BIOSORPTION OF AQUEOUS Pb^{2+}, Cd^{2+}, AND Ni^{2+} IONS BY *Dunaliella salina*, *Oocystis sp.*, *Porphyridium cruentum*, AND *Scenedesmus protuberans* PRIOR TO ATOMIC SPECTROMETRIC DETERMINATION

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ABSTRACT

BIOSORPTION OF AQUEOUS Pb^{2+}, Cd^{2+}, AND Ni^{2+} IONS BY *Dunaliella salina*, *Oocystis sp., Porphyridium cruentum*, AND *Scenedesmus protuberans* PRIOR TO ATOMIC SPECTROMETRIC DETERMINATION

In this study, the possibility of using four different algae for the sorption of heavy metals, namely, Pb, Cd, and Ni, from waters was investigated. *Dunaliella salina, Oocystis sp., Porphyridium cruentum,* and *Scenedesmus protuberans* were shown to be good candidates for the sorption/removal of the metals from waters prior to atomic spectrometric determination. Characterization of the algae was carried out by scanning electron microscopy, FTIR, elemental analysis, and thermogravimetric analysis.

All biomasses behaved similarly in the optimization of sorption parameters. Solution pH of 6.0, sorbent amount of 10.0 mg for 10.0 mL sample volume, shaking time of 60 min, and reaction temperature of 25 ºC were used in the sorption experiments.

It was demonstrated that the primary sorption mechanism is the electrostatic attraction between the negatively charged functional groups on the surface of the biomass and the positively (+2) charged metal ions in the solution. Among the biomasses investigated, *Dunaliella salina* has shown the highest sorption capacity for all the metal ions. It was followed by *Oocystis sp., Scenedesmus protuberans* and *Porphyridium cruentum.* Additionally, the biomasses examined have demonstrated the highest affinity towards Pb^{2+} which was followed by Cd^{2+} and Ni^{2+}.

The competitive biosorption experiments have shown that the uptake of Pb^{2+} ions was not influenced by the presence of other ions for all the algae studied. However, the general trend for the other biomasses was a decrease in their sorption efficiency towards Cd^{2+} and Ni^{2+} ions with the increase in the concentration of the competitive ions.

It can be proposed that the algal biomasses investigated in this study can be utilized successfully in the sorption and selective removal of the studied heavy metal ions from waters.

ÖZET

SUDAKİ Pb^{2+}, Cd^{2+} VE Ni^{2+} İYONLARININ ATOMİK SPEKTROMETRİK TAYİNİ ÖNCESİ *Dunaliella salina, Oocystis sp., Porphyridium cruentum* VE *Scenedesmus protuberans* TARAFINDAN BİYOSORPSİYONU

Bu çalışmada, *Dunaliella salina, Oocystis sp., Porphyridium cruentum* ve *Scenedesmus protuberans*'ın, Pb, Cd ve Ni gibi ağır metallerin sorpsiyonu için uygun olup olmadıkları araştırılmış ve adı geçen iyonların atomik spektrometrik tayini öncesinde sorpsiyon/uzaklaştırma amacıyla kullanılabilecekleri gösterilmiştir. Alglerin karakterizasyonu taramalı elektron mikroskopi, FTIR, elemental analiz ve termogravimetrik analiz teknikleriyle gerçekleştirilmiştir.

Çalışılan tüm algler optimizasyon deneyleri sırasında benzer davranış sergilemiştir. Optimize edilen parametreler, çözelti pH'sı için 6.0, 20.0 mL'lik çözelti için sorbent miktarı 10.0 mg, çalkalama (reaksiyon) süresi 60 dakika ve reaksiyon sıcaklığı 25 ºC'dir.

Artı yüklü ağır metal iyonları ile eksi yüklü alg yüzeyi arasındaki temel sorpsiyon mekanizmasının elektrostatik çekim prensibine dayandığı gösterilmiştir. Denenen algler arasında en yüksek sorpsiyon kapasitesini *Dunaliella salina* göstermiştir. Onu, sırasıyla *Oocystis sp., Scenedesmus protuberans* ve *Porphyridium cruentum* izlemiştir. Ayrıca tüm algler, çalışılan ağır metal iyonları içinde en çok Pb^{2+}'yı tutma eğilimi göstermiştir; Cd^{2+} ve Ni^{2+} daha sonra gelmektedir.

Her üç ağır metal iyonuyla gerçekleştirilen yarışmalı biyosorpsiyon deneyleri, alglerin hiçbirinin Pb^{2+}'yı tutma özelliğinin diğer iyonların varlığından etkilenmediğini işaret etmektedir. Ancak, yarışmacı iyonların derişiminin artması halinde alglerin Cd^{2+} ve Ni^{2+}'yı tutma özellikleri azalmaktadır.

Sonuç olarak, bu çalışmada incelenen alglerin sulardaki ağır metal iyonlarının sorpsiyonu ve seçici olarak uzaklaştırılmasında başarıyla kullanılabilecekleri ileri sürülebilir.

Anyone who has never made a mistake
has never tried anything new…

Albert Einstein

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CHAPTER 1

INTRODUCTION

1.1. Environmental Considerations

Biosorption is emerging as an alternative technology to remove the toxic and pollutant species from aqueous media. To understand the feasibility of the biosorption processes for the removal of toxic metals, it is needed to investigate the modelling of biosorption, and also to test the biosorbent with the industrial effluents. In this study, some new biosorbent materials like algae are presented for commercial utilization owing to the comprehensive research and economical advantage of biosorption.

1.2. Heavy Metals

The term 'heavy metal' can be defined in different ways; one is that the metal has a higher density and can have a potential toxicity even if it has a lower concentration, e.g. Ni. Another definition is that it includes the transition metals, some metalloids, lanthanides and actinides which can show metallic properties. Lead, Cd, Zn, Cu and Hg can be given as the examples of heavy metals.

Some heavy metals like Cu, Zn, Se, Fe and Ni are essential elements which are required for maintaining the metabolism of all living organisms. They are used as co-factors for enzymes or proteins but they are needed in very small amounts. Cadmium, Hg, and Pb are among the non-essential metals. Based on their concentration, not only the essential but also the non-essential metals play a role as cell toxins. This phenomenon comes from the unspecific binding of the metals to important biomolecules and proceeds with:

- blocking of functional groups,
- displacement of essential elements,

- or the change of active conformation (form) of the biomolecule. Hence, heavymetals can have toxicological affects on human body after some concentrations specific for each metal.

Heavy metals are natural components of the Earth's crust. They can enter a water supply by industrial and domestic use, and from acid rain which breaks down soils and releases heavy metals into streams, lakes, rivers, and groundwater. They can be deposited in human body by food, drinking water and air.

All living creatures need essential metals (Cu, Zn, Fe, Ni etc.) to maintain their biological activities. The hazardous effects of heavy metals are the result of their accumulation in the organism. Accumulation causes a rise in the concentration of a chemical in a biological organism in a time interval, hence its concentration becomes greater than its concentration in the environment. This situation makes heavy metals dangerous for living things since chemicals are deposited in living things in any time and are taken up quicker than they are metabolized or excreted.

1.2.1. Lead

Lead, is a soft malleable poor metal, has a bluish white colour; however, it looses its brightness when it is exposed to air. It is found in small amounts in the earth’s crust, but it can also be found in all parts of the environment owing to human activities like burning fossil fuels, mining, and manufacturing. Lead has many uses in, for example, building construction, lead-acid batteries, bullets and shot, weights, devices to shield X-rays and it is also part of solder, pewter, fusible alloys . It is also used in the fuel as an anti-knocking agent in methylated form (tetra-ethyl lead). However, in most industrialized countries their environmental impact has levelled off as legislation aimed at reducing and replacing lead in petrol.

Lead itself does not break down; nevertheless, the change of lead compounds are due to sunlight, air, and water. Once lead is released to the air it generally binds to dust particles and can travel long distances before reaching the soils. Mobility of lead from soils is also based on the type of the lead compound and the characteristics of the soil.

Lead is a pollutant that is present everywhere in the ecosystem. Lead uptake can take place because of consumption of lead containing drinks or foods, and through respiratory system. The lead-based paint in older homes can create a serious

potential risk for most children. Lead can accumulate in soft tissues and bone in over time due to its potential neurotoxicity. Moreover, it can result in the decline of performance. Anemia is another possible result of lead exposure which can replace iron in the hemoglobine.

Serious damage of the brain and kidneys in people can take place upon exposure to high levels of lead and death is inevitable as the end result of the process. In addition, exposure to high levels of lead may account with miscarriage in pregnant women and damage the organs which is responsible from sperm production. EPA limits lead in drinking water to 15 μg/L (Environmental Protection Agency 2008).

1.2.2. Cadmium

Cadmium is one of the natural crustal elements and is generally found in the form of oxides, chlorides, sulfates and sulfides. All rocks and soils such as coal and mineral fertilizers contain cadmium. Batteries, pigments, metal coatings, and plastics also contain cadmium, because it does not corrode easily. Industrial activities, mining, coal-burning and household wastes release cadmium to the environment. Cadmium particles in air have an ability to travel long distances before reaching to the ground or water. It can contaminate soil or water by leakage of hazardous waste sites or from waste disposal. While some cadmium compounds have a solubility in water, it binds strongly to soil particles (Agency for Toxic Substances and Disease Registry 2008). Even though it can change forms it does not break down in the nature. As cadmium has an ability to stay in the body for a very long time, the body is susceptible to long time exposure of low levels of cadmium.

The body can be exposed to cadmium upon breathing contaminated workplace air (battery manufacturing, metal soldering or welding), eating foods even if it has a low concentration in a variety of foods (highest in shellfish, liver, and kidney meats), breathing cadmium in cigarette smoke (doubles the average daily intake), drinking contaminated water, and breathing contaminated air near fossil burning facilities, fuels or municipal waste. If high levels of cadmium are inhaled, lungs are seriously damaged as well as it comes to a conclusion with death. The severe stomach irritation is due to eat food or drink water with very high levels containing cadmium and as it also leads to vomit and diarrhea. Storage of cadmium in the kidneys and buildup of

possible kidney disease is as a result of long-term exposure to lower levels of cadmium in air, food, or water.

The EPA has set a limit of 5 parts of cadmium per billion parts of drinking water (5µg/L) and doesn't allow cadmium in pesticides (Environmental Protection Agency 2008).

1.2.3. Nickel

Nickel, is a naturally occurring element that is a hard silvery-white metal and it inerts to oxidation. Nickel is found in soils, meteorities and on the ocean floor and volcanoes emit nickel.In stainless stell and other metal alloys, pure nickel is used. Comibination of nickel with other metals, such as iron, copper, chromium, and zinc is done to form alloys. In the production of coins, jewelry, and items such as valves and heat exchangers, these alloys are used. Nickel containing compound are formed with elements such as chlorine, sulfur, and oxygen. Great number of nickel compounds have a fairly solubility in water and they have green colour, but they do have netiher taste nor characteristics odour. In the nickel plating, nickel compound are used to colour ceramics, to make some batteries. Nickel compounds are also used as catalyts.

Release of nickel occurs by industries which uses nickel, its alloys and its compounds. Another road of release is by oil-burning power plants, coal-burning power plants .The small particles of dust induce the settle of nickel in the air to the ground or by the aid of rain or snow it binds the soils strongly, in many days. Nickel that is released by industrial waste water winds up in soil or sediment where it is strongly bound to particles including iron or manganese. Accumulation of nickel in animals e.g. fish that is used for food purposes does not seem to happen.

There are lots of exposure roads for nickel to human body which include eating food containing nickel, sking contact with soil, bath or shower water, or nickel containing metals likewise coins handling or touching jewelry. In addition to these, it is exposed by drinking water having lower levels of nickel, breathing air or smoking tobacco containing nickel. People work in ındustries producing or processing nickel have a greater exposure of nickel.

An allergetic reaction can happen in humans is the major outcome of nickel which affects people health harmfully. From the side of the contact, a skin rash can

become evident. Nickel sensitive people can have asthma attacks, but not common and some of them give a reaction consuming food or water containning nickel or breathing dust containing it. Chronic bronchits and reduced lung function problems are the consequences for the people who work in nickel refineries or nickel-processing plants. Together with these problems, workers who drank water containing higher concentration of nickel have stomach ache and suffered adverse effects to their blood and kidneys (Agency for Toxic Substances and Disease Registry 2008). Nasal cancer among workers in Ni refineries was reported in the 1930s, and conclusive evidence of an increased risk of lung and nasal cancer in this group of people was presented in the 1960s (Ebdon, et al. 2001). Nickel sulfide (Ni_2S_3) is considered to be one of the most carcinogenic Ni compounds, as shown in experiments with animals and in human lymphocytes (Ebdon, et al. 2001).

Nickel is an essential element for microorganisms and plants to carry out the reactions which is vital response. By the way, the urease that is an nickel containig enzyme assists in the hydrolysis of urea (Wikipedia 2008).

According to the EPA recommendations, drinking water should contain less than 0.1 mg nickel/L (Environmental Protection Agency 2008).

1.3. Heavy Metal Pollution

Heavy metal pollution is a quickly growing problem for our oceans, lakes, and rivers. It has started to influence our lives by its growing , so it can be emphasized that it has to be awared of the problems due to heavy metals. It can be concluded that the solution for the heavy metal pollution is demanding immediate actions. Since heavy metals has an ability not to decay unlike organic pollutants, hence it is the cause of challenge for remediation. It can be thought that the main cause of heavy metal pollution is due to the industralization and its outcomes, and this brings to threaten to human health, animals, plants, and the planet itself. Fertilizers and sewage also are the another source of heavy metal pollutants, however, the pollution is primarily based on the industralization.

1.3.1. Heavy Metal Determination Techniques

It is needed to determine the amount and the form of heavy metals in sample which is taken up by the area of interest, since the quality of area is relied on these two factors.

The concentration of five soil heavy metals (Pb, Co, Cr, Cu, Hg) was measured in forty sampling sites in central Transylvania, Romania, regions known as centres of pollution due to the chemical and metallurgical activities by the aid of ICP–MS (Inductively Coupled Plasma–Mass Spectrometry) and NAA (Neutron Activation Analysis) (Suciu, et al. 2008). The contents of cadmium, lead, nickel, zinc and copper in bee honey samples were analysed by ICP-OES (Inductively Coupled Plasma–Optical Emission Spectrometry) (Demirezen and Aksoy 2005).

The preconcentration and determination of ultra trace amounts of inorganic mercury and organomercury compounds in different water samples was studied by RP-HPLC (reversed-phase high-performance liquid chromatography) after solid phase extraction on modified C18 extraction disks with 1,3-bis(2-cyanobenzene)triazene (Hashempur, et al. 2008).

Recently, Pei Liang and a co-worker developed a new method for the determination of trace lead by dispersive liquid-liquid microextraction and preconcentration and graphite furnace atomic absorption spectrometry (Liang and Sang 2008). In this study, 1-phenyl-3-methyl-4-benzoyl-5-pyrazo-lone (PMBP) as a chelating agent, which forms complexes with more than 40 metal ions and has found numerous applications in trace element separation and preconcentration. In addition, trace amounts of Pb in human urine and water samples was successfully determined by this method (Liang 2008).

Robles and co-workers studied that phenyl-mercury (Ph-Hg) was selectively preconcentrated by living *Escherichia coli* strain (K-15) and mercury content was determined by CVAAS (cold vapour atomic absorption spectrometry) (Robles, et al. 2000).

Bacteria were used for speciation of Se(IV) and Se(VI) in complex environmental samples. The speciation of soluble inorganic selenium ions, Se(IV) and Se(VI), was studied by living bacterial cells of *E. Coli* and *P. Putida* and the

determination of Se(IV) and Se(VI) was done by electrothermal atomic absorption spectrometry (ETAAS) (Robles, et al. 1999).

1.3.2. Removal of Heavy Metals

Heavy metals have potential risk on the environment depending on their amount and in which they exist, hence it is required to remove the heavy metals from the environment.

For the removal of metal ions from aqueous streams, there are some processes that are applied commonly; reverse osmosis, electrodialysis, ion exchange, precipitation, solvent extraction, and phytoremediation.

Reverse Osmosis is an expensive method in which the separation of heavy metals is achieved by a semi-permeable membrane at a pressure greater than osmotic pressure by the dissolved solids in wastewater.

In electrodialysis, separation of the heavy metals (the ionic components) is performed through semi-permeable ion-selective membranes. Migration of cations and anions towards respective elctrodes takes place when an electrical potential is applied between the two electrodes. The major drawback of this method is clogging the membrane by metal hydroxide formation.

Ion-exchange is a process that metal ions from dilute solutions are replaced with ions held by electrostatic forces on the exchange resin. However, this process has some disadvantages due to the fact that it has high cost and requires partial removal of certain ions.

Precipitation is achieved by the addition of chemicals like as lime, iron salts and other organic polymers, and it needs the conventional solid-liquid removal by sedimentation. The production of large amount of sludge containing toxic compounds during the process is the most significiant problem of this process (Biosorption 2008).

Metal contaminated soil, sediment, and water is cleaned up by the certain plants is called phytoremediation, however the removal of heavy metals takes long time and the regeneration of the plant for the next use is difficult.

Up to now, the conventional methods has some disadvantages like incomplete metal(s) removal, high energy and reagent requirements, generation of toxic sludge or other waste products which makes the careful disposal of waste requisite. This also

leads to develop an economical and environmental friendly waste treatment method which has a higher removal capacity to the heavy metals from aqueous effluents.

The recovery of the heavy metals is a significant importance as well as the removal of them. Hence, the priority of the heavy metals which are to be recovered based on either their removal and/or recovery considered and it is classified into three categories (Volesky 2001) and the heavy metals are assigned in a rank dependent upon these categories (Table 1.1.):

- environmental risk (ER):
- reserve depletion rate (RDR);
- a combination of the two factors.

Table 1.1. Ranking of metal interest priorities
(Source:Volesky 2001).

Relative Priority	Environmental Risk	Depletion Factors	Combined Factors
HIGH	Cd	Cd	Cd
	Pb	Pb	Pb
	Hg	Hg	Hg
	-	Zn	Zn
MEDIUM	Cr	-	-
	Co	Co	Co
	Cu	Cu	Cu
	Ni	Ni	Ni
	Zn	-	-
LOW	Al	-	Al
	-	Cr	Cr
	Fe	Fe	Fe

The assessment of environmental risk could be based on a number of different factors such as combing the threats and understanding the effect on the contemporary life and it could be considered as important, yet.

The significance of metal in terms of its rising market price with time is understood by the utilization of the RDR category. If the RDR is taken into account with the ER, Cd, Pb, Hg, Zn are considered as in high priority. The use of Cd is

growing, whereas, the technological uses of Hg and Pb might be thought as dropping off. These inferences and the outcome of the degree of risk assessment could alter the priorities among the metals of interest.

1.4. Biosorption

The interactions of microorganisms and metals in aqueous media have been studied with an increasing attemptions in recent years (Sağ, et al. 1998, 2000, 2001, De Carvalho 2001, Puranik, et al. 1999, Esposito 2001, Tsezos 2001, Aksu 2002).

Biosorption can be defined as the removal of metals from solution by the certain types of biomass which has an ability to bind and concentrate metals as much as at lower concentrations. Depending on the metabolism or not, large amounts of metals can be accumulated by different processes. Living and dead biomass as well as cellular products such as polysaccharides can be utilized for metal removal. Algae, moss, fungi, bacteria, and fungi are the likely biosorbents to sequester and recover the precious metals or pollutant metals .

1.4.1. Biosorption Mechanisms

Biosorption is becoming the most promising path for the treatment of waste waters. Since microorganisms have complex structure and it makes that there are many routes for the metal uptake by the cell. The biosorption mechanisms are different and classified in the Figure 1.1 (Vegliò 1997).

According to Figure 1.1, biosorption mechanisms can be separated in to groups which are metabolism dependent and non metabolism dependent. Metabolism dependent action of process occurs in the living biomass, while non metabolism dependent one is taking place with dead biomass.

Figure 1.1 indicates the biosorption mechanisms: transport of the metal across the cell membrane occurs with viable cells by intracellular accumulation. In many cases, this action is faced with an active defense system that reacts in the presence of toxic metal.

Precipitation of the metal may take place in the solution together on the cell surface. The precipitation process is favoured when the compounds are produced by microorganisms in the presence of toxic metals and this may be independent upon the

cell metabolism. From the other point of view, precipitation may not be dependent on the cells' metabolism when a chemical interaction happens between the metal and cell surface.

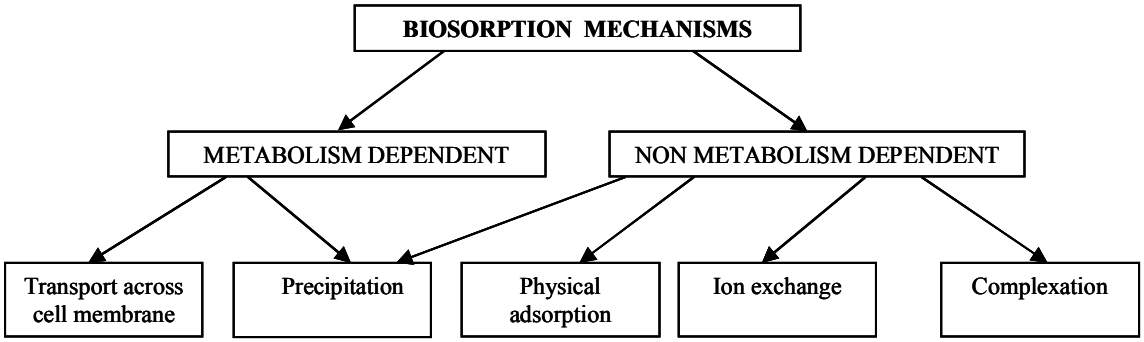


Figure 1.1. Biosorption mechanisms
(Source:Vegliò 1997)

Physico-chemical interaction between the metal and the functional groups present on the microbial cell surface exists throughout non-metabolism dependent biosorption. In this case, biosorption mechanism is relied on physical adsorption, ion exchange and complexation that are independent of metabolism. Cell walls of microbial biomass contains a great degree of polysaccharides, proteins and lipids, and these biomolecules have binding groups such as carboxyl, hydroxyl, sulphate, phosphate and amino groups (Christ, et al. 1981). The functional groups carries negative charge to the cell surface. The binding process of this type of action is mainly a physical nature, usually rapid and reversible,and it also requires minimum energy for activation. Physical adsorption takes place owing to Waals' forces, Kuyucak and Tsezos proved that biosorption of uranium and thorium by *Rhizopus arrhizus* is physical adsorption in th cell-wall chitin structure. Copper biosorption by alga *Chlorella vulgaris* and bacterium *Zoogloea ramigera* was studied and the metal uptake occurs by electrostatic interactions (Aksu, et al. 1992). Polysaccharides are the cell wall constituents of microorganisms and the counter ions (K^+, Na^+, Ca^{2+}, and Mg^{2+}) of the polysaccharides are exchanged with bivalent metal ions. For example, marine algae contains alginate salts of K^+, Na^+, Ca^{2+}, and Mg^{2+} and the biosorption of heavy metals takes place with the replacement of counter ions with the bivalent metal ions (Kuyucak and Volesky 1988). Complex formation is another type of biosorption

mechanism on the cell surface after the interaction between the metal and active groups occurs. This type of mechanism is only valid for Ca, Mg, Cd, Zn, Cu, and Hg (Vegliò 1997). Organic acids such as citric, oxalic, gluonic, fumaric, lactic and malic acids can be be produced by micro-organisms, and chelation of toxic metals with these acids forms metallo-organic molecules. Carboxyl groups found in microbial polysaccahrides and other polymers may give complexation reaction with metals.

It is clear to be understanding that biosorption mechanisms can also take place at the same time.

1.4.2. Factors Affecting Biosorption

In order to understand the quality of the biosorption process and to design the system for the removal of contaminants, we need to evaluate the effect of factors which influences the performance of biosorption. The following factors are thougth to be responsible for biosorption:

1. When the temperature of the sorption media is in the range of 20-35 ^{0}C the biosorption process is not affected by temperature (Aksu, et al. 1992). However, if the increment in the temperature reduces the sorption capacity of biomass (Aksu 2001).
2. The major challenging factor for the biosorptive process is pH seems to be the most important parameter in the biosorptive process. Since, it influences not only the solution chemistry of the metals but also the activity of the functional groups in the biomass and the competition of metallic ions. Table 1.2 shows the functional groups responsible for the binding of metal and their acidity constants. These groups create a negatively charged surface at above the isoelectronic point of the biomass and electrostatic attractions among metals and the cell surface is involved in the biosorption process. At low pH values below isoelectronic point, the functional groups are protonated surface charge. This comes to a conclusion with lower metal uptake when the metal is in the cationic form such as Pb^{2+}, this is due to blocking of functional groups responsible for the metal uptake. However, in the case of Cr(VI) which is anionic in nature, it can bind at lower pH values , that is the surface charge of the cell wall is positive in nature.

3. The metal removal is influenced by the biomass concentration in solution. The lower quantities of biomass is enough to sequester the metals from the solution, if the amount of biomass is increased it influences the solution chemistry of the metals, the activity of the functional groups in the biomass, and also the competition between metallic ions. This phenomenon can be attributed to the interference between the binding sites. This parameter should be undertaken once biosorption is considered for the removal of metals.
4. Biosorption is utilized to treat wastewater if more than one type of metal ions would be present. Hence, the presence of other interfering ions can decline the uptake of the metal which is interested in. In contrast, the presence of Fe^{2+} and Zn^{2+} was found to influence uranium uptake by *Rhizopus arrhizus* (Tsezos and Volesky 1982) and cobalt uptake by different microorganisms seemed to be completely inhibited by the presence of uranium, lead, mercury and copper (Volesky 2007).
5. Cell size and structure , and morphology and also affects the amounts of metal biosorbed by different biomasses. The greater the cell surface area to dry weight ratio, the greater the quantity of metal biosorbed by a cell surface per unit weight.

1.4.3. Advantages of Biosorption

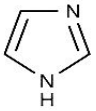
Biosorption has several superiorities over the traditional metal removal methods that makes it is to be more preferential:

1. Cheap: The cost of biosorbents is low as they often are made from abundant or waste materials.
2. Metal selective: The metal-sorbing preformance of different types of biomas can be more or less on different metals. This depends on various factors such as type of biomass, mixture in the solution, and the type of biomass preparation.
3. Regenerative: Biosorbent can be reused after the metal is recycled.
4. No sludge generation: No secondary problems with sludge occurs with biosorption as in the case with many other metal removal technologies.

5. Metal recovery possible: Metals can be recovered after being sorbed from the aqueous media by a suitable eluting solution.
6. Competitive performance: It is capable of performace comparable to the most similar techniques.

Owing to the aforementioned advantages, biosorption based on organisms or plants could be uitlized as an alternative method to remove heavy metals from industrial wastewaters (Pamukoğlu 2006).

Table 1.2. Major binding groups for biosorption
(Source:Volesky 2007)

Binding group	**Structural formula**	**pK_a**	**HSAB classif.**	**Ligand atom**	**Occurence in selected biomolecules**
Hydroxyl	—OH	9.5 – 13.0	Hard	O	PS, UA, SPS, AA
Carbonyl (ketone)	>C=O	-	Hard	O	Peptide bond
Carboxyl	—COOH	1.7 - 4.7	Hard	O	UA, AA
Sulfhydryl (thiol)	—SH	8.3 - 10.8	Soft	S	AA
Sulfonate	—SO_3	1.3	Hard	O	SPS
Thioether	>S	-	Soft	S	AA
Amine	—NH_2	8.0 - 11.0	Intermediate	N	Cto, AA
Secondary amine	>NH	13.0	Intermediate	N	Cti, PG, AA
Amide	—C(=O)—NH_2	-	Intermediate	N	AA
Imine	=NH	11.6 – 2.6	Intermediate	N	AA
Imidazole		6.0	Soft	N	AA
Phosphonate	—P(=O)(OH)—OH	0.9 – 2.1 6.1 – 6.8	Hard	O	PL
Phospodiester	>P(=O)(OH)—OH	1.5	Hard	O	TA, LPS

(PS: polysaccharides, UA: uronic acids, SPS: sulfated PS, Cto: chitosan, PG: peptidoglycan, AA: amino acids, TA: teichoic acid, PL: phospholipids: LPS: lipoPS).

1.5. Algae

Living or dead algal cells are being increasingly used as biosorbents to remove heavy metals from aqueous solutions due to their high sorption uptake and their availibility in practically unlimited quantities in the seas and oceans (Feng and Aldrich 2004).

Algae (singular alga) are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms (Wikipedia 2008). Seaweeds are the largest and most complex marine forms. They are photosynthetic, like plants, and "simple" because they lack the many of the distinct organs found in land plants. All true algae have a nucleus enclosed within a membrane and chloroplasts bound in one or more membranes. Algae constitute a paraphyletic and polyphyletic group: they do not represent a single evolutionary direction or line, but a level or grade of organization that may have developed several times in the early history of life on Earth.

Some organs that are not included in the algae are phyllids and rhizoids in nonvascular plants, or leaves, roots, and other organs that are found in tracheophytes. Since they are photosynthetic, so they are distinguished from protozoa. Even though some groups contain members that are mixotrophic, deriving energy both from photosynthesis and uptake of organic carbon either by osmotrophy, myzotrophy, or phagotrophy, many are photoautotrophic. Some unicellular species rely entirely on external energy sources and have reduced or lost their photosynthetic apparatus.

All algae have photosynthetic parts eventually obtained had origin from the cyanobacteria, moreover they produce oxygen as a byproduct of photosynthesis, unlike other photosynthetic bacteria such as purple and green sulfur bacteria.

Algae are most prominent in bodies of water but are also common in terrestrial environments. However, terrestrial algae are usually rather inconspicuous and far more common in moist, tropical regions than dry ones, because algae lack vascular tissues and other adaptations to live on land. Algae are also found in other situations, such as on snow and on exposed rocks in symbiosis with a fungus as lichen.

The various sorts of algae play significant roles in aquatic ecology. Microscopic forms that live suspended in the water column (phytoplankton) provide the food base for most marine food chains. Some are used as human food or harvested

for useful substances such as agar, carrageenan, or fertilizer. In Table 1.3, protein, carbonhydrates, fats, and nucleic acid percentages in a dry algae is given and is compared with selected conventional foodstuffs (Becker 1994). From Table 1.3, chemical composition varies with the species of the algae. Also, algae has higher protein content such as *Dunaliella salina* in contrast to conventional foodstuffs like egg.

Table 1.3. Gross chemical composition of human food sources and different algae (% of dry matter)

(Source:Becker 1994)

Commodity	**Protein**	**Carbonhydrates**	**Lipids**	**Nucleic acid**
Baker's yeast	39	38	1	–
Rice	8	77	2	–
Egg	47	4	41	–
Milk	26	38	28	–
Meat muscle	43	1	34	–
Soya	37	30	20	–
Scenedesmus obliquus	**50 – 56**	**10 – 17**	**12 – 14**	**3 – 6**
Scenedesmus quadricauda	**47**	**–**	**1.9**	**–**
Scenedesmus dimorphous	**8 – 18**	**21 – 52**	**16 – 40**	**–**
Chlamydomonas rheinhardii	48	17	21	–
Chlorella vulgaris	51 – 58	12 – 17	14 – 22	4 – 5
Chlorella pyrenoidosa	57	26	2	–
Spirogyra sp.	6 – 20	33 – 64	11 – 21	–
Dunaliella bioculata	**49**	**4**	**8**	–
Dunaliella salina	**57**	**32**	**6**	**–**
Euglena gracilis	39 – 61	14 – 18	14 – 20	–
Prymnesium parvum	28 – 45	25 – 33	22 – 38	1 – 2
Tetraselmis maculata	52	15	3	–
Porphyridium cruentum	**28 – 39**	**40 – 57**	**9 – 14**	**–**
Spirulina platensis	46 – 63	8 – 14	4 – 9	2 - 5
Spirulina maxima	60 – 71	13 – 16	6 – 7	3 – 4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43 – 56	25 – 30	4 – 7	–

1.5.1. *Dunaliella salina*

Dunaliella, the unicellular haloterant green alga which is responsible for most of the primary production in concentrated brines. *Dunaliella* is characterized by an ovoid cell volume in the range of 2-8 nm wide x 5-15 μm length, usually in the shape of pear, wider at the basal side and narrow at the anterior flagella top. Known for its anti-oxidant activity because of its ability to create large amount of carotenoids. Its provides the production in the open air for the trans/cis-β-carotene which is more soluble and has better quality as a free radical scavenger (Banerjee, 2005) and potential food additive for enhancing the colour of the fish and egg yolk. It is used in cosmetics and dietary supplements. In such highly saline conditions like salt evaporation ponds, not many organisms can survive. In such conditions, these living things protect against the intense light by using high concentrations of β-carotene in their structures. They have high concentrations of glycerol to protect itself against osmotic pressure. This characteristics give a chance for biological production of these substances commercially.

Dunaliella is found in a wide range of marine habitats such as oceans, brine lakes, salt marshes and salt water ditches near the sea, mainly in water bodies containing more than 2.0 M salt and a high level of magnesium. However, the outcome the presence of magnesium on the distribution of *Dunaliella* is not clear, *Dunaliella* generally grows in a large number of 'bittern' habitats of marine salt producers. The appearance of orange-red algal bloom in marine environments is primarily based on combined sequential growth of *Dunaliella* firstly, and then halophilic bacteria, and is usually found in concentrated saline lakes such as the Dead sea in Israel, the Pink Lake in Western Australia, the Great Salt Lake in Utah, USA. It has been known that *Dunaliella* is the most haloterant eukaryotic organism exhibits a noteworthy extent of adaptation to a variety of salt concentrations, from a salt saturation as low as 0.1M (Cohen 1999).

For β-carotene production, a first pilot plant for *Dunaliella* cultivation founded in the USSR in 1966. From the establishment of plant for *Dunaliella*, one of the success in the halophile technology is the commercial production of Dunaliella for the production of the β-carotene of the globe (Oren 2005). Since, *Dunaliella* allows to the possibility for the cultivation of biomass in open ponds.

1.5.2. *Oocystis sp.*

Oocystis sp. is a green microalgae having unicells or colonies of non-fixed number of cells, and the shape of cell body is variable. The shape of one or more chloroplasts is subjected to change. Autospore or autocoenobium provides asexual reproduction. Colony of 2-8 cells is encircled by cell wall of their mother cell, however, it is sometimes unicellular . It has a broad ellipsoidal cell body (Protist Information Server 2008).

1.5.3. *Porphyridium cruentum*

Porphyridium cruentum, the unicellular red alga with spherical cells lacking a cell wall, is a primitive member of the Rhodophyta, order of Porphyridiales. The diameter of cells ranges from 4 to 9 μm.The cells can live in theirselves or in the form of colonies. The wall-less cells, including a single large chloroplast, which are surrounded by a cover of a water-soluble sulphated polysaccharide. Due to the polysaccharide enveloping the algal cells from the environment, *Porphyridium* can survive under extreme environmental conditions (Arad and Ucko 1989) and it can found in sea water and in humid soils.

From the economical point of view, the useful products of *Porphyridium* such as sulphated polysaccharides and a red proteinaceous pigment as phycoerythrin are obtained possibly. Owing to the production of eicosapentaenoic acid and other polysaturated fatty acids, *Porphyridium cruentum* has come the industrial interest (Banerjee 2005).

1.5.4. *Scenedesmus protuberans*

Scenedesmus protuberans is a small, nonmotile colonial green alga including of cells arranged linearly or zigzag in a flat plate. The colonies usually have two or four cells, but may have 8, 16, or seldom 32 and are sometimes unicellular The cells are generally shaped as cylinder, however might be more lunate, ovoid, or fusiform. In a tyical manner, the end cells each have two flagellates from their outer corners Each cell consists of a single parietal, plate-like chloroplast with a single pyrenoid.

Scenedesmus exists comonly in the plankton of freshwater ponds, rivers, and lakes, and occasionally in brine environments. Its location starting from all of North America from tropical to arctic climates. In nutrient-rich waters, the growth of the biomass might be dense. As in the case of other algae types, it is an leading producer and it has food source property for higher trophic degrees.

The genus, *Scenedesmus*, is frequently utilized as a bioindicator for the detection of nutrients or toxins coming from anthropogenic inputs to aqueous media. For example, *Scenedesmus* produces phytochelatin when the metal content of the medium is increased.

1.6. Use of Immobilized Biomass in Biosorption

One of the major problems in the use of algae for the biological treatment of wastewaters is their recovery from the treated effluent. So as to solve the problems associated with the harvesting the biomass, many systems have been proposed or tried (Olguin 2000). Among the most recent ways of passing this problem are immobilization techniques applied to algal cells. In fact, in the case of photosynthetic cells, over the last 20-25 years considerable progress has been achieved in the immobilization of photobiologhical organisms and organelles such as cyanobacteria, photocynthetic bacteria, algae and chloroplasts.

1.6.1. Immobilization Techniques

Since the microbial biomass is composed of small particles with low density, poor mechanical strength and little rigidity, to use the biomass in the treatment process cell immobilization becomes an emerging path for the elimination of the problem. The cell immobilization is an interesting method to fix and retain biomass on appropriate natural or synthetic materials support by performing a range of physical and biochemical procedures. The immobilization of the biomass in materials leads to the formation of a material having a tunable size, mechanical strength and rigidity and porosity which is required to accumulate the metals. Immobilization of the biomass can produce beads and also granules, this materials could be activated and used repetitively like in a similar way of ion exchange resins and activated carbon.

Various methods are available that can be applied for the biomass immobilization. The principal techniques that are available in literature for the application of biosorption are based on adsorption on inert supports, entrapment in polymeric matrix, covalent bonds in vector compounds, or cell cross-linking (Figure 1.2) (Vegliò and Beolchini 1997).

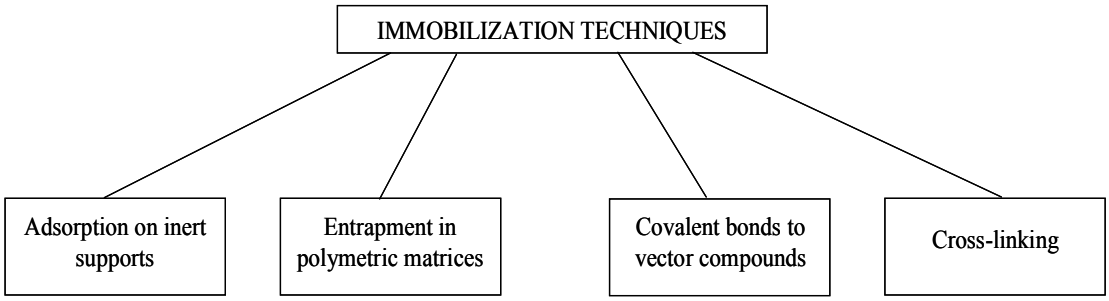


Figure 1.2. Immobilization techniques
(Source:Vegliò and Beolchini 1997)

1.6.1.1. Adsorption on Inert Supports

Sterilization and inoculation processes are applied with starter culture, and the continuous culture are left inside for a period of time. Then, the microorganism (biomass) is supported on the suitable matrices. Many microorganims have an ability to be attached spontaneously to surfaces. Indeed, once a suspension of cells is brought into contact with a surface, the definite amount of adsorption to the solid becomes inevitably. This technique has been used by Zhou and Kiff, 1991 for the immobilization of *Rhizopus arrhizus* fungal biomass in reticulated foam biomass support particles; Macaskie, et al. 1987, immobilised the bacterium Citrobacter sp. by this technique. Scott and Karanjakar (1992), used activated carbon as a support for Enterobacter aerogens biofilm. Bai and Abraham (2003) immobilized *Rhizopus nigricans* on polyurethane foam cubes and coconut fibres. Polyurethane and polyinyl foams have been used as supports for immobilized cells (Olguin 2000).

1.6.1.2. Entrapment in Polymeric Matrices

The polymers matrices used are calcium alginate (Babu et al. 1993, Costa and Leite, 1991, Peng and Koon, 1993, Gulay Bayramoglu et al. 2002), polyacrylamide (Macaskie, et al. 1987), polysulfone (Veglio, et al. 2003, Bai and Abraham 2003). Gel particles are obtained from immobilization in calcium alginate and polyacrylamide. The materials obtained from immobilization in polysulfone and polyethyleneimine are the strongest.

1.6.1.3. Covalent Bonds to Vector Compounds

Silica gel is the most common vector compound (carrier). In this technique, the form of materials are gel type particles. It is generally utilized for algal immobilization (Holan, et al. 1993).

1.6.1.4. Cross-Linking

The addition of the cross-linker leads to the formation of stable cellular aggregates. This technique was found useful for the immobilization of algae (Holan, et al. 1993, Valdman and Leite 2000). The most common cross linkers are: formaldehyde, glutaric dialdehyde, divinylsulfone and formaldehyde - urea mixtures.

1.7. Aim of this Study

The aim of this study was primarily based on the biosorption of aqueous Pb^{2+}, Cd^{2+}, and Ni^{2+} by the algae (biosorbents) such as *Dunaliella salina*, *Oocytis sp.*, *Poprhyridium cruentum*, and *Scenedesmus protuberans* and investigate the biosorption ability of the biosorbents towards the metal ions.

In this sudy, biosorption experiments were with respect to the pH of the metal ion solution, sorption time, initial metal ion concentration, and the amount of the biosorbent used. The desorption of the metals from the biosorbents and the repetitive usability of the biosorbents were also studied. In addition, competitive performance of the metals were also studied in the presence of the other metal ions.

Moreover, there is an additional objective of this study is to perform the experiments with immobilized biosorbents on different suitable matrices like Na-silicate and agarose.

CHAPTER 2

EXPERIMENTAL

2.1. Preparation of Biosorbents

All algae used in this study were obtained from Microalga Culture Collection, Ege University (EGE-MACC). After growth period, the algal cultures were harvested and filtered through Whatman No:1 filter paper. Then, they were washed with deionized water to be made free from the ions of their growing media. The growing media and their contents used in this study are given in Appendix A. The cultures were kept at -24ºC and lyophilized with Christ alpha 1-4 Ld freeze dryer. Lyophilized biomass was powdered and kept in the refrigerator (4ºC) before being used in the related experiments.

2.2. Chemicals and Reagents

All reagents were of analytical grade. Ultra pure water (18MΩ) was used throughout the study. Glassware and plasticware were cleaned by soaking in 10% (v/v) nitric acid and rinsed with distilled water prior to use. The chemicals and the reagents used were tabulated in Table 2.1 and prepared as follows;

1. Standard Pb^{2+} stock solution (4000 mg/L): Prepared by dissolving 1.599 g of $Pb(NO_3)_2$ (Riedel-de Haën, 99%) in ultra pure water and diluting to 250.0 mL. This solution also contains 0.14 M HNO_3 (Merck).
2. Standard Cd^{2+} stock solution (4000 mg/L): Prepared by dissolving 1.630 g of $CdCI_2$ (Fluka, >99%) in ultra pure water and diluting to 250.0 mL. This solution also contains 0.14 M HNO_3 (Merck).
3. Standard Ni^{2+} stock solution (4000 mg/L): Prepared by dissolving 3.539 g of $NiCI_2.6H_2O$ (Carlo Erba, 99%) in ultra pure water and diluting to 250.0 mL. This solution also contains 0.14 M HNO_3 (Merck).
4. Calibration standards: Lower concentration standards were prepared daily from standard stock solutions.

5. pH adjustment: Various concentrations (0.1-1.0 M) of HNO_3(aq) (Merck) and NH_3(aq) (Merck) solutions were used.
6. H_2SO_4 solution (5 %): Prepared by diluting 5.0 mL of concentrated H_2SO_4 (Merck, 96%) to 1000 mL with ultra-pure water.
7. Na-Silicate (6 %): Prepared by diluting the appropriate volumes of stock Na-Silicate (Sigma-Aldrich) solution to 250.0 mL with ultra-pure water.
8. Agarose (2.0g/90.0mL): Prepared by dissolving 2.0 g of agarose (Sigma-Aldrich) in 90.0 mL of ultra-pure water.
9. Triton X-100 (0.01 %): Prepared by diluting 1.0 mL of Triton X-100 to 100.0 mL with ultra-pure water.
10. Na-Citrate (0.1 M): Prepared by dissolving 2.941 g of Na-citrate tribasic dihydrate (Sigma-Aldrich) and diluting to 100.0 mL with ultra-pure water.
11. HCI (0.1 M): Prepared by diluting 0.835 μL of concentrated HCI to 100.0 mL with ultra-pure water.
12. $BaCI_2$ (0.2 M): Prepared by dissolving 1.221 g of $BaCI_2.2H_2O$ (Riedel-de Haën) and diluting to 25.0 mL with ultra-pure water.

Table 2.1. The properties of chemicals and reagents.

Item No	Reagent	Concentration	Company	Product Code	Cas No	Purpose of Use
1	$Pb(NO_3)_2$	4000.0 mg/L	Riedel-de Haën	31137	[1099-74-8]	Stock Solution
2	$CdCI_2$	4000.0 mg/L	Fluka	20899	[10325-94-7]	Stock Solution
3	$NiCI_2.6H_2O$	4000.0 mg/L	Carlo Erba	464645	[7791-20-0]	Stock Solution
4	HNO_3 (65%)	0.01, 0.10, 1.00 M	Merck	1.00456	[7697-37-2]	pH adjustment
5	HNO_3 (65%)	Concentrated	Merck	1.00456	[7697-37-2]	Sample acidification
6	NH_3	0.01, 0.10, 1.00 M	Merck	1.05422	[7664-41-7]	pH adjustment
7	H_2SO_4 (95-97%)	5.0 %	Riedel-de Haën	07208	[7664-93-9]	Silicate synthesis
8	HCI	0.1 M	Merck	1.00314	[7647-01-0]	Eluent
9	Na-Silicate	6.0 %	Sigma-Aldrich	338443	[61981-08-6]	Silicate synthesis
10	Agarose	2.0 g / 90.0 mL	Sigma-Aldrich	A5093	[9012-36-6]	Agarose bead synthesis
11	Na-Citrate tribasic dihydrate	0.1 M	Riedel-de Haën	32320	[6132-04-3]	Eluent
12	$BaCI_2.2H_2O$	0.2 M	Riedel-de Haën	11411	[10326-27-9]	Checking the presence of SO_4^{2-} in biomass immobilization
13	Triton X-100	0.01 %	Fluka	93420	[9002-93-1]	Elimination of oily phase from agarose bead

2.3. Instrumentation and Apparatus

A Thermo Elemental SOLAAR M6 series flame atomic absorption spectrometer (Cambridge, UK) was used in Pb, Cd and Ni determination throughout the study using their hollow cathode lamps at the wavelengths of 217.0, 228.8 and 232.0 nm, respectively. A deuterium lamp was used for background correction in all determinations. Other parameters applied in the determinations were as given in the operating manual of the instrument.

A Varian Liberty Series II Axial view ICP-OES (Palo Alto, CA, USA) was also used in the determinations at the wavelengths of 405.8, 228.8, 231.6 nm for Pb, Cd, and Ni, respectively.

The percentage elemental composition of the algae in terms of C, H, N, and S was determined with LECO-932 elemental analyzer (Mönchengladbach, Germany).

SEM characterization of the algae was carried out using Philips XL-30S FEG (Eindhoven, The Netherlands) prior to analysis.

A Perkin Elmer Spectrum 100 FTIR Spectrometer (Shelton, USA) with Pike MIRACLE Single Reflection Horizontal ATR Accessory at was used to identify the functional groups present in the biosorbents. The Diamond/KRS–5 Lens Single Reflection ATR Plate was used as the sample holder. The spectra of the biosorbents were taken in a 4000.0 cm^{-1} – 450.0 cm^{-1} range with a scan number of 4.0 and a resolution of 4.0 cm^{-1}.

A Perkin Elmer Pyris Diamond TG/DTA Thermogravimetric Analyzer (TGA) (Boston, MA, USA) was used to follow the changes in the weight of algae as a function of temperature.

In sorption studies with batch method, GFL 1083 water bath shaker equipped with a microprocesor thermostat (Burgwedel, Germany) was used to provide efficient mixing and the temperature is set to 25ºC. The pH measurements were performed by using WTW InoLab pH 720 precision pH meter (Weilheim, Germany).

2.4. Biosorption Studies

Biosorption studies were carried out to investigate the effect of initial metal ion concentration, amount of biomass, shaking (reaction) time, solution pH, reaction

temperature, and successive loadings. Sorption experiments were carried ot in 50.0 mL polyethylene centrifuge tubes. Solution pH was adjusted with dilute NH_3 and HNO_3. The volume of metal ion solutions was 10.0 mL, the amount of biomass was 10.0 mg, and the temperature was 25ºC, unless stated otherwise. Sorption studies with batch method was carried out by using GFL 1083 water bath shaker equipped with a microprocesor thermostat. After the sorption step, the biomass was separated from the solution by filtration and the filtrate was acidified with HNO_3 so as to contain 1.0 % HNO_3. Finally, the solutions were analyzed by flame atomic absorption spectrometry (FAAS).

For the evaluation of the performance of the biomasses in the removal of heavy metals, successive loading experiments were performed in a similar way as in the biosorption studies. The shaking time was 30 min, the amount of biosorbent was 10.0 mg, the solution volume was 10.0 mL, and the metal ion concentration was 10.0 mg/L. After each shaking, the mixture was filtered and the same biomass was used in the next run after being washed with ultra-pure water. This process was repeated for 5 consecutive cycles.

2.5. Desorption Studies

In recent years, biosorption is assumed to be an alternative route to the classical techniques for the treatment of wastewater. Therefore, the regeneration of the biomasses can have a crucial importance in lowering the running costs. In addition, regeneration of the biomass after the sorption of pollutants (e.g., heavy metal ions) with a suitable desorption solution will make it available for the next cycle. Of course, the desorption process must also be able to preconcentrate the metals, to be used repetitively and be free from decomposition and physical changes.

In the desorption of metals from the biomass, dilute solutions of mineral acids like hydrochloric, sulphuric, nitric, and acetic acid can be utilized (de Rome and Gadd 1987, Zhou and Kiff 1991, Luef, et al. 1991, Holan, et al. 1993, Pagnanelli, et al. 2003, Bai and Abraham, 2003). In this study, desorption studies were performed in a way that, 10.0 mg biosorbent were shaken with 10.0 mL of 10.0 mg/L metal ion solution for 30 minutes. After shaking and filtration steps, biosorbed metals were tried to be desorbed, in separate experiments, with 0.1 M HNO_3, 0.1 M HCI, and 0.1

M Na-citrate. Finally, the concentration of the metal ions in the filtrate was determined by FAAS.

2.6. Immobilization Studies

2.6.1. Immobilization of Biosorbents into Sodium-Silicate.

The method for biomass immobilization within the polysilicate matrix was the same as that reported by Karunasagar (2005) and Torresdey (1999). Briefly, 3.0 mL of 5.0 % H_2SO_4 were mixed with sufficient volume of 6.0 % sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. Here, the solution pH was checked with pH paper in order not to damage the electrode system of a pH meter due to use of silicate solution. Then, 200.0 mg of biomass were added to the silicate solution and stirred for 15 min. The pH was raised to 7.0 by a slow addition of 6.0 % sodium silicate. Meanwhile, a polymer gel was formed and washed with ultra-pure water thoroughly until the addition of barium chloride did not result in a cloudiness ($BaSO_4$) due to the presence of sulphate ion. The polymer gel with immobilized algae was dried overnight at 40ºC and ground by mortar and pestle. A blank silicate containing no algae was also prepared. Then, the particles were used in the sorption experiments. The typical structure of silica gel surface is shown in Figure 2.1.

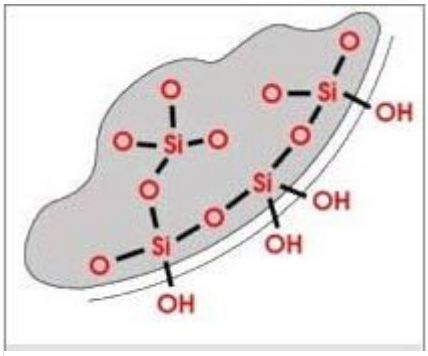


Figure 2.1. The structure of silica gel

2.6.2. Immobilization of Biosorbents into Agarose

Another immobilization method was also applied in which the algae was fixed in agarose (Lopez et al. 1997) (Figure 2.2 shows the structural unit of agarose (1 4)-3,6-anhydro-α-L-galactopyranosyl-(1 3)-β-D-galactopyranan). For this purpose, 2.0 g of agarose were dissolved in 90 mL of distilled water by heating at 100°C and after cooling to 40°C, 10 mL of cell suspension (1 g dry weight/10 mL) were added and mixed. The aqueous phase and the oily phase (vegetable oil, e.g. olive or sunflower), in a proportion of 10:1, were placed in a funnel (15 cm diameter) connected to a plastic tube closed with forceps (Figure 2.3). The cell-polymer mixture was added dropwise rapidly into the oil-water mixture, and the polymerization/solidification took place in the oily phase. When all gel-beads had been passed through the interface they were collected directly in the aqueous phase at the end of the plastic tube attached to the funnel. The gel-beads and a part of the aqueous phase were collected in a container. To eliminate the oily phase, the gel-beads were washed once with Triton X-100 (0.01 %) and three times with ultra-pure water. Blank agarose beads were also prepared without the addition of algae. Then, the beads were dried at 40ºC in an oven, and agarose immobilized with algae was used in the sorption experiments.

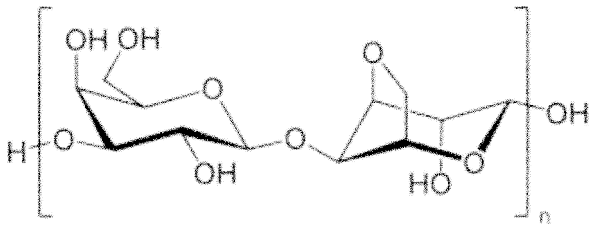


Figure 2.2. The structural unit of agarose.

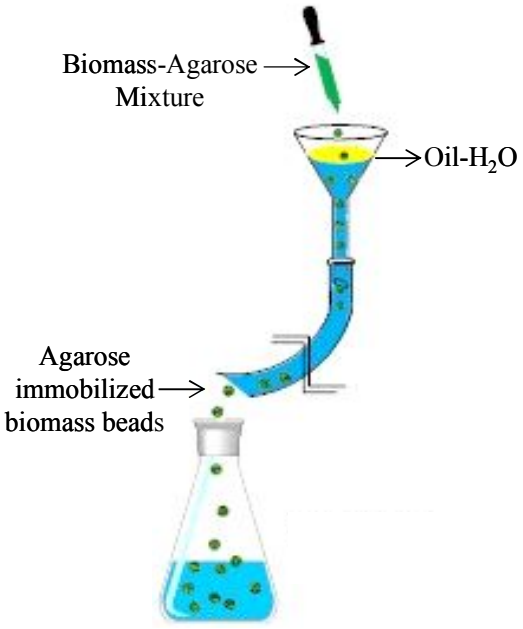


Figure 2.3. Apparatus used in the immobilization of algae into agarose by interphase technique

2.7. Sorption Isotherm Models

The sorption isotherms are the mathematical models which provides an explanation about the behaviour of adsorbate species between solid and liquid phases. Langmuir, Freundlich, and Dubinin-Radushkevich (D-R) isotherm models were studied for the investigation of biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Dunaliella salina*, *Oocystis sp.*, *Porphyridium cruentum*, and *Scenedesmus protuberans*.

Langmuir isotherm model is based on the complete monolayer coverage on the adsorbent surface. Langmuir isotherm model is expressed by the following equation 2.1 (Mumin, et al. 2007):

$$q_e = \frac{q_m K_a c_e}{1 + K_a c_e} \tag{2.1}$$

The above equation can be rearranged to the following linear form:

$$\frac{1}{q_e} = \frac{1}{K_a c_m} * \frac{1}{c_e} + \frac{1}{c_m} \tag{2.2}$$

where c_e is the equilibirium concentration (mg/L), q_e the amount of Pb^{2+}, Cd^{2+}, and Ni^{2+} ions biosorbed (mg/g), c_m is q_e for a complete monolayer sorption capacity (mg/g), K_a is a sorption equilibrium constant (L/mg). The values of c_m and K_a were determined from the intercept and the slope of $1/q_e$ versus $1/c_e$; the linearized form of Langmuir equation 2.2.

Freundlich isotherm model is based on the assumption of heterogeneous surfaces as well as multilayer sorption and also being the binding sites are not equivalent. It is expressed by the equation (Şeker, et al. 2008):

$$q_e = K_f c_e^{\,n} \tag{2.3}$$

The above equation can be rearranged to the following linear form:

$$\log q_e = \log K_f + n \log c_e \tag{2.4}$$

where K_f and n are isotherm constants, respectively. The values of K_f and n are calculated from the intercept and the slope of $\log q_e$ versus $\log c_e$; the linear form of Freundlich equation 2.4.

Another isotherm model applied in this study is Dubinin-Radushkevich isotherm model. The basic principles of this model is the Polany's adsorption potential theory and Dubinin's minipore filling theory. Dubinin-Radushkevich isotherm model can be given by the equation (Shahwan, et al. 2005):

$$q_e = c_m \exp(-k\varepsilon^2) \tag{2.5}$$

where

$$\varepsilon = RT \operatorname{Ln}(1 + 1/c_e) \tag{2.6}$$

The equation (5) can be rearranged to the following linear form:

$$\operatorname{Ln} q_e = \operatorname{Ln} c_m - k\varepsilon^2 \tag{2.7}$$

where c_m and k are the constants of Dubinin-Radushkevich isotherm model,respectively, the maximum adsorption capacity (mol/g) and the adsorption

energy. The constant k is related to the mean free energy of sorption per mole of the adsorbent as it is moved from infinite distance in the solution to the surface of the biomass, E, which can be calculated using the following relation:

$$E = 1/(2k)^{1/2} \tag{2.8}$$

The values of c_m and k are obtained from the slope and the intercept of $\ln q_e$ versus ε plot.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Characterization of Biosorbents

3.1.1. SEM Results

In a typical SEM analysis, the samples were introduced as powders fixed onto metal disks and then exposed to the electron beam. As seen from the micrographs given in Figure 3.1, the algae used in this study possess different textures and unit sizes.

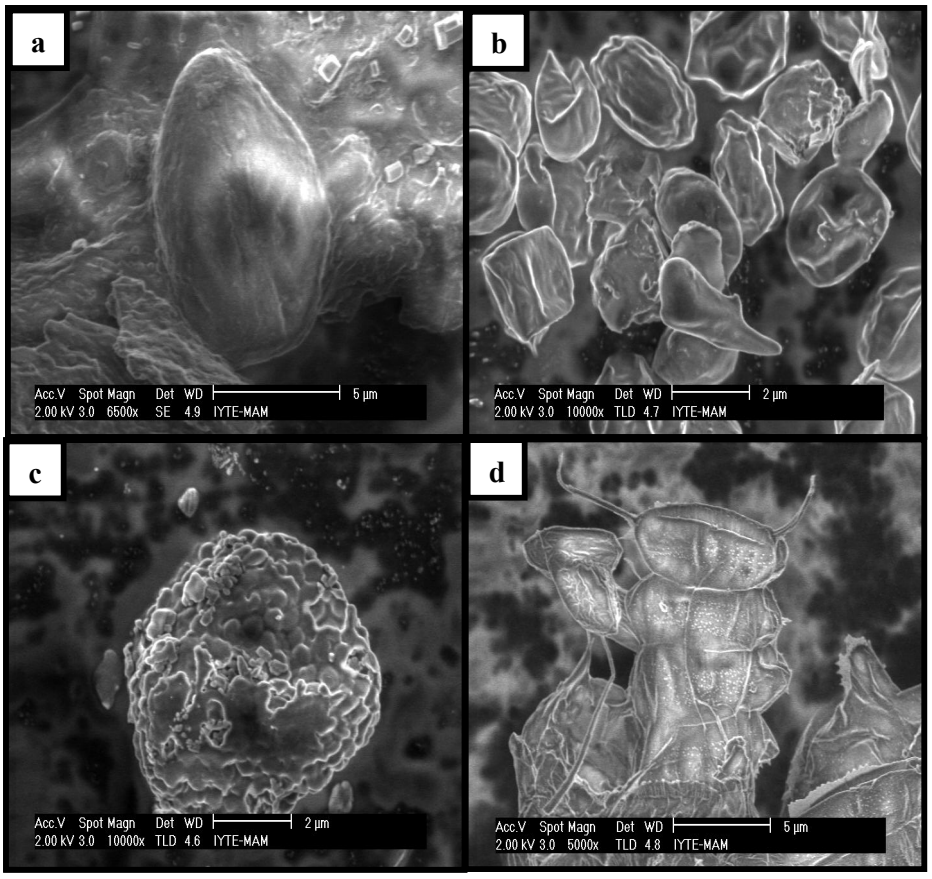


Figure 3.1. SEM images of the algae: (a) Dunaliella salina (b) Oocystis sp. (c) Porphyridium cruentum (d) Scenedesmus protuberans

3.1.2. FTIR Results

FTIR spectroscopy gives valuable information about the nature of the bonds present and allows identification of functional sites such as carboxyl, sulfonate, hydroxyl, and amino groups on the cell surface. These groups have been proposed to be responsible for the biosorption of metals by algae. Their relative importance in the biosorption of metals might rely on the parameters such as the quantity and availability of the binding sites, chemical site, and affinity between the metal and the functional site.

FTIR technique has been widely utilized to detect vibrational frequency changes in seaweeds (Park, et al. 2004; Sheng, et al. 2004; Figuera, et al. 1999). The organic functional groups and the corresponding IR frequencies observed in seaweeds (literature values) and in the biomasses used in this study are tabulated in Table 3.1. Almost all of the frequencies obtained from *Dunaliella salina, Oocystis sp., Porphyridium cruentum,* and *Scenedesmus protuberans* are in accordance with each other and with the literature values. Strong similarities among the FTIR spectra can also be seen in Figure 3.2. The region between 3200-3500 cm^{-1} exhibits the stretching vibration of O-H and N-H which also confirms the presence of hydroxyl and amine functional groups in the algal structure. The region between 3000-2800 cm^{-1} shows the C-H stretching vibrations of sp^3 hybridized C in CH_3 and $>CH_2$ functional groups. The peaks at 1652 cm^{-1} (for *Dunaliella salina*), 1642 cm^{-1} (for *Oocystis sp.*), 1638 cm^{-1} (for *Porphyridium cruentum*), and 1647 cm^{-1} (for *Scenedesmus protuberans*) reveal the presence of carbonyl group. The presence of amide in the structure of each alga is confirmed by the peak at 1545 cm^{-1} (for *Dunaliella salina*), 1544 cm^{-1} (for *Oocystis sp.*), 1542 cm^{-1} (for *Porphyridium cruentum*), and 1542 cm^{-1} (for *Scenedesmus protuberans*). The absorption peaks around 1240 cm^{-1} and 1150 cm^{-1} indicate the phosphate esters in *Dunaliella salina*, *Oocystis sp.*, and *Scenedesmus protuberans*. The phosphate esters are the source of phosholipids. The absorption peaks at the respective frequencies of 1075 cm^{-1} and 1076 cm^{-1} confirm the presence of sulfoxides in *Dunaliella salina* and *Porphyridium cruentum*. The observed frequencies in FTIR spectra of the algae used indicate the presence of amine ($R–NH_2$), amide ($R_1(CO)NR_2R_3$) (aminoacids, proteins, glycoproteins, etc.), carboxylic acids (fatty acids, lipopolysaccharides, etc.), sulfoxides and phosphates.

Table 3.1. IR stretching frequencies for seaweeds and the algae used in this study

Assignment	Wavenumber (cm^{-1})				
	Seaweeds[a-f]	***Dunaliella salina***	***Oocystis sp.***	***Porphyridium cruentum***	***Scenedesmus protuberans***
·Bonded –OH, –NH stretching[a]	3280	3280	3280	3320	3279
·Asymmetric stretching of aliphatic chains (–CH)[b]	2920	2921	2922	2925	2920
·Symmetric stretching of aliphatic chains (–CH)[b]	2854	—	2852	—	2851
·C=O stretching of COOH[c]	1740	—	—	—	—
·Asymmetric C=O[c]	1630	1652	1642	1638	1647
·Amide II[a] C–N stretching	1530	1545	1544	1542	1542
·Symmetric C=O[c]	1450	1462	1453	1420	1452
·Asymmetric $–SO_3$ stretching[d]	1371	1374	—	—	—
·C–O stretching of COOH[c], ·Phosphate esters[e]	1237	1262	1243	—	1242
·Symmetric $–SO_3$ stretching[d], ·P=O stretching (aliphatic)[e]	1160	—	1150	—	1150
·C–O (ether)[a], ·Amine (C–N)[f], ·S-O (sulfoxides)[e],	1117	1075	—	1076	—
·C–O (alcohol)[a], ·P-O-C (aliphatic)[e]	1033	1020	1019	1032	1022
·S=O stretching[d]	817	850	—	—	—

[a] Sheng, et al. 2004
[b] Pons, et al. 2004
[c] Fourest and Volesky 1996
[d] Figuera, et al. 1999
[e] Silverstein and Webster 1997
[f] Solomons and Fryhle 1998

3.1.3. Elemental Analysis

The elemental compositions of the algae used in this study are given in Table 3.2. *Dunaliella salina*, Oocystis sp. and *Scenedesmus protuberans* have higher carbon percentages as 40.19%, 47.66%, and 47.52%, respectively. *Dunaliella salina* has the highest nitrogen content among all. When compared with the other biosorbents a higher sulphur percentage (1.57%) is found in *Porphyridium cruentum* which is due to the presence of sulphated polysaccharides in the biosorbent structure.

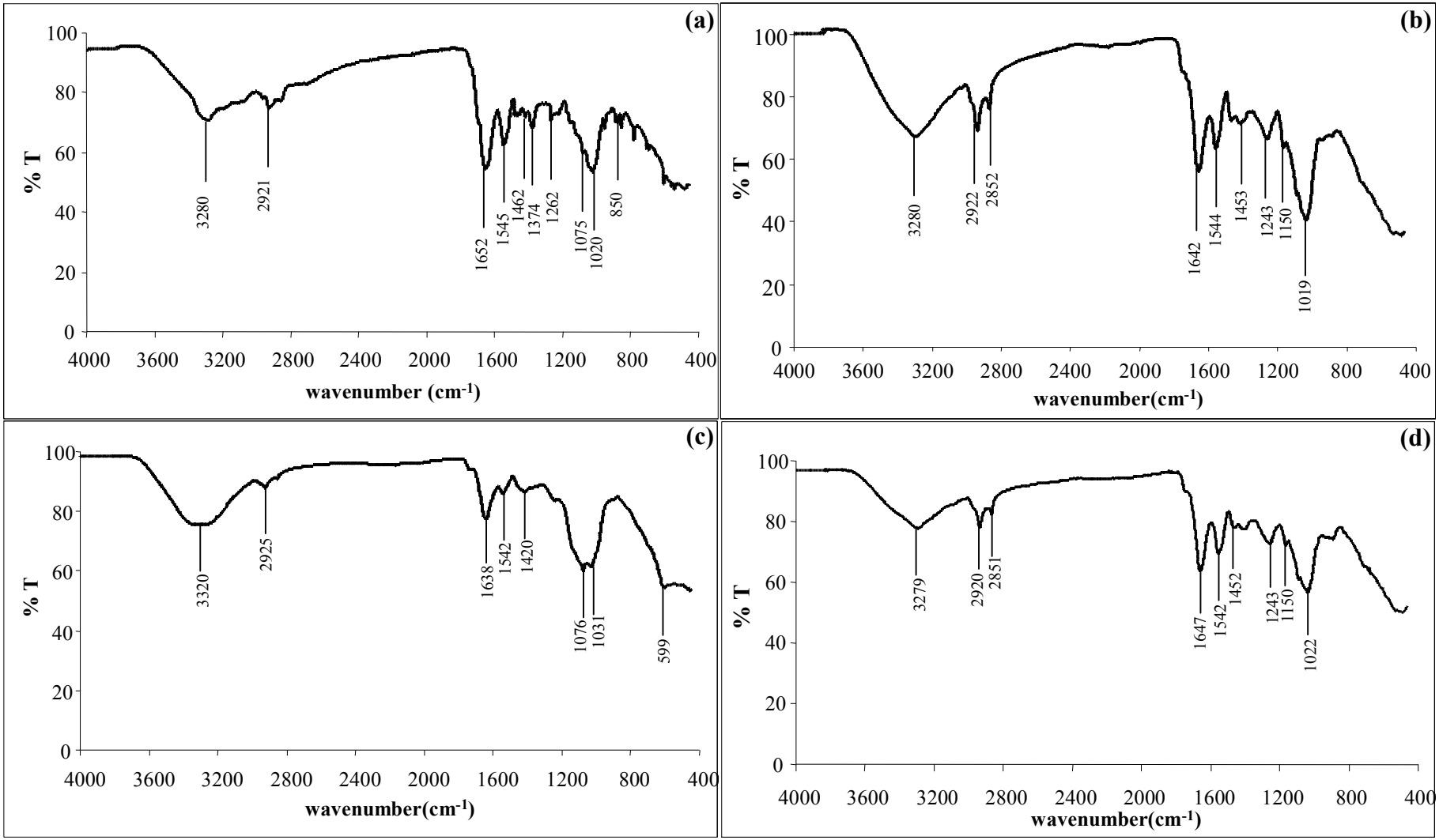


Figure 3.2. IR Spectra of (a) *Dunaliella salina*, (b) *Oocystis sp.*, (c) *Porphyridium cruentum*, (d) *Scenedesmus protuberans*

Table 3.2. Elemental composition of biosorbents in terms of C, H, N, and S

Biosorbents	% C	% H	% N	% S
Dunaliella salina	40.19	5.79	**9.19**	0.76
Oocystis sp.	**47.66**	**7.18**	5.49	0.57
Porphyridium cruentum	14.45	3.11	1.30	**1.57**
Scenedesmus protuberans	**47.52**	**7.02**	**7.18**	0.66

3.1.4. Thermo Gravimetric Analysis (TGA)

Thermal stability of the algae was investigated using TGA in which the mass loss of the sample is monitored as a function of temperature. The solid samples were heated from 25ºC to 750ºC in 10 minute intervals in N_2 atmosphere. The plots are given in Figure 3.3–3.6.

In Figure 3.3, the first mass loss of *Dunaliella salina* which is less than 10% in the temperature range of 25ºC-100ºC is due to the release of bound water molecules. The largest loss can be seen in 240ºC-560ºC, and then in 560ºC-630ºC which can be attributed to the decomposition of the organic part of the sample.

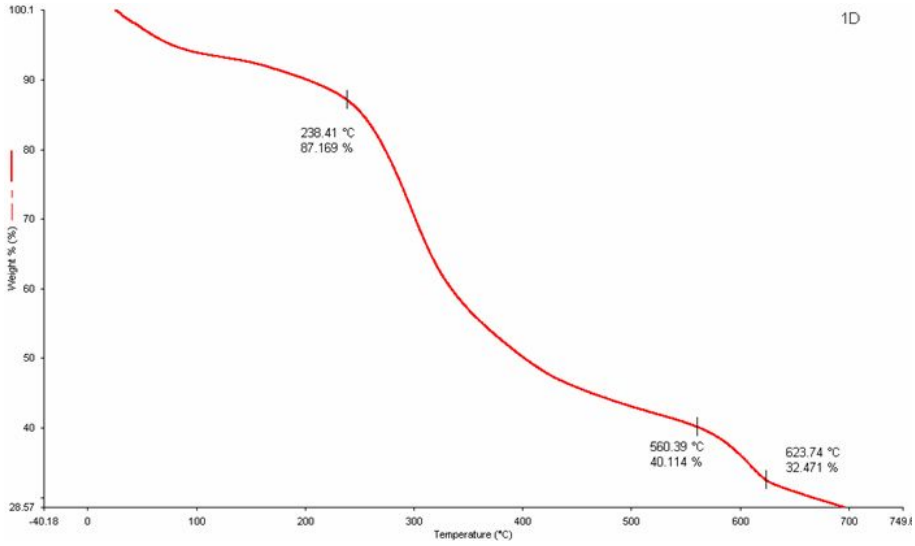


Figure 3.3. TGA decomposition curve of *Dunaliella salina*

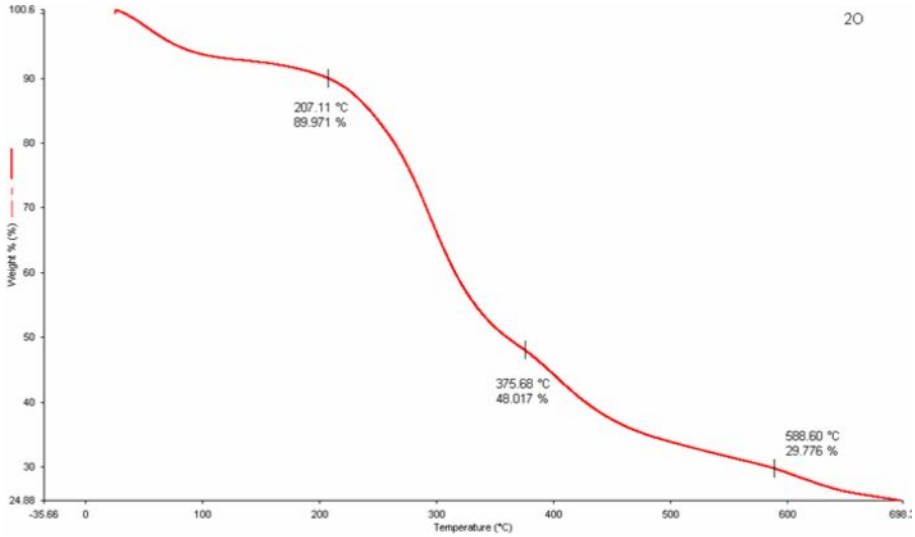


Figure 3.4. TGA decomposition curve of *Oocystis sp.*

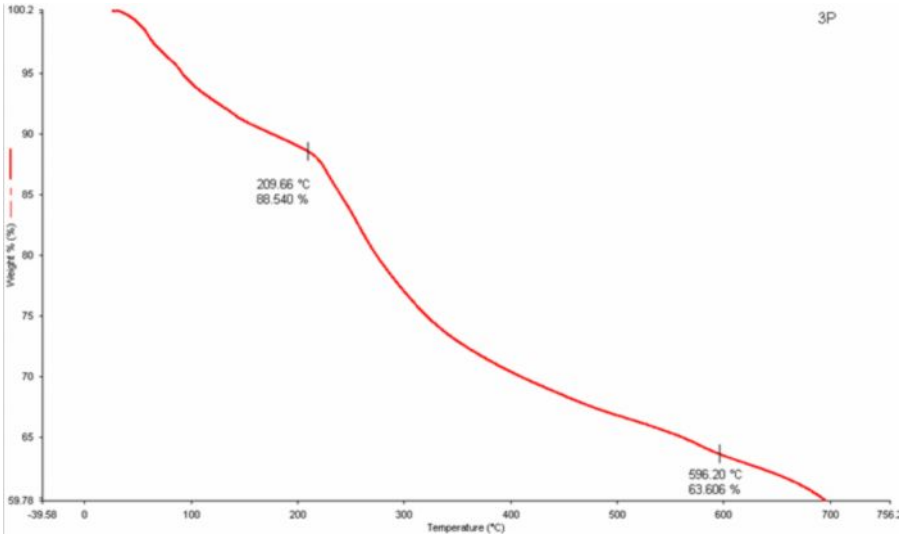


Figure 3.5. TGA decomposition curve of *Porphyridium cruentum*

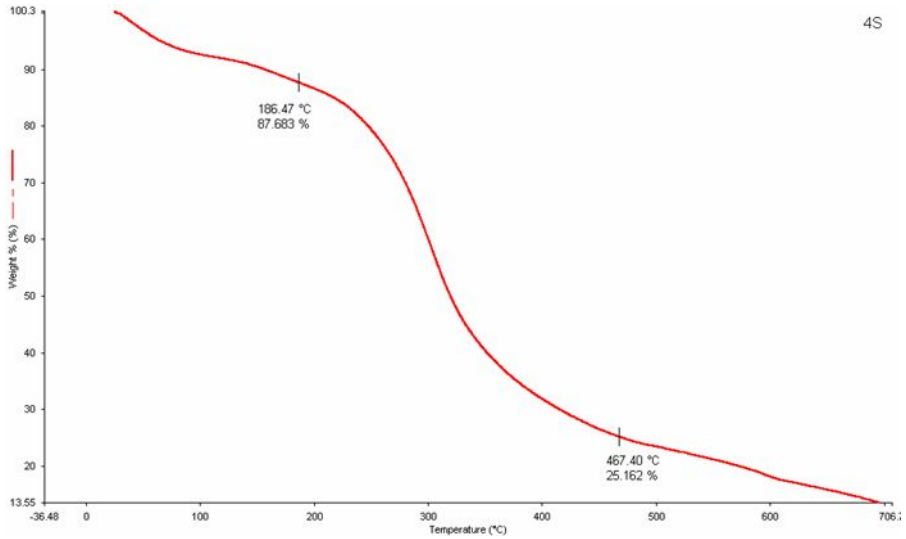


Figure 3.6. TGA decomposition curve of *Scenedesmus protuberans*.

Figure 3.6, the decomposition curve of *Scenedesmus protuberans*, shows that the mass loss in this alga in the same temperature range is higher than any of the other algae. As with the others, the first decrement in mass observed in 25ºC-100ºC belongs to the loss of water and is about 10%.

3.2. Parameters Affecting Biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+}

Apart from the principal parameters affecting biosorption, such as the type and the form of the biomass and the metal ions, the conditions under which the biosorption process occurs are accounted on the efficiency of the process. The predominant parameter of all is the pH of the solution. Moreover, the other factors such as the type and the amount of the competing ions present also influence the sorption of the analyte metal ion(s). This situation either increase or decrease the metal uptake.

3.2.1. Effect of Solution pH

Solution pH should be taken into account as one of the most important parameters that affect biosorption since the pH of the solution may change the form, thus the chemistry of the target metal ion as well as the binding site(s) on the biomass. In order to examine the effect of solution pH, sorption experiments were performed at

different pHs while keeping the other parameters constant. Solution temperature, solution volume, biosorbent amount, initial metal ion concentration, and shaking time were 25ºC, 10.0 mL, 10.0 mg, 10.0 mg/L, and 60 min, respectively. For Pb^{2+}, pH of the solution was varied from 2.0 to 6.0, while for Cd^{2+} and Ni^{2+} the range was 2.0 to 12.0.

Three of the algae, namely *Dunaliella salina*, *Oocystis sp.* and *Scenedesmus protuberans* have demonstrated a very similar sorption characteristics towards the metal ions investigated. The "sorption % vs. pH" plots have reached a plateau around pH=4.0 (Figure 3.7). After this pH, the change in the sorption percentage was not very significant. On the other hand, the maximum sorption with *Porphyridium cruentum* could only be obtained after a pH of 10.0 for both Cd ve Ni. As with the other metal ions, even *Porphyridium cruentum* exhibited a very efficient sorption for Pb^{2+} at pH=4.0. Another critical point with Pb^{2+} ions was that the pH values greater than 6.0 was not applied in the sorption experiments due to the possibility of formation of Pb-hydroxides. Actually, during the initial stages of optimization for Pb sorption, when the pH of the solution was adjusted to 8.0, an immediate formation of a white cloudiness, possibly due to the precipitation of $Pb(OH)_2$, was observed.

Although it has been shown that any pH greater than 4.0 could be used for sorption, pH of 6.0 was selected as a compromise. As explained before, higher pHs were not applied due to the possibility of formation of metal hydroxides. It can be said that if the objective is the removal of toxic metal ions from the solutions, it does not matter whether it is the biosorption of the metal ions or the precipitation of metal hydroxides causing the removal. However, in a study like this, the aim is to enlighten the actual mechanism responsible for the elimination of the pollutants. Therefore, the subsequent studies were focused on the understanding of the characteristics of sorption by the biomasses. A possible mechanism might be the electrostatic attraction between the metal ions in the solution and the functional groups of the biomass. It has been reported that the isoelectronic point (IEP) of algae lies between 3.0–4.0, and at pH values greater than the IEP of that specific alga, functional groups on its surface will be negatively charged (Christ, et al. 1981, Forster 1997). This leads to electrostatic attraction between the metal cations and the negatively charged functional groups on the biomass. The speciation diagrams of Pb, Cd, and Ni given in Figure 3.8 indicate the pH dependent forms of these metals in the solution. As can be seen from the diagrams, the predominant forms of the metals at pH 6.0 are +2 oxidation state; namely, Pb^{2+}, Cd^{2+}, and Ni^{2+}. These diagrams, together with the "% sorption vs. pH graph" have proven that

the underlying mechanism of sorption is the electrostatic attraction between (+2) charged ions and (–) charge of the surface.

3.2.2. Effect of Shaking Time

In order to find out the time required for the sorption equilibrium to be reached, biosorption experiments were carried out as a function of shaking time. A quantity of 10.0 mg of biomass was added to 10.0 mL of 100.0 mg/L Pb^{2+}, Cd^{2+}, and Ni^{2+} solutions having a pH of 6.0 at 25ºC and the samples were shaken for 1, 5, 15, 30, and 60 min. The results given in Figure 3.9 demonstrate the very fast kinetics of sorption. Less than 5 min is sufficient for the attainment of the equilibrium for the metal ions by all the algae employed. Still, a shaking time of 60 min was used to be on the safe side.

3.2.3. Effect of Initial Metal Ion Concentration

The extent of removal of heavy metals from aqueous solution depends strongly on the initial metal ion concentration. In order to assess this, sorption experiments were performed at the initial metal ion concentration of 10.0, 50.0, 100.0, 150.0, 200.0, 250.0, and 500.0 mg/L at pH 6.0 with 10.0 mg of biosorbent added into 10.0 mL solutions at 25ºC. According to Figure 3.10–3.13, metal sorption firstly raises with the increase in the metal ion concentration after which a saturation point is approached at a certain point. *Dunaliella salina* has a capability to biosorb the metal ions in greater amount compared with the other biosorbents, and this behaviour also indicates a very high sorption capacity towards Pb^{2+} ions. *Oocystis sp.*, *Scenedesmus protuberans,* and *Porphyridium cruentum* follow *Dunaliella salina* in terms of sorption capacity. The surface of the algal cell wall contains several functional groups which play a role in the sorption process. The number of available functional groups decreases with the increase in the initial metal ion concentration and this is confirmed by the decrease in the percentage sorption with an increase in the initial metal ion concentration, although the amount of sorbed metal increases in the meantime. Compared to the other metal ions, Ni has always shown a lower sorption with any of the algae applied. This behaviour can be explained by the lower selectivity of the algae for Ni.

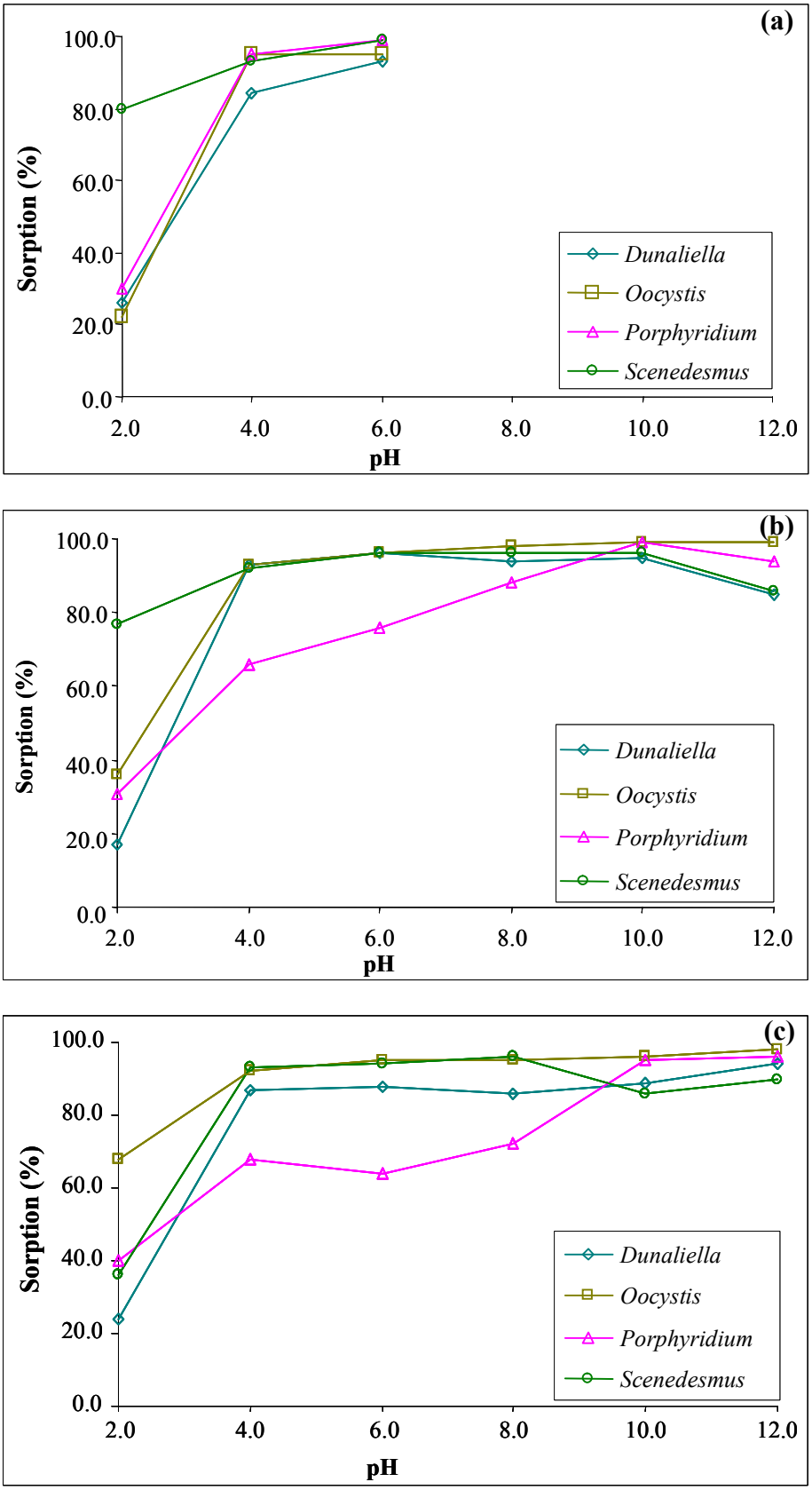


Figure 3.7. Effect of solution pH on the biosorption of (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} (10.0 mg of biosorbent, 10.0 mL metal ion solution of 10.0 mg/L concentration, sorption time of 60 min)

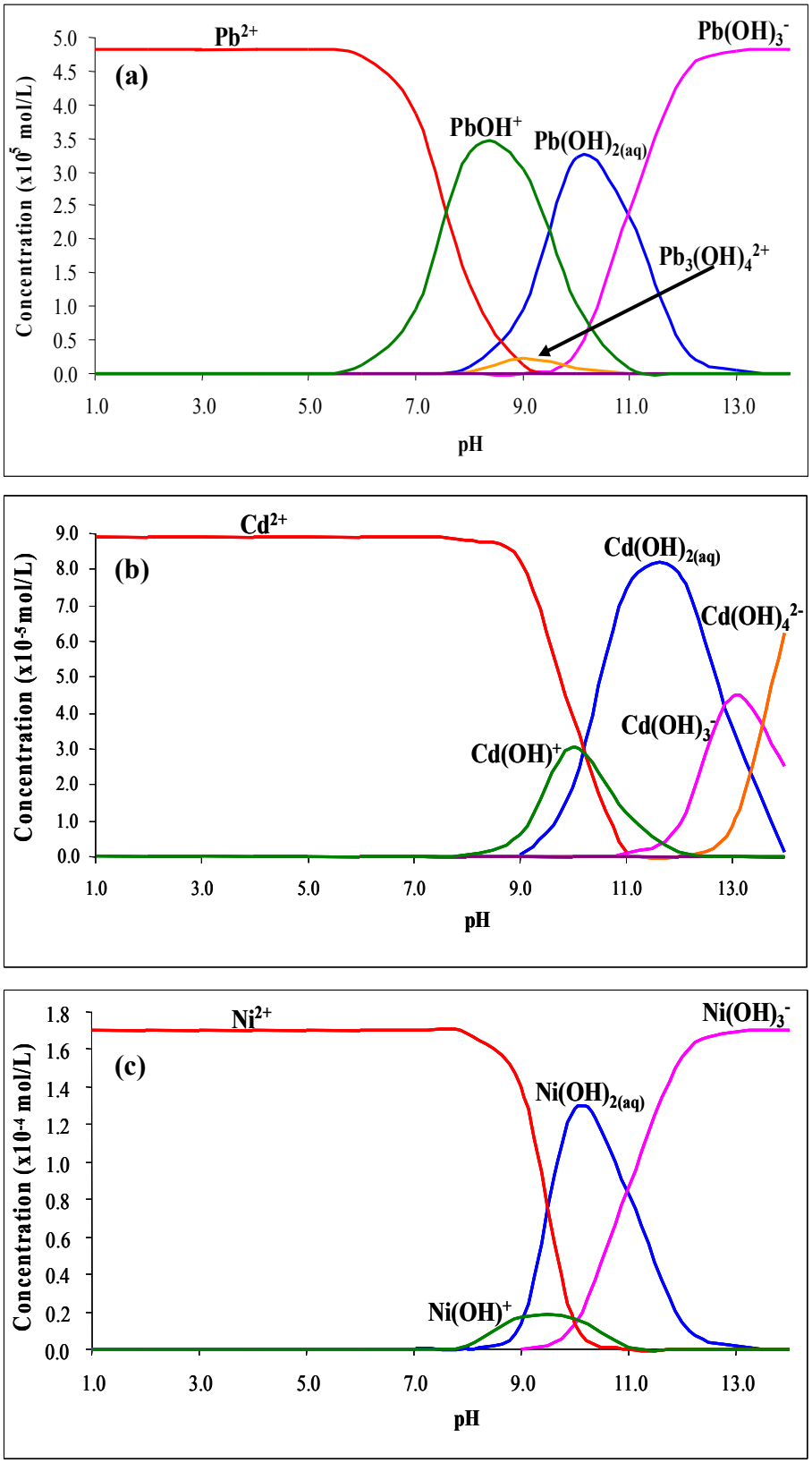


Figure 3.8. The speciation diagrams of (a) Pb, (b) Cd, and (c) Ni (4.83×10^{-5} mol Pb^{2+}/L=10.0 mg Pb^{2+}/L, 8.90×10^{-5} mol Cd^{2+}/L=10.0 mg Cd^{2+}/L, 1.70×10^{-4} mol Cd^{2+}/L=10.0 mg Cd^{2+}/L)
(Source:Visual Minteq Program)

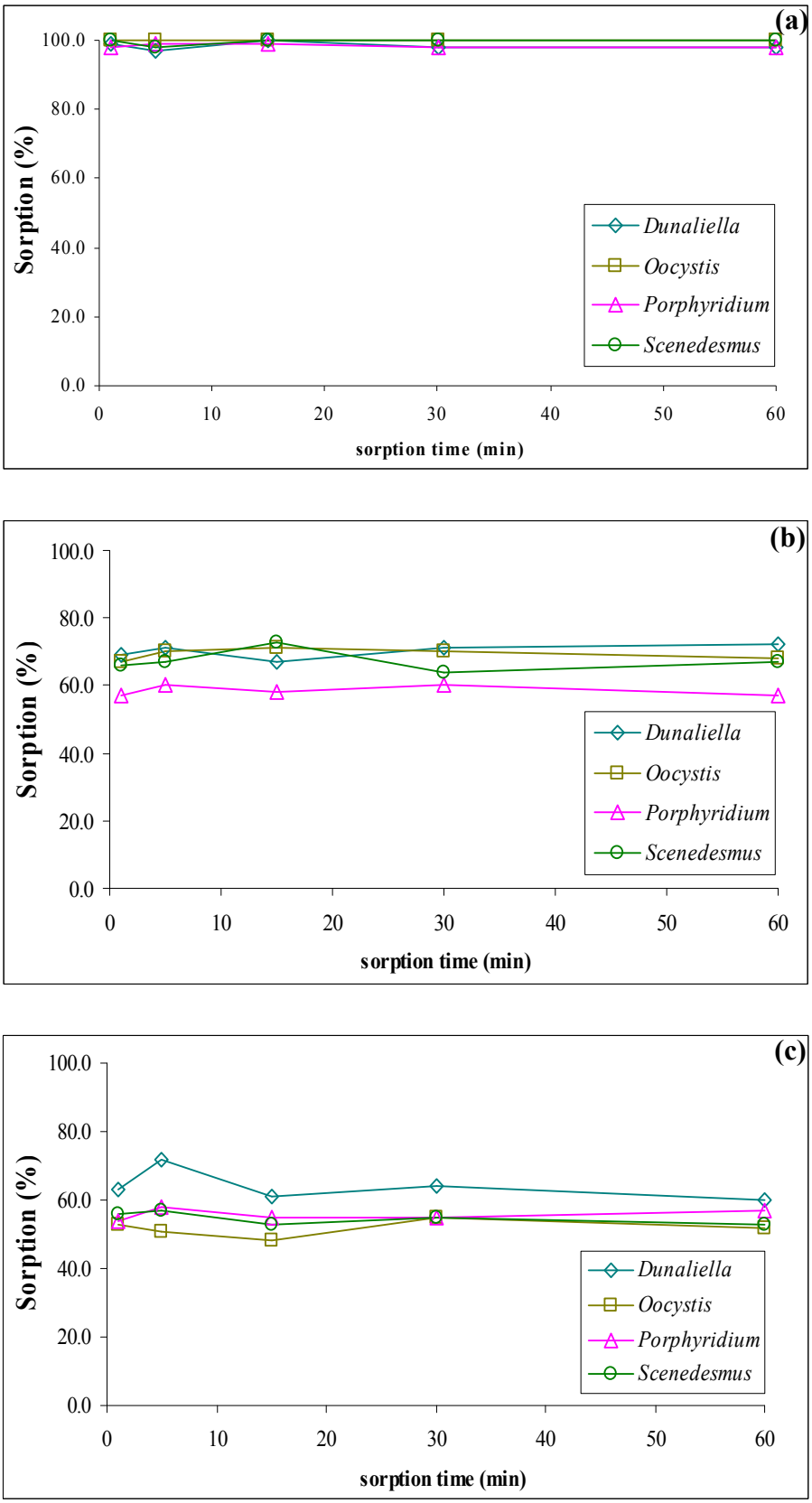


Figure 3.9. Effect of shaking time on biosorption of (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} by algae (10.0 mg of biosorbent, 10.0 mL metal ion solution of 100.0 mg/L metal ion concentration, solution pH of 6.0)

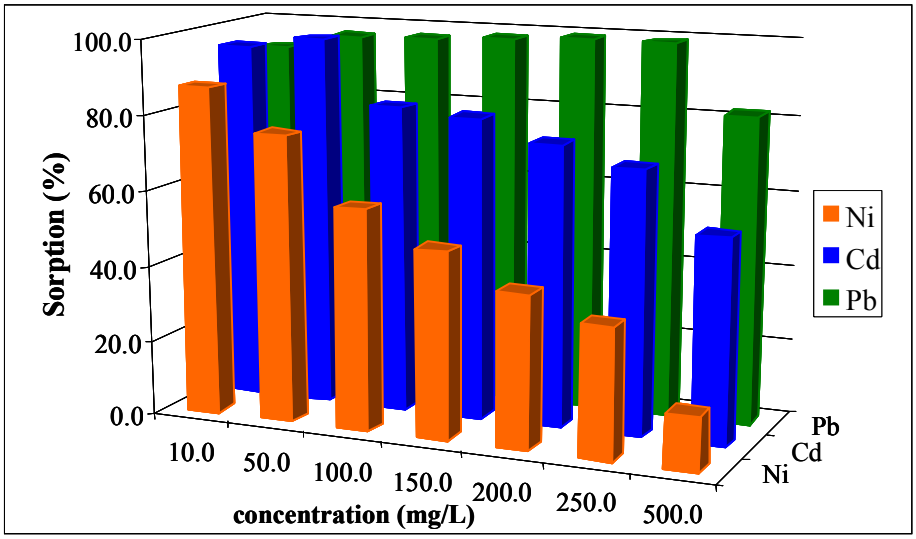


Figure 3.10. Effect of initial metal ion concentration on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Dunaliella salina* (10.0 mg of biosorbent, sorption time of 60 min, solution pH of 6.0, 10.0 mL of solution with different metal ion concentrations)

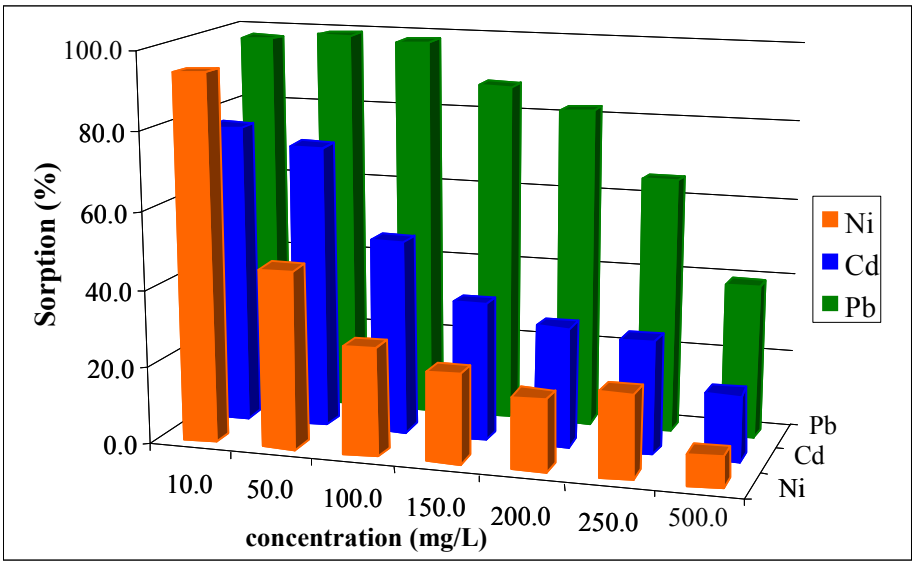


Figure 3.11. Effect of initial metal ion concentration on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Oocystis sp.* (10.0 mg of biosorbent, sorption time of 60 min, solution pH of 6.0, 10.0 mL of solution with different metal ion concentrations)

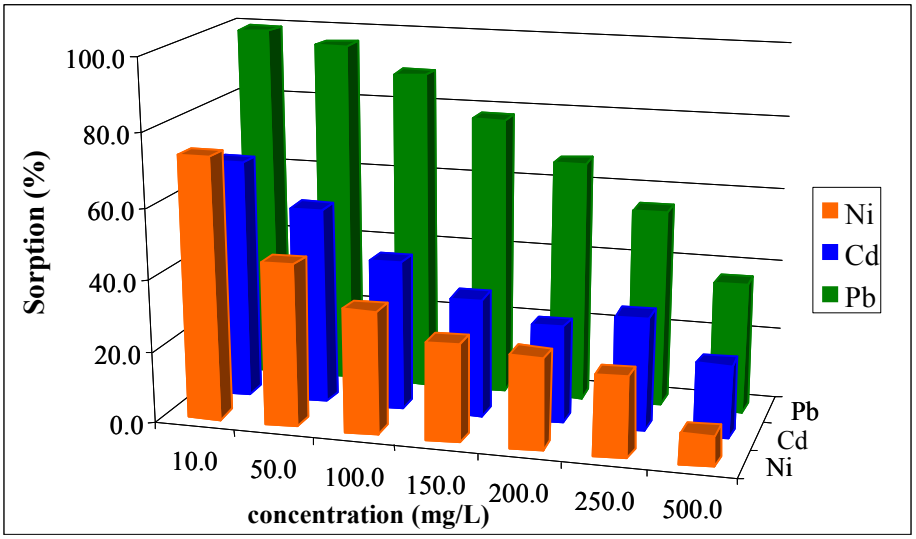


Figure 3.12. Effect of initial metal ion concentration on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Porphyrdium cruentum* (10.0 mg of biosorbent, sorption time of 60 min, solution pH of 6.0, 10.0 mL of solution with different metal ion concentrations)

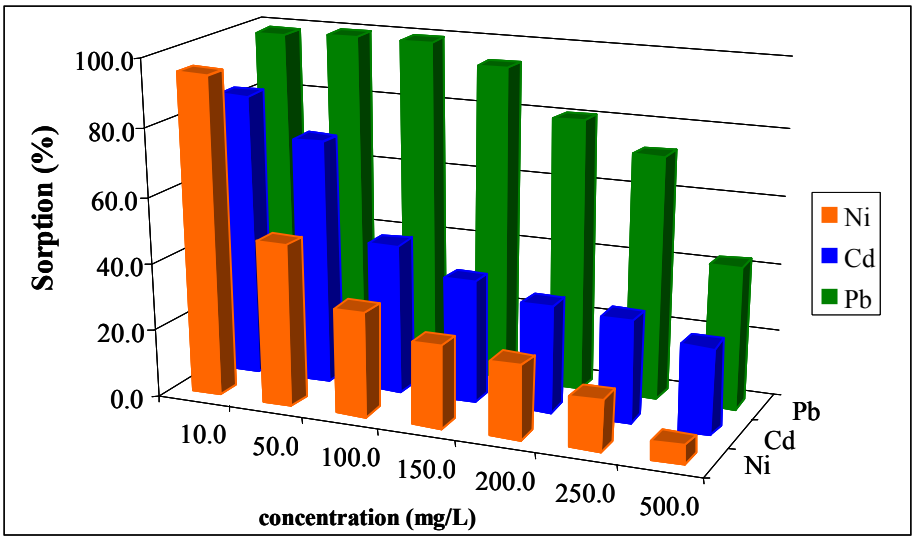


Figure 3.13. Effect of initial metal ion concentration on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Scenedesmus protuberans* (10.0 mg of biosorbent, sorption time of 60 min, solution pH of 6.0, 10.0 mL of solution with different metal ion concentrations)

3.2.4. Effect of Biomass Amount

A part of the experiments was performed by changing the biosorbent dose to figure out how the sorption process could be affected. Different amounts of biosorbents

such as 10.0, 20.0, 50.0, 100.0, and 200.0 mg were shaken with 10.0 mL solution of 250.0 mg metal/L at 25ºC for 60 min, and the results are given in Figure 3.14-3.17. The results showed that the percentage sorption of Pb^{2+} ions by *Dunaliella salina* increases 20.0 mg biosorbent dose, then it stays constant from 20.0 mg to 200.0 mg biosorbent dose. The percentage uptake of Pb^{2+} ions by *Oocystis sp., Porpyridium cruentum, and Scenedesmus protuberans*, shows an exponential growth. Cd^{2+} and Ni^{2+} sorption is lower in comparison with Pb^{2+}. A relationship can also be given between a biomass amount and sorption due to the fact that the availability of the metal ion might be limited with increased electrostatic interactions, interference between binding sites, and reduced mixing at higher biosorbent dose. Furthermore, electrostatic interactions between cells could be considered as significant for the biomass-dependent metal uptake. With a large amount of metal ion which is biosorbed if the distance between the cells is greater, otherwords lower amount of biosorbent is used which is enough for the sorption takes place. Even though an increased biosorbent dose has an reducing role on the sorption capacity of a biosorbent, and in this study, the total removal of metal ion by a biosorbent is higher at higher biosorbent dose. Hence, this behaviour can be explained that there is no such a correlation between biosorbent amount and metal removal (Madrid and Camara 1997).

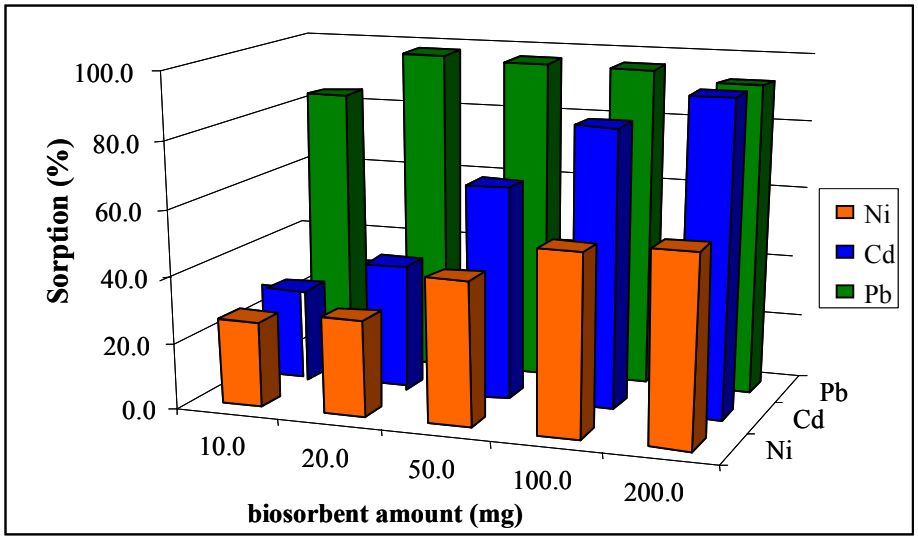


Figure 3.14. Effect of biosorbent amount on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Dunaliella salina* (10.0 mL solution of 250.0 mg/L, solution pH of 6.0, sorption time of 60 min, and different amounts of biomass)

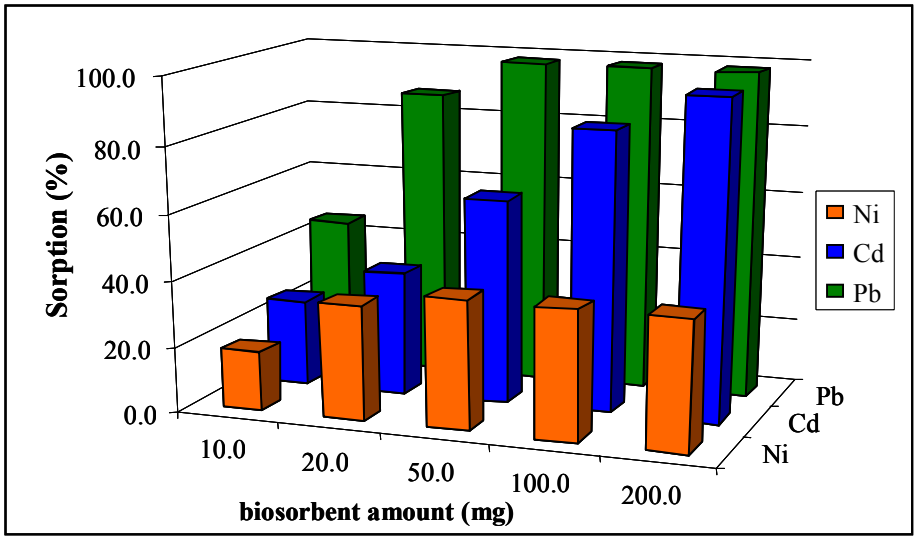


Figure 3.15. Effect of biosorbent amount on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Oocystis sp.* (10.0 mL solution of 250.0 mg/L, solution pH of 6.0, sorption time of 60 min, and different amounts of biomass)

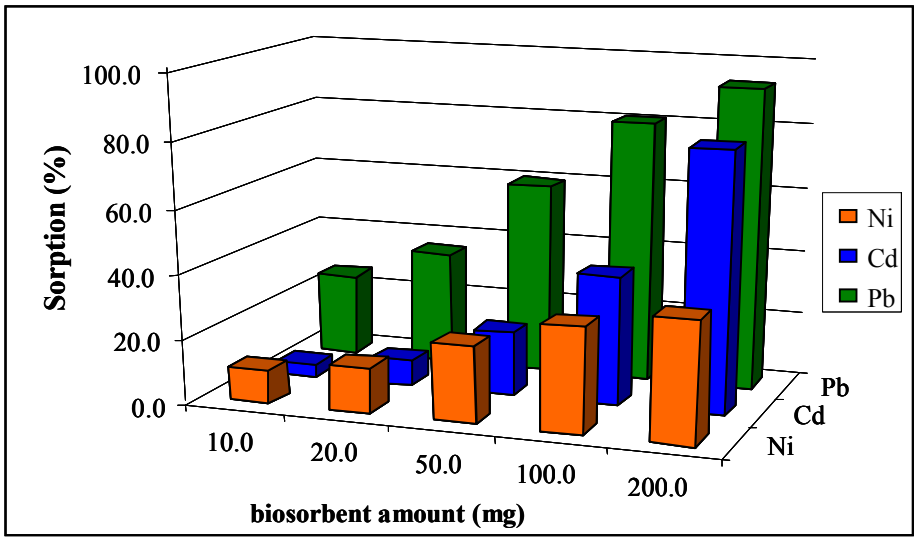


Figure 3.16. Effect of biosorbent amount on biosorption of Pb^{2+}, Cd^{2+} , and Ni^{2+} by *Porphyridium cruentum* (10.0 mL solution of 250.0 mg/L, solution pH of 6.0,sorption time of 60 min, and different amounts of biomass)

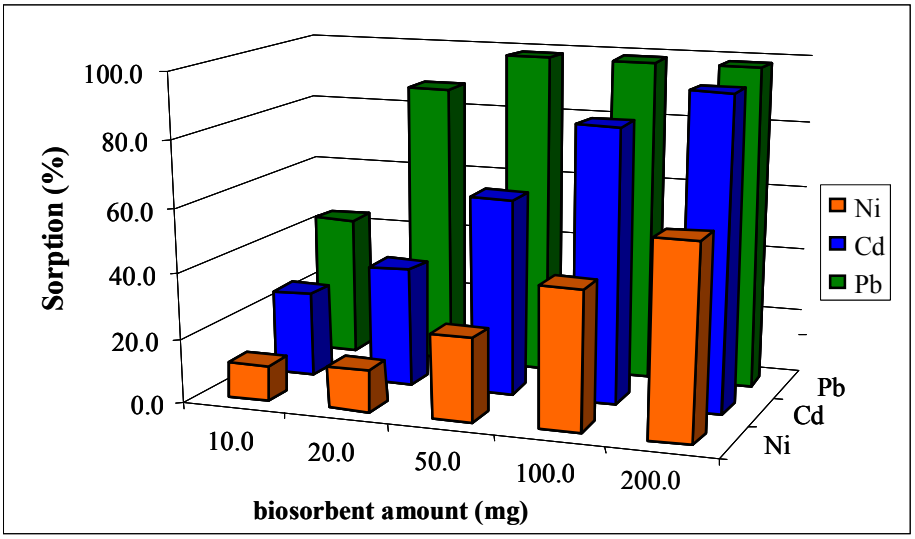


Figure 3.17. Effect of biosorbent amount on biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Scenedesmus protuberans* (10.0 mL solution of 250.0 mg/L, solution pH of 6.0, sorption time of 60 min, and different amounts of biomass)

3.3. Desorption Studies for *Oocystis sp.* and *Scenedesmus protuberans*

For the evaluation of the efficiency and feasibility of a metal removal procees, the regeneration and reusability of the biosorbent should be considered. In addition to this, the recovery of the metals which has either an economical importance or a vital role in the environment should be emphasized. Hence, the desorption of metal ions bound on the biosorbent is applied to re-solubilise biosorbent-bound metals by suitable eluant or desorbing solution. Therefore, biosorbent can be utilized in multiple sorption – desorption cycles. A common example method for the desorption of heavy metal from the biomass is the treatment of biomass after sorption cycle in acidic pH. Increasing the acidity of the metal-loaded biosorbent suspension leads to separation of metal cations from biosorbent by protons from the binding sites (Mehta and Gaur 2005). Notwithstanding, at extremely low pH values, the biomass can be damaged. On the other hand, organic and mineral acids, bases, salts, and metal chelators have been studied for their ability on the desorption of metals (Kuyucak and Volesky 1989, Hu and Reeves 1997, Esteves and Valdman 2000). Hence, as the different eluents should be screened for their ability for both maximum recovery of metal ions and the protection of biomass after sorption-desorption cycle (Mehta and Gaur 2005).

For the recovery of metal ions from the biosorbent, at first sorption experiment was performed using 10.0 mL of 100.0 mg/L metal ion solution at pH 6.0 contacted with 10.0 mg of biosorbent at 25ºC. Then, the desorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} was carried out with 10.0 mL of 0.100 M HNO_3, 0.100 M HCI, and 0.100 M Na-citrate for 15 min and the obtained results are given in Figure 3.18-3.19.

From the result, the desorption of Pb^{2+} and Ni^{2+} from *Oocystis sp.* is higher when compared with Cd^{2+}. In the case of *Scenedesmus protuberans*, Pb^{2+} and Cd^{2+} recovered in greater amount than Ni^{2+}. Lower recovery of Ni^{2+} from *Scenedesmus protuberans* can be due to the higher affinity of Ni^{2+} for this algae.

However, the treatment of *Oocystis sp.* and *Scenedesmus protuberans* with HNO_3 and HCI damaged the algae, while Na-citrate treatment for the recovery of Pb^{2+}, Cd^{2+}, and Ni^{2+} from *Oocystis sp.* and *Scenedesmus protuberans* did not damage the biosorbents.

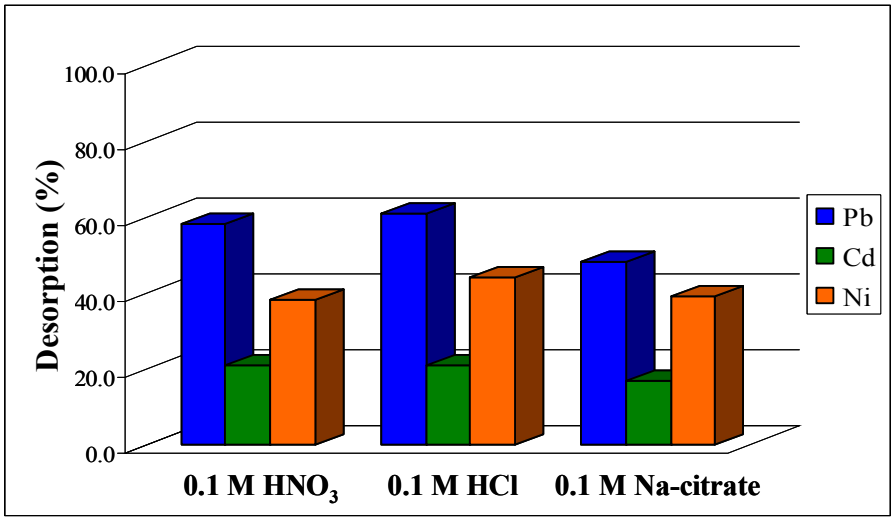


Figure 3.18. Desorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} from *Oocystis sp.* using 0.100 M HNO_3, 0.100 M HCI, and 0.100 M Na-citrate

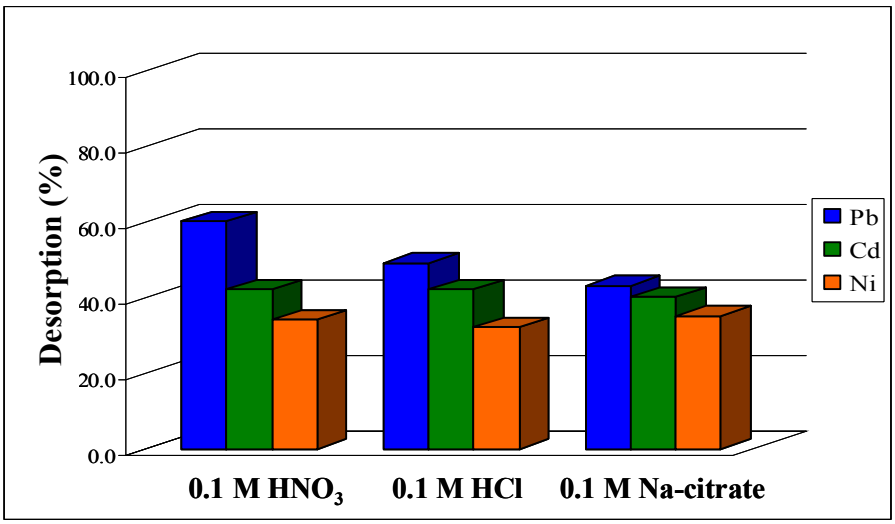


Figure 3.19. Desorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} from *Scenedesmus protuberans* using 0.100 M HNO_3, 0.100 M HCI, and 0.100 M Na-citrate

3.4. Competitive Biosorption: Three-Metal Ion System

Competitive biosorption of Pb^{2+}, Cd^{2+}, Ni^{2+} by the biosorbents were carried out at three different metal ion concentrations. In the experiments, a 10.0 mg of biosorbent was allowed to be treated with a 10.0 mL mixed metal ion solution at 25ºC. The results are given in Table 3.3 - 3.6 with the sorption of a single metal ion system for the sake of comparison.

In general, the presence of other ions in the solution influences the biosorption of target metal ion. From the results, at low cocentration of metal ions present in the mixed system, the biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by all biosorbents does not seem to be affected much. However, when the concentration of three metal ions in the mixed solution is raised the percentage sorption declines. In all cases, the removal of Pb^{2+} by all biosorbents from the solution is not influenced, and this result can be due to the higher selectivity of the biosorbents towards Pb^{2+}.

Table 3.3. Competitive biosorption of Pb^{2+} + Cd^{2+} + Ni^{2+} *by Dunaliella salina* (10.0 mL solution of different mixed-metal ion concentration, solution of 6.0, and sorption time as 60 min)

Initial concentration of Pb^{2+} + Cd^{2+} + Ni^{2+} (mg/L)	**% Sorption**					
	Pb^{2+}		**Cd^{2+}**		**Ni^{2+}**	
	Single	**Mixed**	**Single**	**Mixed**	**Single**	**Mixed**
10.0	92.6 ± 0.0	97.9 ± 0.2	95.2 ± 0.6	100.0 ± 0.0	87.2 ± 0.1	91.3 ± 0.3
50.0	96.5 ± 0.5	100.0 ± 0.0	98.2 ± 1.6	79.3 ± 1.7	76.3 ± 0.7	58.7 ± 1.0
100.0	96.9 ± 0.5	100.0 ± 0.0	81.8 ± 1.5	50.9 ± 4.0	58.7 ± 1.1	33.8 ± 2.5

Table 3.4. Competitive biosorption of Pb^{2+} + Cd^{2+} + Ni^{2+} *by Oocystis sp.* (10.0 mL solution of different mixed-metal ion concentration, solution pH of 6.0,and sorption time as 60 min)

Initial concentration of Pb^{2+} + Cd^{2+} + Ni^{2+} (mg/L)	**% Sorption**					
	Pb^{2+}		**Cd^{2+}**		**Ni^{2+}**	
	Single	**Mixed**	**Single**	**Mixed**	**Single**	**Mixed**
10.0	97.8 ± 0.8	100.0 ± 0.0	77.4 ± 0.4	94.0 ± 0.0	94.3 ± 0.2	77.8 ± 0.1
50.0	99.3 ± 0.3	98.9 ± 0.1	73.4 ± 2.2	33.7 ± 0.5	46.0 ± 2.3	27.4 ± 0.6
100.0	98.2 ± 1.0	92.8 ± 0.6	50.5 ± 0.6	14.6 ± 1.2	28.1 ± 1.0	13.3 ± 1.1

Table 3.5. Competitive biosorption of Pb^{2+} + Cd^{2+} + Ni^{2+} *by Porphyridium cruentum* (10.0 mL solution of different mixed-metal ion concentration, solution pH of 6.0, and sorption time as 60 min)

Initial concentration of Pb^{2+} + Cd^{2+} + Ni^{2+} (mg/L)	**% Sorption**					
	Pb^{2+}		**Cd^{2+}**		**Ni^{2+}**	
	Single	**Mixed**	**Single**	**Mixed**	**Single**	**Mixed**
10.0	99.6 ± 0.1	99.7 ± 0.3	67.0 ± 0.2	73.5 ± 0.1	73.3 ± 1.0	61.7 ± 0.2
50.0	96.3 ± 1.5	92.1 ± 0.5	55.1 ± 1.0	26.0 ± 0.7	45.3 ± 1.0	28.1 ± 0.7
100.0	89.4 ± 2.6	74.2 ± 2.8	42.1 ± 0.6	8.3 ± 5.0	34.5 ± 1.0	13.3 ± 1.8

Table 3.6. Competitive biosorption of Pb^{2+} + Cd^{2+} + Ni^{2+} *by Scenedesmus protuberans* (10.0 mL solution of different mixed-metal ion concentration, solution of 6.0, and sorption time as 60 min)

Initial concentration of Pb^{2+} + Cd^{2+} + Ni^{2+} (mg/L)	**% Sorption**					
	Pb^{2+}		**Cd^{2+}**		**Ni^{2+}**	
	Single	**Mixed**	**Single**	**Mixed**	**Single**	**Mixed**
10.0	98.6 ±0.1	100 ± 0.0	84.6 ± 0.2	100.0 ±0.0	95.0 ± 0.1	87.5 ± 0.2
50.0	99.6 ± 0.2	99.8 ± 0.2	73.2 ± 0.0	40.4 ± 0.6	48.5 ± 0.4	28.7 ± 0.3
100.0	99.5 ± 0.5	94.8 ± 0.6	44.8 ± 1.1	13.0 ± 1.2	31.5 ± 0.8	10.1 ± 1.2

3.5. Successive Loading

Successive loading of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Dunaliella salina*, *Oocystis sp.* *Porphyridium cruentum*, *Scenedesmus protuberans* for initial metal ion concentration as 10.0 mg/L was examined as explained in section 2.4. The results are shown in Figure 3.20-3.23. *Dunaliella salina* has an ability to continue to biosorb Pb^{2+}, Cd^{2+} ions from the solution in higher than Ni^{2+} ions after five successive runs performed. However, in the third cycle, biosorption of Pb^{2+} by *Dunaliella salina* is lowered.

Repetitive loading of Pb^{2+} and Cd^{2+} on the biosorbents *Oocystis sp.* and *Scenedesmus protuberans* does not appear to be affected significantly even after five successive loadings. On the contrary, biosorption of Ni^{2+} by *Oocystis sp.* starts to decline at the fifth successive loading, whereas that of Ni^{2+} by *Scenedesmus protuberans* does at the fourth cycle.

In the case of *Porphyridium cruentum*, at the first cycles, Cd^{2+}, and Ni^{2+} ions are removed in lower amount, then at the later runs, Cd^{2+}, and Ni^{2+} removal increases. This behaviour can be attributed to the presence of Na^{+} ions present in the biomass of *Porphyridium cruentum*. In Pb^{2+} case, the biosorbent continues to hold most of its original uptake capacity over five cycles of use in the biosorption of Pb^{2+} (removes the ions almost totally for five cycles). To confirm the lower sorption of Cd^{2+} and Ni^{2+} ions by *Porhyridium cruentum*, the experiments were continued at 10.0 - 100.0 mg/L metal ion concentrations for 10 successive loadings. The results are shown in Figure 3.24-3.25. It can be seen that, after eight successive runs were applied the sorption of Cd^{2+} ions levelled off. This situation can also be seen for the sorption of Ni^{2+} ions at fourth successive runs passed. If the concentration of the metal ions was increased, the sorbed

metals ions was started to pass the solution. This pheneomenon started to take place at the tenth successive loading for Cd^{2+} and at the ninth successive loading for Ni^{2+} were performed.

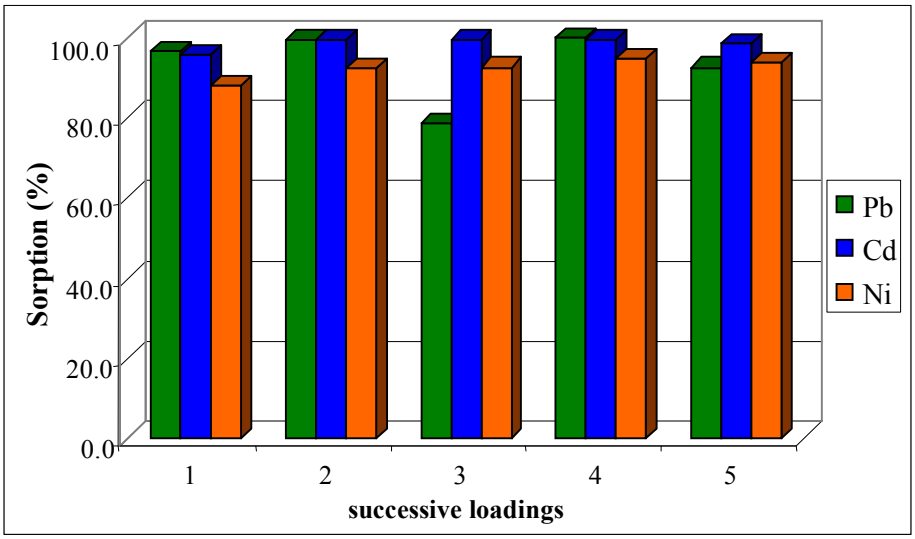


Figure 3.20. Repetitive biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Dunaliella salina* (10.0 mL solution of 10.0 mg/L metal ion concentration, solution pH of 6.0, sorption time of 30 min., and successive loadings)

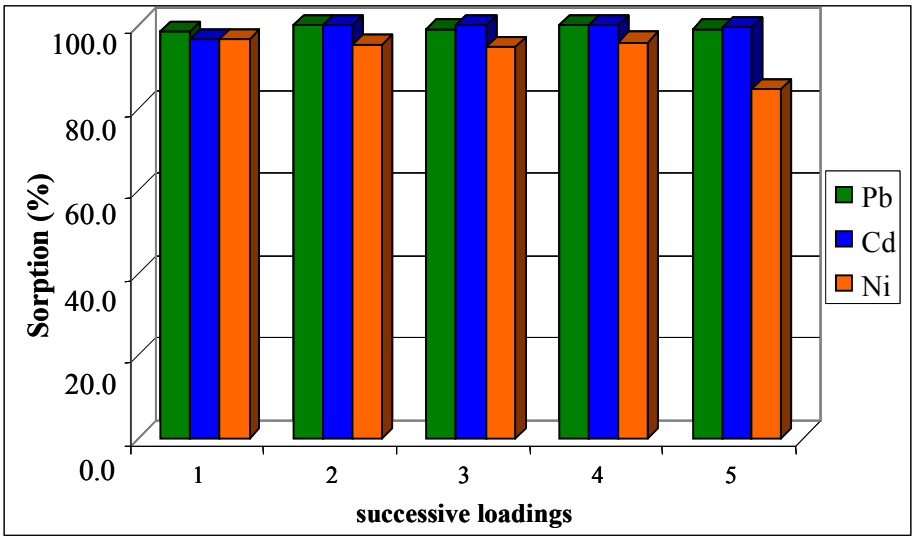


Figure 3.21. Repetitive biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Oocystis sp.* (10.0 mL solution of 10.0 mg/L metal ion concentration, solution pH of 6.0, sorption time of 30 min., and successive loadings)

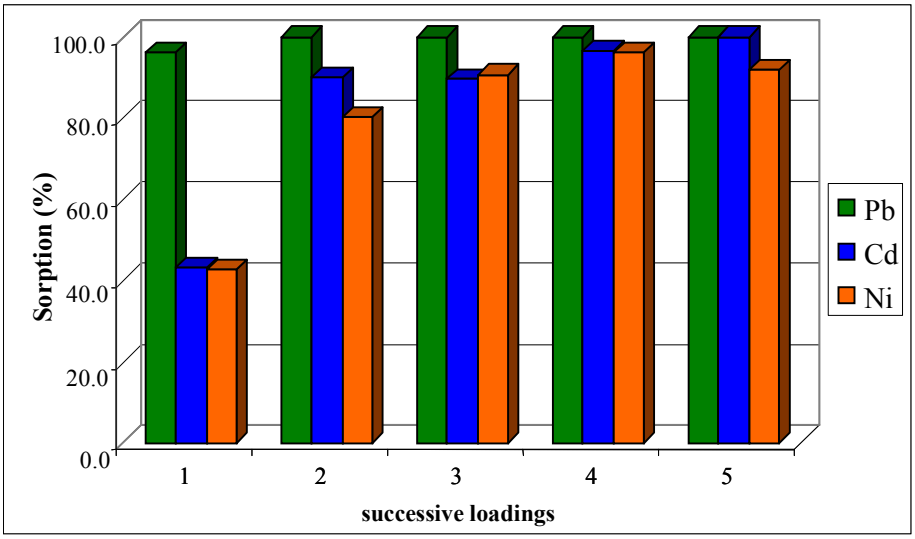


Figure 3.22. Repetitive biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Porphyridium cruentum* (10.0 mL solution of 10.0 mg/L metal ion concentration, solution pH of 6.0, sorption time of 30 min., and successive loadings)

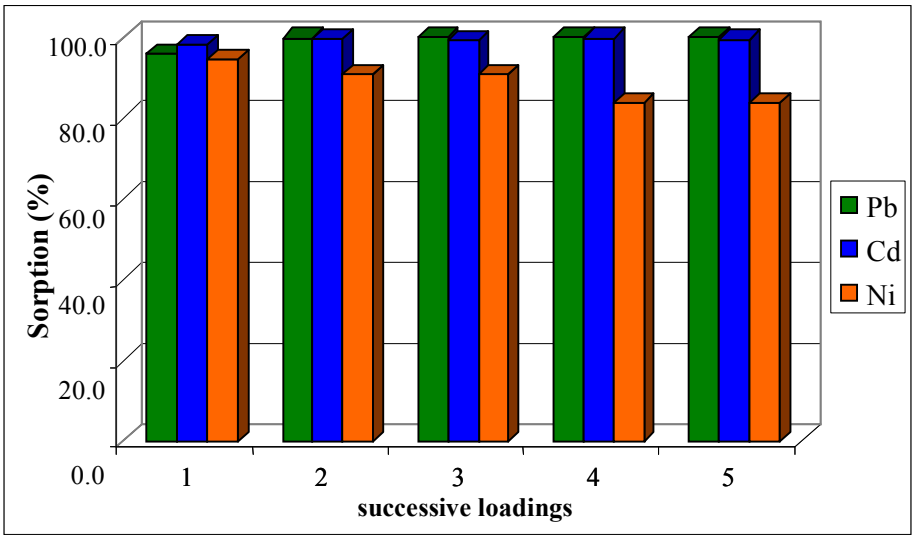


Figure 3.23. Repetitive biosorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by *Scenedesmus protuberans* (10.0 mL solution of 10.0 mg/L metal ion concentration, solution pH of 6.0, sorption time of 30 min., and successive loadings)

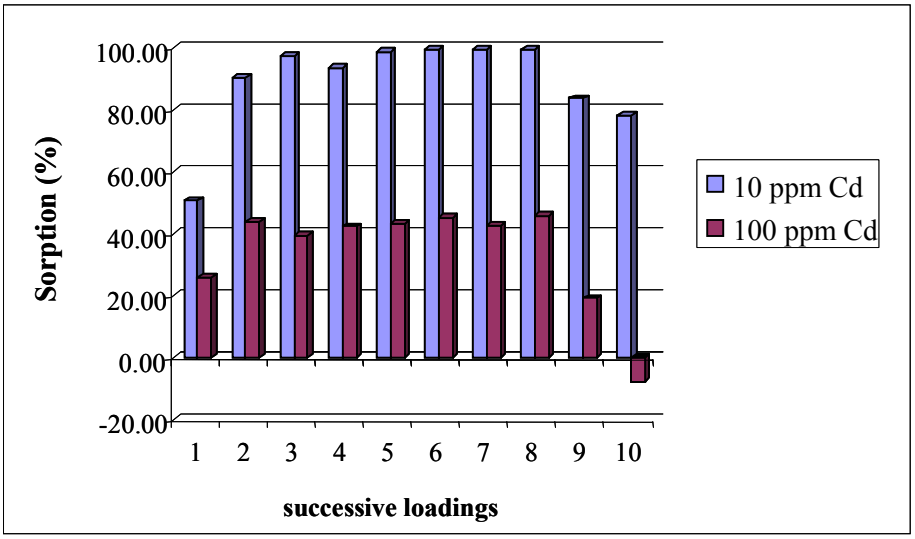


Figure 3.24. Percent biosorption of Cd^{2+} by *Porphyridium cruentum* (10.0 mL solution of 10.0-100.0 mg/L metal ion concentrations, solution pH of 6.0, sorption time of 30 min., and 10 successive loadings)

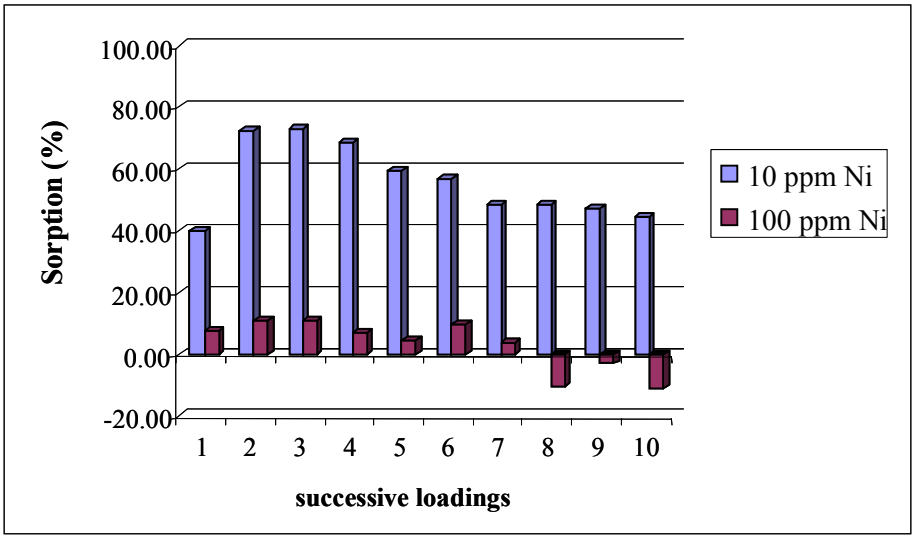


Figure 3.25. Percent biosorption of Ni^{2+} by *Porphyridium cruentum* (10.0 mL solution of 10.0-100.0 mg/L metal ion concentrations, solution pH of 6.0, sorption time of 30 min., and 10 successive loadings)

3.6. Immobilization Studies

Biosorption studies were carried on with immobilized algae, *Oocystis sp.* and *Scenedesmus protuberans* into Na-silicate and agarose.

3.6.1. Immobilization of *Oocystis sp.* and *Scenedesmus protuberans* into Sodium-Silicate.

In this study, *Oocystis sp.* and *Scenedesmus protuberans* were immobilized into sodium-silicate matrix. The SEM images (Figure 3.26) of Na-silicate immobilized *Oocystis sp. Scenedesmus protuberans*. It can be observed that the algal cells were fixed to Na-silicate matrix. From Table 3.7,The results demonstrates that the immobilization of the *Oocystis sp.* and *Scenedesmus protuberans* into sodium silicate appears to increase the biosorption of Pb^{2+} in great manner in comparison to that of Cd^{2+},and Ni^{2+} (In Table 3.7., the results of the sorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by both Na-silicate immobilized and free algae).

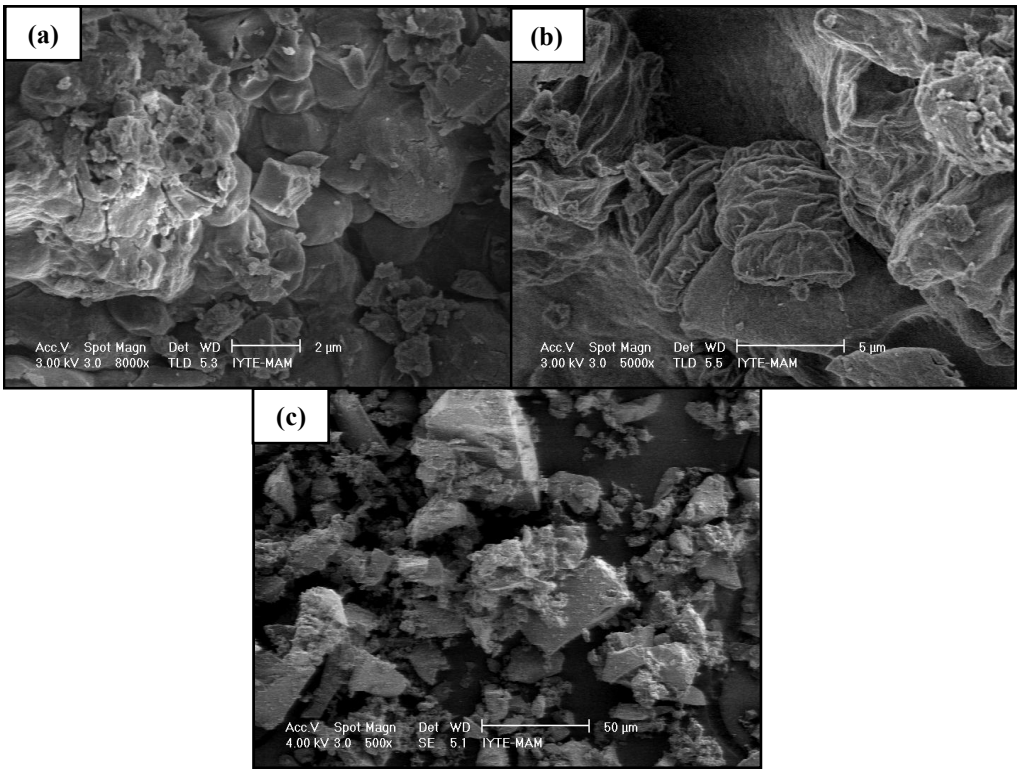


Figure 3.26. SEM images of Na-silicate immobilized biosorbent: (a) *Oocystis sp.,* (b) *Scenedesmus protuberans*, and SEM image of (c) blank Na-silicate

Table 3.7. Biosorption data of Pb^{2+}, Cd^{2+},and Ni^{2+} by both immobilized with Na-silicate and free algae (10.0 mL solution of 250.0 mg/L metal ion concentration, soltion pH of 6.0, sorption time as 60 min)

	Sorption (%)		
Biosorbents	**Pb**	**Cd**	**Ni**
Na-silicate	100.0 ± 0.0	33.0 ± 2.0	41.0 ± 2.0
Oocystis sp.	67.6 ± 3.4	29.2 ± 1.7	21.7 ± 3.5
Na-silicate+*Oocystis sp.*	97.0 ± 3.0	47.0 ± 0.0	43.0 ± 3.0
Scenedesmus protuberans	72.3 ± 1.4	31.3 ± 2.8	15.3 ± 2.8
Na-silicate+*Scenedesmus protuberans*	98.0 ± 1.0	45.0 ± 1.0	41.0 ± 2.0

3.6.2. Immobilization of *Oocystis sp.* and *Scenedesmus protuberans* into Agarose

In Table 3.8., the results correspond to the sorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} by both agarose immobilized and free algae. The immobilized *Oocystis sp.* into agarose appears to not change the sorption ability of *Oocystis sp.* towards Pb^{2+} and Ni^{2+} ions so much. However, in the case of Cd^{2+} , the sorption by *Oocystis sp* seems to be affected, and the immobilization has a rising effect on the sorption of $Cd^{2+}$by *Oocystis sp.*. It has been indicated that agarose immobilized *Scenedesmus protuberans* shows no change in the sorption of Pb^{2+} and Cd^{2+} ions. It can be seen that, from Table 3.8, Ni^{2+} sorption was not altered so much by the agarose immobilization of *Scenedesmus protuberans*.

Table 3.8. Biosorption data of Pb^{2+}, Cd^{2+},and Ni^{2+} by both immobilized with agarose and free algae (10.0 mL solution of 250.0 mg/L metal ion concentration, solution pH of 6.0, sorption time as 60 min)

	Sorption (%)		
Biosorbents	**Pb**	**Cd**	**Ni**
Agarose	57.0 ± 3.0	26.0 ± 1.0	27.0 ± 0.0
Oocystis sp.	67.6 ± 3.4	29.2 ± 1.7	21.7 ± 3.5
Agarose + *Oocystis sp.*	62.0 ± 5.0	46.0 ± 2.0	27.0 ± 2.0
Scenedesmus protuberans	72.3 ± 1.4	31.3 ± 2.8	15.3 ± 2.8
Agarose + *Scenedesmus protuberans*	72.0 ± 4.0	28.0 ± 3.0	28.0 ± 1.0

3.7. Sorption Isotherm Results

The Langmuir, Freundlich, and Dubinin-Radushkevich isotherm models were employed for the investigation of the sorption equilibrium between the metal ion solution and the biomass, in this study. The data obtained from the sorption experiments are used in the linear fits (3.27-3.38.) of the isotherm models. The constants obtained from the linear fits are tabulated in the Table 3.9. with their correlation coefficients, R^2, and are used for the evaluation of the nonlinear fits (Figure 3.39.-3.42.) of the isotherm models. The nonlinear fits are given with the experimental results to understand the behaviour of the sorption isotherm model with the expermental result.

Dunaliella salina has an ability to biosorb Pb^{2+} from the aqueous medium, almost totally, so it could not be mentioned about which isotherm model is fitted to the experimental data. The Dubinin-Radushkevich isotherm model fits the experimental data for the sorption of Cd^{2+} ions that can be seen from the nonlinear fit of the model with the experimental data.The applicability of the Dubinin-Radushkevich isotherm to the sorption of Ni^{2+} ions by *Dunaliella salina* is supported by the linear (R^2 as 0.9982) and nonlinear fits of the isotherm model.

The obtained results demonstrates that the Langmuir isotherm model is able to fit the sorption of Pb^{2+} ,Cd^{2+} and Ni^{2+} by *Oocystis sp.* with the correlation coefficient, R^2, as 0.8148, 0.9958, and 0.9365, respectively. These results can also be confirmed by the nonlinear fits of the isotherm.

The sorption of Pb^{2+} by *Porphyridium cruentum* follows Dubinin-Radushkevich isotherm with the correlation coefficient, R^2, 0.9960, while that of Cd^{2+}, and Ni^{2+} by *Porphyridium cruentum* obeys Langmuir isotherm, with the correlation coefficients, 0.9904 and 0.9836, respectively.

It has been observed that the Dubinin-Radushkevich isotherm is able to fit the experimental data for the sorption of Pb^{2+} by *Scenedesmus protuberans*. According to the data obtained from the sorption of Cd^{2+} and Ni^{2+} by *Scenedesmus protuberans*, the Langmuir isotherm correlates with the experimental results.

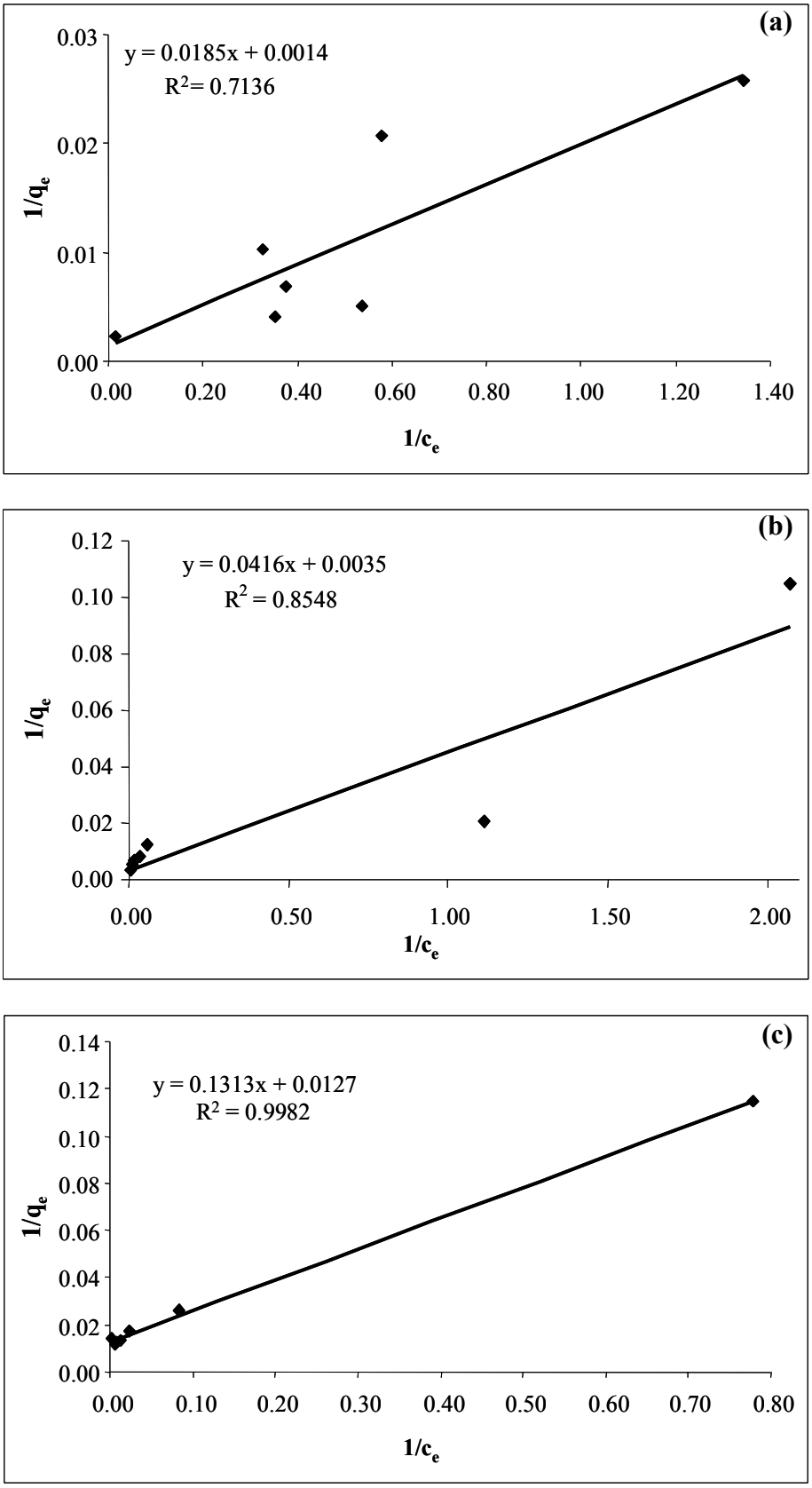


Figure 3.27. Langmuir isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions by biosorbed by *Dunaliella salina*.

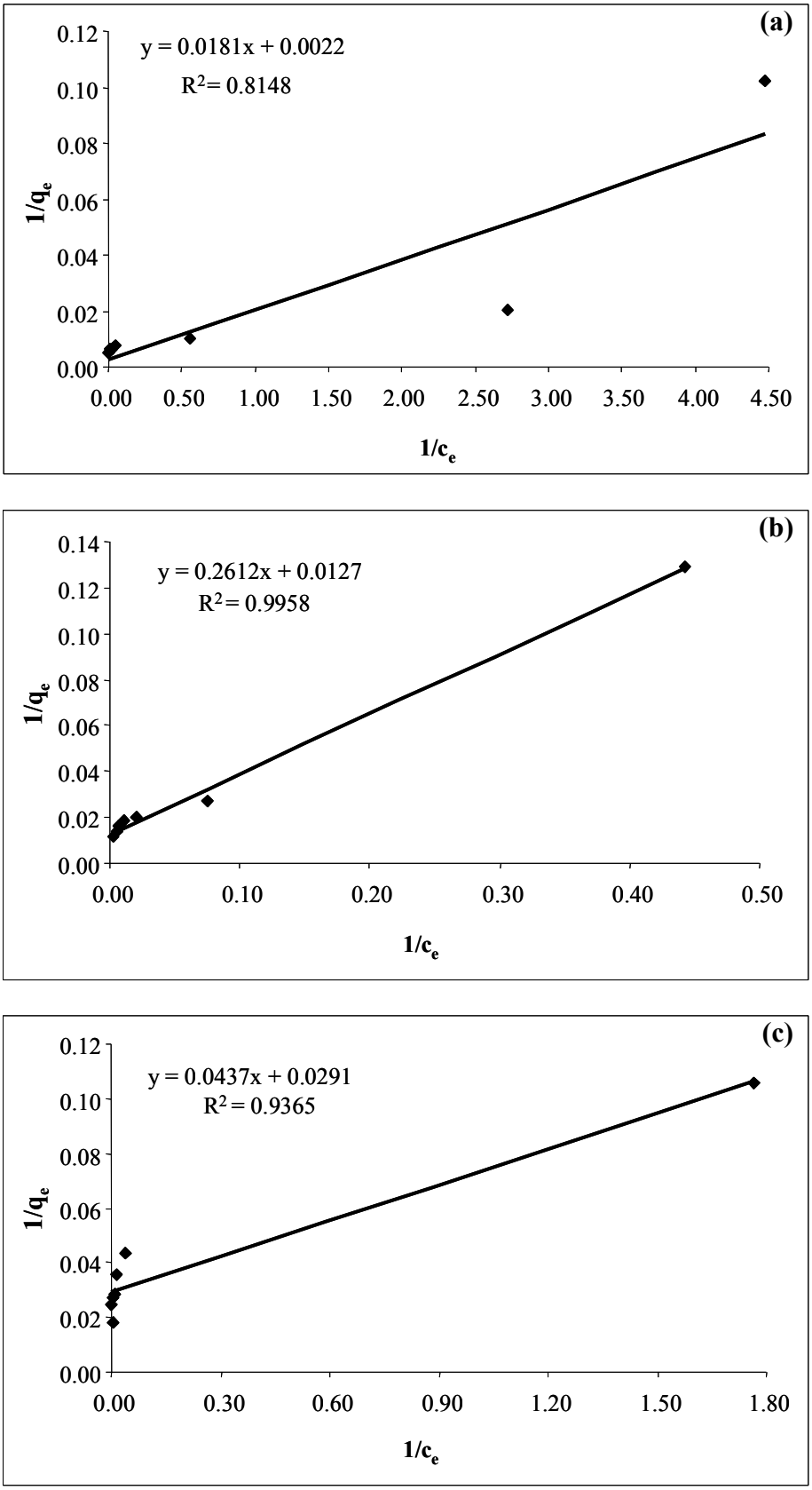


Figure 3.28. Langmuir isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Oocystis sp.*

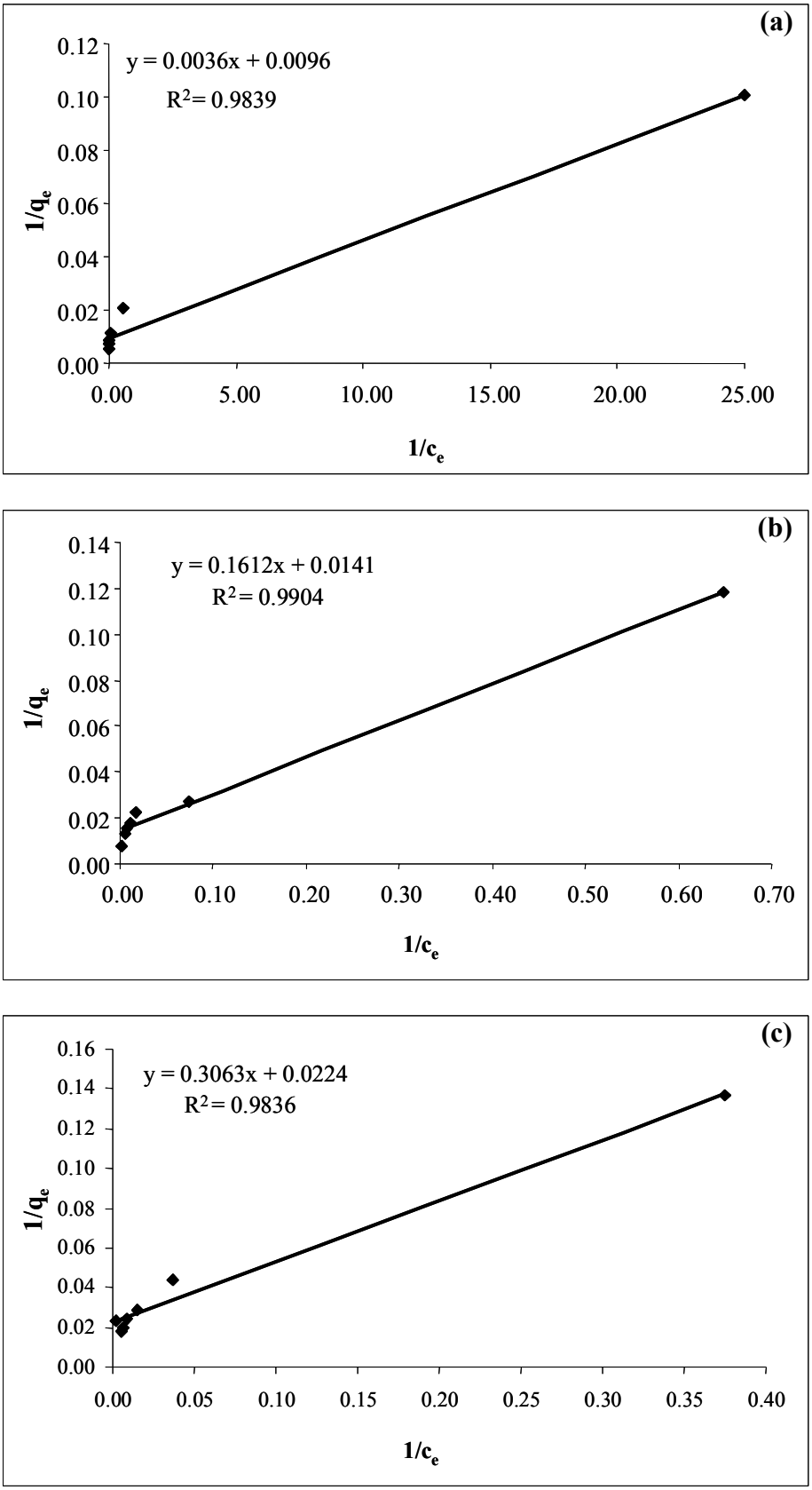


Figure 3.29. Langmuir isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Porphyridium cruentum*.

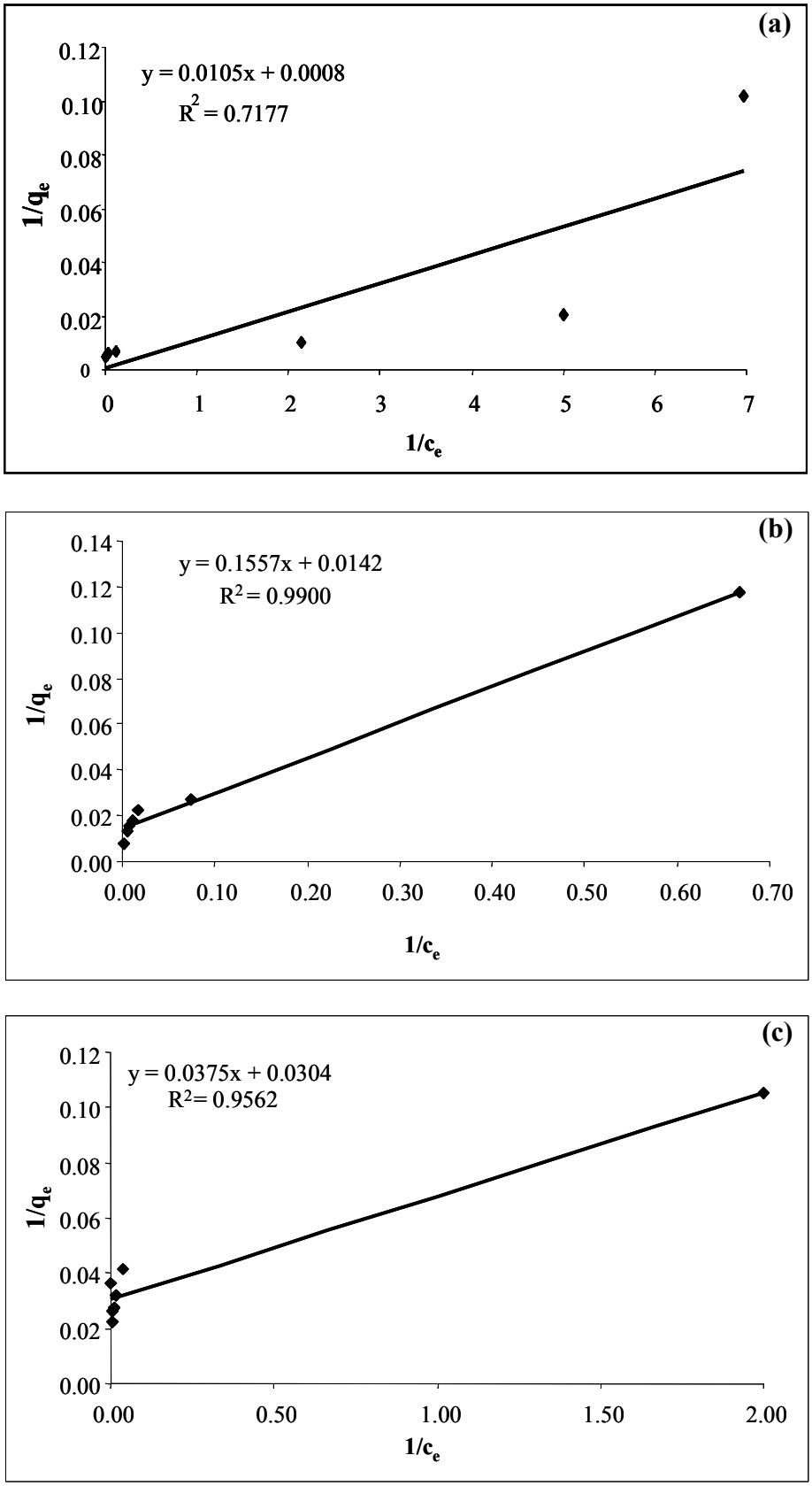


Figure 3.30. Langmuir isotherm plot for (a) Pb^{2+}, (b) Cd^{2+},and (c) Ni^{2+} ions biosorbed by *Scenedesmus protuberans*

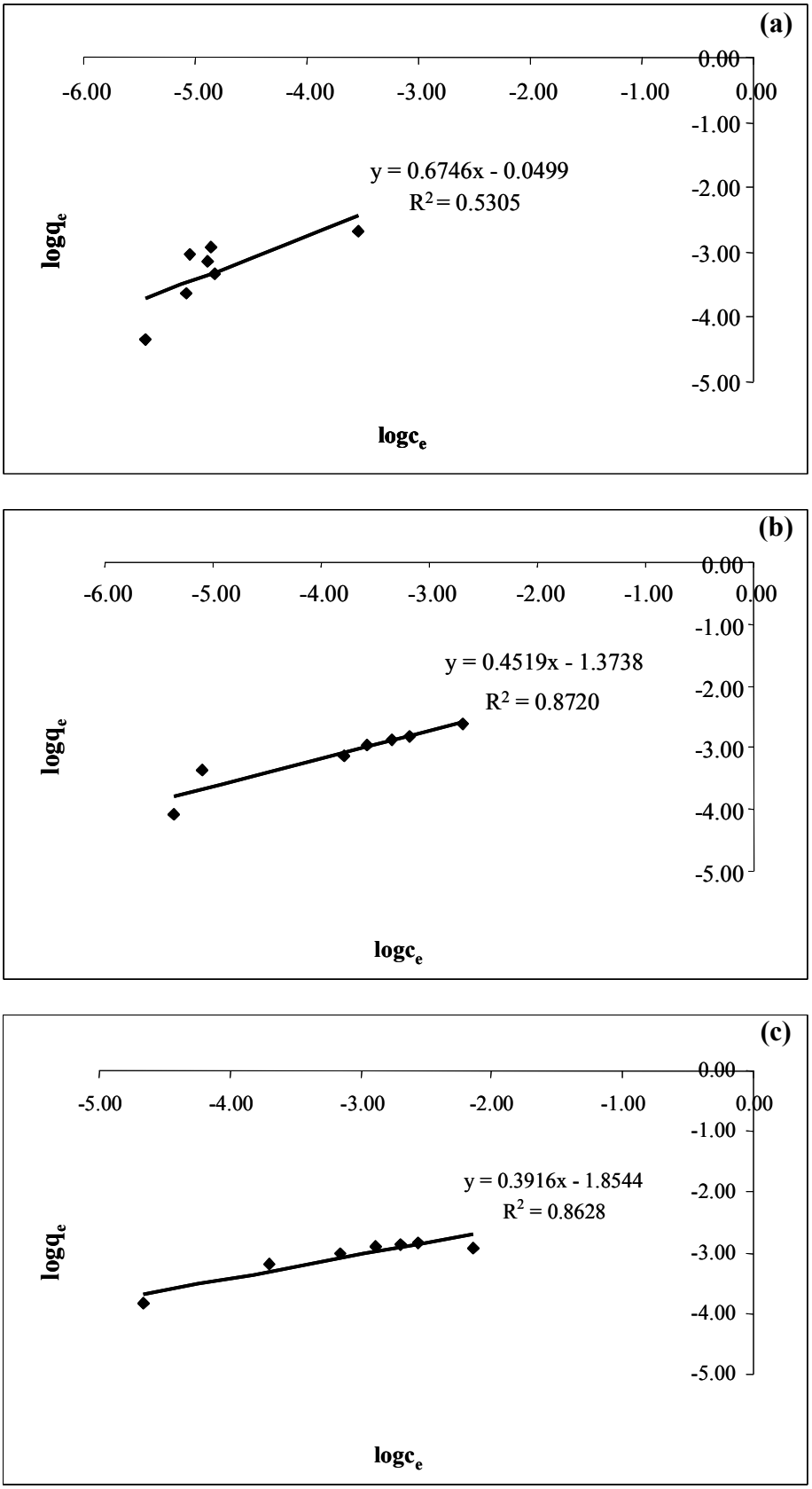


Figure 3.31. Freundlich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Dunaliella salina*

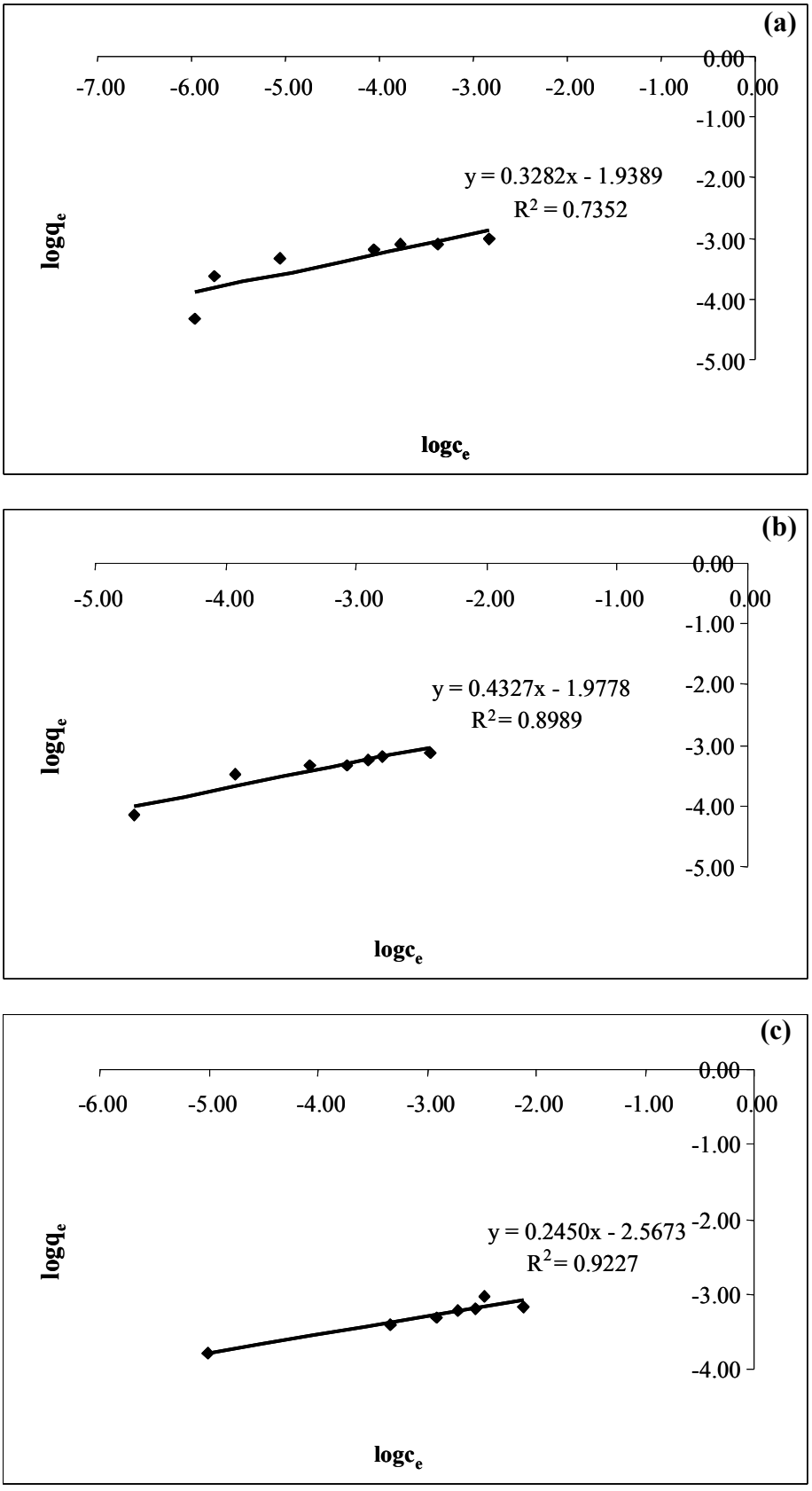


Figure 3.32. Freundlich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Oocystis sp.*

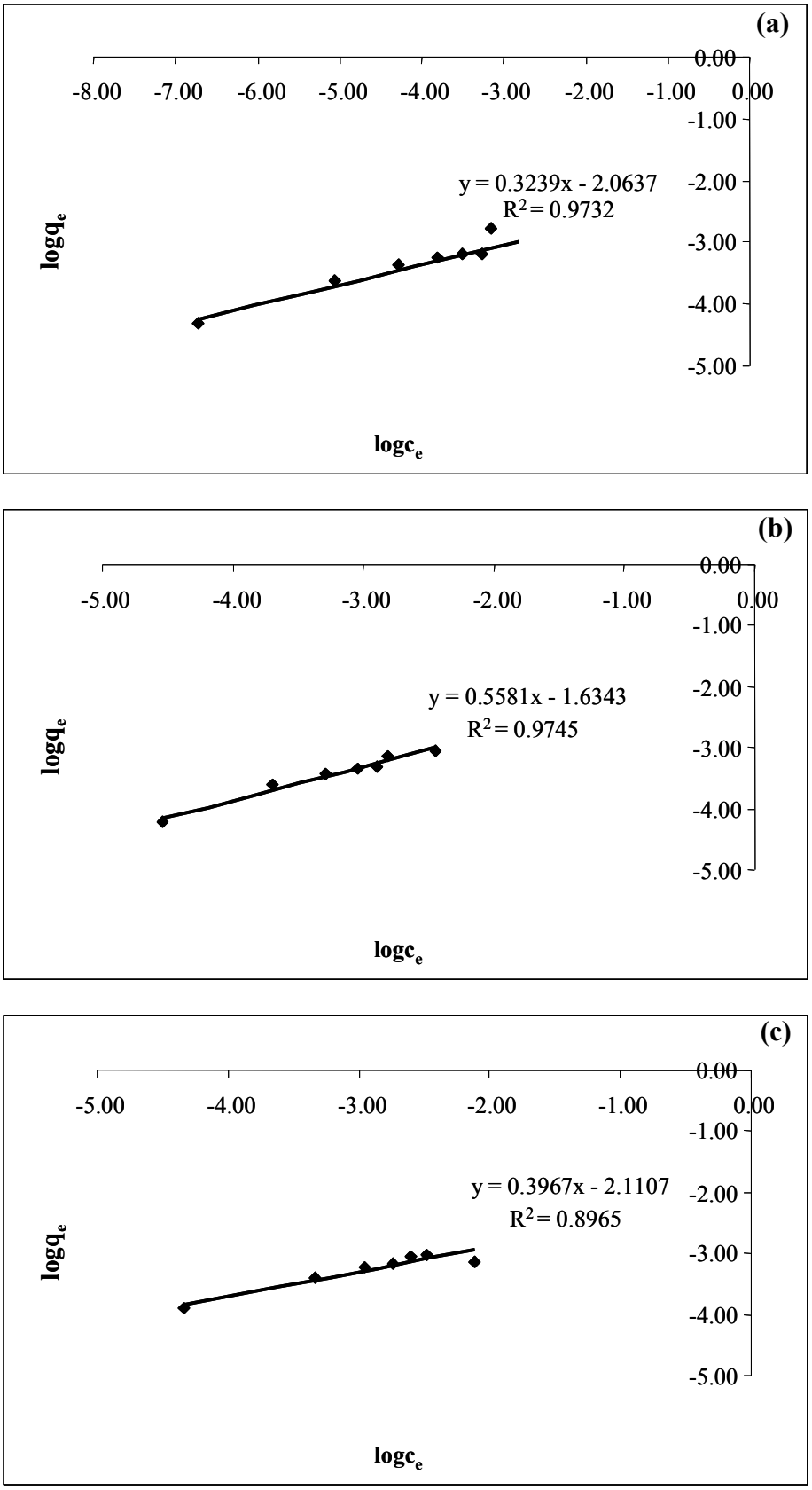


Figure 3.33. Freundlich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Porphyridium cruentum*

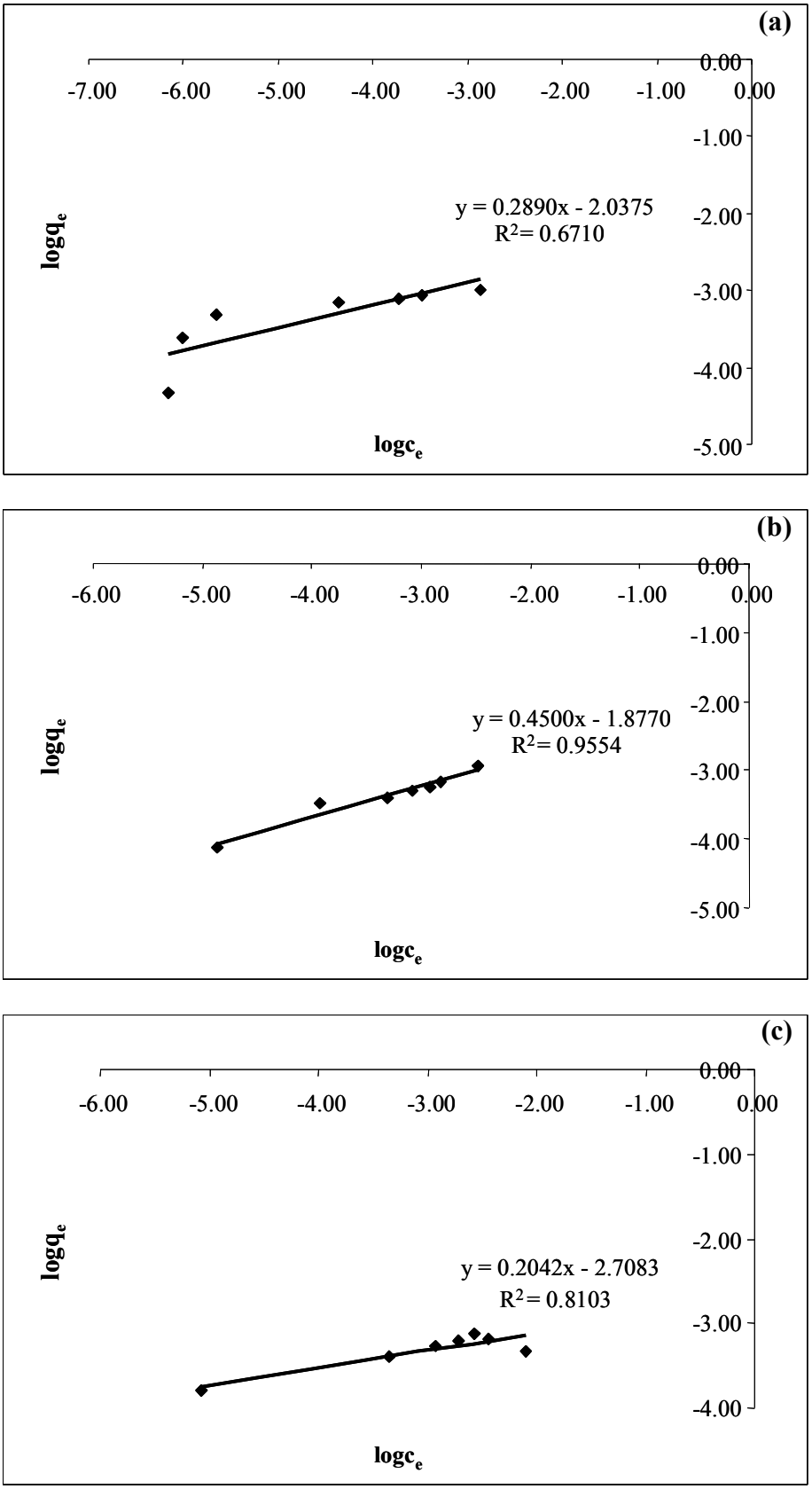


Figure 3.34. Freundlich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Scenedesmus protuberans*

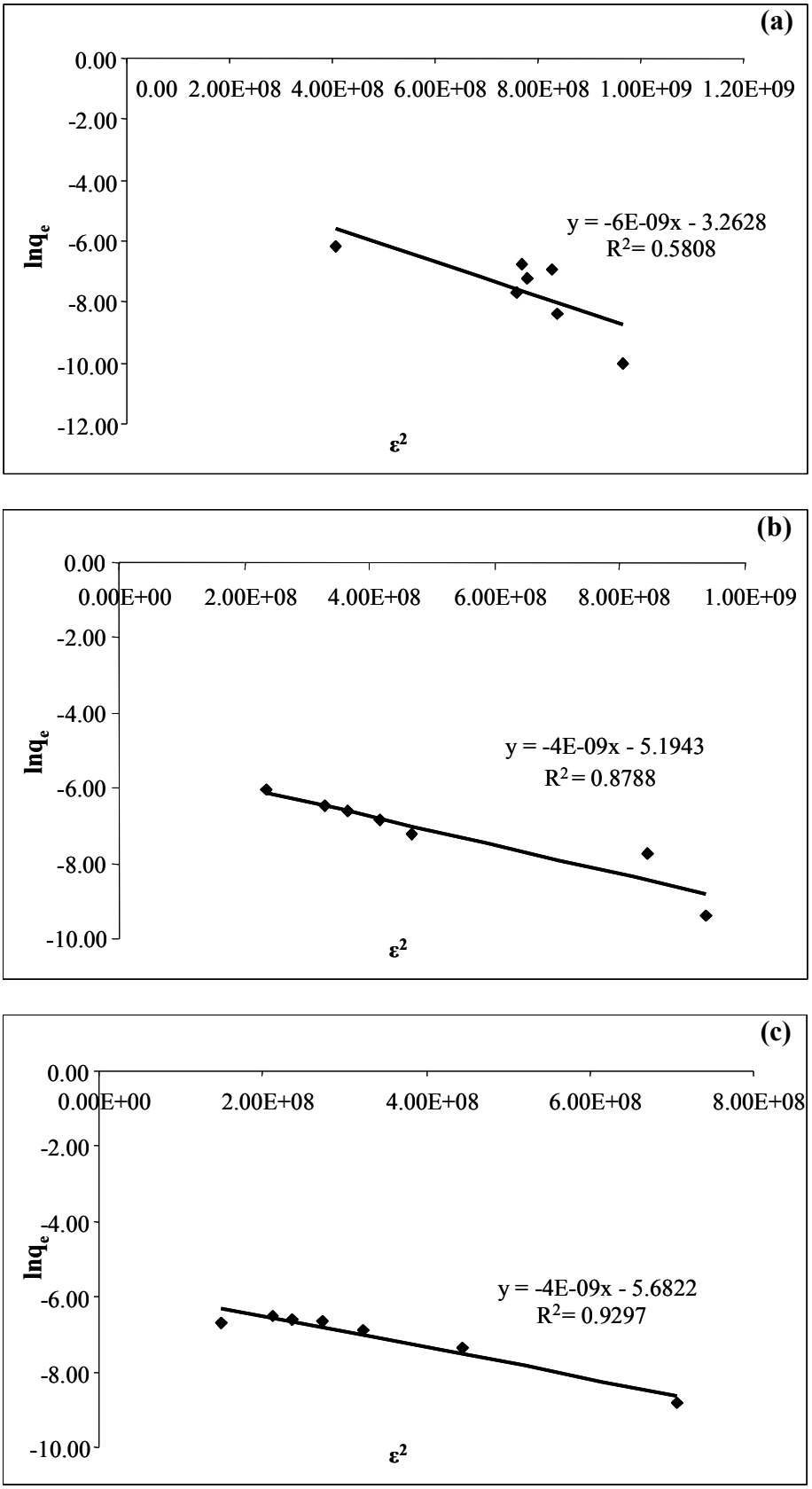


Figure 3.35. Dubinin-Radushkevich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Dunaliella salina*

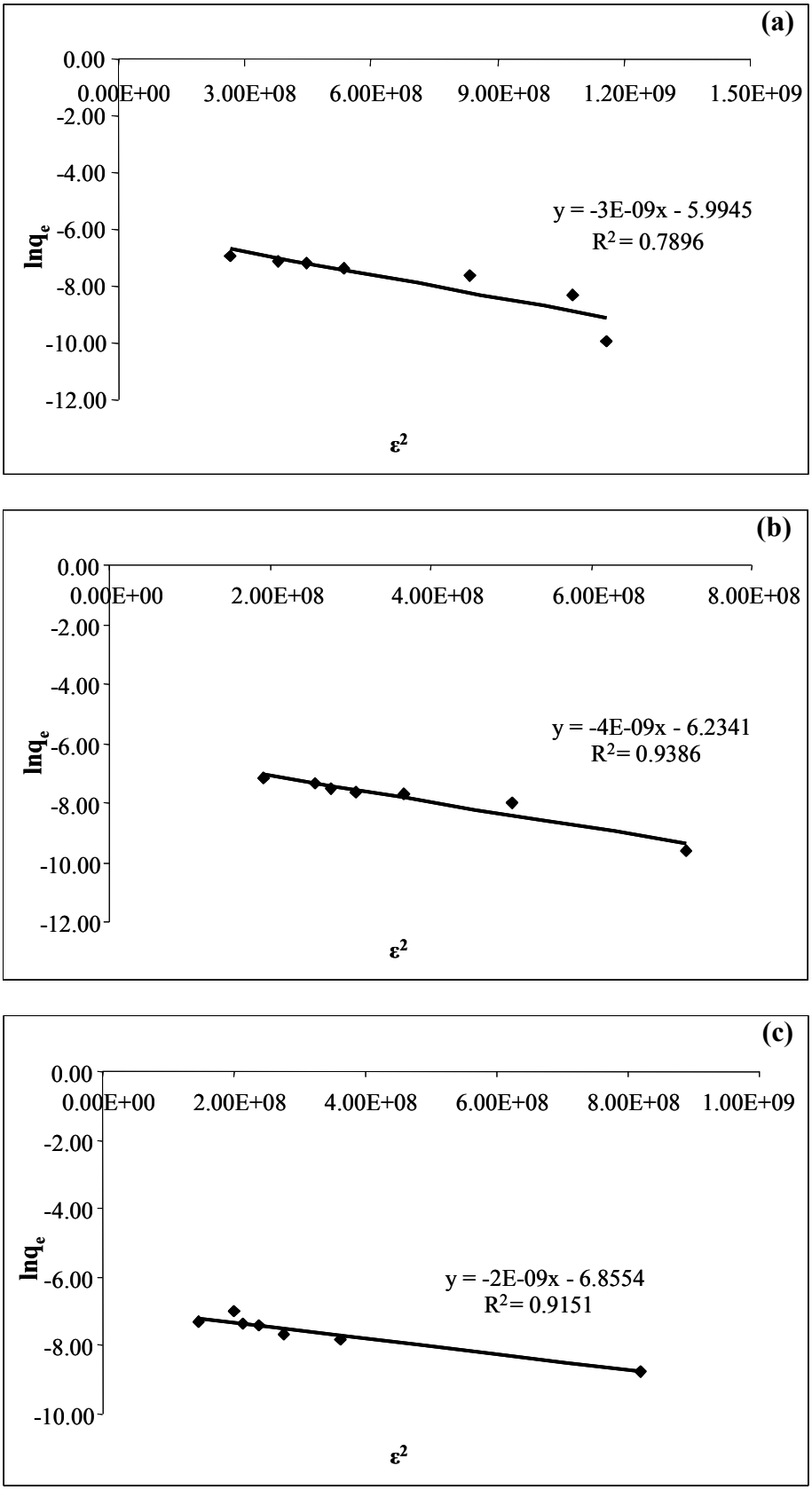


Figure 3.36. Dubinin-Radushkevich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Oocystis sp.*

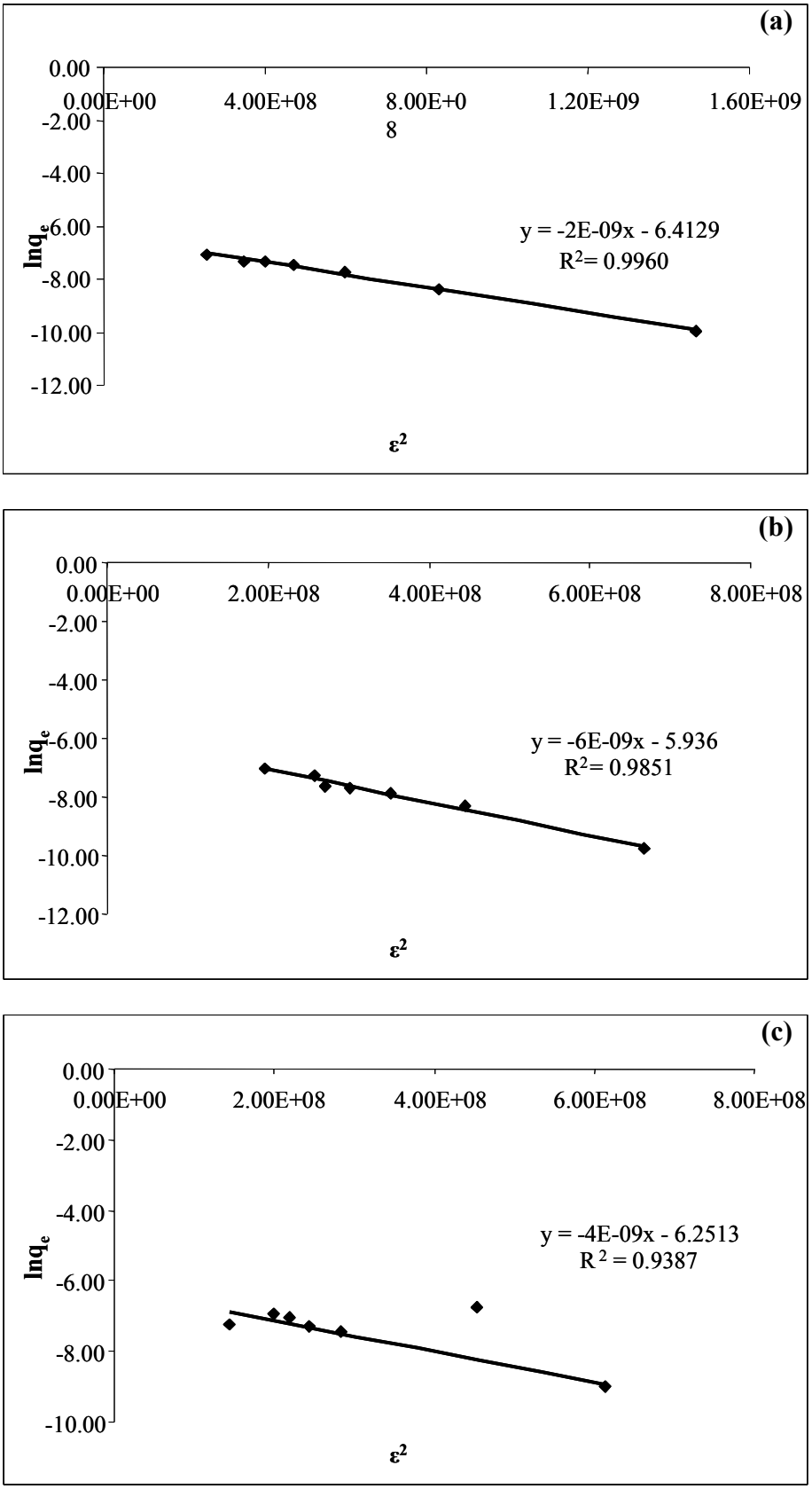


Figure 3.37. Dubinin-Radushkevich isotherm plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions by biosorbed by *Porphyridium cruentum*

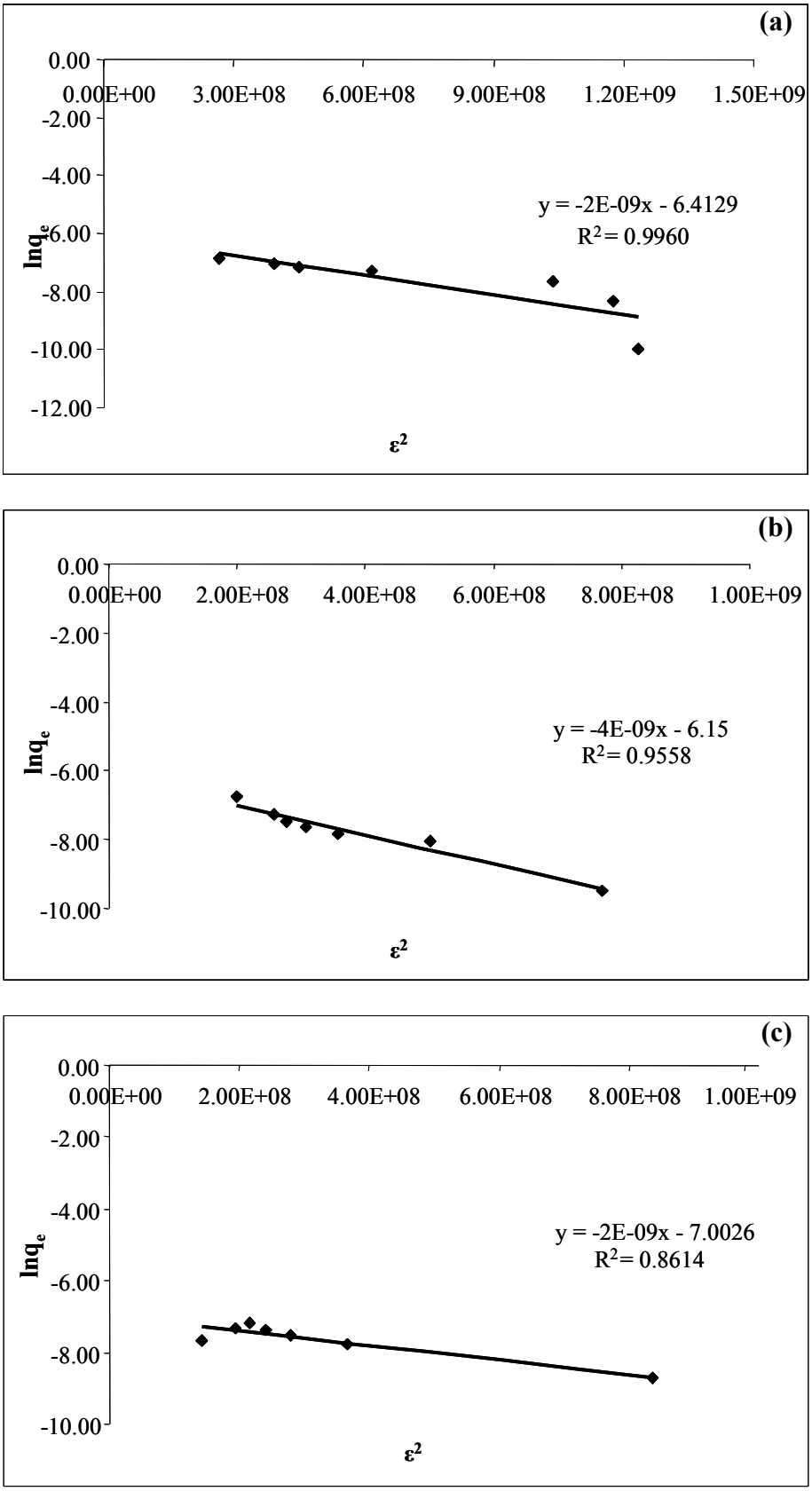


Figure 3.38. Dubinin-Radushkevich plot for (a) Pb^{2+}, (b) Cd^{2+}, and (c)Ni^{2+} ions biosorbed by *Scenedesmus protuberans*

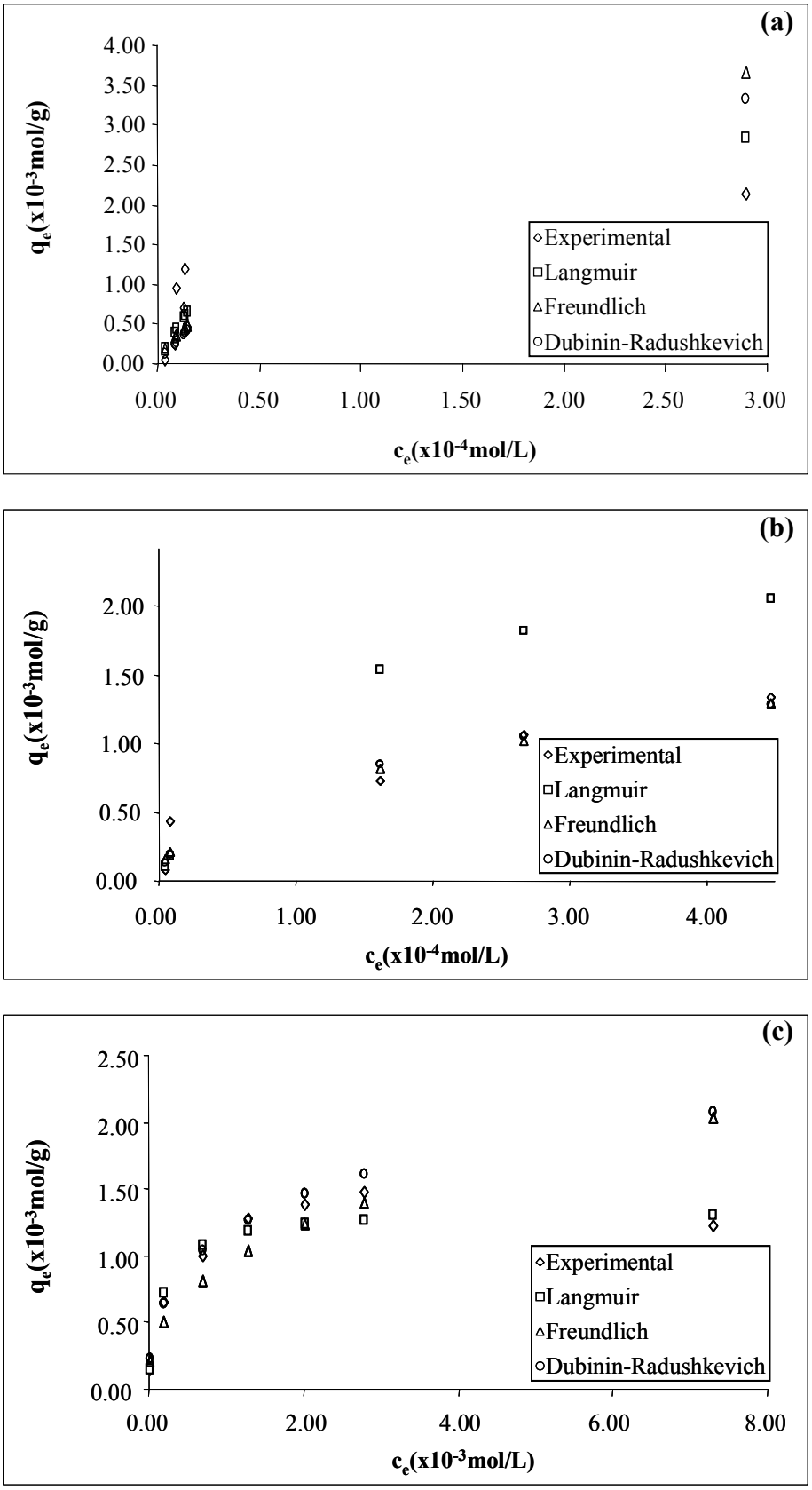


Figure 3.39. Isotherms of (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Dunaliella salina* (◊:Experimental, □:Langmuir, Δ:Freundlich, ○:Dubinin-Radushkevich).

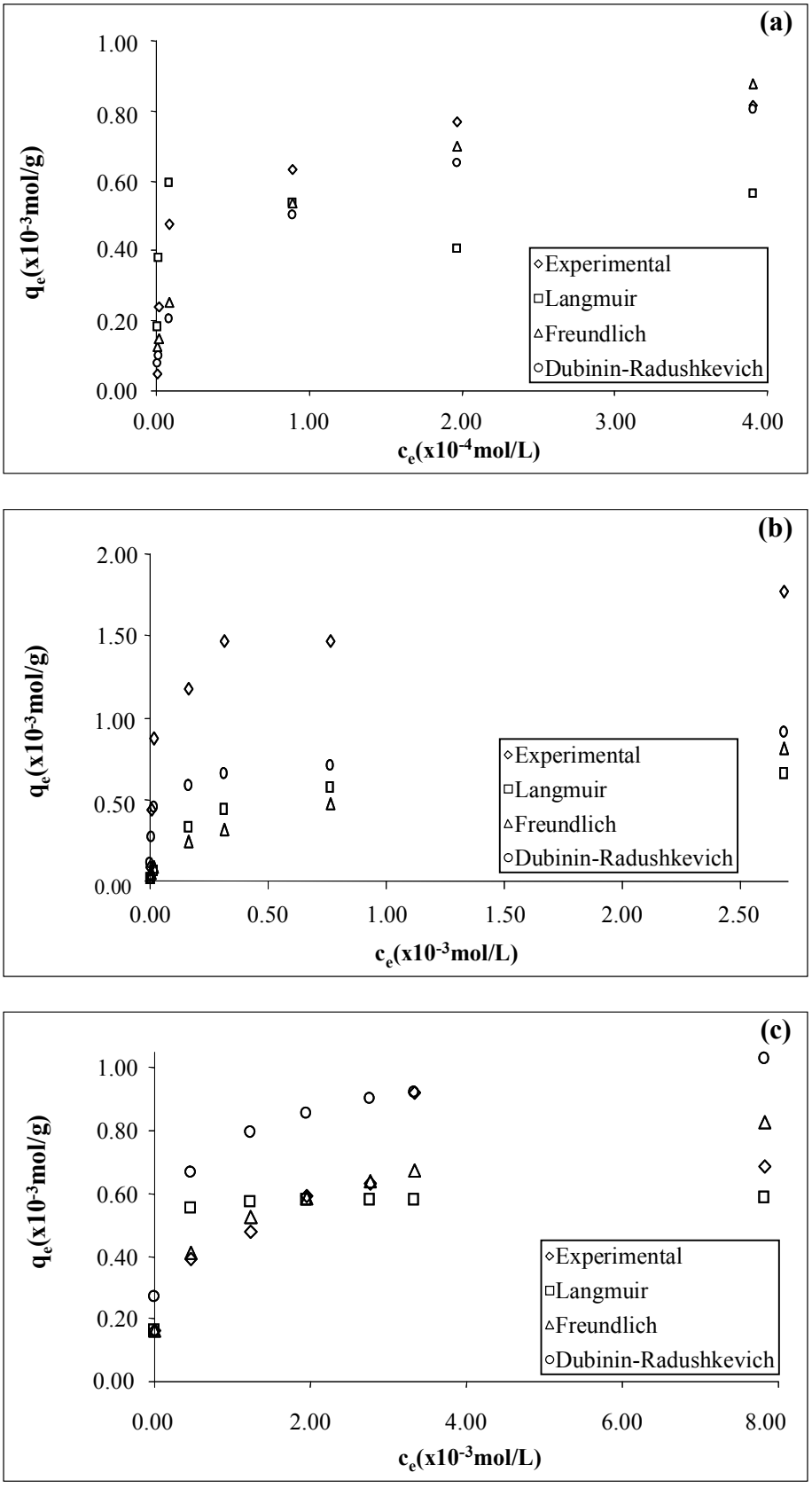


Figure 3.40. Isotherms of (a) Pb^{2+}, (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Oocystis sp.* (◊:Experimental, □:Langmuir, Δ:Freundlich, ○:Dubinin-Radushkevich)

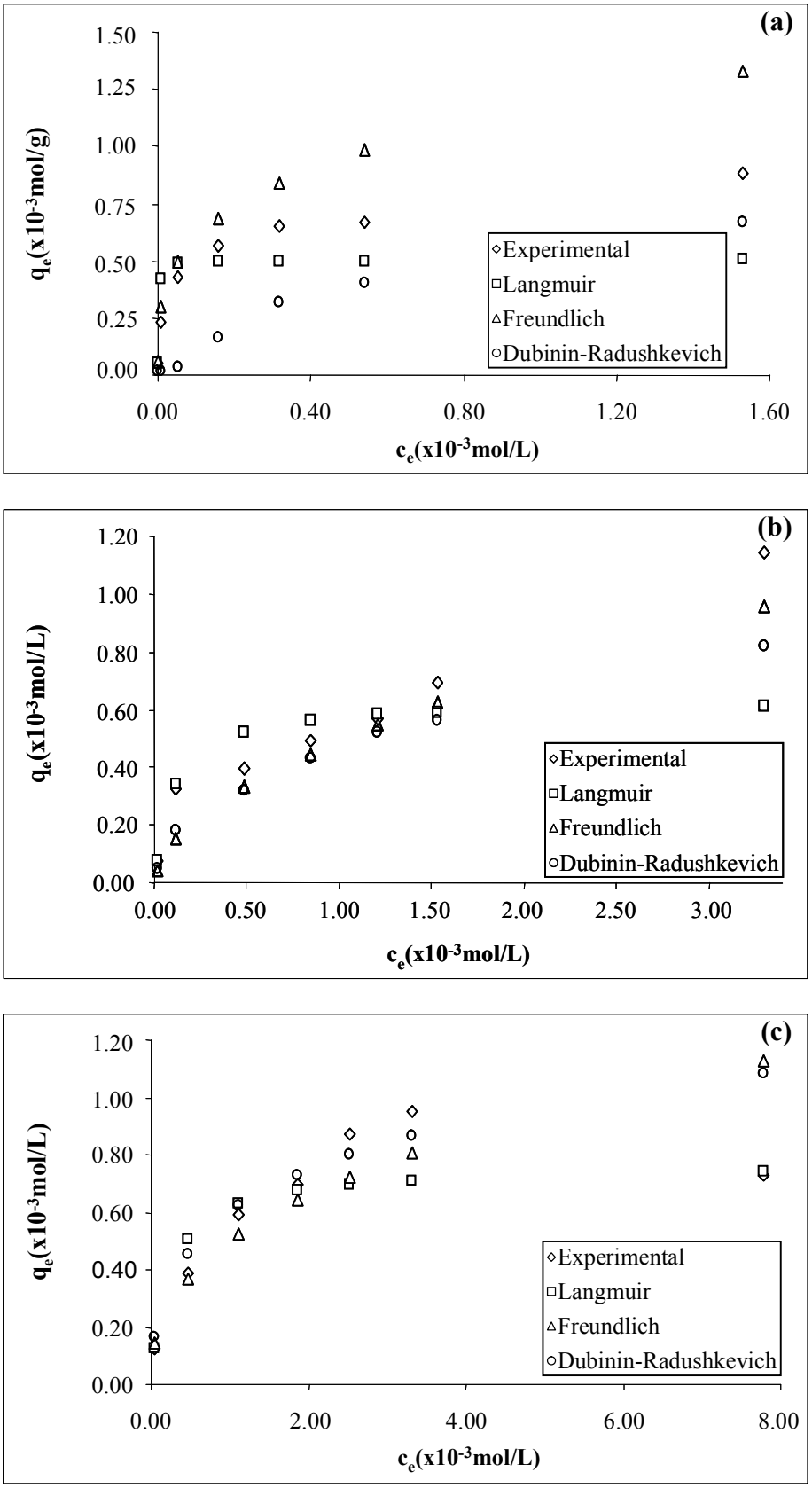


Figure 3.41. Isotherms of (a) Pb^{2+} , (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Porphyridium cruentum* (◊:Experimental, □:Langmuir, Δ:Freundlich, ○:Dubinin-Radushkevich).

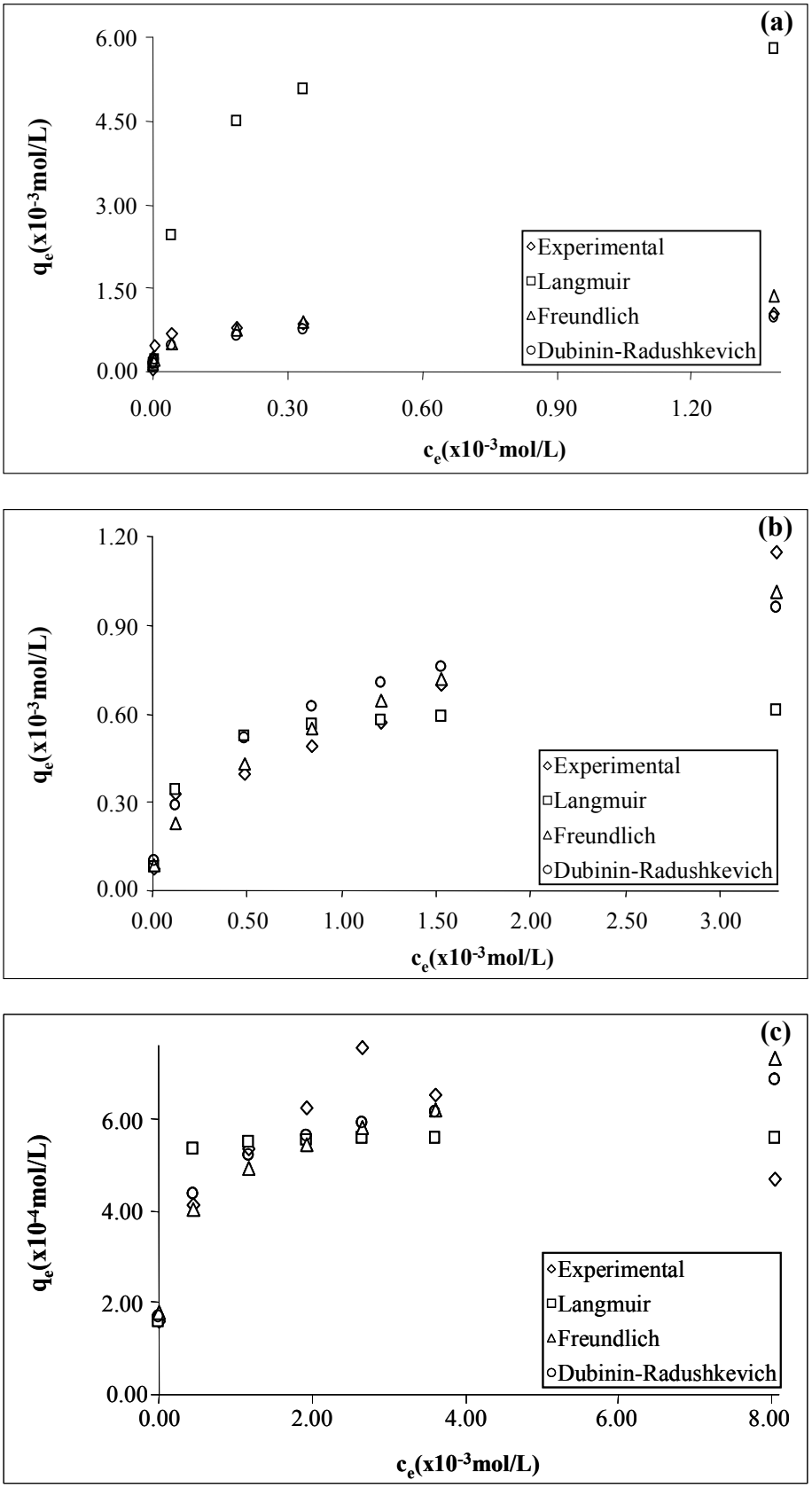


Figure 3.42. Isotherms of (a) Pb^{2+} , (b) Cd^{2+}, and (c) Ni^{2+} ions biosorbed by *Scenedesmus protuberans* (◊:Experimental, □:Langmuir, Δ:Freundlich, ○:Dubinin-Radushkevich)

Table 3.9. Isotherm constants for Pb^{2+}, Cd^{2+}, and Ni^{2+} sorption by algae.

Algae	Metal ion	Langmuir			Freundlich			Dubinin-Radushkevich		
		K_a	C_m (mol/g)	R^2	n	K_f	R^2	C_m (mol/g)	E (kJ/mol)	R^2
Dunaliella salina	Pb^{2+}	0.0757	0.0034	0.7136	0.6746	0.8915	0.5305	0.0383	9.1	0.5808
	Cd^{2+}	0.0841	0.0025	0.8548	0.4519	0.0423	0.8720	0.0055	11.2	0.8788
	Ni^{2+}	0.0967	0.0013	0.9982	0.3916	0.0140	0.8628	0.0038	11.2	0.9297
Oocystis sp.	Pb^{2+}	0.1215	0.0022	0.8148	0.3282	0.0115	0.7352	0.0025	12.9	0.7896
	Cd^{2+}	0.0486	0.0007	0.9958	0.4327	0.0105	0.8989	0.0020	11.2	0.9386
	Ni^{2+}	0.6659	0.0006	0.9365	0.2450	0.0027	0.9227	0.0011	15.8	0.9151
Porphyridium cruentum	Pb^{2+}	2.6667	0.0005	0.9839	0.3239	0.0086	0.9732	0.0016	15.8	0.9960
	Cd^{2+}	0.0875	0.0006	0.9904	0.5581	0.0232	0.9745	0.0026	9.1	0.9851
	Ni^{2+}	0.0731	0.0008	0.9836	0.3967	0.0077	0.8965	0.0019	11.2	0.9387
Scenedesmus protuberans	Pb^{2+}	0.0762	0.0060	0.7177	0.2890	0.0092	0.6710	0.0016	15.8	0.9960
	Cd^{2+}	0.0912	0.0006	0.9900	0.4500	0.0133	0.9554	0.0021	11.2	0.9558
	Ni^{2+}	0.8107	0.0006	0.9562	0.2042	0.0020	0.8103	0.0009	15.8	0.8614

CHAPTER 4

CONCLUSION

It has been shown that *Dunaliella salina, Oocystis sp., Porphyridium cruentum,* and *Scenedesmus protuberans* can be utilized for the sorption of Pb^{2+}, Cd^{2+}, and Ni^{2+} ions prior to flame atomic absorption spectrometric determination. In the first part of the study, the characterization of *Dunaliella salina, Oocystis sp., Porphyridium cruentum,* and *Scenedesmus protuberans* was investigated with SEM images, FTIR spectra, elemental analysis, and TGA decomposition curves of algae. SEM images show that each alga has different unit structures and sizes. It can be derived from the FTIR spectra that the functional groups present in the algae are responsible for the metal uptake. Percentage concentrations of C, H, N, and S was determined by elemental analyzer and each alga was found to have different elemental composition. The thermal stability of the algae was investigated through the TGA plots and *Porphyridium cruentum* was found to be the most stable one among the others.

Optimization of the biosorption parameters, such as solution pH, initial metal ion concentration, shaking time, biomass amount were carried out to elucidate the sorption characteristics of the algae. Solution pH was adjusted to 6.0 as a compromised point which is both sufficiently higher than the isoelectronic point of the algal cells that guarantees the surface is negatively charged and also reasonably lower than the pHs where the metal hydroxides start to be formed. It was demonstrated that the primary sorption mechanism is the electrostatic attraction between the negatively charged functional groups on the surface of the biomass and the positively (+2) charged metal ions in the solution.

Uptake of metal ions is affected from the shaking time and it can be said that all algae had a very fast kinetics towards the metal ions. Even 5 min have been shown to be adequate to reach equilibrium under the optimized conditions; however, a shaking time of 60 min was applied in all studies to be on the safe side.

Among the biomasses investigated, *Dunaliella salina* has shown the highest sorption capacity for all the metal ions. It was followed by *Oocystis sp., Scenedesmus*

protuberans and *Porphyridium cruentum.* Additionally, the biomasses examined have demonstrated the highest affinity towards Pb^{2+} which was followed by Cd^{2+} and Ni^{2+}.

Biosorption experiments were performed to find the optimum amount of the biomass for a fixed sample volume of 10.0 mL for the metal ion concentration of 250.0 mg/L. Increase in the amount of biosorbent was expected to increase also the metal sorption since the number of available functional sites would be higher. As mentioned before, *Dunaliella salina* has shown a better sorption performance than the other sorbents and it was found that even 20.0 mg of *Dunaliella salina* was sufficient to obtain maximum sorption for Pb^{2+} while 100.0 mg was required for Cd^{2+} and Ni^{2+}.

To recover the metal ions from *Oocystis sp.* and *Scenedesmus protuberans* desorption experiments were performed and it was found that acidic eluents can be used to release the previously sorbed metal ions from the algae with a desorption percentage of around 60%. The disadvantage of using acidic eluents and the inevitable damage to the algae can be compensated by the economical production of the biomasses. Sodium citrate can also be used for the same purpose with a lower recovery. However, in contrast to acidic eluents, its use eliminates the risk of damaging the biomass.

The biosorption experiments were performed in the presence of the other metal ions to evaluate the performance of biosorbents in a competitive environment. Uptake of Pb^{2+} ions was not influenced by the presence of other ions for all the algae studied. However, the general trend for the other biomasses was a decrease in their sorption efficiency towards Cd^{2+} and Ni^{2+} ions with the increase in the concentration of the competitive ions.

From environmental point of view, it can be said that the algal biomasses are natural, highly abundant, and easy to produce materials which can be utilized successfully in the selective removal of toxic metal ions from waters.

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APPENDIX A

A1. EM (Erdschreiber's Medium) (Growing Medium for *Dunaliella salina*)

EM (Erdschreiber's Medium)	
Contents	**Concentration**
Pasteurized seawater	3.0L
P-IV metal solution	36.0mL
$NaNO_3$ (autoclave before adding)	59.5 mg
$Na_2HPO_4.7H_2O$ (autoclave before adding)	53.6 mg
Soilwater: GR+ Medium	150.0mL
Vitamin B_{12}	3.0mL

P-IV Metal Solution	
Contents	**Concentration**
$Na_2EDTA.2H_2O$	0.750 g/L
$FeCI_3.6H_2O$	0.097 g/L
$MnCI_2.4H_2O$	0.041 g/L
$ZnCI_2$	0.005 g/L
$CoCI_2.6H_2O$	0.002 g/L
$Na_2MoO_4.2H_2O$	0.004 g/L

Soilwater: GR+ Medium	
Contents	**Concentration**
Green house soil	1tsp/200mL H_2O
$CaCO_3$ (optional)	1mg/200mL H_2O

A2. Bold's Basal (BB) Medium (Growing Medium for *Oocystis sp.* and *Scenedesmus protuberans*)

Bold's Basal (BB) Medium	
Contents	**Concentration**
$NaNO_3$ (5.0g/200mL)	10.0mL
$CaCI_2.2H_2O$ (0.5g/200mL)	10.0mL
$MgSO_4.7H_2O$ (1.5g/200mL)	10.0mL
K_2HPO_4 (Dibasic) (1.5g/200mL)	10.0mL
KH_2PO_4 (3.5g/200mL)	10.0mL
NaCI (0.5g/200mL)	10.0mL
$FeSO_4.7H_2O$ (4.98g/L)	1.0mL
H_3BO_3 (11.42g/L)	1.0mL
KOH solution	1.0mL
Trace Metals	1.0mL
Distilled water to	1.0L

BBM KOH Solution	
Contents	**Concentration**
KOH	3.1g/100mL
EDTA	5mg/100mL

Trace Metals	
Contents	**Concentration**
$ZnSO_4.7H_2O$	0.882g/100mL
$MnCI_2.4H_2O$	0.144g/100mL
MoO_3	0.070g/100mL
$CuSO_4.5H_2O$	0.157g/100mL
$Co(NO)_3.6H_2O$	0.049g/100mL

A3. F/2 Medium (Growing Medium for *Porphyridium cruentum*)

F/2 Medium	
Contents	**Concentration**
$NaNO_3$ (7.5g/L)	1.0mL
$NaH_2PO_4.H_2O$ (5.0g/L)	1.0mL
$Na_2SiO_3.9H_2O$ (30.0g/L)	1.0mL
F/2 Trace Metal Solution	1.0mL
F/2 Vitamin Solution	0.5mL
Filtered sewater to	1.0L

F/2 Trace Metal Solution	
Contents	**Concentration**
$FeCI_3.6H_2O$	3.15g
$Na_2EDTA.2H_2O$	4.36g
$CuSO_4.5H_2O$ (9.8g/L)	1.0mL
$Na_2MoO_4.2H_2O$ (6.3g/L)	1.0mL
$ZnSO_4.7H_2O$ (22.0g/L)	1.0mL
$CoCI_2.6H_2O$ (10.0g/L)	1.0mL
$MnCI_2.4H_2O$ (180.0g/L)	1.0mL
Distilled water to	1.0L

F/2 Vitamin Solution	
Contents	**Concentration**
Vitamin B_{12} (1.0g/L)	1.0mL
Biotin (0.1g/L)	10.0mL
Thiamine HCI	200.0mg
Distilled water to	1.0L