that have been tested. Similarly, as shown in *Figure 15*, t/qt was plotted versus time according to the linearized pseudo-second-order equation. It is clear to indicate that this model was the best model for the removal of studied metal since the R^2 values were 0.9867, 0.9943, and 0.9943 for Al^{3+}, Ni^{2+}, and Cu^{2+}. After linearizing the Elovich equation, qt was plotted in terms of ln(t) for which the a and b parameters were identified from the slope and intercept of the plot in *Figure 16*. Finally, the intraparticle diffusion model was plotted by qt in terms of time and the constant parameters were found by the slope and intercept of the plot in *Figure 17*. However, after comparing all the values of R^2 for all model, the pseudo-second-order model was the best model to remove Cu^{2+}, Ni^{2+}, and Al^{3+} from industrial wastewater solution by *Spirulina sp.*. The results of this experiment is similar to the adsorption kinetics of Al^{3+}, Ni^{2+}, and $Cu^{2+}$by other different algae species adsorbents, such as *Spirulina platensis* (Şeker et al. 2008, Chojnacka, Chojnacki and Górecka 2005), *Sargassum filipendula* (Kleinübing et al. 2010), and *Euglena gracilis* (Winters, Gueguen and Noble 2016). However, it is considered to be much faster compared to many types of adsorbents, such as ZnS nanocrystals (Xu et al. 2016), magnetic hydroxyapatite nanorods (Nguyen Thanh et al. 2017), and nanoporous carbon (Bakhtiari et al. 2015).

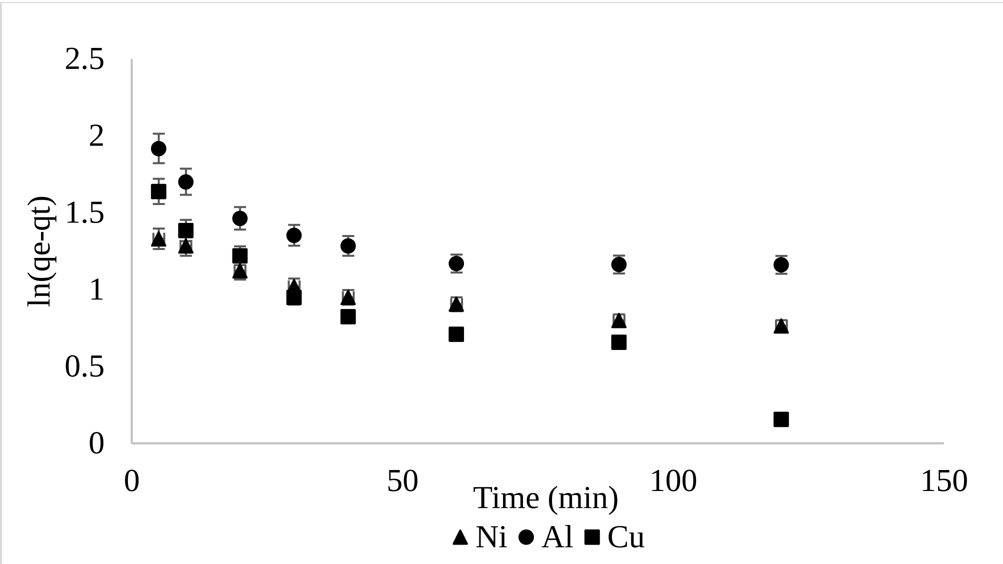

Figure 14: Pseudo-first-order kinetics of the adsorption of Cu^{2+}, Ni^{2+}, Al^{3+} (1 g/L, 26 °C, and 10 mg/L of the initial concentration of heavy metal).

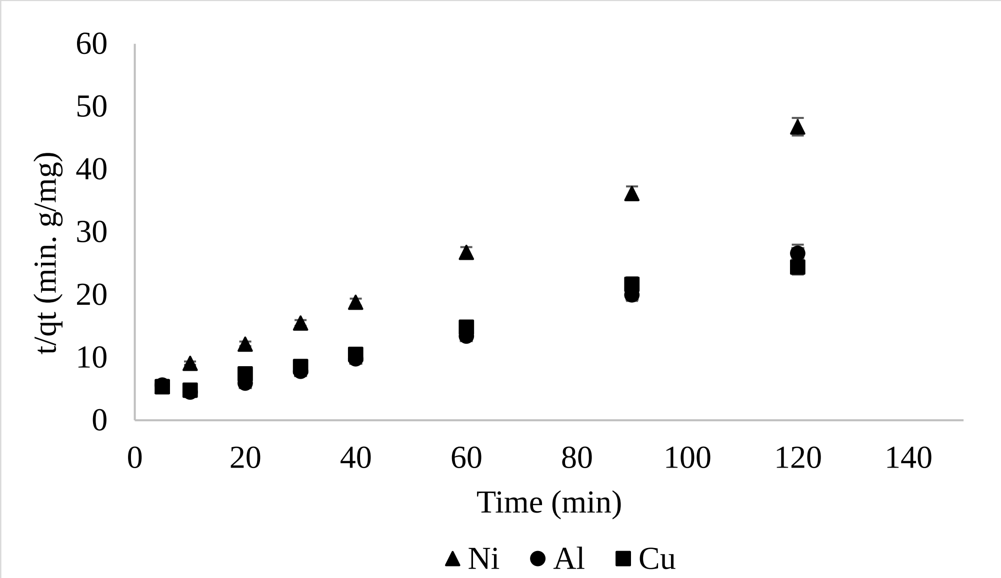

Figure 15: Pseudo-second-order kinetics of the adsorption of Cu, Ni^{2+}, Al^{3+} (1 g/L, 26 °C, and 10 mg/L of the initial concentration of heavy metal).

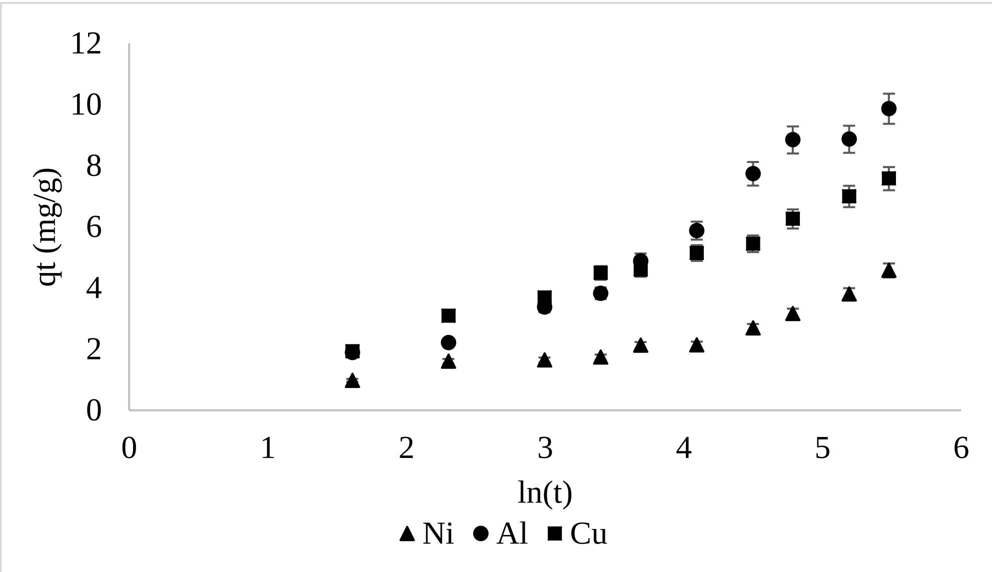

Figure 16: Elovich's equation for the adsorption of Cu^{2+}, Ni^{2+}, Al^{3+} (1 g/L, 26 °C, and 10 mg/L of the initial concentration of heavy metal

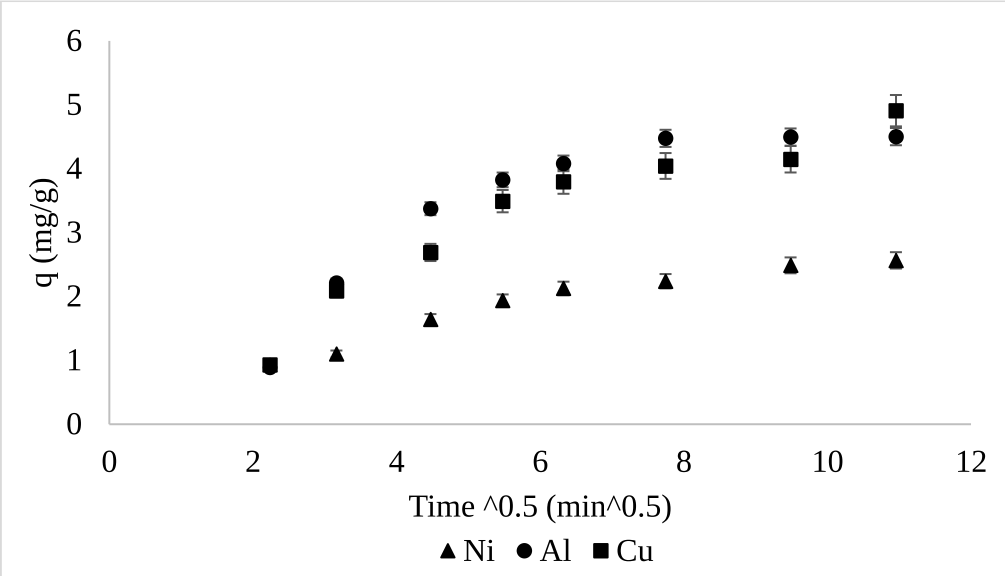

Figure 17: Intraparticle diffusion model of the adsorption of Cu^{2+}, Ni^{2+}, Al^{3+} (1 g/L, 26 °C, and 10 mg/L of the initial concentration of heavy metal).

Table 3: A Comparison Between The Different Adsorption Kinetics for The Removal of Cu^{2+}, Ni^{2+}, Al^{3+}.

Model	Parameters	Al^{3+}	Ni^{2+}	Cu^{2+}
Pseudo-first order	K_1 (min^{-1})	0.02	0.03	0.02
	q_e (mg/g)	5.73	4.13	2.49
	R^2	0.91	0.87	0.87
Pseudo-second order	K_2 (min^{-1})	0.01	0.01	0.03
	q_e (mg/g)	6.74	5.52	2.85
	R^2	0.99	0.99	0.99
Elovich model	A	0.73	0.42	1.53
	B	0.56	1.38	0.87
	R^2	0.95	0.91	0.97
Intrapa-rticle diffusion model	k_d	0.37	0.19	0.34
	θ	1.17	0.68	1.04
	R^2	0.75	0.91	0.78

4.3 Living algae experiment

4.3.1 Algae strains screening test

As shown in *Figure 18*, the highest optical density values by the end of day 7 were 0.698, 0.596,0.547, 0.493, and 0.538 *for Mychonastes, Chlorella, Chlorophyta, Desmodesmus*, and *Scenedesmus* at initial Al^{3+} concentration of 10 mg/L. Thus, the behavior of scale up to 200 mL and 500 mL that was conducted only by these microalgae species is shown in *Figure 19 a and b*. Therefore, the second part of the experiment, where different initial concentrations of Al^{3+} were tested, the top 5 algae species were used for Al^{3+} removal from industrial wastewater samples.

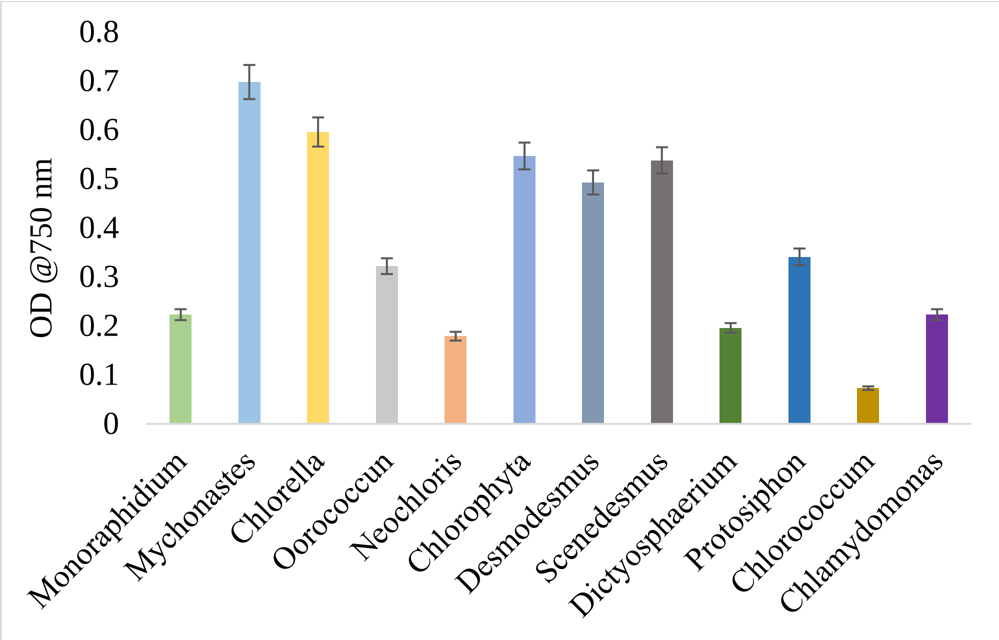

Figure 18: The values of OD for 12 microalgae strains in 100 mL (initial OD @750nm = 0.1)

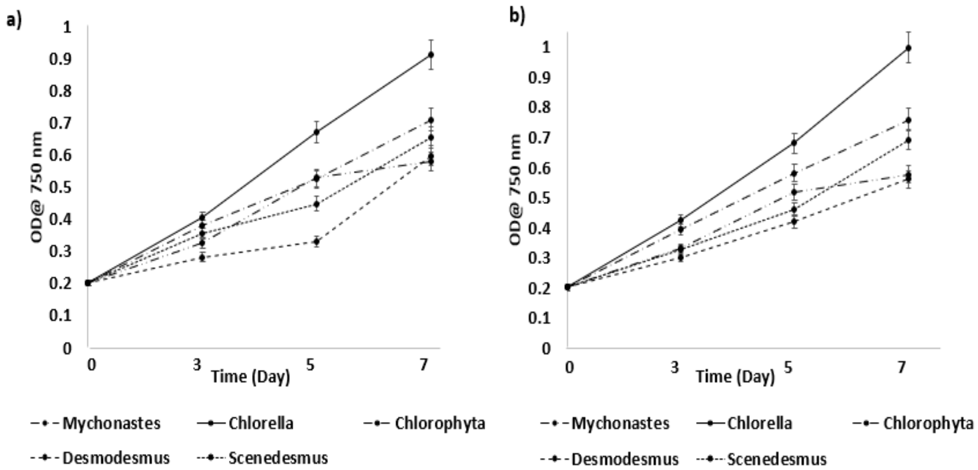

Figure 19: The values of OD for top 5 algae strains in a) 200 mL b) 500 mL (initial OD @750nm = 0.2)

4.3.2 Aluminum test

The graphical representation of the average Aluminum concentrations that present the industrial wastewater samples for each sample during different test days at an initial concentration of 5, 10, and 15 mg/L are shown in *Figure 20a, 21a, 22a*, respectively. At initial Al^{3+} concentrations of 15 and 10 mg/L, it could be seen from these graphs that the initial concentration of Al^{3+} decrease rapidly between day 0 and day 3, after that the uptake be more slower during day 5 till day 7. This rapid decrease comes as a proof of the two phases of the heavy metal uptake by algae process. According to many previous research works, there are two phases of remove metals by algae; firstly, the metal will rapidly bound to the surface of algal cell by metabolism independent mechanism, secondly, the binding will be much slower which is due to simultaneously increase in the growth and adsorption on the algal surface (Rao et al. 2010). *Figure 20b, 21b, 22b* show the final the %removal of Al^{3+} from wastewater sample by the end of Day 7 at the initial Al^{3+} concentration of 5, 10, and 15 mg/L, respectively. At an initial concentration of 5 mg/L, the highest percentage value of Al^{3+} content was recorded to be 58% with *Chlorophyta.* However, the lowest percentage of removal was found to be 11% with chlorella. On the other hand, at the initial concentration of 15 mg/L, all the algae species show a close variation in Al^{3+} content removed which is between 21% (*Scenedesmus*) and 45% (*Chlorophyta*). Although all samples of initial Al^{3+} concentration of 10 mg/L recorded above 52 percent in Al^{3+} content removed. Overall, the best and highest removal percentage of Al^{3+} from industrial wastewater was found to be at 10 mg/L of initial concertation of Al^{3+} which made this concentration to be the optimum concentration to remove Al^{3+} from industrial wastewater samples by the tested algae species. (Chojnacka, Chojnacki and Górecka 2004) studied the removal of Al^{3+}

by *Spirulina sp.* from industrial effluent. The maximum percentage of Al^{3+} removal was found to be 48.6% after 24 hours. It can be noticed that our average results of using *Spirulina sp.*to remove Al^{3+} at different initial concentrations within 2 hours is higher compared to Chojnacka *et. al.* results.

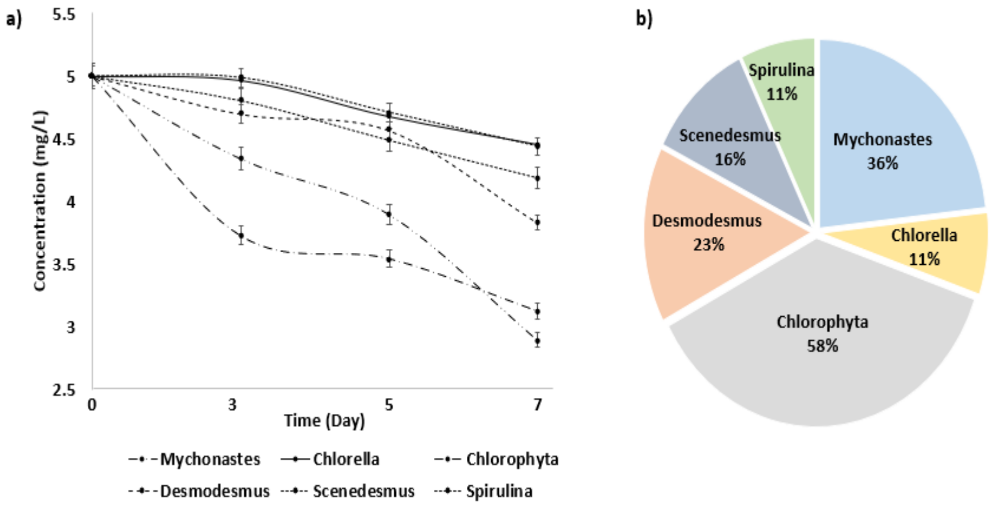

Figure 20: a) Graphical trend of Aluminum concentrations and b) final %Removal by the end of day 7 at the initial concentration of 5 mg/L

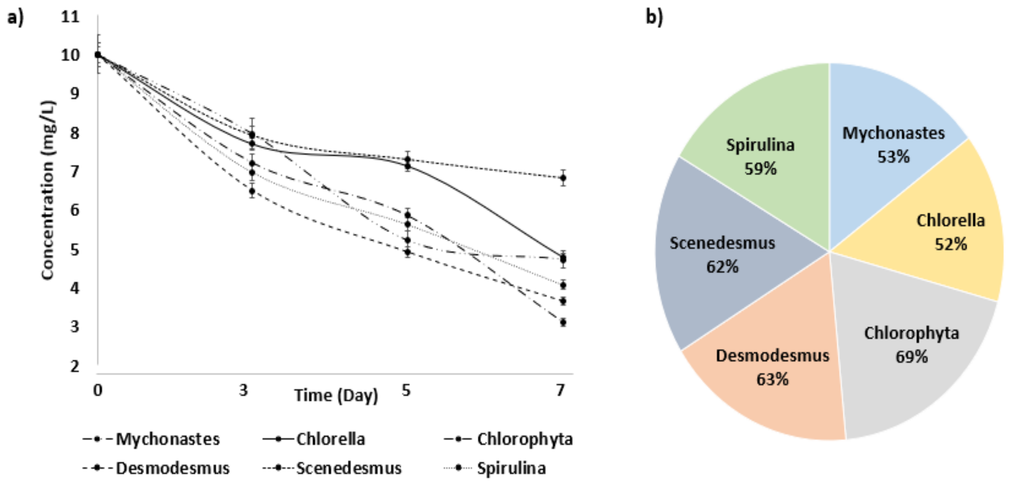

Figure 21: a) Graphical trend of Aluminum concentrations and b) final %Removal by the end of day 7 at the initial concentration of 10 mg/L.

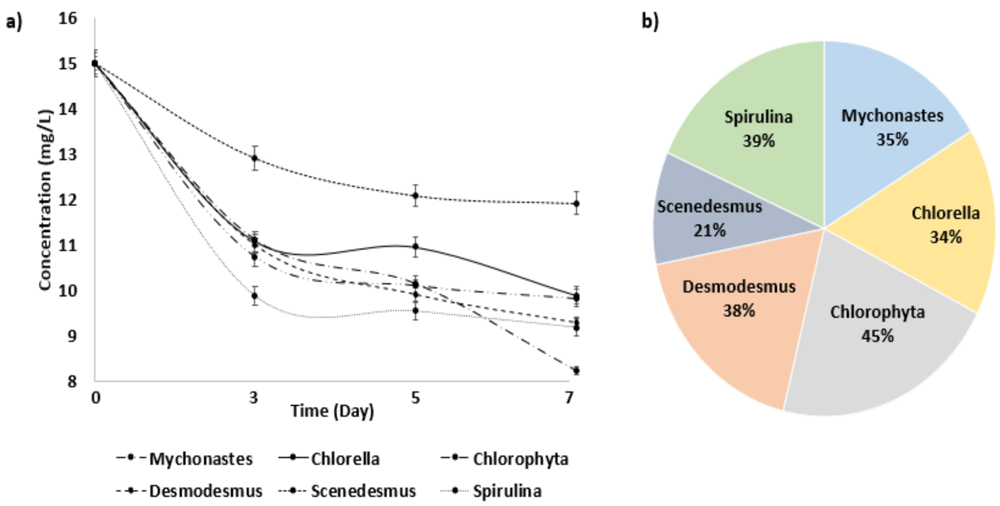

Figure 22: a) Graphical trend of Aluminum concentrations and b) final %Removal by the end of day 7 at the initial concentration of 15 mg/L

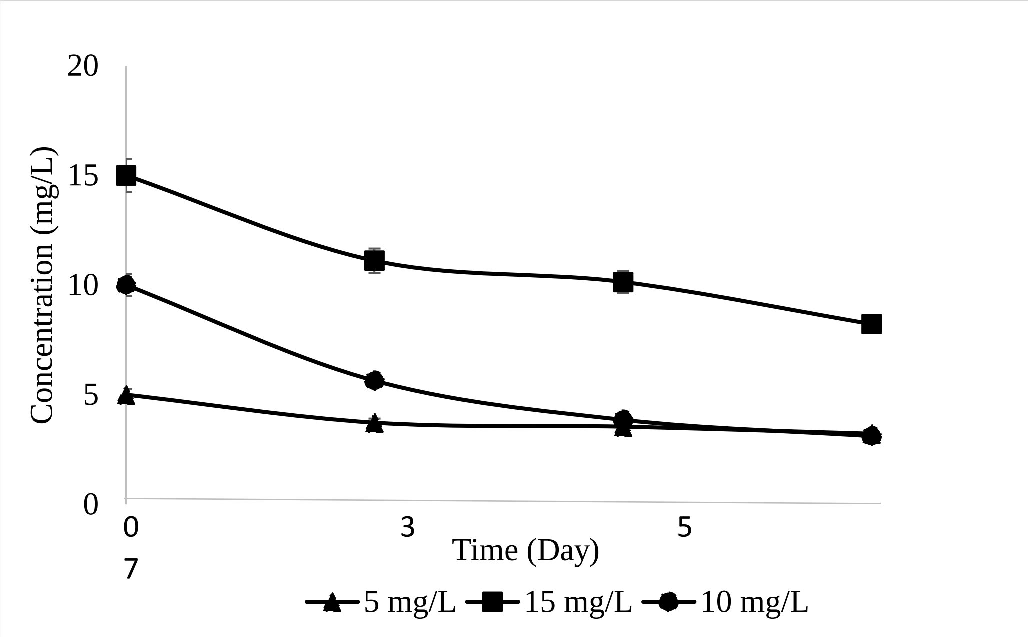

Figure 23: Graphical trend of Aluminum concentrations in industrial wastewater samples removed by Chlorophyta during the test days at the initial concentrations of 5, 10, and 15 mg/L.

4.3.3 Observation during Aluminum removal test

The growth of algae species was observed by measure the OD @750 nm to remove Al^{3+} from industrial wastewater samples at different initial concentrations 5, 10, and 15 mg/L are shown in *Figure 24 a, b, and c*, respectively. The experiment was conducted in large scale (700 mL) as for the all initial Al^{3+} concentrations it is clear to see that all the species showed a very good growth based on the measurements of optical density. It could then be concluded that once heavy metals are successfully accumulated in the algae biomass, it does not affect the biomass growth.

Figure 25 a, b, and c show the pH values of Al^{3+} samples during test days with initial concentrations of 5, 10, and 15 mg/L, respectively. As mentioned in the previous chapters, the accumulation process of heavy metals by algal species is highly dependent on pH. Typically, the cell wall of the algae is considered to be anion which means that it possesses a negative charge, however, the metal ions carries a net positive charge. Wherefore, higher pH (maximum 8, otherwise the metals will precipitate) is preferable in the algal cultures for heavy metals removal (Fraile et al. 2005, Romera et al. 2007). The pH values in removing Al^{3+} (10 mg/L) were between 7.072 and 7.850 which is the best range to remove heavy metals by algal species and this explain the high percentage removal of Al^{3+} as shown in *Figure 25*. However, the low percentage removal of Al^{3+} with an initial concentration of 15 mg/L can be explained by the values of pH in which in most of the samples the pH value was less than 7. This means it was an acidic solution where the metal ions will compete with hydrogen ions in the binding on the algal wall that leads to reducing the removal of Al^{3+} from industrial wastewater samples. On the other hand, the solution of Al^{3+} with an initial concentration of 5 mg/L is considered to be a basic solution based on the pH values in all samples which were above 8 that explain the very low percentage removal under this initial concentration of Al^{3+}.

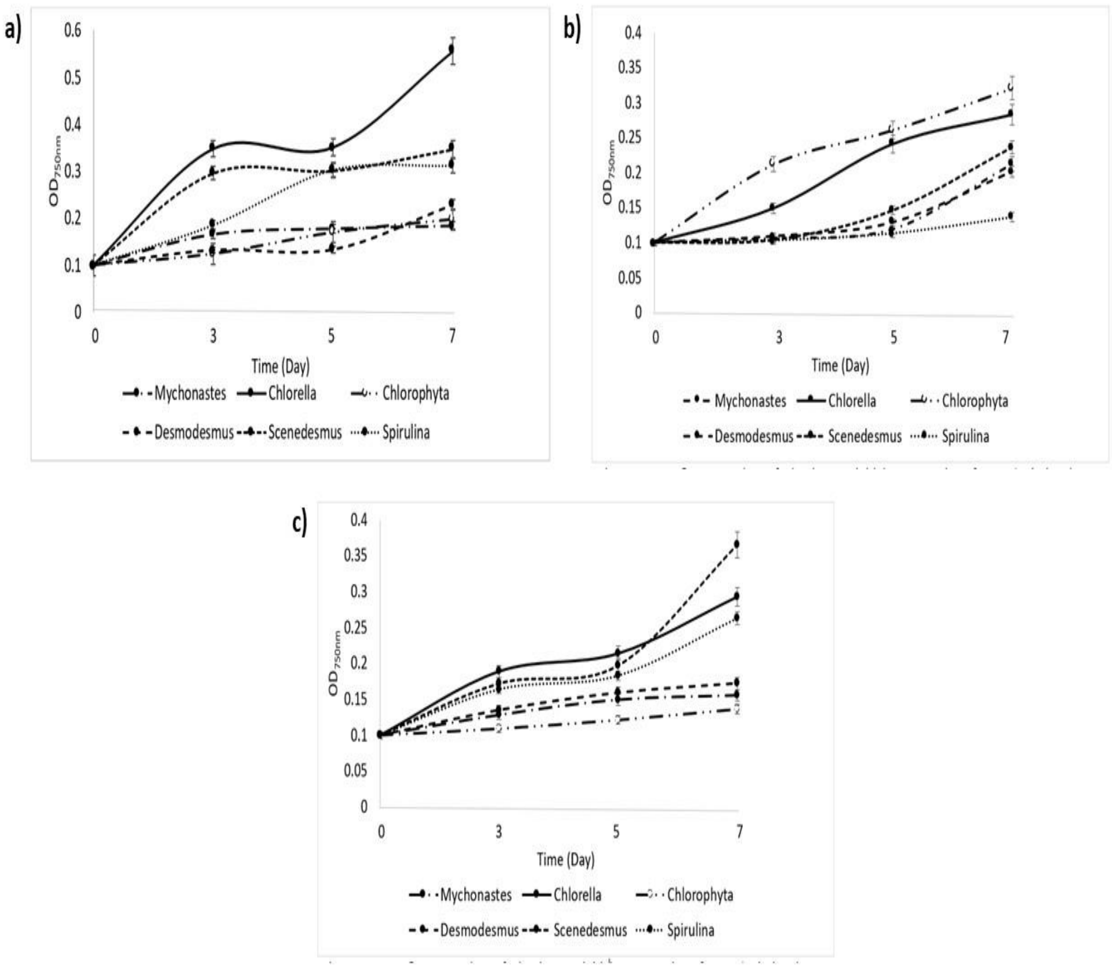

Figure 24: OD@750nm values of Aluminum at the initial concentration of a) 5 mg/L b) 10 mg/L c) 15 mg/L during the test days.

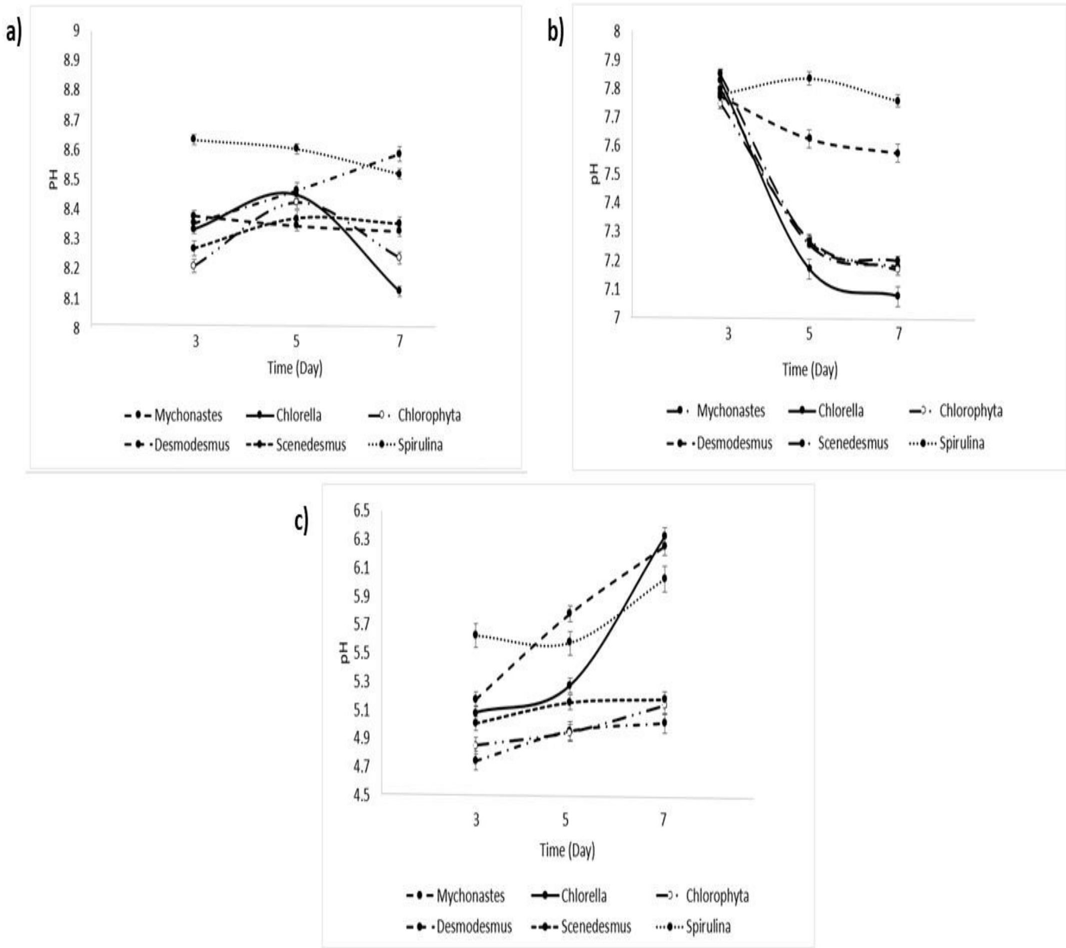

Figure 25: pH values of Aluminum at the initial concentration of a) 5 mg/L b) 10 mg/L c) 15 mg/L during the test days.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The main objective of this research is to test the ability of living and non-living algae species to remove Copper, Nickel, and Aluminum from industrial wastewater samples and to find the optimal operating conditions such as pH, biomass dosage, contact time, and initial metal concentration. The results showed that all the tested algae strains are applicable to be used for the removal of heavy metal ions.

The optimal operating conditions were tested for Copper, Nickel, and Aluminum removal by non-living *Spirulina sp.* biomass. Each parameter was studied independently, by fixing the other parameters to be constant. The impact of contact time was studied, and it was clearly to see that increasing the contact time leads to increase the removal percentage of heavy metal ions up to 90 min and it remains stable till the end of time period indicating the reach of saturation point of *Spirulina sp.* biomass. The results showed that the optimum pH, biosorbent dosage, and initial metal concentration are 5.5, 0.5 g/L, and 10 mg/L, respectively. The maximum removal% of heavy metals from industrial wastewater samples by non-living *Spirulina sp.* biomass was found to be 57.38% for Nickel, 38.24% for Copper, and 93.46% for Aluminum within 4 hours.

In order to fit the experimental data, Langmuir and Freundlich isotherms models have been used. The results showed that the Langmuir isotherm was much better able to fit the linearized experimental data points compared to the Freundlich isotherm with all metal based on the value of correlation coefficient (R^2). All the metals were linear with a R^2 more than 0.9 which are 0.9903 for Aluminum, 0.9853 for Copper, and 0.9847 for

Nickel. It was concluded that all metals have the same optimal operating conditions, although, all operating conditions showed positive results.

Finally, a preliminary screening was conducted for 12 different living algae strains to find the top 5 algae strains that were able to growth with 10 mg/L of initial concentration of Aluminum ions. The results showed that the best growth of algae was with *Mychonastes*, *Chlorella*, *Chlorophyta*, *Desmodesmus*, and *Scenedesmus* with OD@750nm values of 0.698, 0.596,0.547, 0.493, and 0.538, respectively. These algae species were used to conduct different experiment with different initial Aluminum concentration of 5, 10, and 15 mg/L. During these experiment OD@750 nm, pH values, and the percentage removal of Aluminum were reported. *Chlorophyta* showed the highest percentage removal of Aluminum at all initial concentrations; 58%, 69%, and 45% at 5, 10, and 15 mg/L of the initial Aluminum ions concentration. However, the results showed that the highest removal% by the top 5 algae species was reached at the initial Aluminum concentration of 10 mg/L where the reason is that the pH values were between 7.072 and 7.850 which is the best range to remove heavy metals by algal species as mentioned in the literature.

In order to improve this work, further investigations may be needed. This include study the impact of the other different parameters for living algae experiment such as the effect of light, temperature, and CO_2 & O_2 availability. In addition, it very important to study the remaining parameters that affect the non-living algae experiment such as agitation speed, temperature, and adsorbent particle size.

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APPENDIX

The Strains Of Algae Used in The Test (Saadaoui et al. 2016)

Algae strain name	Accession number	Location of isolation (GPS)
Monoraphidium sp.	[GenBank: KM985382]	N 2548′53.00″, E 5121′23.00″
Mychonastes sp.	[GenBank: KM985381]	N 25 9′41.73″, E 5121′40.21″
Chlorella sp.	[GenBank: KM985384]	N 25 9′41.73″, E 5121′40.21″
Oorococcun sp.	[GenBank: KM985388]	N 2522′19.94″, E 5129′28.08″
Neochloris sp.	[GenBank: KM985397]	N 2517′42.25″ E 5126′39.70″
Chlorophyta sp.	[GenBank: KM985402]	N 25 0′28.46″, E 5111′55.57″
Desmodesmus sp.	[GenBank: KM985425]	N 25 2′8.48″, E 51 9′36.06″
Scenedesmus sp.	[GenBank: KM985405]	N 2522′23.81″ E 5129′21.96″
Dictyosphaerium sp.	[GenBank: KM985416]	N 25 9′41.73″, E 5121′40.21″
Protosiphon sp.	[GenBank: KM985423]	N 2548′6.23″, E 5121′7.08″
Chlorococcum sp.	[GenBank: KM985396]	N 2522′19.94″, E 5129′28.08″
Chlamydomonas sp.	[GenBank: KM985374]	N 2548′24.99″, E 5120′51.01″