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Institute of Water and Energy Sciences (Including Climate Change)

**EXPERIMENTAL DESIGN FOR OPTIMAZING THE
REMOVAL OF HEAVY METALS ABILITY FROM
WASTEWATER BY FICUS BENJAMINA'**

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DECLARATION

I hereby declare that ‘EXPERIMENTAL DESIGN FOR OPTIMIZING THE REMOVAL OF HEAVY METALS ABILITY FROM WASTEWATER BY FICUS BENJAMINA’ is my own unaided work. It is being submitted for the degree of Master in Water Engineering to the Pan African University. It has not been submitted before for any degree or examination to any other University /Institution.

Signature.....

Ms. Naima Hamdaoui

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SUMMARY

Excessive and cumulative release of toxic metals from industrial effluents due to rapid industrialization has posed hazard to aquatic ecosystem integrity and environmental/ human health. The inadequacy and high cost of traditional metal treatment technologies coupled with the imposition of stricter environmental regulations and guidelines for industrial point source discharges have increased the demand for economically feasible alternative methods.

Application of natural and abundant sorption material known as biosorbents comprising microbial biomass, agriculture waste, and industrial waste biomass, has gained international attention in scientific world as a low-cost and effective method for The removal of heavy metals from waste water. However, this method has not been able to gain the attention of industry for large-scale applications thus far.

This research was aimed to further evaluate the potential of biosorption performance of Cd^{2+} and Pb^{2+} from aqueous solutions using readily available dried plant biomasses namely: *Ficus benjamina*. The objectives of this work are as following: i) to investigate the effects of pH, temperature and initial concentration, ii) determine the factors impacting the biosorption process besides interaction between factors using full factorial design, iii) To validate the result optimization model for the removal of Cd^{2+} and Pb^{2+} from real industrial wastewater.

The removal of Cd^{2+} and Pb^{2+} was studied separately, using the factorial design 2^3 . The three factors considered are pH, temperature and metal ion concentration at two markedly different levels: pH (2.0 and 6.0), Temp (20°C and 45°C) and metal ion concentration (Cd^{2+} : 5 and 300 mg l⁻¹; Pb^{2+} : 10 and 500 mg l⁻¹). The experiments were carried out in a batch type reactor system with 0.2g of biosorbent (dead dried biomass of *Ficus Benjamina*), 50 ml of Cd^{2+} and Pb^{2+} solutions , and the blending rate (300 rpm)and. The removal efficiencies of both ions during an exposition time of 6 h were then evaluated. The results were statistically analyzed using analysis of variance to

define the most important process variables affecting the metal removal efficiency. The most significant effects for Cd^{2+} are pH and X while The largest effect of the main factors in Pb^{2+} biosorption was ascribed to initial metal ion concentration(X).

The best percentage removal of Cd^{2+} was 81.2% at pH=6, Temp=45°C and X=5mg/l but the best percentage of Pb^{2+} was 81.4% at pH=2, Temp=20°C and X=10mg/l. When applying these optimum conditions on real wastewater sample collected from El Mahmoudia channel, affected by Industrial, agricultural and sewage discharges. The best Removal results were 99.9% for Cd^{2+} and 92.4% for Pb^{2+} .

These results indicated that *Ficus Benjamina* can be used as a low cost adsorbent for the removal of heavy metals from waste water.

Keywords: Biosorption, heavy metals, *Ficus benjamina*, full factorial design.

Résumé

La libération excessive et cumulative de métaux toxiques provenant des effluents industriels en raison de l'industrialisation rapide a posé un risque pour l'intégrité de l'écosystème aquatique et la santé environnementale / humaine. L'insuffisance et le coût élevé des technologies traditionnelles de traitement des métaux, conjugués à l'imposition de réglementations et de directives environnementales plus strictes pour les rejets industriels de sources ponctuelles, ont augmenté la demande de méthodes alternatives économiquement réalisables.

L'application d'un matériau de sorption naturel et abondant connu sous le nom de biosorbants comprenant la biomasse microbienne, les déchets agricoles et la biomasse des déchets industriels a gagné l'attention internationale dans le monde scientifique en tant que méthode peu coûteuse et efficace pour l'élimination des métaux lourds des eaux usées. Cependant, cette méthode n'a pas été en mesure d'attirer l'attention des industries pour une application à grande échelle jusqu'à présent.

Cette recherche visait pour plus évaluer le potentiel de biosorption du Cd^{2+} et le Pb^{2+} à partir de solutions aqueuses en utilisant des biomasses de plantes séchées facilement disponibles, comme : *Ficus benjamina*. Les objectifs de ce travail sont les suivants: i) étudier les effets du pH, de la température ainsi que la concentration initiale, ii) déterminer les facteurs qu'influent sur le processus de biosorption en plus de l'interaction entre les facteurs utilisant la conception factorielle complète; iii) valider le modèle d'optimisation des résultats pour L'élimination du Cd^{2+} et Pb^{2+} des eaux usées industrielles réelles.

L'élimination du Cd^{2+} et du Pb^{2+} a été étudiée séparément, en utilisant la conception factorielle 2^3 . Les trois facteurs considérés étaient la concentration en pH, en température et en ions métalliques à deux niveaux nettement différents: pH (2 et 6), température (20 ° C et 45 ° C) Et la concentration en ions métalliques (Cd^{2+} : 5 et 300 mg l⁻¹, Pb^{2+} : 10 et 500 mg l⁻¹). Les expériences ont été réalisées dans un système de réacteur de type discontinu avec 0,2 g de biosorbant (biomasse séchée de *Ficus Benjamina*), 50 ml de solutions Cd^{2+} et Pb^{2+} , et la vitesse de mélange (300 tr / min).

L'efficacité d'élimination des deux ions pendant un temps d'exposition de 6 h a ensuite été évaluée. Les résultats ont été analysés statistiquement en utilisant l'analyse de variance pour définir les variables de processus les plus importantes affectant l'efficacité de l'élimination des métaux. Les effets les plus importants pour le Cd^{2+} sont le pH et X tandis que le plus grand effet des principaux facteurs dans la biosorption Pb^{2+} a été attribué à la concentration initiale en ions métalliques (X).

Le meilleur pourcentage d'élimination du Cd^{2+} était de 81,2% à pH = 6, Temp = 45 ° C et X = 5mg / l mais le meilleur pourcentage de Pb^{2+} était de 81,4% à pH = 2, Temp = 20 ° C et X = 10mg / l. Lors de l'application de ces conditions optimales sur l'échantillon réel d'eaux usées prélevé sur le canal El Mahmoudia, affecté par les rejets industriels, agricoles et des eaux usées. Les meilleurs résultats de suppression ont été de 99,9% pour Cd^{2+} et 92,4% pour Pb^{2+} .

Ces résultats indiquent que *Ficus Benjamina* peut être utilisé comme adsorbant à faible coût pour l'élimination des métaux lourds des eaux usées.

Mots-clés: Biosorption, métaux lourds, *Ficus benjamina*, conception factorielle complète.

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CHAPTER ONE INTRODUCTION

1.1 Overview

The availability of water resources are becoming increasing scare; the consumption and exploitation of water resources, along with exponential increase in population have caused water pollution (Ahmed et al., 2012; Ashraf et al., 2011 Barcelo et al., 2011 Seolatto et al., 2012; Sharma et al., 2013) as water used in industry creates as wastewater which harm our environment due to introducing various contaminants into water bodies (Surchi, 2011). Major water pollution sources are domestic, industrial, agricultural as well as solid waste pollution, thermal pollution, shipping water pollution and radioactive wastes (Ashraf et al., 2011). Discharge of inorganic and organic pollutants into water bodies have greatly affected the ecological balance and cause harmful effects on flora and fauna (Ahmed et al., 2012). For example, oil spills happened in 2010 (British petroleum oil spillage in the Gulf of Moxico) and the latest in 2012 (Edfu sugar factory's oil spillage in the Nile River, Egypt).

Human activities in urban, industrial and mining areas contaminate water with heavy metals. Toxic heavy metals of particular concern in treatment of industrial wastewaters include: mercury, lead, cadmium, zinc, copper, nickel and chromium (Fenglian & Wang, 2011; Mousavi et al., 2010). Many industries, especially pulp and paper industries, pigments, petroleum and batteries manufacturing, fertilizer and pesticide industry and application, leatherworking, photography discharge heavy metals into water bodies in high levels (Murthy et al., 2012; Seolatto et al., 2012; Wang & Chen, 2009). The heavy metals, such as lead, copper, cadmium, zinc and nickel ions are among the most common pollutants found in industrial effluents (Mousavi et al., 2010).

Metals are ubiquitous constituents in the biosphere, dynamic to our industry, infrastructure and daily life. Since the industrial revolution, metals have progressively been redistributed in the environment, with accumulation in terrestrial and aquatic habitats being associated with adverse effects on the biota and human health (Sparks, 2005). Under certain conditions, either vital micronutrients, such as Cu and Zn, or toxic elements such as Hg, Pb, and Cd, in the environment might accumulate to a toxic concentration level, leading to ecological harms (Park et al., 2011).

Heavy metals released into the environment pose a significant threat to the environment and human health because of their toxicity and persistence (Salma et al., 2017). Because of non-biodegradability of heavy metals in the environment and their harmfulness to living organisms, their removal becomes so important with regard to protection of ecosystem and human health.

It is a great challenge to remove cadmium and lead ions from wastewater. Over a few decades, a variety of methods, e.g. chemical precipitation, evaporation, coagulation, ion exchange, membrane processing, electrolytic and adsorption technologies are used to remove these toxic substances from industrial wastewaters and other effluents (Rakhshae et al., 2005; Park et al., 2011). However, these techniques have certain disadvantages such as incomplete metal removal, high reagent and energy requirements and generation of toxic sludge that require disposal. However, to attain the toxic specie residual concentration in the effluent with acceptable contents according with the legislation, other operations are required. Due to the high costs of commercial adsorbents, the search for alternate and innovative treatment techniques has focused attention on the use of biological materials for heavy metal removal and recovery technologies (Biosorption).

Biosorption describes any system which includes a sorbate working together with a biosorbent resulting in an accumulation at the sorbate–biosorbent interface, and therefore a decrease of sorbent concentration in the solution (Sasaki et al., 2013).

Biosorption is a property of both living and dead organisms, and has been exploited as a promising biotechnology because of its simplicity (Abiona et al., 2016).

One of the heavy metals in water is Cadmium (II); it is highly toxic to humans, plant, sand, animals and it is responsible for causing kidney damage, renal disorder, high blood pressure, bone fractures, and destruction of red blood cells (Purkayastha et al., 2014). According to the World Health Organization (WHO), the maximum accepted level of Cd (II) in water is 0.005 mg/l (Abaliwano et al., 2008).

Another heavy metal is Lead (II): it is one of the most dangerous contaminants; the overload amount of lead in water causes severe problems such as anaemia, encephalopathy, hepatitis and kidney disease (Abdel-Aty et al., 2013). According to World Health Organization (WHO) the highest desirable limit of Pb(II) is 0.05 mg/l (Mataka et al., 2006).

This research will consider the possibility of using an experimental design technique to investigate the biosorption of cadmium and Lead ions from waste water using *Ficus benjamina* biomass as a biosorbent material, to help in finding an alternative methods to treat water that could be economically and environmental friendly techniques.

1.2 Problem Statements

The problem formulation and the offered solution in this work are based on the challenges associated with heavy metals in the environment and their proper remediation methods as summarized below:

- Anthropogenic activities have disrupted the natural biogeochemical cycles of metals in the environment.

- In case of discharging untreated wastes, accumulated levels of toxic heavy metals in the environment can poison the biosphere and threaten our water resources.
- Heavy metals such as lead and Cadmium are toxic.
- More stringent environmental regulations are being in force to reduce metal discharge in the environment and avert the adverse health impacts.
- Environmental process costs usually add up to overall costs and minimizing such costs are always desirable.
- Although effective, the traditional metal removal methods appear to be costly and inadequate.
- A preponderance of biosorption data reported in the literature refers to results from batch experiments.
- An industrially relevant biosorption method for removal of toxic metals has not been achieved thus far.
- We need to assist and provide proper, cost effective and eco-friendly technology to reduce the concentration of toxic metals from wastewater to match the accepted discharge limits.

Therefore, to address the above problems and the associated challenges listed above in removal of heavy metals from wastewater, we need to assist and provide proper, cost effective and eco-friendly technology to reduce the concentration of toxic metals from wastewater to match the accepted discharge limits.

1.3 Objectives

The main objective of the proposed study is:

To utilize the factorial experiment design to optimize the adsorption of heavy metals by *Ficus benjamina* biomass.

The specific objectives are:

- To explore how pH, temperature and initial concentration of heavy metals interacted and ultimately affected Cd^{2+} and Pb^{2+} removal efficiency from aqueous solutions.
- To evaluate the adsorption performance of locally derived sorbate for the removal of Cd^{2+} and Pb^{2+} from single aqueous solution using a factorial design 2^3 scheme.
- To test the validity of the result optimization model for the removal of Cd^{2+} and Pb^{2+} from real industrial wastewater
- To find the best conditions that can be used for Cd (II) and Pb (II) removal from water.

1.5 Significance

The results of this project will serve in treating water by removing cadmium and Lead ions and other heavy metals, using natural and environmentally friendly materials. This research opens the possibility of using *Ficus Benjamina* biomass to be employed in the treatment of industrial effluents and agricultural waste waters before being delivered into the environment.

1.6 Dissertation Summary

This dissertation includes five chapters. Chapter one is the introduction chapter, which included the background of the study, problem statements, research objectives and significance.

Chapter two is also proposed to cover scientific literature on the study including the following headlines: the heavy metals, their sources and impact, Methods used for the removal of heavy metals from contaminated waters, Biosorption of heavy metals, Factors Affecting Biosorption. And factorial experimental design. Also, Previous works using factorial experimental design for Cd²⁺ and Pb²⁺ biosorption

Chapter three will present step by step procedures of the thesis study involving the materials and methods including the Instruments, Reagents, Experimental Procedures (Batch biosorption procedure, Statistical design of experiments, Application of real wastewater samples).

Chapter four covered the results and discussion. The final chapter will highlight the thesis study conclusions and the future recommendations from the current study.

CHAPTER TWO LITERATURE REVIEW

2.1 Background

2.1.1 Heavy metals

Every day, there are thousands of chemicals discharged directly and indirectly into water bodies as industrial waste causing serious air, soil, and water contamination without further treatment for elimination of the included harmful compounds (Salim et al., 2002; Khairy et al., 2014). Heavy metals are without doubt well thought-out as the most hazardous and harmful metals even if they are present as traces, since they accumulate in the tissue of living organisms (Rao et al., 2010). Most of the metals are carcinogenic, teratogenic and cause severe health problems like organ damage, reduced growth and development, nervous system impairments and oxidative stress. Heavy metals introduced into water by several industries such as mining, electroplating, petroleum refining (Rao et al., 2010) and other industries with its toxicity which is presented in Table 2.1.

Table 2.1: Heavy metal sources and toxicity

Metal	Source	Toxic effect	WHO limit (mg/l)	MOH limit (mg/l)	References
Cadmium	Electroplating, smelting alloy manufacturing, pigments, plastic and mining	Itai–Itaidisease, carcinogenic, renal disturbances, lung insufficiency, bonelesions, cancers, hypertension, weight loss	0.005	0.003	Sharma and Bhattacharyya, 2005; Momodu and Anyakora, 2010; Singh et al., 2005; MOH, 2015)
Lead	Manufacturing of batteries, pigments Electroplating, Ammunition	Anaemia, brain damage, anorexia, malaise, loss of appetite	0.05	0.01	(Low et al., 2000; Ali et al., 2013; Mataka et al., 2006; MOH, 2015)
Chromium	Electroplating, paints and pigments, metal processing, steel fabrication and canning industry	Epigastric pain, nausea, vomiting, severe diarrhoea, lung tumours, Carcinogenic, mutagenic, teratogenic	0.03	0.05	(Ali et al. 2013; Rao et al., 2010; Singh et al., 2005; MOH, 2015)
Copper	Electronics plating, paint manufacturing, wire drawing, copper polishing	Reproductive and developmental toxicity, neurotoxicity, and acute toxicity, dizziness, diarrhoea	1.5	1	(Bilal et al., 2013; MOH, 2015)
Arsenic	Smelting, mining, energy production from fossil fuels, rock sediment's	Bone marrow depression, haemolysis, liver tumours, gastrointestinal symptoms,	0.02	0.01	(Momodu and Anyakora, 2010; MOH, 2015)

2.1.2 Sources and Impacts of Heavy metals

Heavy metals are the main group of inorganic contaminants, and a considerable large area of land is contaminated with them due to use of sludge, pesticides, fertilizers, and emissions from municipal waste incinerators car exhausts and smelting industries (Garbisu & Alkorta, 2003; Halim et al., 2003) cited from (Singh et al., 2010). For example, heavy metals in air emissions are brought down into water during rainfall, leached from solids into surface streams and rivers, and effluents from industries are often discharged directly into the river or other receiving surface water bodies (Wang & Shamma, 2009)

Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major causes of soil contamination with heavy metals, and an increased metal uptake by food crops grown on such contaminated soils is often observed. In general, wastewater contains substantial amounts of beneficial nutrients and toxic heavy metals, which are creating opportunities and issues for agricultural production, respectively (Chen et al., 2008 ; Singh et al., 2005).

Extreme accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also lead to elevated heavy metal acceptance by crops, and thus affect food quality and safety (Marcovecchio, et al., 2007). Heavy metal accumulation in soils and plants is of increasing concern because of the potential human health risks. This food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body. Heavy metal buildup in plants depends upon plant species, and the efficiency of different plants in absorbing metals is assessed by either plant uptake or soil-to-plant transfer factors of the metals (Ramachandra et al., 2005).

Vegetables cultivated in wastewater-irrigated soils take up heavy metals in large enough quantities to cause potential health risks to the consumers. In order to measure the health risks, it is essential to identify the potential of a source to introduce risk agents into the environment, estimate the amount of risk agents that come into conta

ith the human-environment limitations, and quantify the health consequence of the exposure (Mahvi et al., 2005).

European Union defined two lists (Table 2.2). Black list which contains those compounds considered so toxic, persistent or bio-accumulative within the environment that priority should be given to eliminating pollution by them. Grey list that contains compounds which are environmentally harmful but less so than those in Black list (Forster & Wase, 2003).

Table2.2: Black and Grey List Metals

Black List	Grey List
Cadmium	Lead
Mercury	Chromium
	Copper
	Nickel
	Zinc
	Arsenic
	Cobalt
	Silver
	Selenium

Cd^{2+} and Pb^{2+} are frequently used industrial processes such as metallurgy, mining, painting, smelting, batteries, and alloys industry so their products remain a challenge for developing countries which lack the capacity to manage and dispose of the substances in products in an environmentally sound manner (Piper & Caicedo Restrepo, 2013). They are listed among the 25 hazardous substances that pose the

most significant potential threat to human health. The act as a cumulative poison and all their compounds are potentially harmful (Fawzy, 2007).

As awareness of the adverse impacts of them has increased, many uses have been reduced significantly in industrialized countries. In additions, as public awareness has grown, waste management systems have increasingly been put in place in industrialized countries to reduce releases of lead and cadmium to the environment (Piper Caicedo Restrepo, 2013).

2.1.3 Cadmium (Cd^{2+})

Cd^{2+} is one of the most toxic heavy metals and is considered non-essential for living organisms (Mudhoo et al., 2012). Due to its acute toxicity, Cd^{2+} has joined lead and mercury in the most toxic “Big Three” category of heavy metals with the greatest potential hazard to humans and the environment (Wang & Shamma, 2009). The natural sources of Cd^{2+} release into the environment are due to natural mobilization of Cd^{2+} from the Earth’s crust and mantle, such as volcanic activity including hydrothermal vents and weathering of rocks. The major anthropogenic sources of Cd^{2+} release into the environment are mobilization of cadmium impurities in raw materials such as phosphate minerals due to runoff from agricultural areas treated with phosphate fertilizers, loss of recyclable Ni-Cd batteries through the waste disposal system with consequent release of cadmium to the environment, electroplating, pain pigments, smelting and alloy manufacturing (Benaissa, 2006; HELCOM, 2005; UNEP, Version of December 2010). An increasing part of the global cadmium consumption is used for nickel-cadmium (Ni-Cd) batteries, which in 2005 accounted for 82% of the total cadmium consumption according to the International Cadmium Association, (ICDA, 2007) the estimated cadmium usage in Ni-Cd batteries was 83 % of total cadmium consumption (UNEP, Version of December 2010).

Cd^{2+} which widely used and extremely toxic in relatively low dosages, is one of the principal heavy metals responsible for causing severe risks to human health. Acute

overexposure to cadmium fumes may cause pulmonary damage after acute inhalation in the 1930's. After the Second World War, bone effects and protein-uria were reported from Japan, in descriptions of a bone disease (itai-itai disease), with fractures and severe pain, linked to cadmium exposure and related to a low-calcium diet (UNEP, Version of December 2010). The International Agency for Research on Cancer (IARC) classifies Cd^{2+} in Group I: carcinogenic to humans (IARC, 1993), and the US Environmental Protection Agency (EPA) has determined that Cd^{2+} is a probable human carcinogen by inhalation (UNEP, Version of December 2010). Also, when ingested by human beings, Cd^{2+} that is not excreted immediately has a long half-life of several hundred days (Mudhoo et al., 2012).

2.1.4 Lead (Pb^{2+})

Pb^{2+} is the most common of the heavy elements (Wang & Shammash, 2009). This metal exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals (Tangahu et al., 2011). Pb^{2+} is one of the most dangerous contaminants, it is present in tap water as a result of dissolution from natural sources or from household plumbing systems containing lead in pipes (Mudhoo et al., 2012). Pb^{2+} is released to the environment by various anthropogenic activities from industrial wastewaters due to its extensive use as raw material for storage battery manufacturing, printing, electroplating, pigments, Lead smelting, a coloring element in ceramic glazes; notably in the colors red and yellow, fuels photographic materials and explosives manufacturing (Hasan et al., 2008; Hasan et al., 2009; Ray et al., 2006). Also, from internal combustion engines, lead solder in food cans, lead-arsenate pesticides (Mudhoo et al., 2012; Ray et al., 2006). International Lead and Zinc Study Group (ILZSG) representing about 86 % of the total global consumption of lead from batteries (UNEP, Version of December 2010). Dust in homes and streets as well as soil may contain high concentrations of lead; this is particularly the case for dust in homes where paint with lead pigments has been used, and soil around lead-emitting industries (UNEP, Version December 2010).

Lead poisoning in humans causes severe damage to the kidneys, nervous system, reproductive system, liver, and can cause sickness or death. Severe exposure to Pb^{2+} has been associated with sterility, abortion, stillbirth, anemia, nephritic syndrome, hematological damage and neonatal deaths (Hasan et al., 2008; Ray et al., 2006). Perhaps no other metal, not even arsenic, has had its toxicology so extensively studied as has Pb^{2+} . Its poisoning has been actually linked to the fall of the Roman Empire. The high lead content in bones from roman period supports the hypothesis that the use of lead water pipes (Wang & Shamma, 2009).

Pb^{2+} can be absorbed by the body through inhalation, ingestion, dermal contact (mainly as a result of occupational exposure), or transfer via the placenta. Once lead is absorbed, it enters either a “rapid turnover” biological pool with distribution to the soft tissues (blood, liver, lung, spleen, kidney, and bone marrow) or a “slow turnover” pool with distribution mainly to the skeleton. Of the total body lead, approximately 80-95% in adults and about 73% in children accumulate in the skeleton. The biological half-life of lead is approximately 16-40 days in blood and about 17-27 years in bones (Wang & Shamma, 2009).

2.2 Methods used for the removal of heavy metals from contaminated waters

Remediation of heavy metals is necessary to protect the environment from their toxic effects and preserve the environment for future generations (Fulekar, 2010). Most of the previous work has focused on the removal of higher concentrations of pollutants, relying on the more traditional and more expensive absorbents materials. However, as the discharge regulations become more and more stringent due to development of advanced analytical technique and better health monitoring technologies, the importance of the low concentration pollutants is consequently becoming an essential issue (Carvalho et al., 2011). It is a great challenge to remove cadmium and lead ions from wastewater. Over a few decades, Several physicochemical techniques have been assumed for the elimination of heavy metals, which is regarded as a challenging job with respect to cost and technical complexity

(Barcelo and Poschenrieder, 2003 ; Sheoran et al., 2011). A variety of methods, e.g. chemical precipitation, evaporation, coagulation, ion exchange, membrane processing, electrolytic and adsorption technologies are used to remove these toxic substances from industrial wastewaters and other effluents (Gadd, 2009). However, these techniques have certain disadvantages such as incomplete metal removal, high reagent and energy requirements and generation of toxic sludge that require disposal. Therefore Biological treatment is suggested to be a more cost-effective and economic alternative. This method entails the usage of biological material that has the Review ability to bind metals through processes such as biosorption (Sheoran et al., 2011). Table 2.3, illustrates the advantages and disadvantages of some techniques to remove heavy metals.

Table 2.3: Advantages and disadvantages of several techniques used currently to Remove heavy metal from water (O'Connell et al., 2008).

Method	Advantages	Disadvantages
Chemical Precipitation	Inexpensive; Simple. Most of the metals can be removed.	Disposal problems. High solid waste produced.
Ion-exchange	Effective Possible metal recovery	Sensitive to the presence of particules Expensive resins
Chemical coagulation	De watering. Sludgesettling. High efficiency (>95) Less solid waste produced Less chemical consumptio	Large consumption of chemicals. High cost. High running cost. Low flow rates.

2.3 The other alternatives for the heavy metals removal:

To attain the toxic specie residual concentration in the effluent with acceptable contents according with the legislation, other operations are required. Due to the high costs of commercial adsorbents, the search for alternate and innovative treatment techniques has focused attention on the use of biological materials for heavy metal removal and recovery technologies (Biosorption). This technique is favored because of abundance of biomass, low cost, reduced sludge compared to conventional treatment techniques and better decontamination efficiency from highly diluted solutions.

Plants and microorganisms are used to remediate toxic contaminants from the environment; this is recognized as biological remediation (Singh et al., 2010). Biological remediation is considered as the most effective method of toxic metal removal because it is a natural process, environmentally friendly, has a low cost, and high public acceptance (Ding et al., 2012). Biological remediation techniques include bioremediation, phytoremediation, bioventing, bioleaching, land forming, bioreactors, composting, bioaugmentation and biostimulation. Among these methods, bioremediation and phytoremediation are the most useful techniques. These methods have advantages over physicochemical methods because they preserve natural soil properties and depend on solar energy (El Zokm et al., 2012).

Phytoremediation is an developing green technology that is used for the uptake of vast quantities of heavy metals from soil and storage of these in a harvestable (removable) component (Singh et al., 2012). Plants usually cope with contaminants by changing the properties of soil, which conserves soil efficacy and fertility (Mousavi et al., 2010). After growing the hyper-accumulating plants on a contaminated site, the biomass can be treated to recover the contaminant (Ha et al., 2011). Phytoremediation could be a feasible option to remediate heavy-metal polluted soils, particularly when the biomass produced during the phytoremediation process can be economically valorized in the form of bio-energy, e.g., biodiesel production. The use of metal

accumulating bio-energy crops (such as Brassica spp.) might be suitable for this purpose. Certain plants are capable of accumulating contaminants in their tissues (Juwarkar et al., 2010), for example, *Eleocharis acicularis* can accumulate As, Cu, Zn and Pb (Ha et al., 2011).

2.4 Biosorption of heavy metals

Biosorption is a physico-chemical process, which comprises the removal of various pollutants, such as metal or metalloid kinds, compounds and particulates from solution by biological material (Fulekar and Gavrilescu, 2010). It is metabolically passive process and is performed by material of biological origin, not by living biomass (Chojnacka, 2010). Also, it is defined also as the passive binding of trace elements on a biosorbent having chemically active sites or functional groups (Lichtfouse et al., 2012a). On the other hand, bioaccumulation is metabolically active and is performed by living cells (Chojnacka, 2010). It is the accumulation of contaminant via all routes available to the organism (Bradl, 2005).

The process of biosorption includes a solid phase (sorbent) and a liquid phase (solvent) containing a dissolved species to be sorbed (Sud et al., 2008). It can describe any system where a sorbate (e.g. an atom, molecule, a molecular ion) interacts with a biosorbent (i.e. a solid surface of a biological matrix) resulting in an accumulation at the sorbate biosorbent interface, and therefore a reduction in the solution sorbate concentration (Gadd, 2009).

Biosorption process contains four steps: (i) migration of metal ions from the bulk solution to the surface of the adsorbent; (ii) diffusion through boundary layer to bio-carbon surface; (iii) adsorption at a binding site and (iv) intra particle diffusion into the interior of the sorbent surface (Singanan and Peters, 2013).

Nowadays, there are many reasons for studying biosorption process. Treatment of wastewater and the purification of water for human purposes by conventional means are very expensive (Barka et al., 2013). In many countries, the technology of

wastewater treatment or water purification has to be simple, as there is a lack of skilled labor to safely perform more sophisticated technologies such as chemical precipitation. In these countries the use of plant biomasses for the removal of metal ions promises to be an inexpensive another treatment process (Zimmermann and Wolf, 2010). Table 2.4 represents the advantages and disadvantages of common biosorbents.

Table 2.4: Advantages and disadvantages of common biosorbents

Advantages	Disadvantages
Low operation costs if low-cost sorbents are used. (Fu and Wang, 2011)	Shorter lifetime of biosorbents when compared with conventional sorbents. (Fu and Wang, 2011)
Low quantity of sewage sludge disposed. (Gadd, 2008)	Fast saturation i.e. when metal interactive sites are occupied.(Gadd, 2008)
COD of wastewater does not increase. (Sahmoune et al., 2011)	Recyclable and decomposable properties of biomass are delaying their long-term
The process is simple in operation and very rapid. (Sahmoune et al., 2011)	applications in adsorption processes. (Sahmoune et al., 2011)
Biosorbents are selective and regenerable. (Fu and Wang, 2011)	The characteristics of the biosorbents cannot be biologically controlled.(Ahalya et al., 2003)

2.4.1 Cd²⁺ and Pb²⁺ removal using different types of biosorbents

Different types of biomass have been investigated for the biosorption characteristics of Pb²⁺ and Cd²⁺ from aqueous solution. The seaweed, *Sargassum* sp, was used by Park et al., (1999) for removal of lead and cadmium ions from wastewater. Vecchio et al.,(1998) studied the removal of Cu²⁺, Pb²⁺ and Cd²⁺ ions by biosorption on bacterial cells. Also, Srivastav et al.,(1993) used the aquatic plants for removal of lead and zinc ions from waste water. The adsorption of lead ions on nonliving *Penicilliumchrysogenum* biomass was also investigated (Niu et al., 1993).Seolatto et al., (2012) studied the removal of Lead, Cadmium and Chromium by the Pequi Fruit. Sulaymon et al., (2012) studied the biosorption of Cd²⁺ and Pb²⁺using rice hurk. Al-Qahtani (2012) studied the biosorption of Cd²⁺ and Pb²⁺ on *Cyperuslaevigatus*. Moreover Muhammad & Nwaedozi, (2012) studied the removal of Lead and Cadmium using marine algae-seaweed, *Ascophyllumnodosum*, as adsorbent.

2.4.2 Factors Affecting Biosorption

Many parameters were found to affect the biosorption process of heavy metals. Amongst these parameters that could be cited are temperature, pH, and initial metal concentration:

i) Effect of the pH

One of the most important parameter that affects biosorption process is pH. There are three ways in which the pH can influence metal biosorption:

First, the state of the active sites may be changed. When the binding groups are acidic, the availability of free sites depends on pH: at lower pH the active sites are protonated and therefore competition between protons and metal ions for the sorption site occurs (Naja et al., 2005). At low enough pH, virtually all sites become protonated and complete desorption of the bound metal ions is possible (Hansen et al., 2006), which is

why acid treatment is a method for metal elution and regeneration of the sorbent material. At higher solution pH, the solubility of metal decreases sufficiently allowing precipitation, which may complicate the sorption process (B. Volesky, 2003).

ii) Effect of Temperature

Temperature influences the rate of biosorption because of its effect on: (I) the stability of the metal species in solution, (II) the stability of the biosorbent – metal complex dependent upon biosorption sites, and (III) the cell wall configuration (Demirbas, A. 2008). For endothermic reactions, higher temperatures enhance sorption due to the increase in surface activity and kinetic energy of the solute (Vijayaraghavan and Yun, 2008). In contrast, an increase in heat would cause a decrease in the biosorption capacity of the biosorbents in the system where binding of the metal ion is exothermic.

iii) Initial Metal Ion Concentration

The Initial Metal Ion concentration is another factor that affects biosorption process. Studies have verified that at higher concentration of metal ions the amount of adsorbed ions is greater than the amount at lower concentration since there are more binding sites for interaction (Mukhopadhyay et al., 2008). There is a greater driving force at higher concentration between the solid and liquid interface thus enabling mass transfer. Biosorption efficiencies decreased with the increasing of initial metal concentrations. The initial metal concentration provides a driving force that overcomes mass transfer resistance of metal ion between the adsorbent and the biosorbent solution (Nemr, A.E. 2009). The biosorption percentage decrease may be due to lack of enough surface area to absorb the available metal in the solution (King at al., 2008).

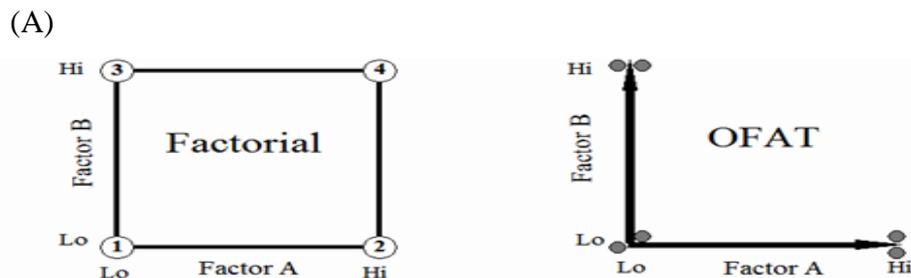
2.5 Factorial Experimental Design

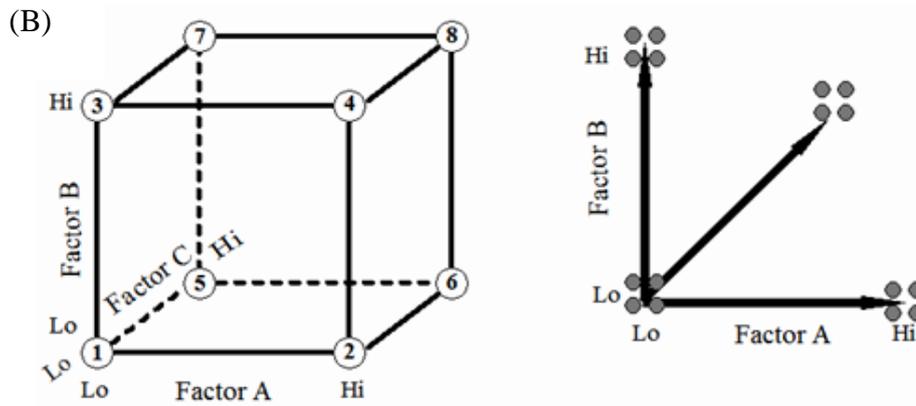
2.5.1 The Use of factorial designs to optimize metal removal efficiency

The analysis in which the evaluation of more than one factor can be done is called full factorial analysis. Factorial design is employed to define the most important process variables affecting the metal removal efficiency. It is also used to reduce the total number of experiments in order to achieve the best overall optimization of the system. The factorial experimental design methodology involves changing all variables from one experiment to the next to determine which factors have important effects on the response as well as how the effect of one factor varies with the level of the other factors. The determination of factor interactions could only be attained using statistical designs of experiments, since it cannot be shown when the system optimization is carried out by varying just one factor at the time and fixing the other. Few studies employed the factorial design method for evaluating the influence of the operation variables on biosorption processes. The biosorption of Cd^{2+} and Pb^{2+} was optimized using 2^3 factorial designs by (Peternele et al., 1999; Barros et al., 2003). The biosorption of Cr^{3+} and Cr^{6+} using 2^3 and 2^4 factorial designs, respectively was studied (Carmona et al., 2005; Brasil et al., 2006).

2.5.2 Overview of Factorial Experimental Design

The simplest factorial design involves two factors at two levels. The one factor at a time (OFAT) design is shown in Figure 1 (a) (Anderson and Whitcomb, 2007). However, Figure (B) shows the points for the factorial designs that are starting with low levels and ending with high levels.





(C)

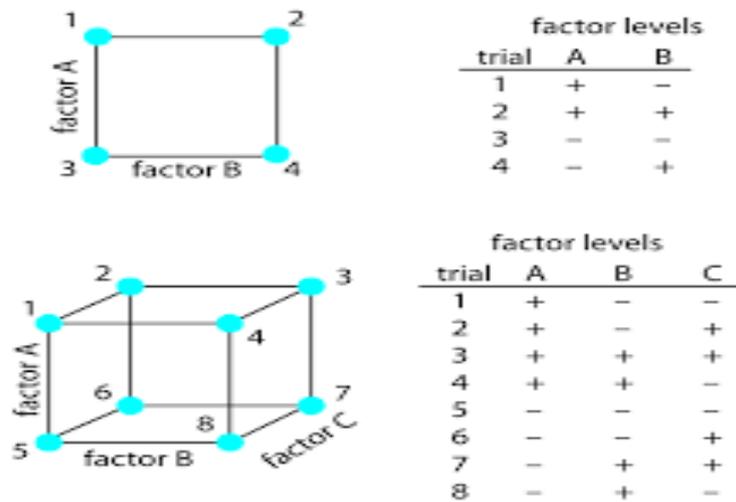


Figure 1.1 : (A) One-factor-at-a-time (OFAT), (B) two-level factorial design (Anderson and Whitcomb, 2007), (C) Optimizing the Experimental Procedure

For example, full 2^3 factorial design needs only 8 runs versus 16 for an OFAT experiment. Hence, the ratio of the number of observations needed in a one-at-a-time experiment to the number required in a full 2^k factorial experiment raises linearly according to equation (1) and Figure 2 (Spall, 2010).

$$\frac{k+1}{2} \dots\dots\dots (1)$$

Where: k exemplifies the number of factors in factorial design.

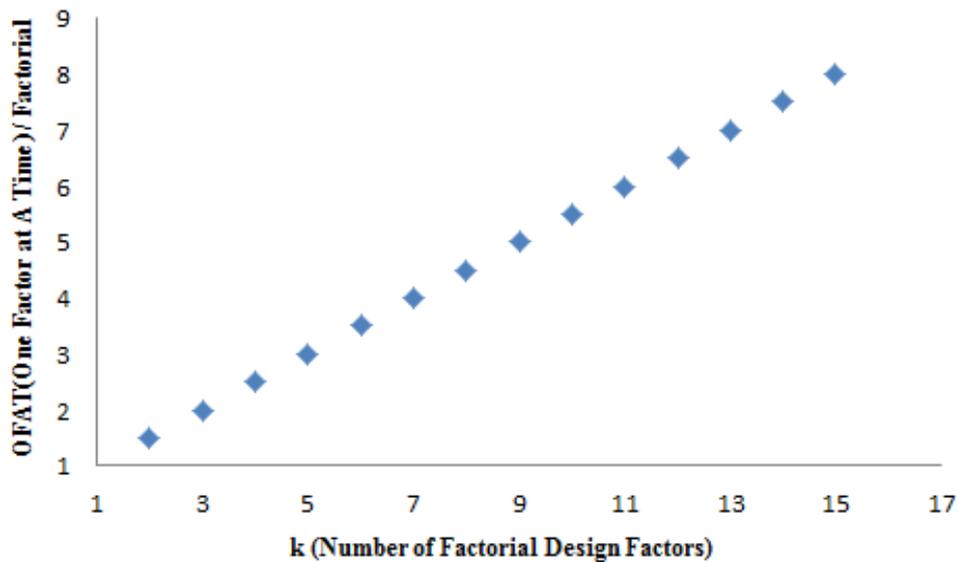


Figure 1.2: Relative adeptness of one-at-a-time and factorial design

2.5.3 Previous works using factorial experimental design for Cd^{2+} and Pb^{2+} biosorption

Badr (2007) studied the removal of Cd^{2+} and Pb^{2+} by *Eichhornia crassipes* from synthetic wastewater using factorial design (2^3). Experiments were carried out at two markedly different levels of three factors: pH (2.0 and 6.0), Temperature (T) (20 and 45° C), and Metal ion concentration (X) (10 and 1200 mg/L). Maximum removal was observed near pH = 6 for Cd^{2+} while that for Pb^{2+} was observed near pH = 2. The most major effect for Cd^{2+} and Pb^{2+} biosorption by *Eichhornia crassipes* was ascribed to pH and The interaction effects of T, pH and X. pH that have a significant influence on the Cd^{2+} and Pb^{2+} exclusion efficiency. The best removal percentage of Cd^{2+} was 85.0% when pH= 6, X= 10 mg/L and T= 20°C while for Pb^{2+} , it was 84.0% when pH= 2, X= 1200 mg/L and T= 20°C

The potential of *Phragmites australis* biomass for the removal of Cd^{2+} and Pb^{2+} from synthetic wastewater using factorial design (2^3) was studied by Fawzy (2007). Experiments were carried out at two distinctly different levels of three factors: pH (2.0 and 6.0), Temperature (T) (20 and 45°C), and Metal ion concentration (X) (10 and 1200 mg/L). The most significant effect for Cd^{2+} and Pb^{2+} biosorption was ascribed to

pH. The interaction effects of T, pH and T, X have a significant influence on the Cd²⁺ removal efficiency while, the main factors (T and X) exert significant effect on biosorption of Pb²⁺. The best removal percentage of Cd²⁺ was 83.5% when pH= 6, X=10 mg/L and T= 20°C while for Pb²⁺, it was 72.5% when pH= 2, X= 1200 mg/L and T= 80 °C.

Varma et al. (2010) studied the removal of cadmium from aqueous solutions using *Psidiumguajava* leaves powder. The experiments were designed by Box Behnken design method (3-1 fractional factorial design). The aspects in this study are metal concentration (50,100,150 mg/L), pH of aqueous metal solution (2, 4, and 6) and biomass dose (0.25, 0.5 and 0.75g). Others variables such as speed of shaker was adjusted at 160 rpm, volume of the aqueous solution 50 ml and temperature 30°C and optimum agitation time 60 min were kept constant. The removal percentage of Cd²⁺ was 95.11% at initial concentration=90 mg/l, pH=4 and biomass dose= 1g. Increase in pH resulted in an increase in Cd uptake. Authors found that low sorption of Cd²⁺ in the pH<4. They attributed could have been due to competition with the H ions for metal binding sites on the biomass cells, while the increase in pH favors metal sorption mainly because of negatively charged groups.

Seolatto et al. (2012) studied the removal of Lead, Cadmium, and Chromium by the Pequi Fruit Skin (*Caryocarbrasiliense* Camb.) biomass and considered factors such as biomass dose (0.15, 0.75g), pH (3, 5) and biomass size (0.2, 0.7mm) that were studied using a factorial statistical design. The results showed that Pb²⁺ ions logged the highest biosorption with an average of 16.78 mg/g and up to 80% removal percentage. However, there was less removal of chromium and cadmium. The dose of biomass and pH were found to be the most important factors in the biosorption while grain size did not influence the biosorption process.

Al-Qahtani (2012) studied the biosorption of Cd²⁺ and Pb²⁺ on *Cyperus laevigatus* using the factorial design (2³). The three factors were screened at two distinctly different levels were pH (2.0 and 6.0), temperature (T) (20 and 45°C) and metal ion concentration (X) (20 and 800 mg/l). The most significant effect for Cd²⁺ and Pb²⁺ biosorption was ascribed to (T). The best percentage of Cd²⁺ remedial

was 85% when pH= 2, T= 450C and X= 800 mg/L but the best percentage of Pb²⁺ remedial was 82% when pH= 6, T= 450C and X= 20 mg/L.

Muhammad and Nwaedozie (2012) studied the removal of Lead and Cadmium using marine algae-seaweed (*Ascophyllum nodosum*) as adsorbent for metal elimination at two temperatures (23.5°C and 37°C) and four pH values (2, 5, 7 and 10). The determined removal of Lead was 93.41% at pH 2 and 53.13% for cadmium at pH 10. Temperature was found to have no significant effect on the adsorption process.

CHAPTER THREE MATERIALS AND METHODS

3.1 Instruments

- Kitchen Grinder (Moulinex),
- Siever 0.5 mm (US Standard Sieve Series) DUAL MFG.CO,
- Analytical Balance (Sartorius, d=0.01 g) with Maximum weight=810g,
- Electronic Oven (Binder),
- pH/mV Hand-held meter (Crison pH meter, pH meter, pH 25),
- Orbital Shaker (HAF, Teck) ; made in Egypt model A22 was used for experimental work,
- Varian Inductively Coupled Plasma (ICP-AES)

3.2 Glassware and Other Apparatus

- Measuring Flasks,
- Cylinder,
- Erlenmeyer Conical Flasks (250ml),
- Beakers,
- Aluminium foil,
- Aluminium dishes,
- Pipette,
- Syringe Pipette,
- Spatula,
- Dropper,
- Microdropper,
- Whatman Glass Microfiber Filters(GF/A),
- Funnels, Sampling Plastic Bottles, Marker

3.3 Reagents

- Lead A.A.Standard (1000ppm) (CP Achem),
- Cadmium A.A.Standard (1000ppm) (CP Achem),
- 0.1M Nitric acid HNO₃ (Panreac, 65% pure),
- 0.1M Sodium Hydroxide NaOH (ADWIC),
- Double Distilled Water

3.4 Experimental Procedur

3.4.1 Biosorbent Preparation

The *Ficus benjamina* biomass, weeping fig, was used as a biosorbent material. It is a very popular houseplant in temperate areas, due to its elegant growth and tolerance of poor growing conditions. It does best in bright, sunny conditions requires a moderate amount of watering in summer, and only enough to keep it from drying out in the winter. The plant is sensitive to cold and should be protected from strong drafts. When grown indoors, it can grow too large for its situation, and may need drastic pruning or replacing. *Ficus benjamina* has been shown to effectively remove aseous formaldehyde from indoor air (Kim et al., 2008).



Figure 3.1: Ficus Benjamina ‘Weeping fig’

This biomass was washed with tap water to remove any dust or foreign particles attached to biomass and then rinsed with double distilled water (DDW). The washed biomass was dried at 60°C in oven for one day, then grounded to powder, sieved through a sieve with a mesh size of 0.5 mm and finally packed in plastic bottles for further usage (Figure 3.2).

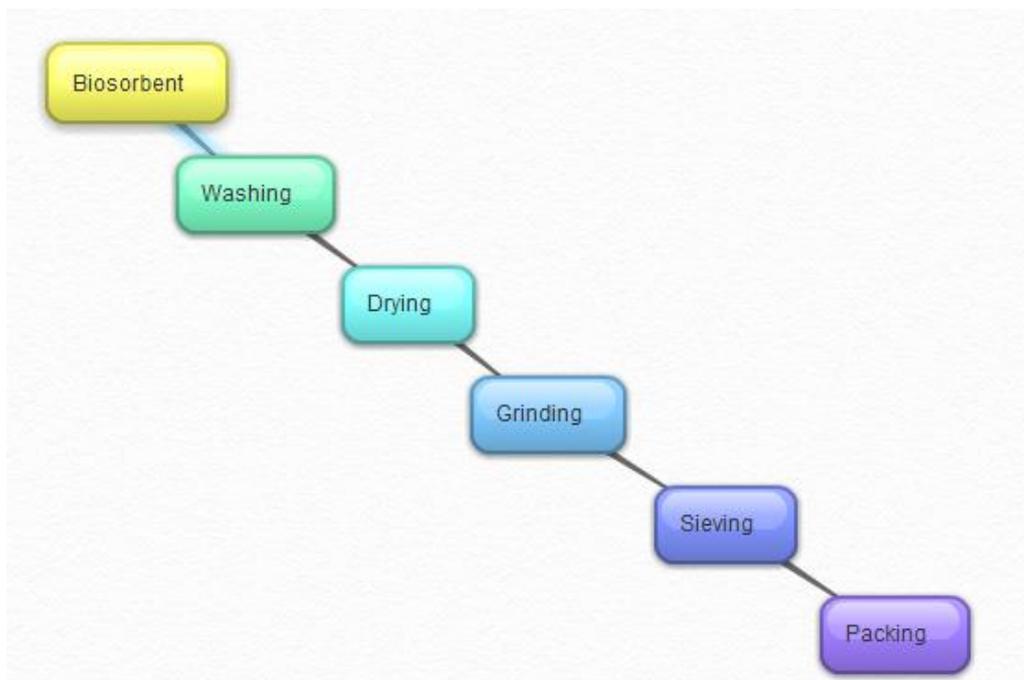


Figure 3.2: Procedure for preparation of biomass

3.4.2 Preparation of Heavy Metal Solution

Standard solutions of Cd^{2+} and Pb^{2+} , with initial concentration of 1000 ppm, were used to prepare experimental concentration of 5 and 300 ppm (for Cd^{2+}) and 10 and 500 (for Pb^{2+}) using distilled water. The concentration is measured as mass/ volume so the equation for dilution to obtain experimental concentration is:

$$M_1 V_1 = M_2 V_2$$

Where: M_1 is molarity, V_1 is volume and the subscripts 1 and 2 refer to initial and final values.

3.4.3 pH Adjustment

- I. Acid solution of 0.1M HNO₃ was prepared according to the following formula:

$$\text{Molarity of acid} = \frac{(\% \text{ age purity})(\text{Specific gravity})1000}{\text{Molecular weight}}$$
$$M = \frac{0.65 \times 1.42 \times 1000}{63.01} = 14.48$$

Dilution of concentration acid by:

$$M_1 V_1 = M_2 V_2$$
$$14.48 \times V_1 = 0.1 \times 100$$
$$V_1 = 0.69 \text{ml}$$

This volume was taken by syringe pipette, transferred to measuring flash(100 ml) and the volume was completed by Distilled Water.

- II. Basic solutions of 0.1 and 1M NaOH were prepared according to the following formula:

$$\text{Molarity} = \frac{\text{Weight} \times 1000}{\text{Molecular weight} \times \text{Volume}}$$
$$0.1 = \frac{Wt \times 1000}{40 \times 500}$$
$$Wt = 2 \text{ g}/500 \text{ml}$$

And

$$1 = \frac{Wt \times 1000}{40 \times 100}$$
$$Wt = 4 \text{g}/100$$

3.5 Batch biosorption procedure

Batch experiments were carried out under the following conditions:

- 0.2 g of *Ficus benjamina* biomass, 50 ml of Cd²⁺ and Pb²⁺ solution higher, an agitation speed of 200 rpm (round per minute) on orbital shaker. The pH, temperature, and initial Cd²⁺ and Pb²⁺ concentration employed.
- The experiments were carried out with the values of pH (2, 6) that were not be influenced by the metal precipitation, as metal hydroxide. The maximum temperature employed in the present study is 45 ° C (Table 3.1 & Figure 3.2). Samples were collected after 6 hours to reach equilibrium for the sorption system.

Table 3.1: High and low levels of factors

*Factor	Element			
	Cd²⁺		Pb²⁺	
	Low level	High level	Low level	High level
Temp (°C)	20.0	45.0	20.0	45.0
X (mg/L)	5.0	300.0	10.0	500.0
pH	2.0	6.0	2.0	6.0

*T: Temperature; X: Initial concentration

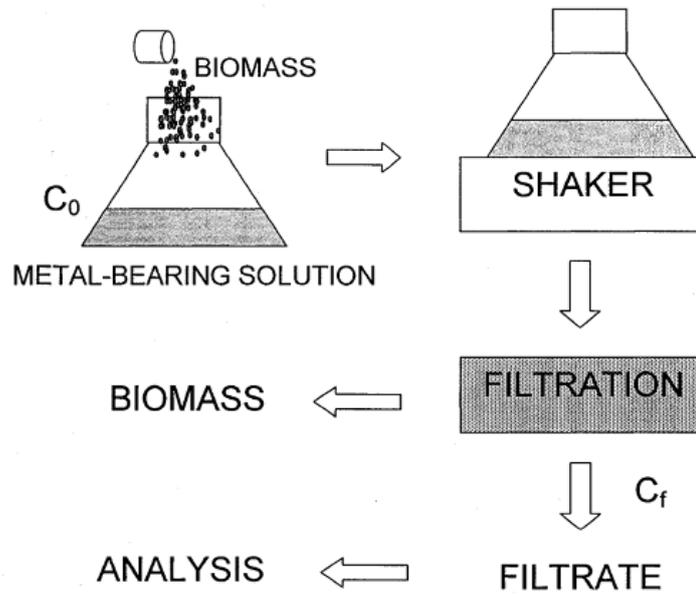


Figure 3.3: Scheme of Batch Experiments

- Aliquots for analysis were filtered using Whatman Glass microfiber filters (GF/A) and stored in plastic bottles for further analysis (Benaissa, 2006). The residual Cd^{2+} and Pb^{2+} concentration were measured by Varian ICP-AES.
- Control samples were made in the same process of normal samples but in absence of any metal.
- Sixteen duplicate experiments were carried out: eight for Cd^{2+} and eight for Pb^{2+} . All possible combinations of variables, called factors in the jargon, were used, and a matrix was established according to their high and low levels, represented by (+1) and (-1), respectively (Table 3.2).

The percentage of removal efficiency (R) of Cd^{2+} and Pb^{2+} from aqueous solution was defined as:

$$R(\%) = \frac{C - C_F}{C} \cdot 100 \quad (2)$$

Where: C and C_F are, the initial and final concentrations of Cd^{2+} and Pb^{2+} , respectively.

Table 3.2: Possible interactions of the studied factors levels. X: metal ion concentration pH: hydrogen ion concentration; T: temperature; (-1): low level; (+1): high level.

Interaction of factors levels		
X	pH	Temp
-1	-1	-1
-1	-1	+1
-1	+1	-1
-1	+1	+1
+1	-1	-1
+1	-1	+1
+1	+1	-1
+1	+1	+1

3.7 Statistical design of experiments (full factorial design)

Experiments, however, are time consuming and money intensive. It is desirable to achieve the goal with a minimum of experiments (Fink, 2009). Factorial design is employed to define the most important process variables affecting the metal removal efficiency. It is also used to reduce the total number of experiments in order to achieve the best overall optimization of the system. The factorial experimental design methodology involves changing all variables from one experiment to the next to determine which factors have important effects on the response as well as how the effect of one factor varies with the level of the other factors. The determination of factor interactions could only be attained using statistical designs of experiments (Table 4), since it cannot be shown when the system optimization is carried out by varying just one factor at the time and fixing the other

Two levels of the three studied factors were used, high level “+1” and low level “-1” (Table 3.2). In a full factorial experiment, Cd²⁺ and Pb²⁺ remedial percentage are measured at all combinations of the experimental factor levels

according to equation (2). The combination of factor levels signifies the conditions at which responses will be measured (Varma et al., 2010).

3.8 Response surface methodology (RSM)

Response surface methodology (RSM) was conducted to study the optimum factors for Cd²⁺ and Pb²⁺ biosorption. RSM was used in this study instead of a conventional method ‘change one factor at a time’. The levels of each factor used in this experimental design were taken from full factorial experimental design experiments.

For treatment of data, The MINITAB statistical software (release 16) was employed throughout in order to obtain the main effects, coefficients, standard deviation of coefficients and other statistical parameters of the final model.

3.9 Metal analysis

The metal ions contents in all the samples prior to and after batch biosorption experiments were analyzed by Agilent 5100 Inductively Coupled Plasma (ICP-OES).

3.10 Quality control/ Quality assurance

Poor results can occur during sample preparation and while storage. These errors are due to cross-contamination from glassware or used chemicals and metal ions loss owing to sorption or volatilization (EPA, 2007; Mortimer et al., 2007; Zhang, 2007). In this examination, metal solutions were kept in high density polyethylene bottles till their metal content was tested. Polyethylene or Teflon bottles are used in inorganic analysis to minimize loss of metal ions on their surfaces (Salim and Cooksey, 1980; Mortimer et al., 2007).

3.11 Application of biosorption on Real Wastewater Samples

The wastewater sample was collected from El Mahmoudia channel. It is considered as the main water source for Alexandria and Behiera governorate that locate at northern west of Egypt (receive about 15 Mm³/day). This channel receives water from Rosetta branch at Mahmoudia city. The canal receives domestic and agriculture wastes from Zarcon Drain and other non-point sources. It is also affected by Industrial, agricultural and sewage discharges (Egyptian Environmental Affairs Agency (EEAA), 2002). Few studies were published about Mahmoudia canal, Galal (1983) and EEAA(2002) reported that Lindane, DDE and DDT were found in Mahmoudia canal and the concentrations were ranged from 0.91 to 2.32 µg/l. They also cited that the heavy metals (Cu, Pb, Cd, Zn, Mn and Fe) were found in the canal at levels more than the maximum permissible limits. The same researchers found that the total dissolved solids (TDS), total alkalinity, total hardness (TH) and sulphates were less than the maximum permissible limits.



Figure 3.4: Al-Mahmoudia Channel

The sample was stored in a container in a refrigerator at a temperature below 4°C until analyzed. The sample was taken into Volumetric flask 100 ml for ICP analysis to measure Cadmium Cd(II) and Lead Pb(II) ions concentration before and after treatment. Optimum design came-out from the laboratory experiment has to be applied on this wastewater sample to calculate the removal efficiency by the applied biosorbent.

CHAPTER FOUR RESULTS AND DISCUSSION**4.1 Results**

The harmful effects of organic and inorganic pollutants on ecosystems and on human health are well known and much expenditure is devoted to industrial treatment methods to avoid or limit discharges (Gadd, 2009). Sorption technology is presently being used extensively for the removal of organic and inorganic micropollutants, in soluble or insoluble forms from aqueous solutions (Lin and Juang, 2009). Biosorption, in particular, can be deliberated as a safe, cost active and ecofriendly technology for heavy metals removal from aqueous solutions (Singanan and Peters, 2013).

Metallic ion uptake by a biosorbent in a batch system usually depends on several factors, such as acidity of the medium (pH), initial metallic ion concentration, time of contact between the metallic ion and the biosorbent, speed of shaking, etc.

The optimization of all those variables using the univariate procedure is very tedious and the best condition could not be attained, because the interactions among all the factors are neglected. Also, it is not known if the set of other fixed variables were kept at other levels, the results would lead to the same optimization. In addition, the total number of experiments to be carried out in the univariate procedure is much higher when compared with statistical design of experiments.

For studying the Cd²⁺ and Pb²⁺ biosorption on *Ficus benjamina*, the removal efficiency depends on the factors: acidity of the medium (pH), initial metallic ion concentration (X) and temperature (Temp) using a batch adsorption system. Other variables such as biosorbent concentration and speed of agitation were kept constant. A full 2³ factorial design and results for removal efficiency percent are shown in Table (4.1).

Table 4.1: Experimental factorial design results for Cd²⁺ and Pb²⁺ biosorption

Factor			Element					
Temp	X	pH	Cd ²⁺			Pb ²⁺		
			Removal Efficiency (%)*	Average		Removal Efficiency (%)*	Average	
1	1	1	46.7	47.5	47.1	24.6	25.4	25
1	1	-1	41.7	42.3	42	28	29.1	28.6
1	-1	1	80.8	81.5	81.2	10	10.9	10.5
1	-1	-1	42	43.4	42.7	38	38.6	38.3
-1	1	1	55.3	56.4	55.9	34	34.8	34.4
-1	1	-1	18.3	19.2	18.8	6	6.6	6.3
-1	-1	1	68.8	69.2	69	48	48.9	48.5
-1	-1	-1	53.4	54.2	53.8	81	81.7	81.4

* Experiment in duplicate

The effect of a factor is defined as the change in response produced by a change in level of the factor. This is frequently called main effect, as it refers to the primary factors of interest in the experiment (Montgomery, 2001). The regression coefficients are calculated by dividing the net effects by two. The t-test values were obtained by the regression coefficients by standard error (Saadat&Karima-Jashni, 2011).

4.1.2 Cadmium (Cd²⁺) biosorption

The main interaction effect, coefficients of the model, standard error of each coefficient and the probability for the full 2³ factorial designs for Cd²⁺ are presented in tables 4.1.

Table 4.2: Statistical parameters for 2^3 design of Cd^{2+}

Term	Effect	Coefficient	S.E. of Coefficient	p
Constant		51.31	0.388	0.006
Main factors				
Temp	3.88	1.94	0.388	0.083
X	-20.72	-10.36	0.388	0.029
pH	23.98	11.99	0.388	0.017
Interaction of two factors				
Temp.X	3.33	1.66	0.388	0.118
Temp.pH	-2.17	-1.09	0.388	0.500
X.pH	-2.88	-1.44	0.388	0.085
Interaction of three factors				
Temp.X.pH	-13.83	-6.91	0.388	0.550

The codified mathematical model employed for the 2^3 factorial design is:

$$R = A_0 + A_1Temp + A_2X + A_3pH + A_4Temp.X + A_5Temp.pH + A_6X.pH + A_7Temp.X.pH \quad (3)$$

Where: A_0 : represents the global mean

A_i : the other regression coefficients.

Substituting the coefficients A_i in Equation (3) by their values from Table 6 we get the following equations:

$$RCd^{2+} = 51.31 + 1.94Temp - 10.36X + 11.99pH + 1.66TX - 1.09Temp.pH - 1.44XpH - 6.91TempXpH \quad (4)$$

The effects of the main factors (Temp, X, pH) represent deviations of the average between high and low levels for each one of them. In case of Cd^{2+} , a change in X value from low to high level results in 20.72 % increase in the removal efficiency

(Table 4.2). If a variation from high to low is made for Temp and pH, increases of 3.88% and 23.98 % in the removal efficiency are observed, respectively.

As can be seen from Tables (4.2), some main factors and their interactions were significant at 5% of probability level ($p < 0.05$). On the other hand, some effects were discarded, because they did not exhibit any statistical significance. As such, the resultant models can be represented by:

$$RCd^{2+} = 51.31 - 10.36X + 11.99pH \quad (5)$$

Cube plot (Figure 4.1) starts with all low levels and ending with all high levels. For example, 53.8 and 42.7 represent factor (pH) at the low level while 69.0 and 81.2 represent the factor (pH) at the high level. The effect of (pH) and other factors can be explained by the same sequences.

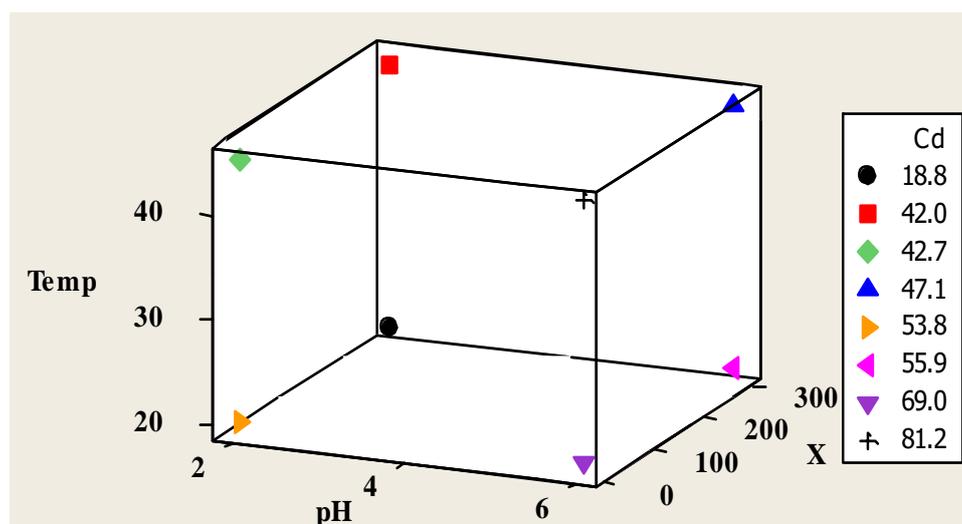


Figure 4.1: Cube plot for (RF %)

In order to better evaluate each factor and its interaction in case of Cd^{2+} , the normal probability plot of standardized effects is represented by (Figure 4.2). The graph of Cd^{2+} could be divided in two regions: the region with percent below 50%, where the factors and their interactions presented negative coefficients (X, Temp.pH, X.pH, Temp.X.pH), and the region with percent above 50%, where the factors presented positive coefficients (Temp, pH, Temp.X). All these factors and interactions

which were represented as a square were significant figures while the effects represented by a circle were not significant (Fig 4.2).

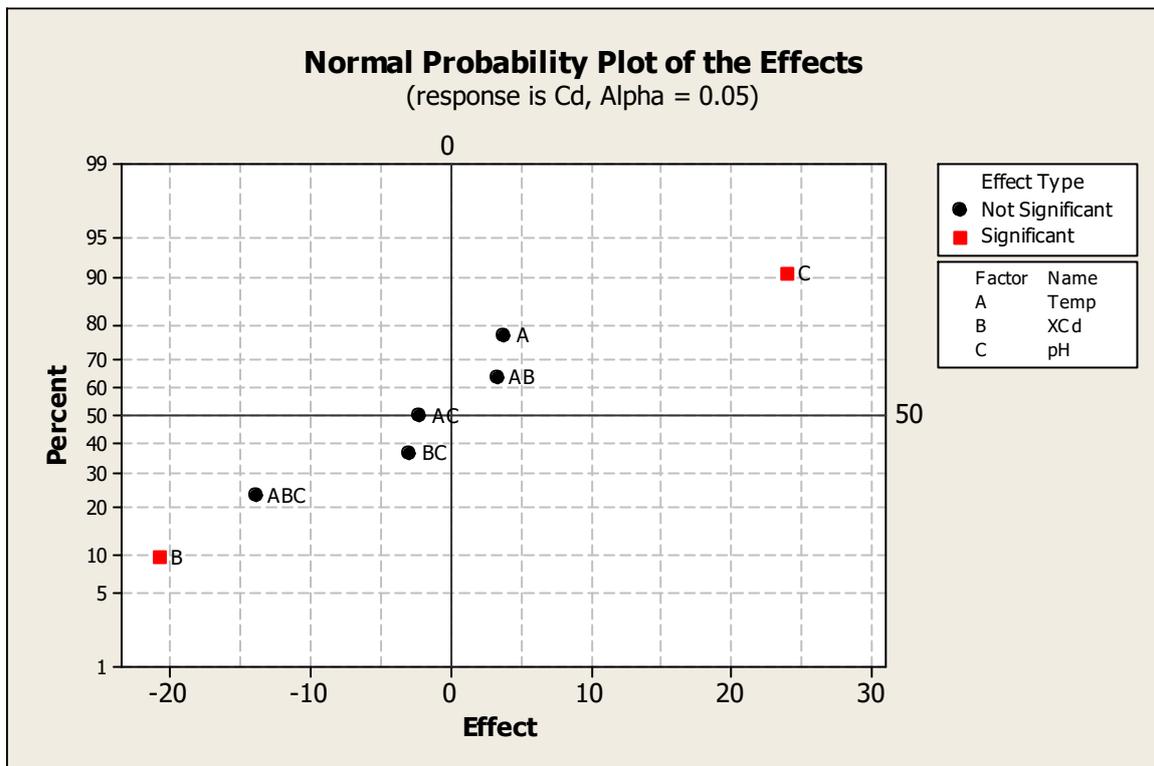


Figure 4.2: Normal Probability Plot of the Standardized Effects of Cd²⁺

The Pareto Chart of the Standardized Effects of Cd²⁺ at $p = 0.05$ is shown in (Figure 4.3). In order to determine whether calculated effects were significantly different from zero, t-test was employed. It was observed that for a 95% confidence level and eight degrees of freedom, the t-value was equal to 18.77.

All the values that presented an absolute value higher than 18.77 ($p = 0.05$), which were located at right of the line, were significant. The absolute standardized value of the effect of each factor and its interaction appeared at the right of each bar. The vertical line indicates minimum statistically significant effect magnitude for a 95% confidence level and values are shown in the horizontal columns are t-test values for each effect (Carmona et al., 2005; Saadat&Karimi-Jashni, 2011). The T factor is not significant at $p = 0.05$ level which was located at left of the line and also it was shown in (Figure 4.3).

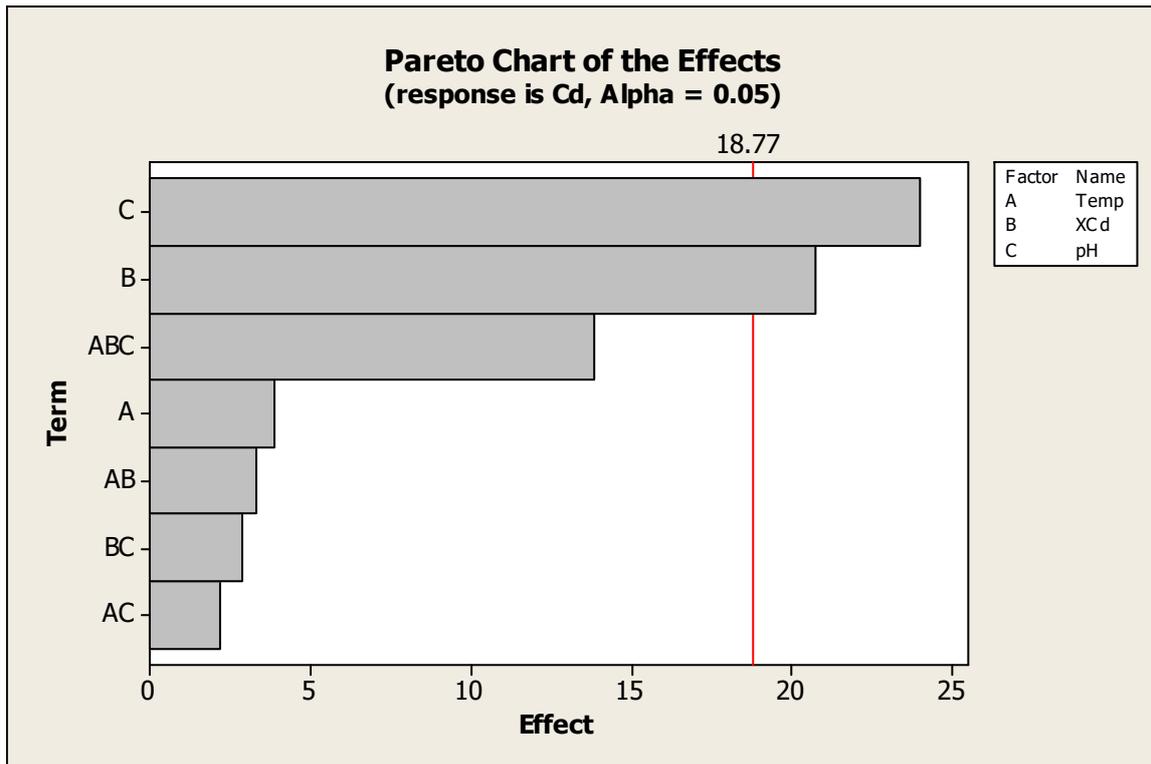


Figure 4.3: Pareto plot of standardized effect (absolute value) at $p=0.05$

The results of Cd^{2+} biosorption as in (Table 4.2, Figure 4.2, Figure 4.3) demonstrated the order of the factors that affecting the Cd^{2+} biosorption in descending order (pH, X). The results indicated that the factor (pH) has the largest effect on biosorption of Cd^{2+} by *Ficus benjamina*. It was noticed that the response increased when this factor is changed from the low level to high level. This meant that Cd^{2+} sorption was favored at high pH value (positive coefficient). The second factor is (X) and the negative value of its coefficient means that Cd^{2+} sorption by were favored at low dose level.

The interaction plot does not show the within group variability, so only the p-value can be used to determine if the interaction is significant. After estimating the factors of main effect, the determination of the significant factors affecting the removal efficiency was followed by performing analysis of variance (Minitab 16) as shown in (Table 4.3) (Abdel-Ghani et al., 2009). Table (4.3) presented the analysis of variance for the factorial design 2^3 without the insignificant three-way interactions. As

can be seen, the main factors and two-way interactions were significant at 5% of probability level ($p < 0.05$), as discussed above.

Table 4.3: Analysis of variance for removal efficiency of Cd^{2+} - full 2^3 factorial design (coded units)

Source	d.f.	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	2450.91	2450.91	816.97	680.10	0.028
2-Way Interactions	3	845.26	845.26	281.76	234.55	0.048
Residual Error	1	1.20	1.20	1.20		
Total	7	3297.38				

d.f.: degree of freedom. Seq SS: sequential sum of squares, Adj.MS: adjusted sum of squares, F= factor F and p: probability.

ANOVA shows the sum of squares used to estimate the factor's effect and the F-ratios defined as the ratio of the respective mean-square-error. As there are two levels for each factor, each ANOVA main effect and interaction effects have one degree of freedom (Abdel-Ghani et al., 2009; Carmona et al., 2005). From the P values defined as the smallest level of significance leading to rejection effects are statistically significant when $P < 0.05$). Also, it was absolutely vital to verify the conclusions drawn from Normal Probability plot of the Standardized Effects for (RF%) and Pareto chart (figure 4.2 & 4.3). By doing ANOVA (Anderson & Whitcomb, 2007) to determine whether various observed nonzero main and interaction effects are statistically significant so using ANOVA (Spall, 2010).

Validation model (Figure 4.4) presents the normal probability plot of predicted removal efficiency for Cd^{2+} where the observed values (experimental) were set closely to calculated values (model) that allowed suggesting a normal distribution with ($R^2=$

0.9882). Homogeneously speed data either side of zero (line) indicated the suitability of the model for the present study.

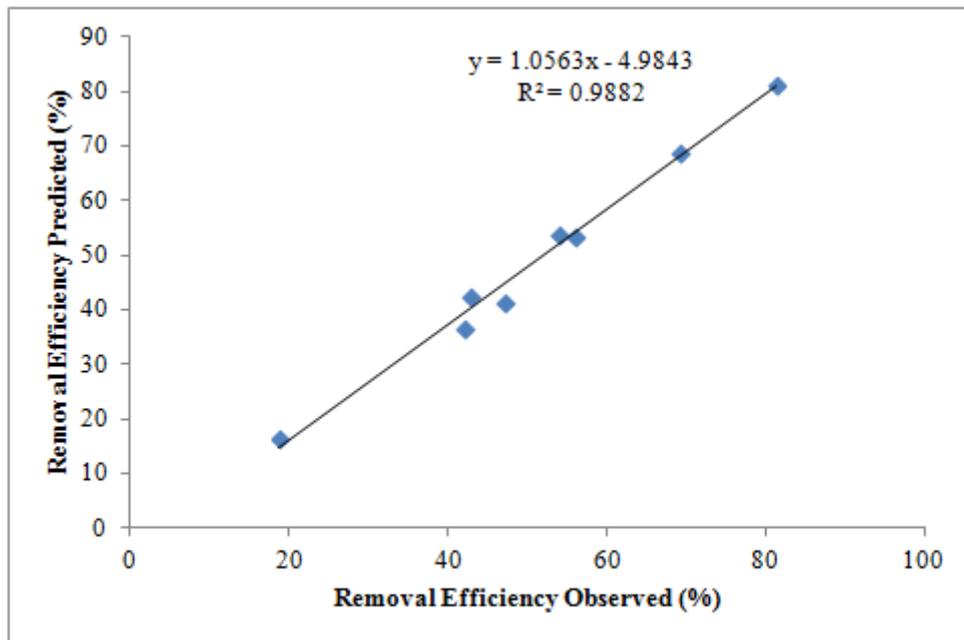


Figure 4.4: Normal probability plot for the removal efficiency of Cd^{2+}

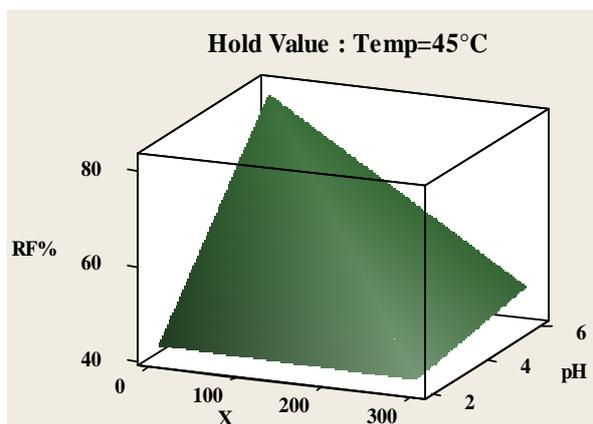
Response surface methodology (RSM) is a collection of mathematical and statistical technique useful for analyzing the effects of several independent variables on the response. RSM has an important application in the process design and optimization as well as the improvement of existing design (Amini et al., 2008). The three- dimensional response surface plots confirmed the results obtained by factorial experimental design. The response surface plots represented in the study were obtained by keeping one factor constant while varying the other two factors (Freitas et al., 2009). Its importance may be to find factor settings (operating conditions) that produce the best response and model a relationship between the quantitative factors and the response (Montgomery, 2001).

Data obtained from the response surface plots are exemplified in Figures (4.5, 4.6, 4.7). The removal efficiency of Cd^{2+} by *Ficus benjamina* (RF) over independent variables pH and X was depicted in (Figure 4.5). The points giving the maximum removal of Pb^{2+} were found to be at pH= 6 and X= 5 ppm when temperature was at its high level of 45°C. Also, the best removal efficiency of Pb^{2+} at its low level of 20°C were found to be at pH= 6 and X= 5ppm.

From Figure 4.6, the highest Cd^{2+} removal percentage was achieved at Temp=20°C and pH=6 when X was at its low and high levels (5 and 300 ppm).

Figure 4.7 showed that the highest Cd^{2+} removal percentage was achieved at Temp=20°C and X=300 ppm.

(A)



(B)

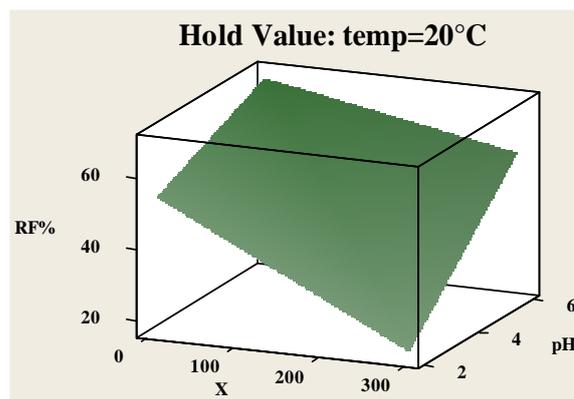
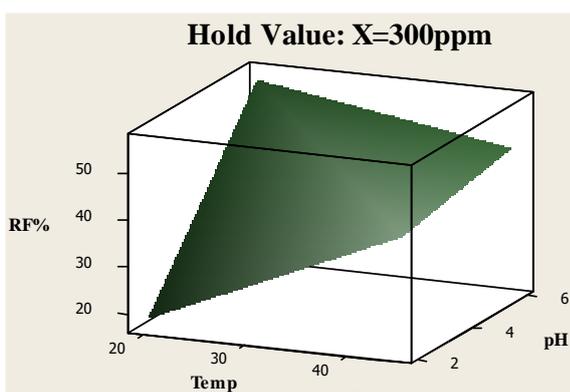


Figure 4.5: Response surface plots showing the effect of pH and X on Cd²⁺ removal% by *Ficus benjamina* at Temperature (Temp): (A) 45°C and (B) 20°C.

(C)



(D)

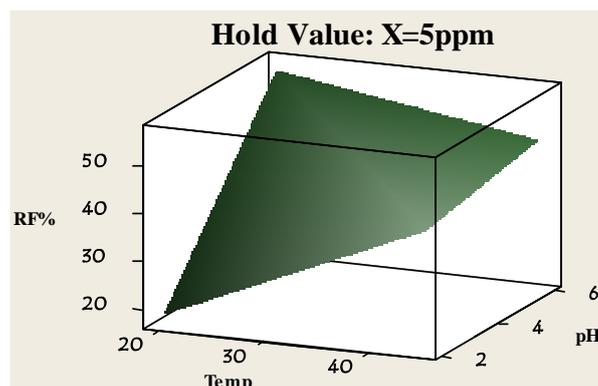
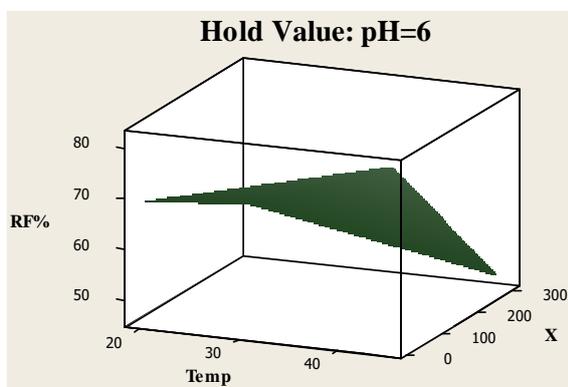


Figure 4.6: Response surface plots showing the effect of pH and Temp on Cd²⁺ removal% by *Ficus benjamina*. At X: (C) X=300ppmand (D) X=5ppm.

(E)



(F)

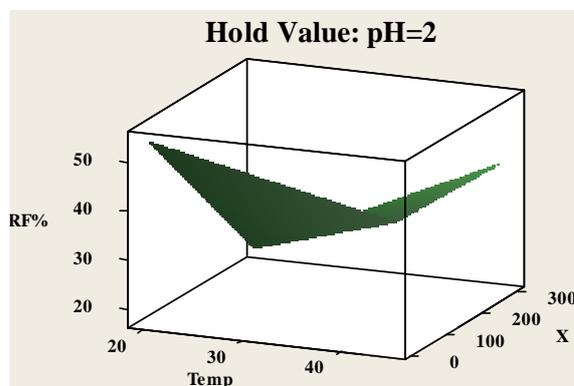


Figure 4.7: Response surface plots showing the effect of (X) and (Temp) on Cd²⁺ removal% by *Ficus benjamina*. (E) pH=6 and (F) pH=2.

4.1.2 Lead (Pb²⁺) biosorption

Eight duplicate experiments were carried out; '+1' for the higher level and '-1' for the lower level. In this study, the factors screened were initial metal ion concentration (X), pH of the solution and temperature (Temp). Removal efficiency (RF %) is calculated according to eq (2) (page 29). The results for % removal efficiency of Pb²⁺ biosorption by *Ficus benjamina* are shown in (Table 4.4)

Table 4.4: Experimental factorial design results for Pb²⁺

Factors			Removal Efficiency (%)*		Average %
Temp	X	pH			
1	1	1	24.6	25.4	25
1	1	-1	28	29.1	28.6
1	-1	1	10	10.9	10.5
1	-1	-1	38	38.6	38.3
-1	1	1	34	34.8	34.4
-1	1	-1	6	6.6	6.3
-1	-1	1	48	48.9	48.5
-1	-1	-1	81	81.7	81.4

* Experiment in duplicat

The effects, regression coefficients (Coef) and the associated standard errors (SE Coef) of biosorption are shown in (Table 4.5). Also, there is *p* that represents the probability values.

Table 4.5: Statistical parameters for 2^3 design (Pb^{2+})

Term	Effect	Coefficient	S.E. of Coefficient	<i>p</i>
Constant		34.13	4.50620	0.020
Main factors				
Temp	-17.05	-8.52	0.004	0.085
X	-21.10	-10.55	0.124	0.043
pH	-9.05	-4.53	0.936	0.091
Interaction of two factors				
T.X	23.50	11.75	0.001	0.253
T.pH	-6.65	-3.33	0.001	0.031
X.pH	21.30	10.65	0.025	0.037
Interaction of three factors				
T.X.pH	-9.2	-4.60	0.001	0.932

From Eq (3) (page 37)

Substituting the coefficients A_i in Equation (3) by their values from Tables we get the following equations:

$$RPb^{2+} = 34.13 - 8.52Temp - 10.55X - 4.53pH + 11.75Temp.X - 3.33Temp.pH + 10.65X.pH - 4.60Temp.X.pH \quad (6)$$

$$RPb^{2+} = 34.13 + 21.10X + 23.50X.Temp + 21.30X.pH \quad (7)$$

Cube plot (Figure 4.8) starts with all low levels and ending with all high levels. For example, 81.4 and 38.3 represent factor (pH) at the low level while 48.5 and 10.5 represent the factor (pH) at the high level to determine the effect of (pH) and other factors can be explained by the same sequences.

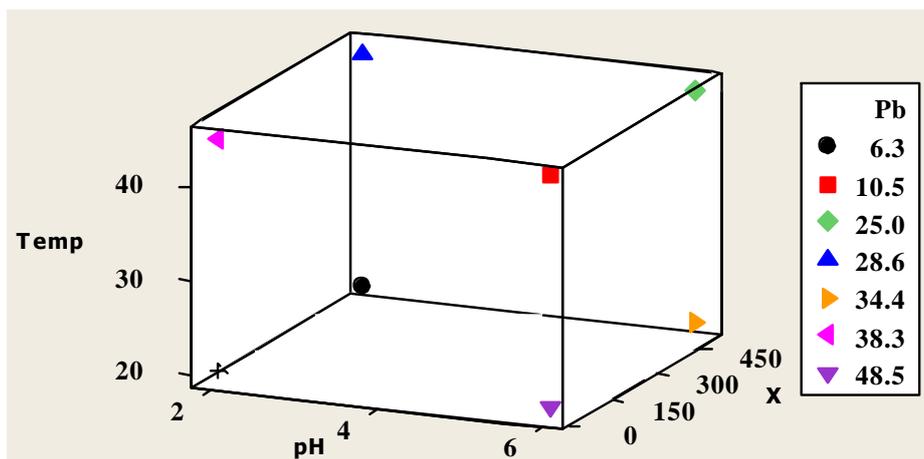


Figure 4.8: Cube plot for (RF %)

In order to better evaluate each factor and its interaction in case of Pb^{2+} , Fig. 4.9, presented the normal probability plot of standardized effects. The graph of Pb^{2+} could be divided in two regions: the region with percent below 50%, where the factors and their interactions presented negative coefficients (Temp, X, pH), and the region with percent above 50%, where the factors presented positive coefficients (Temp.X, Temp.pH, X.pH, Temp.X.pH,). All these factors and interactions which were represented as a square were significant figures while the effects represented by a black circle were not significant (Fig 4.9)

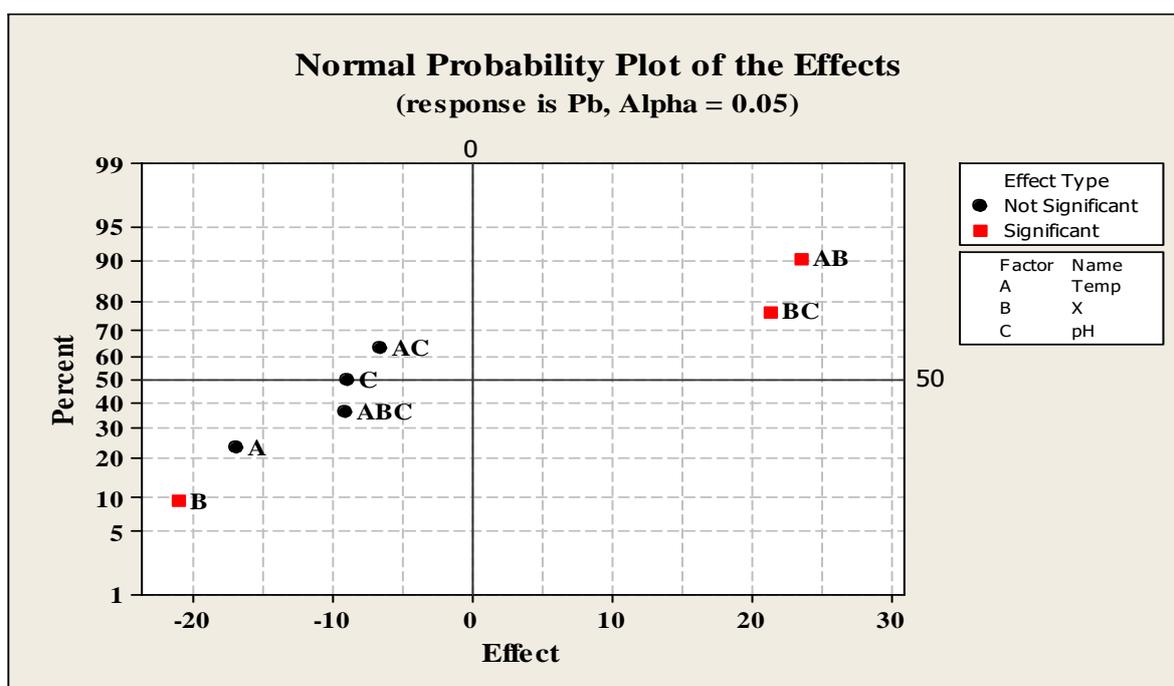


Figure 4.9: Normal Probability Plot of the Standardized Effects of Pb^{2+}

The Pareto Chart of the Standardized Effects of Pb^{2+} ($p = 0.05$) is shown in (Fig. 4.10). In order to determine whether calculated effects were significantly different from zero, t-test was employed. It was observed that for a 95% confidence level and eight degrees of freedom, the t-value was equal to 20.30.

All the values that presented an absolute value higher than 20.30 ($p = 0.05$), which were located at right of the line, were significant. The absolute standardized value of the effect of each factor and its interaction appeared at the right of each bar. All factors are significant at $p = 0.05$ and also they were shown in (Figure 4.10).

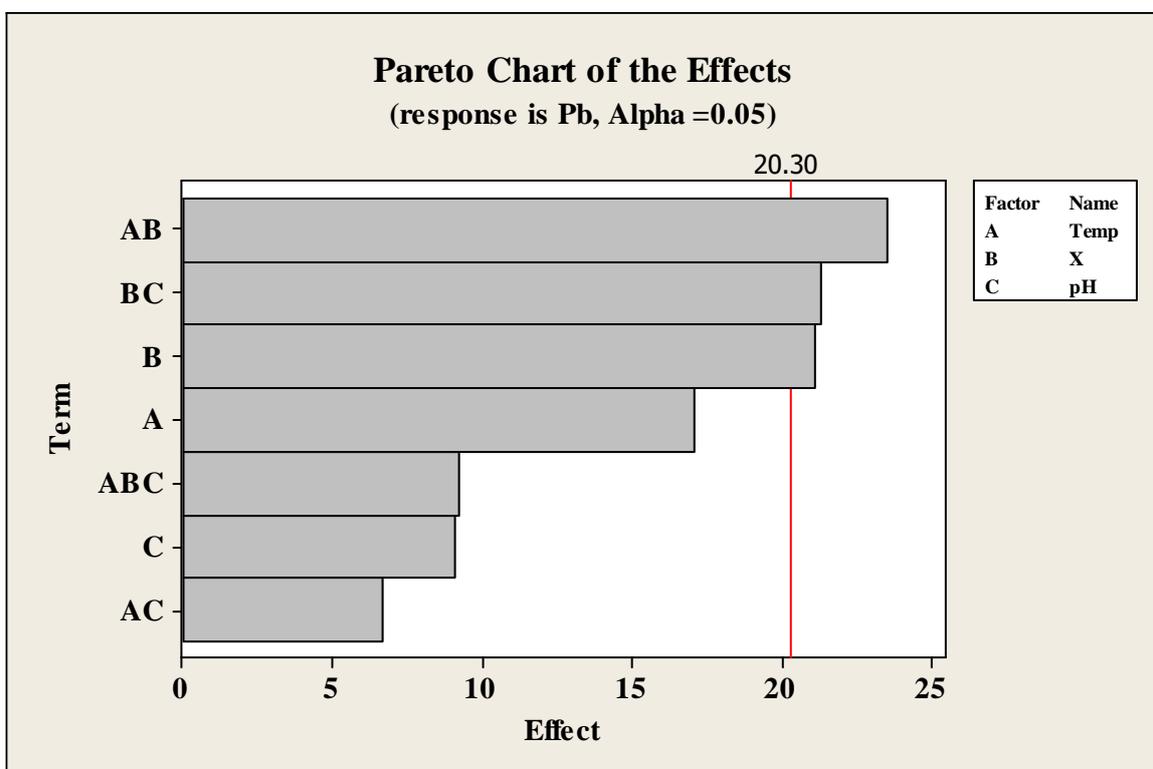


Figure 4.10: Pareto plot of standardized effect of Pb^{2+} (absolute value) at $p=0.05$

The results of Pb^{2+} biosorption as in (Table 4.6, Figure 4.9, Figure 4.10) demonstrated the order of the factors that affecting the Pb^{2+} biosorption in descending order (X.Temp, X.pH, X). The results indicated that the interaction factor (Temp.X) has the largest effect on biosorption of Pb^{2+} by *Ficus benjamina*. It was noticed that the response increased when this factor is changed from the low level to high level (positive coefficient). The second interaction that affects the biosorption is (X.pH) and

the value of its coefficient means that Pb^{2+} sorption by were favored at high level. The third factor is (X) which acquires a positive coefficient.

The interaction plot does not show the within group variability, so only the p-value can be used to determine if the interaction is significant. After estimating the factors of main effect, the determination of the significant factors affecting the removal efficiency was followed by performing analysis of variance (Minitab 16) as shown in (Table 4.6) (Abdel-Ghani et al., 2009). Table (4.6) presented the analysis of variance for the factorial design 2^3 without the insignificant three-way interactions. As can be seen, the main factors and two-way interactions were significant at 5% of probability level ($p < 0.05$), as discussed above.

Table 4.6: Analysis of variance for removal efficiency of Pb^{2+} - full 2^3 factorial design (coded units)

Source	d.f.	Seq SS	Adj SS	Adj MS	F	p
Main Effects	3	1635.6	1635.6	987.61	309.81	0.042
2-Way Interactions	3	2100.3	2100.3	1053.22	330.39	0.040
Residual Error	1	3.19	3.19	3.19		
Total	7	3905.2				

d.f.: degree of freedom. Seq SS: sequential sum of squares, Adj.MS: adjusted sum of squares, F= factor F and p: probability.

ANOVA shows the sum of squares used to estimate the factor's effect and the F-ratios defined as the ratio of the respective mean-square-error. As there are two levels for each factor each ANOVA main effect and interaction effects have one degree of freedom (Abdel-Ghani et al., 2009; Carmona et al., 2005). From the P values defined as the smallest level of significance leading to rejection effects are statistically significant when $P < 0.05$). Also, it was absolutely vital to verify the conclusions drawn from Normal Probability plot of the Standardized Effects for (RF) and Pareto

chart (figure 13). By doing ANOVA (Anderson & Whitcomb, 2007) to determine whether various observed nonzero main and interaction effects are statistically significant so using ANOVA (Spall, 2010).

Validation model (Figure 4.11) presents the normal probability plot of predicted removal efficiency for Pb^{2+} where the observed values(experimental) were set closely to calculated values (model) that allowed suggesting a normal distribution with ($R^2=0.979$). Homogeneously speed data either side of zero (line) indicated the suitability of the model for the present study.

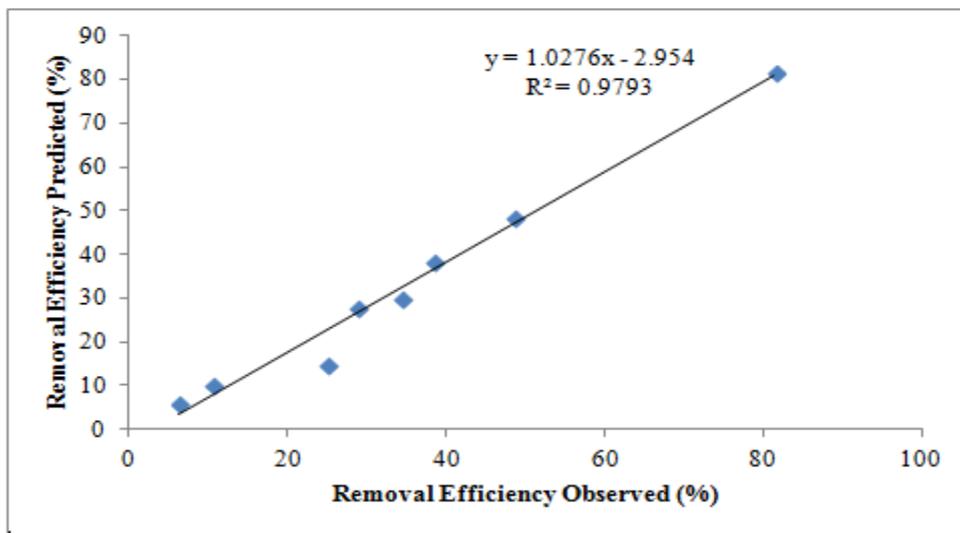


Figure 4.11: Normal probability plot for the removal efficiency of Pb^{2+}

Response surface methodology (RSM) is a collection of mathematical and statistical technique useful for analyzing the effects of several independent variables on the response. RSM has an important application in the process design and optimization as well as the improvement of existing design (Amini et al., 2008). The three- dimensional response surface plots confirmed the results obtained by factorial experimental design. The response surface plots represented in the study were obtained by keeping one factor constant while varying the other two factors (Freitas et al., 2009). Its importance may be to find factor settings (operating conditions) that

produce the best response and model a relationship between the quantitative factors and the response (Montgomery, 2001).

Data obtained from the response surface plots are exemplified in Figures (4.12, 4.13, 4.14). The removal efficiency of Pb^{2+} by *Ficus benjamina*(RF) over independent variables pH and X was depicted in (Figure 4.12). The points giving the maximum removal of Pb^{2+} were found to be at pH= 2 and X= 10ppm when temperature was at its high level of 45°C. Also, the best removal efficiency of Pb^{2+} at its low level of 20°C were found to be at pH= 2 and X= 10 ppm.

From Figure 4.13, the highest Pb^{2+} removal percentage was achieved at Temp=45°C and pH=6 when X was at its low level (10ppm). Figure 4.14 showed that the highest Pb^{2+} removal percentage was achieved at Temp=20°C and X=10 ppm when pH was at its low level (pH=2).

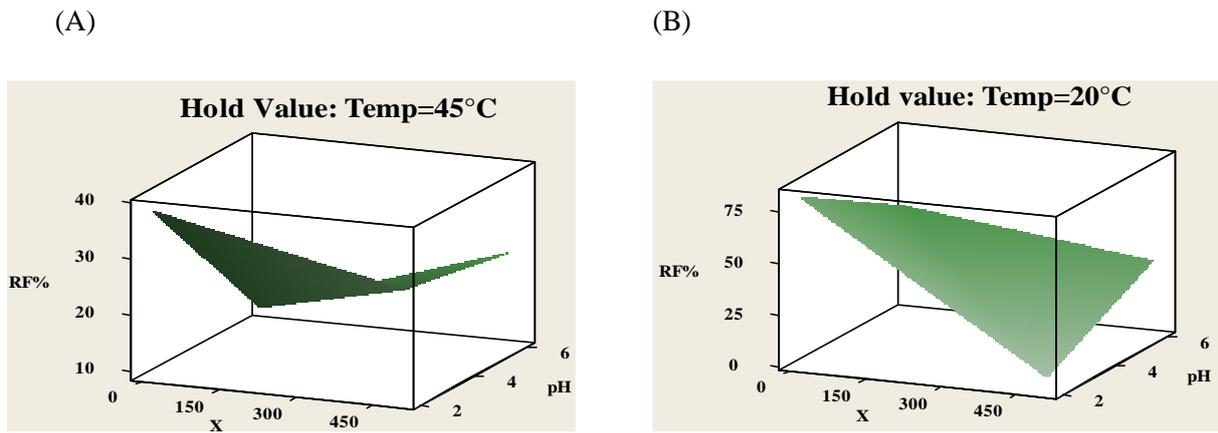


Figure 4.12: Response surface plots showing the effect of pH and X on Cd²⁺ removal% by *Ficus benjamina* at Temperature (Temp): (A) 45°C and (B) 20°C.

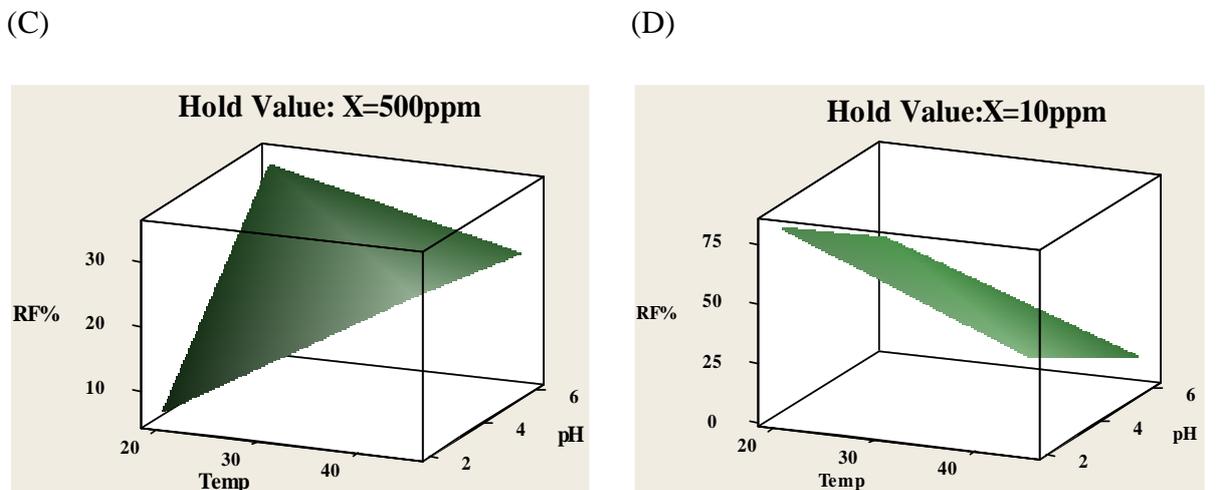


Figure 4.13: Response surface plots showing the effect of pH and Temp on Pb²⁺ removal% by *Ficus benjamina*. At X: (C) X=500ppm and (D) X=10ppm.

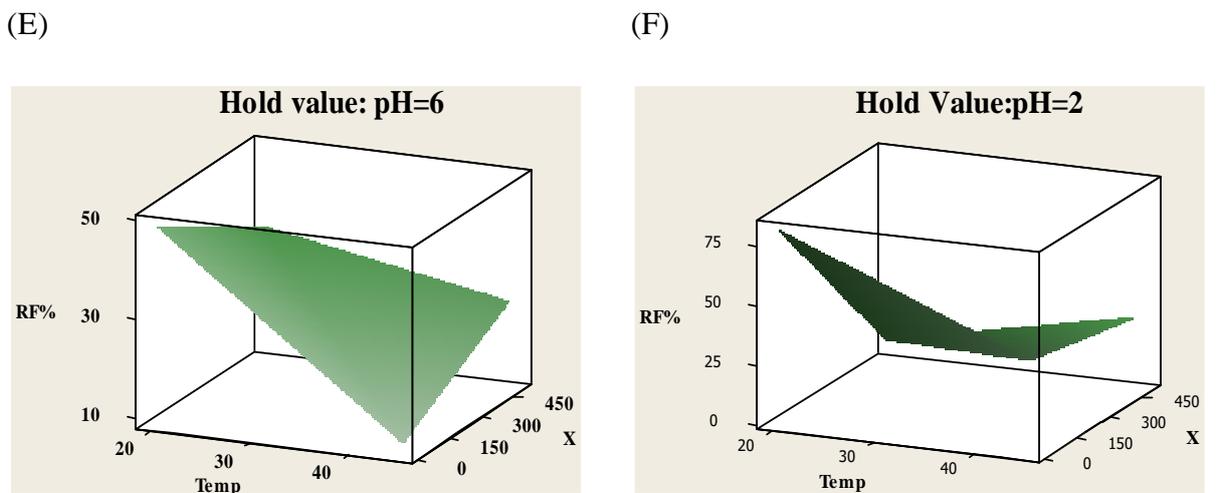


Figure 4.14: Response surface plots showing the effect of (X) and (Temp) on Pb²⁺ removal% by *Ficus benjamina*. (E) pH=6 and (F) pH=2.

4.1.3 Real wastewater Samples

Results achieved from the applications of *Ficusbenjamina* for the removal of Pb^{2+} and Cd^{2+} from real filtered wastewater sample are shown in Table 4.7. Commonly, the removal percentage of Pb^{2+} reached 92.04%. While, the uptake percentage of Cd^{2+} reached up to 99.9%.

Table 4.7: Experimental factorial design results for Cd^{2+} and Pb^{2+} biosorption from real filtered waste water

Factor			Element			
Temp	X	pH	Cd^{2+}		Pb^{2+}	
			Result ppm	Average	Results ppm	Average
1	1	1	0.034	96.8	0.4	87.2
1	1	-1	0.001	99.9	0.75	75.9
-1	1	1	0.035	96.7	0.27	92.4
-1	1	-1	0.075	92.9	0.41	86.8

* Experiment in duplicate, initial Pb concentration is 3.11 ppm, initial Cd concentration is 1.06 ppm.

4.2 Discussion

The harmful effects of organic and inorganic pollutants on ecosystems and on human health are well known and much expenditure is devoted to industrial treatment methods to avoid or limit discharges (Gadd, 2009). Sorption technology is presently being used extensively for the removal of organic and inorganic micropollutants, in soluble or insoluble forms from aqueous solutions (Lin and Juang, 2009). Biosorption, in particular, can be deliberated as a safe, cost active and eco-friendly technology for heavy metals removal from aqueous solutions (Singanan and Peters, 2013).

In the last few decades, Contamination of water bodies by heavy metals is becoming a serious environmental threat due to increasing industrialization. So,

attention has been dedicated to identify new, effective and economical methods for its removal from waste water (Siyal et al., 2012). For example, Perié et al., (2004) used clay minerals such as zeolite structure Na^+ , Ca^{2+} , K^+ , Mg^{2+} and the ingoing ionic charge Zn^{2+} or Cu^{2+} or Pb^{2+} shows that the total quantity of ingoing ions is lower than the total quantity of outgoing ions. The problem of ion exchange can be explained by higher hydration radius of heavy metal ions, as well as by adsorption of their positive monovalent hydroxyl-species on the inner surface. This considerably blocks heavy metals adsorption. Also, coal fly ash was used as low cost adsorbent to remove heavy metals as Zn^{2+} , Pb^{2+} , Cd^{2+} , Mn^{2+} and Cu^{2+} by Mohan & Gandhimathi, (2009). The removal percentage was 42%, 74%, 71%, 28%, and 39% respectively.

The research done by Taty –Costodes(2003) using sawdust of *Pinus sylvestris* for biosorption of Cd^{2+} and Pb^{2+} from aqueous solution solutions. The capacity of the metal ions to bind onto the biomass was 96% for Cd^{2+} , and 98% for Pb^{2+} using rice husks. The maximum removal percentage of studied biosorption of Cd^{2+} and Pb^{2+} were found to be 28.4% and 84.6%, respectively. Hussein et al., (2004) used nonliving biomass of different *Pseudomonas* species to remove Cu^{2+} , Cd^{2+} and Ni^{2+} isolated from Western Alexandria Sewage Treatment Plant, Alexandria, Egypt. The best removal percentage

Cu^{2+} ranged between 50 and 93% while in case of Cd^{2+} and Ni^{2+} the removal percentage was ranged between 35% and 88% by three other strains from the species *P. putida*. Montanher et al., (2005) used rice bran to remove Cd^{2+} , Cu^{2+} , Pb^{2+} and Zn^{2+} from aqueous solutions of pulp and paper industry effluent by water hyacinth (*Eichhornia crassipes*). The higher adsorptive capacity for Pb^{2+} and Zn^{2+} was found to 80.3% and 73.4%, respectively.

Garg et al., (2008) used various agricultural residus such as sugarcane bagasse (SCB), maize corncob (MCC) and *Jatropha* oil cake (JOC) of the removal of Cd^{2+} from aqueous solutions. The maximum removal percentage of Cd^{2+} was 99.5%, 99% and 85% by JOC, MCC, and SCB, respectively.

There are many factors that affect the biosorption of any heavy metals such as: metal ion concentration, hydrogen ion concentration, temperature, biomass dose, particle size of biosorbent, contact time and agitation rate. In some researches, they test the effect of the temperature on biosorption process. Martín-Lara et al. (2011) found that temperature variation between 25°C and 60°C practically had no influence on the Pb²⁺ uptake capacity by dissimilar wastes generated from olive-oil production (olive stone, olive mill solid and also olive tree pruning waste). Muhammad and Nwaedozi (2012) studied the effect of temperature (23.5°C and 37°C) on the adsorption process for Cd²⁺ and Pb²⁺ ions. However, they did not find any significant effect on biosorption process. Also, Panda et al. (2008) reported that temperature factor has no significant effect on biosorption of Cd²⁺ using husk of *Lathyrussativus*. In contrary, Loureiro et al. (2006) and Singh et al. (2010) presented that as the temperature increases from 20°C to 40°C an increase in the adsorption of Cd²⁺, Pb²⁺ and Cu²⁺ were measured when using *Trichodermaviride* as biosorbent. However, Badr (2007) noted that the biosorption of Cd²⁺ and Pb²⁺ was decreased with the rise in temperature. She described the results to either the damage of active binding sites in the biomass or increasing tendency to desorb metal ions from the interface to the solution.

One of the latest researches done by Salma Mtaallah, et al., (2017), for the adsorption of cadmium on activated alumina using factorial experimental design. The aim of this research is to evaluate the main effects and interactions among dose of activated alumina, initial cadmium concentration, pH of the solution and temperature. The results indicated that, under optimal conditions, cadmium removal from industrial effluent samples was >98%.

In this investigation, particle size of the biosorbent was constant at (0.5mm). The increase in external surface area due to small particle size outcomes in increased number of binding sites and contact surfaces. Similar results observed by Abd-Elnaby and Egorov (2012). The contact time between adsorbent and adsorbate has a significant function to reach equilibrium in biosorption experiments. In this research the optimum contact time was 6hrtoreach equilibrium for the soption system (Carmona

et al; 2005). This is concomitant with Benaissa (2006), Liu et al. (2012) and Singanan and Peters (2013). They found that the rate of adsorption is higher at the first hour of the biosorption process due to availability of a large number of active sites on the biosorbents' surface, then it becomes slower in the range of hour to three hours as these sites are exhausted by the rate at which the adsorbate is moved from the exterior to the interior sites of the biosorbents. Finally, the adsorption capacity or removal percentage did not vary significantly after 3h.

Agitation rate is also an important factor in biosorption process because mass transfer resistance can minimize the uptake of metal ions from the aqueous solutions. The boundary layer resistance could be affected by the rate of agitation (Singanan and Peters, 2013). When increasing the blending rate, the diffusion rate of a solute from the bulk liquid to the liquid boundary layer surrounding particles becomes higher due to the enhanced turbulence and the decrease in the thickness of the liquid boundary layer. Hence, at higher agitation rates, the boundary layer becomes very thin, which typically enhances the rate at which a solute diffuses over the boundary layer and increase the mobility of ions (Lichtfouse et al., 2012). As such, in the current study the agitation rate was accustomed at 300 rpm. Wong et al. (2003) found that the uptake of Pb^{2+} and Cu^{2+} increased with increasing blending rate and the maximum uptake was at 250 rpm.

Results came-out from this study showed that the removal efficiency (RF%) of Cd^{2+} and Pb^{2+} are highly affected by initial metal ion concentration (X), hydrogen ion concentration (pH) and temperature (Temp). Other factors such as particle size, biomass dose (BD), contact time and speed of agitation were reserved constant.

Factorial experimental design is broadly used in experiments involving numerous factors where it is necessary to study the joint effect of the factors on a response (Montgomery, 2001). This design permits the simultaneous study of the effects of factors levels that may have on the optimization of biosorption process with less number of investigation (Calero et al., 2013). In a factorial design, if a coefficient is large, then the corresponding experimental variables has a big influence on the

response. If the coefficient is small, then it has only a small influence on the response (Fink, 2009).

4.2.1 Cadmium (Cd²⁺) Biosorption

In this study, according to the model obtained by applying factorial design (2³) and (Table 4.2, Figures 4.2 & 4.3), it was found that the largest effect of the key factors in Cd²⁺ biosorption by *Ficus benjamina* was attributed to pH values. The positive value of its coefficient meant that the Cd²⁺ uptake by *Ficus Benjamina* biomass was favored at high pH values (pH 6.0). In order to avoid a disruption of the *Ficus Benjamina* biomass at pH lower than 6.0, this value was fixed for continuing the optimization of this work.

It was found that the pH is one of the important factors that have a significant influence on metal uptake in biosorption process of heavy metals using agricultural wastes (Sharma & Bhattacharyya, 2005; Sharma et al., 2012). The pH value of solution strongly influences not only the site dissociation of the biomass surface, but also the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation, of heavy metals. It was demonstrated that the suitable pH ranges for the various metal ions were slightly different (Sawyer et al., 2002).

As pH increased the negative charge density on the adsorbent surface increases because of de-protonation of the metal binding sites (Şahan and Öztürk, 2013). Related comments were made by Anirudhan and Sreekumari (2011). They concluded that the increase in metal removal with increase in pH can be described on the basis of a decrease in competition between proton and the metal cations for the same functional groups and by the decrease in positive charge of the adsorbent which results in a lower electrostatic repulsion between the metal cations and the surface. However, at lower pH the adsorption of many heavy metals usually took place with low removal

efficiency. This occurred because there was a high concentration of proton in the solution and this proton competed with metal ions informing a bond with active sites on the surface the biomaterials. These bonded active sites thereafter became saturated and was inaccessible to other cations (Kewsarn P, 2000; Pavasant, 2006). The results of the present study are in agreement with the results found by Badr (2007) and Fawzy (2007). They found that maximum removal percentage of Cd^{2+} was at the higher pH values.

The second important factor for overall optimization Cd^{2+} biosorption was the initial concentration of Cd^{2+} (X). The negative value of its coefficient justifies that low concentration of Cd^{2+} in the solution (5 mg/l) lead to highest biosorption of Cd^{2+} . (Figures 4.2 & 4.3).

This result is in line with Gopal et al. (2002) and Ajmal et al. (2003). They stated that the metal ion concentration is the most key factor in Pb^{2+} and Zn^{2+} biosorption using different biosorbents. Furthermore, Senthil Kumar et al. (2010) found that at lower metal ions concentration, the percentage uptake was higher due to larger surface area of adsorbent being available for adsorption. When the concentration of metal ions became higher, the percentage removal decreased since the available sites for adsorption became less due to saturation of adsorption sites. At a higher concentration of metal ions, the ratio of initial number of moles of metal ions to the adsorption sites available was higher, resulting in lower adsorption percentage. Moreover, with increasing metal ion concentration, the functional groups on biomass surface could be saturated and there were a few existing active sites on the biomass surface so the metal ions are competed for the existing binding sites (Singh et al., 2010; Lopez-Mesas et al., 2011; Surchi, 2011; Singh and Gadi, 2012; Abdel-Aty et al., 2013).

4.2.2 Lead (Pb^{2+}) Biosorption

The results of Pb^{2+} biosorption as in (Table 4.5, Figure 4.9, Figure 4.10) demonstrated that the order of the factors (decending order) affecting the Pb^{2+} biosorption was (X.Temp), (X. pH) and (X).

According to the model obtained in our results by applying factorial design (2^3), the results indicated that the interaction factor (X.Temp) has the most significant and the largest effect on biosorption of Pb^{2+} by *Ficus benjamina*. Each factor has negative coefficients; the interaction between the two factors has a positive coefficient. Only the achievement of this result emphasizes the merit of using the statistical design of experiments over the conventional univariate process of optimization of the system. This information would not be acquired in a univariate of optimization in biosorption system. The positive value of (X.Temp) coefficient tells us that both factors should be increased in order to achieve the highest response, contrary to each factor alone. Otherwise, if the system were being optimized by using univariate procedure, a small dimension of the X of the solution associated with a small dimension of the (Temp) could lead to a misinterpretation of the results achieved.

These results were in line with other studies. Azouaou et al., (2010); Mausumi et al., (2006) and Mehdi et al., (2011) found that the rate of adsorption is a function of initial concentration of metal ions where the highest adsorption was found to take place at lower concentrations. This may be due to the interaction of all metal ions present in the solution with binding sites. At higher concentrations, more metal ions are left unabsorbed in solution due to saturation of adsorption sites. By comparing with other studies done by Senthil Kumar et al. (2010), at lower metal ions concentration, the percentage uptake was higher due to larger surface area of adsorbent being available for adsorption. When the concentration of metal ions became higher, the percentage removal decreased since the available sites for adsorption became less due to saturation of adsorption sites.

Temperature has also an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature, which indicates that ion exchange mechanism exists in biosorption to some extent (Wang & Chen, 2006). In the present investigation, the effect of temperature on the biosorption capacity for Pb^{2+} ions has been investigated at low level ($20^{\circ}C$). Comparing with other studies done by Ali et al., (2015), who studied the biosorption of Lead by *Spirulina platensis* biomass. They

found that maximal adsorption was obtained at low level of temperature 25°C (90.8 %). This revealed the exothermic nature of Pb²⁺ on to the studied plant (Kapoor & Viraraghavan, 1997). Further it was also noted that at higher temperatures the rate of lead adsorption declined rapidly due to either the damage of active binding sites in the biomass (O'zer, A & D. O'zer, 2003) or increasing tendency to desorb metal ions from the interface to the solution (Sari et al., 2012). The increase in the equilibrium adsorption of lead (II) with temperature indicated that removal of Pb (II) ions by *S. platensis* biomass is enhanced at ambient temperatures. Therefore, 25°C was selected as the optimum temperature for further experiments. The results observed in this study were similar to the results obtained for pb²⁺removal by green alga *Ulvalactuca* biomass(Sari and Tuzen2008).They showed a remarkable decrease in adsorption yield from 95 to 63% for Pb (II) ions in response to increase in temperature from 20°C to 50°C.

The second interaction factor that affects the biosorption of Pb²⁺ by *Ficus benjamina* is (X.pH).Each factor has negative coefficients; the interaction between the two factors (X and pH) has a positive coefficient that should be increased to enhance the removal efficiency of Pb²⁺biosorption. These results justify that low pH led to the highest removal efficiency.

Many studies concerning the sorption of Pb²⁺ by different biomaterials indicated that pH has an influence on the removal efficiency. It was found that pH is one of the most important environmental factors in biosorption of heavy metals (Esposito et al., 2002). Badr (2007) and Fawzy (2007) found that the maximum removal percentage of Pb²⁺ was at lower pH value. They studied the removal of Pb²⁺ by *Eichhornia crassipes* and *Phragmites australis* respectively, and they found that the maximum removal was observed near pH=2. Olu-Owolabi et al. (2012) found that Pb²⁺ exists in aqueous solution at the range of pH from 5-6 without getting precipitated. In addition, Reddy et al. (2010a) stated that when pH is higher than 6, a high decrease in the sorption of Pb²⁺ ion by *Moringa oleifera* bark was obtained and there will be the formation of Pb²⁺ hydrolysis products.

The third important factor affecting overall optimization of the batch system was the (X) factor. The negative value justifies that low metallic ion concentration led to high removal efficiency of Pb^{2+} .

4.2.3 Real wastewater Samples

In the current study, the biosorption of Pb^{+2} and Cd^{+2} by the biomass under investigation was studied using real wastewater samples. The average concentration of Pb^{2+} in waste water was 3.11 mg/L while Cd^{2+} average concentration in water was 1.06 mg/L.

In this study, the factors screened were pH with two levels (2, 6) and Temperature (20°C, 45°C) for the removal efficiency of Cd^{2+} and Pb^{2+} by *Ficus Benjamina* biomass from the filtered wastewater samples. The Optimum biomass dose (0.2 g), the time period (6 Hrs) and the blending rate (300 rpm) were fixed as used in the optimization experiments. Results achieved from the application of *Ficus Benjamina* biomass for the removal Cd^{2+} and Pb^{2+} from the real filtered wastewater sample, was 99.9% (0.001 ppm) for Cd^{2+} at pH= 2 and Temp=45°C. While that for Pb^{+2} was 92.4% (0.27 ppm) at pH=6 and Temp=20°C.

In this study, results confirmed that *Ficus Benjamina* could be considered as an effective biosorbent for Cd^{2+} , Pb^{2+} and for the other heavy metals. However, the removal efficiency of Cd^{2+} from polluted water samples was slightly higher than that of Pb^{2+} . The biomass removed up to 99.9% of Cd^{2+} from the polluted wastewater sample while 92.4% of Pb^{2+} was treated at the same conditions.

In this account, the biosorption results for mono-metal solutions of Cd^{2+} or Pb^{2+} (prepared samples) were differed from that of the real wastewater samples. While biomass removed up to 81.2% of the dissolved Cd^{2+} in the prepared water samples. However, the removal percentage reached 99.9 % when using real polluted wastewater samples. This is could be attributed to the relatively lower concentration of Cd^{2+} by influence metal uptake by plant biomass (Kabata and Pendias, 2001). However the Pb^{2+} removal reached 81.4% when using the dissolved Pb^{2+} in an aqueous solution and

92.4% for the real waste water samples. This is could be because of the high metal ions concentration into the water (3.11 ppm) as comparing to Cd²⁺ ions concentration (1.06 ppm). From these results, we conclude that the concentration of biomass has an important role in metal uptake. Therefore, this factor should be taken into account.

CHAPTER FIVE CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The concept of this study is based on a waste to treat another waste; Biosorption is the ability of inactive and dead biomass to bind heavy metals from even very dilute aqueous solutions. The biosorbents that were used for biosorption process should be eco-friendly, economic and available to be a viable alternative for the treatment of industrial and agricultural wastewater.

The present study was undertaken to optimize the biosorption performance of Pb^{2+} and Cd^{2+} from aqueous solutions using dried readily available plant biomass namely: *Ficus benjamina*. The objectives are to investigate the effects of initial metal ion concentration

(X), acidity of the medium (pH), and temperature (Temp) on the biosorption process, determine which factor exerts more effects on the biosorption process as well as the interaction between factors using full factorial experimental design, investigate the validity of the optimized models and test the possibility of recovery of the investigated biosorbent for further usage.

Sixteen batches in duplicate experiments were formulated for full factorial experimental (2^3) designs. The following factors were kept constant The Optimum biomass dose (0.2 g), the time period (6 Hrs) and the blending rate (300 rpm) were fixed as used in the optimization experiments The biosorption of Cd^{2+} and Pb^{2+} were evaluated and optimized by 2^3 full factorial experimental design using MINITAB® 16 software.

In the this study, the largest effect of the main factors in Cd^{2+} biosorption by *Ficus benjamina* was ascribed to (pH) and (X). The largest effect of the main factors in Pb^{2+} biosorption by the same biosorbents was ascribed to initial metal ion concentration(X).

The best percentage removal of Cd^{2+} was 81.2% at $\text{pH}=6$, $\text{Temp}=45^\circ\text{C}$ and $X=5\text{mg/l}$ but the best percentage of Pb^{2+} was 81.4% at $\text{pH}=2$, $\text{Temp}=20^\circ\text{C}$ and $X=10\text{mg/l}$. When applying these optimum conditions on real wastewater samples, the best results were 99.9% for Cd^{2+} and 92.4% for Pb^{2+} .

The biosorbent tested in this study did not undergo any chemical or physical alterations, making it suitable for removal of metals from wastewaters.

5.2 Recommendations for Future Work

Biosorption is gaining increasing attention as a promising metal remediation method. More work is needed to improve our understanding of biosorption process, its complex mechanism, and its applicability in large-scale systems. The following recommendations are made for future work:

- The optimization of specific biosorption process applications has to be done in conjunction with industrial sectors as a field application as an efficient method for heavy metal removal from waste water solution;
- Since the concentration of both metal ions in various industrial wastes differs extremely, thus for each case, it is recommended that researchers treat each problem individually and find its appropriate model;
- Studying the removal efficiency of this biomass with the other heavy metals using multi metal system;
- Investigating the effect of the other factors on the removal efficiency particularly: the biosorbent concentration, time of contact between the metallic ion and the biosorbent, speed of shaking;
- The desorption technique should be implemented to recover metals and recycle the biosorbents for subsequent usage;
- Future researches should be done to explore the performance of the examined plants with multi-metal solutions;

- Additional researches are required to extend the biosorbent methods to additional metals and with different factors and levels;
- Sites has to follow the maximum allowable limits for each type of generated waste to select the appropriate treatment method whenever required;
- Full biological treatment (bioremediation and phytoremediation) of sewage, which oxidizes or/ and degrades organic matter, beside precipitation and removal of nutrients by suitable waste treatment processes became necessary;
- International standards for different types of water quality has to be implemented and monitored.

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