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SORPTION OF HEAVY METALS BY THE GREEN MACROALGA, *Caulerpa lentillifera*

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งานวิจัยนี้เกี่ยวข้องกับการศึกษาการดูดซับโลหะหนัก (ตะกั่ว, สังกะสี, ทองแดง และ แคดเมียม) โดยใช้สาหร่ายช่อพริกไทยซึ่งเป็นสาหร่ายทะเล โดยนำมาอบแห้งเพื่อใช้เป็นตัวดูดซับทางชีวภาพ การทดลองในเบื้องต้นแสดงให้เห็นถึงช่วงของค่าความเป็นกรดด่างที่ใช้ในการทดสอบการดูดซับ ซึ่งเป็นช่วงความเป็นกรดด่างที่โลหะหนักต่าง ๆ เหล่านี้สามารถละลายอยู่ในรูปของสารละลายได้ การทดสอบการดูดซับโดยใช้สาหร่ายช่อพริกไทยกับสารประกอบโลหะหนักรูปแบบต่าง ๆ เช่น คลอไรด์ ซัลเฟต และไนเตรต พบว่าค่าการดูดซับของโลหะหนักต่างรูปแบบกันมีค่าใกล้เคียงกัน การทดสอบการชะล้างด้วยน้ำร้อนและ EDTA แสดงให้เห็นว่าโลหะหนักไม่สามารถถูกชะล้างด้วยน้ำร้อนแต่สามารถถูกชะล้างได้ดีด้วย EDTA โดยผลการทดลองนี้แสดงให้เห็นว่าพันธะการดูดซับไม่น่าจะเป็นแรงแวนเดอร์วาล์ล แต่ควรเป็นพันธะที่มีความแข็งแรงกว่า เช่น แรงไอออนิกหรือโควาเลนซ์ การศึกษาลักษณะทางจลนศาสตร์ของการดูดซับด้วยมวลชีวภาพพบว่าการดูดซับเป็นไปอย่างรวดเร็ว โดยโลหะหนักถูกกำจัดได้ 80-90% ภายในเวลา 20 นาที ซึ่งเป็นผลที่เน้นว่ากลไกการดูดซับที่เกิดขึ้นเป็นกลไกแบบ passive transport นอกจากนี้การวิเคราะห์ด้วยเทคนิค FT-IR แสดงให้เห็นว่าแคดเมียมสามารถเกิดพันธะแบบโควาเลนซ์กับ C-O ในหมู่ฟังก์ชันคาร์บอกซิล ส่วนโลหะอื่นๆ เช่น ตะกั่ว, สังกะสี และ ทองแดง นอกจากสามารถเกิดพันธะโควาเลนซ์ กับ C-O แล้ว ยังสามารถเกิดพันธะอิเล็กโทรสแตติกหรือพันธะไอออนิกกับหมู่คาร์บอกซิล ซัลโฟนิล และอะมิโน อย่างไรก็ตามการศึกษาถึงผลของขนาดของสาหร่ายพบว่าการดูดซับสังกะสีมีค่าเพิ่มขึ้นเมื่อขนาดของสาหร่ายเล็กลงซึ่งไม่เกิดขึ้นกับโลหะอื่น ๆ นั้นแสดงว่าการดูดซับสังกะสีด้วยมวลชีวภาพน่าจะเกิดขึ้นกับตำแหน่งเป้าหมายบนสาหร่ายที่แตกต่างจากโลหะอื่น ๆ และจากการศึกษาความสามารถในการดูดซับพบว่าสาหร่ายช่อพริกไทยสามารถดูดซับแคดเมียมได้สูงสุด ทำให้สามารถวิเคราะห์ได้ว่า (ก) ตำแหน่งเป้าหมายของแคดเมียมแตกต่างจากโลหะอื่นๆ หรือ (ข) ตำแหน่งเป้าหมายของแคดเมียมอาจจะเหมือนกับตำแหน่งสำหรับโลหะอื่น แต่ตำแหน่งนี้สามารถสร้างพันธะกับแคดเมียมได้ง่ายกว่าโลหะอื่น ๆ การศึกษายังพบว่าโลหะส่วนมากสามารถถูกดูดซับได้ดีที่ค่าความเป็นกรด/ด่างสูงขึ้น ยกเว้นการดูดซับตะกั่วที่การดูดซับเป็นไปได้ดีในช่วงค่าความเป็นกรด/ด่างต่ำ และเมื่อค่าความเป็นกรด/ด่างสูงขึ้นจะให้ค่าการดูดซับที่ต่ำลง นอกจากนี้พบว่าการดูดซับของโลหะทั้ง 4 เป็นไปตามไอโซเทอมของ Langmuir

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KEY WORD: SORPTION / ALGAE / *CAULERPA* /ENVIRONMENTAL MANAGEMENT

VIMONRAT SUNGKHUM: SORPTION OF HEAVY METALS BY THE GREEN MACROALGA, *Caulerpa lentillifera* THESIS ADVISOR: ASSIST. PROF. PRASERT PAVASANT, Ph.D. THESIS CO-ADVISOR: ASSIST. PROF. SURAPHONG WATTHANACHIRA, D.Eng. 130 PP. ISBN 974-17-1725-3

This work investigated the adsorption of heavy metals (Pb, Zn, Cu and Cd) using the biosorbent derived from the biomass of marine algae, *Caulerpa lentillifera*. Preliminary experiments showed that the heavy metals of interest had sufficient solubility at the pH range examined in this work. It was found that the biomass could adsorb various forms of heavy metals, i.e. chloride, sulphate, and nitrate, at a similar adsorption capacity. The desorption tests with hot water and EDTA demonstrated that all heavy metals could not be desorbed with hot water but could well be leached out using EDTA. This suggested that the adsorption bonding should not be Van Der Waals, but should in fact involve a stronger force such as ionic or covalent bonding. The kinetic profiles of adsorption by the biomass was rapid with 80-90% of the heavy metals removed within 20 minutes, which suggested that the adsorption occurred due to a passive transport mechanism. The analysis with FT-IR indicated that Cd could form a covalent bond with the C-O stretching in carboxyl functional group. Other metals, i.e. Pb, Zn, and Cu could form a covalent bond with the soft C-O and it was also possible that they formed an electrostatic or ionic bond with a hard carboxyl group (COO^-), sulfonate group (SO_3^{2-}) and amino (NH_2^-) group. However, the investigation on the effect of particle size indicated that Zn adsorption was enhanced with a smaller algal size whereas the effect of particle size was not apparent for other heavy metals. This meant that the adsorption of Zn with this biomass might have taken place on different active sites than those for other metals. The adsorption capacity was found to be the greatest for Cd. Two possible conclusions could be drawn from these results: (i) the active site for Cd was different from that of other metals, and (ii) Cd was more easily bonded to the active site than other metals. Most heavy metals were found to adsorb better at high pH except the adsorption of Pb which was found to increase with pH only at low pH range. Further investigation suggested that the adsorptions of these heavy metals follow Langmuir isotherm.

Department Environmental Management Student’s signature……………………

Field of study Environmental Management Advisor’s signature…………………...

Academic year 2002 Co-Advisor’s signature……………….

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ABBREVIATIONS

AA = Atomic Absorption Spectrophotometer
b = constant value of Langmuir isotherm
C_i = initial heavy metal concentrations
C_f = equilibrium (final) heavy metal concentrations
°C = degree celcius
C_e = equilibrium concentration
C=O = Carbonyl
COOH = Carboxyl
EDX = Electron dispersive X-ray spectroscopy
EDTA = Ethylenediaminetetraacetic acid
FT-IR = Fourier Transform Infrared Spectrometer
g = gram
g/l = gram per litre
K_f = constant value of Freundlich isotherm
KBr = potassium bromide
l = litre
M = molar
mg = milligram
mg/g = milligram per gram
mg/l = milligram per litre
ml = millilitre
mmol/g = millimole per gram
mmol/l = millimole per litre
μm = micrometre
No. = number
NH_2 = amino
1/n = intensity indicator
ppm = part per million
q_{max} = the maximum adsorption capacity
R^2 = linear line
rpm = round per minute

SEM = Scanning Electron Microscopy
S=O = sulfonyl
x/m = solute (metal) concentration in the sorbent (algae)
V = volume of the heavy metal concentration
W = dry weight of the biomass-added

CHAPTER 1

INTRODUCTION

1.1 Motivations

Electroplating processes have been known as significant sources of extremely toxic wastewaters. The most important toxic contaminants are acids and heavy metals e.g. copper, lead, cadmium, chromium, zinc, nickel and tin. These contaminants if allowed to enter natural watercourses will affect health and welfare of human beings. Therefore it is necessary that heavy metals in industrial wastewater be appropriately removed before being discharged into the environment. Heavy metal bearing effluents can be treated effectively by several methods. Conventional techniques for removing dissolved heavy metals include chemical precipitation, carbon adsorption, ion exchange, evaporation and membrane processes [Kojima and Lee, 2001]. The aim of these technologies is to reduce the heavy metal content in the effluent to below the permissible discharge limits. The selection of a particular treatment technique depends notably on a number of factors including waste type and concentration, other constituents, level of clean-up required and economics of the process. One similar characteristic of these technologies is that all of them are usually practical and cost-effective only when the wastewater contains large amounts of heavy metals. On the other hand, these techniques are often ineffective when applied to dilute wastes with heavy metal concentrations less than 100 ppm [Volesky, 1990].

In recent years biosorption has emerged as a cost effective and efficient alternative for the removal of heavy metals from low strength wastewaters. Biosorption is the uptake of heavy metals and radionuclides from aqueous solutions by biological materials. Microorganisms, including algae, bacteria, yeast, fungus, plant leaves and root tissues can be applied as bioadsorbents for detoxification and recovery of toxic or valuable metals from industrial discharged water. One of the most promising bioadsorbents is “algae”.

Caulerpa lentillifera is a marine green macroalga cultivated as food for animals and humans, and also commonly used to treat wastewater from shrimp farms. Due to its rapid growth rate, farmers often have trouble with the over populated algae.

Turning excess *Caulerpa lentillifera* into bio-adsorbent might present a viable answer to this problem.

This work focuses on the biosorption of heavy metals by *Caulerpa lentillifera* Various factors such as pH, particle size, concentration of algae and heavy metal are included in the study.

1.2 Objectives

- To study the efficiency of heavy metal adsorption by *Caulerpa lentillifera*
- To investigate the effect of pH on such adsorption process.
- To examine if the heavy metal concentrations would affect the removal rate by *Caulerpa lentillifera*

1.3 Scope of this work

- Cu, Zn, Pb and Cd adsorptions by *Caulerpa lentillifera* were investigated.
- The range of pH was controlled at 1-7 depending on the type of heavy metals.
- The experiment was conducted with synthetic heavy metal solution and the range of concentration of the heavy metal was 0-250 mg/l.
- Only batch experiment was carried out.

1.4 Contributions of this work

- Establishment of the basic knowledge on the use of algae in adsorbing heavy metals.
- Alternative technology for the removal or recovery of heavy metals.
- Potential development of local technology for the management of wastewater from electroplating industry.

CHAPTER 2

TREATMENT TECHNOLOGIES FOR WASTEWATER CONTAINING HEAVY METALS: OVERVIEW

2.1 Electroplating industry

Electroplating or electrodeposition is an electrochemical process, which involves the deposition of relatively thin metallic coating on an electrically conductive surface. The metal-plating industry provides protection against corrosion, increases erosion resistance and can be used for decoration. Facilities in this industry segment are located worldwide and vary in size and plating capacity. All of the methods for chemical surface treatment involve dipping metal objects into a bath to apply the metal coating through electrode deposition [Ha, 1993]. Metals most commonly used for these applications include Cr, Cu, Cd, Ni, Pb, Zn and Ag.

The major metal-bearing waste categories in the metal-plating industry include rinsed water, bath drag-outs and residues from work pieces and spent plating baths. The wastewater treatment often has to deal with a large volume of hazardous waste and usually contributes to the generation of hazardous sludge containing heavy metals. The metal concentrations in the waste streams vary with the different processing sequences. Example of industries that are the main uses of heavy metals are listed in Table 2-1 [Kaewsarn, 2000].

2.2 Heavy metals

The term heavy metal generally has been used to describe those metals having atomic numbers higher than iron (molecular weight > 59) or having density greater than 5 g/ml. From the environmental standpoint, metals may be classified into two groups. The first is essential metals, which are required in trace amount for microorganisms as nutritional components, but are toxic in greater amount. This group consists of As, Cr, Co, Cu, Ni, Se, Va and Zn etc. The second group is highly poisonous and is not known to have any nutritional value. This group consists of Pb, Hg, Cd, Ur, Ag and Be etc [Kojima and Lee, 2001].

Problems with heavy metals differ greatly from most other forms of pollution. This is primarily because metals do not undergo degradation. Moreover, most heavy metals only require relatively low dosages to cause toxicity. Examples of the threshold limits and the effects caused by heavy metal uptake are given in Table 2-2.

2.3 Metals removal and recovery technologies from wastewater

Because of the strict regulations, effluent or water contaminated with heavy metals must be treated. Table 2-3 provides a list of possible technologies for the removal of various heavy metals. This section presents concise evaluation of various metal removal and recovery technologies including chemical precipitation, membrane separation, ion exchange, evaporation, carbon adsorption and biosorption.

2.3.1 Precipitation

Precipitation is a method for heavy metal removal. Usually the effluent was adjusted at high pH to reduce the solubility of the metal compounds. The sedimentation characteristics of the precipitate are improved by adding a flocculating or coagulating agent where a subsequent sedimentation and filtration removes the precipitation of the metals.

This process can handle relatively high flowrates of wastewater containing high concentrations of heavy metals and so it is suitable for the treatment of many mining effluents. The efficiency of the treatment depends on (1) the concentration and type of metals, (2) the concentration of total dissolved solids, (3) the concentration of complexing agents and (4) the concentration of organics, oil and grease. The rates of formation of reaction are usually low in water with low metal content leading to an increase in the consumption of chemicals used. In addition, in precipitation systems containing more than one metal ion species, optimum removals may not occur for a given metal species when another metal has been treated for maximum removal. Precipitation of metals is depend upon two factors: the concentration of the metal and the pH of the water as illustrated in Figure 2.1-2.2 [Ayres *et al*., 1994]. High concentration of oil and grease may result in a longer settling time for the precipitation due to a formation of emulsion. In addition, it was reported that the removal of toxic metals by chemical precipitation from dilute effluent is not economical [Matheickal, 1998].

Some disadvantages of precipitation are as follows [Banerjee, 2000]:

- Excess amounts of chemicals required

- Often high concentration of metal ions in supernatant or resulting effluent
- Metal cyanide complexes cannot be treated directly
- Increasing cost of land or lagoon
- Poor settling and filtering properties of the sludges

2.3.2 Membrane separation

Membrane separation processes consist of four major techniques; Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF), and Reverse Osmosis (RO). These are used to recover plating compounds from rinse water and to enable reuse of rinse water [Kaewsarn, 2000].

UF can be used as a pre-treatment method for RO units to avoid fouling of the RO membranes. Typical MF or UF treatment systems involve chemical precipitation followed by pressurized flow of the underflow though the filter membrane to remove the precipitate. The pressure exerted on the solution forces clean solution through the membrane.

RO is a treatment technique that reverses the direction of the water flow under osmotic pressure. The technology has been applied to the metal finishing industry for the recovery and reuse of both plating chemicals and rinse water. The pH range in which the membrane can be operated limits the application of RO to the treatment of metal-containing wastes.

Disadvantages of membrane processes [Banerjee, 2000]:

- Lower rejection by membranes at higher metal concentrations
- High energy requirement for treatment of concentrated solution
- Short life span of expensive membranes
- Affected by various chemical and pH
- Skilled operator required
- Prohibitive cost in wastewater treatment

2.3.3 Ion exchange

Ion exchange is a reversible chemical reaction wherein an ion (an atom or molecule that has lost or gained an electron and thus acquired an electrical charge) in the solution is exchanged with a similarly charged ion attached to an immobile solid particle [Remco Engineering, 1981]. Ion exchange is a treatment technology applicable to

(1) metals in wastewater where the metals are present as soluble ionic species (e.g. Cr^{+3} and CrO_4^{-2})
(2) nonmetallic anions such as halides, sulfates, nitrates and cyanides and
(3) water-soluble, ionic organic compounds including
    (a) acids such as carboxylic, sulphonics and some phenols
    (b) amines when the solution acidity is sufficiently acidic to form the corresponding acid salt
    (c) quaternary amines and alkyl-sulfates

The following waste characteristics affect the performance of ion exchange: (1) the concentration and valance of the contaminant, (2) the concentration of competing ionic species, (3) the concentration of interfering inorganic and organics, (4) the concentrations of dissolved and suspended solids, oil and grease, and (5) the corrosiveness of wastewater relative to the ion exchange resin material. Treatable waste streams should not contain high solids or high organic levels because solids will foul the resin column and cause treatment inefficiencies due to channeling. Detail about this can be found in [Kunin, 1985].

Disadvantages of ion exchange [Banerjee, 2000]:

- Chemical regeneration can occur, which breaks the polymer network or modification of functional groups by a metal species in the solution
- The metal removal capacity is usually affected by the presence of calcium or magnesium ions in the solution
- Resin can be fouled by precipitates such as calcium sulphate or ferric hydroxide
- Ion exchange resins are more expensive than biosorbents

2.3.4 Evaporation

Evaporation is a simplified recovery system for the separation of substances based on volatility differences. Although the technology is established, recent advancements have made mechanical evaporation a more costly method for metal recovery. Evaporation involves evaporating the wastewater to a concentrated solution of metal bearing water, which may be recycled back to the process. Evaporation has many benefits, e.g. easy to operate, little operator attention required [Matheickal, 1998].

Disadvantages of evaporation [Banerjee, 2000]:

- Feasible only if sufficient metal is to be recovered
- Considerable operation cost
- Metals cannot be individually isolated, usually a sludge containing the mixture of metals and perhaps other concentrated compounds is obtained.

2.3.5 Carbon adsorption

Adsorption of a substance involves its accumulation at the interface between two phases, such as a liquid and solid or a gas and solid. Common adsorbents in water treatment include activated carbon, ion exchange resins, adsorbent resins, metal oxides, hydroxides and carbonates, activated alumina, clays and other solids that are suspended in or in contact with water [Matheickal, 1998]. The most commonly used adsorbent is activated carbon.

Disadvantages of carbon adsorption is that the adsorbent has to be regenerated after use and after the regeneration step, the activated carbon loses some 10% of its weight and 15% of its uptake capacity. These are also added costs of operation [Banerjee, 2000].

2.3.6 Biosorption

Biosorption is a method for removal of heavy metals from wastewater. The technology is more effective and economical for removing heavy metals particularly at low concentration and also it enables easy recovery and recycle of metals.

Advantages of biosorption [Banerjee, 2000]:

- Metals at low concentration can be selectively removed.
- Biosorbent has very low affinity for calcium and magnesium ions.
- The system operates ever a broad pH range (pH 3 to 9).
- The system is effective over a temperature range of 4 to 90°C.
- The system offers low capital investment and low operating costs.
- The cost and liability of toxic sludge disposal are eliminated by converting pollutant metals to a metal product.

One of the disadvantages of biosorption is the very limited knowledge on some species of biological materials. To date, there is not a sufficiently detailed study of each potential species to clarify the mechanism of metal uptake. The system inherent dependence on factors like pH, temperature and nutrient concentration etc., makes the method for each biomaterial rather species specific. The comparison of metal uptake capacities between different biosorbents are shown in Table 2-4 [Kaewsarn, 2000].

2.3.7 Concluding remarks: Evaluation of various treatment techniques.

Conventional physical and chemical technologies such as adsorption, reverse osmosis, electrodialysis and precipitation have been used as the effecitve treatment method for heavy metal-containing wastewater effectively when concentrations of the metal ions are high. However, these techniques are usually ineffective or too expensive when metal concentrations in wastewater are low compared to biological technologies. The qualitative comparison between various treatment technologies regarding the costs and efficiency are summarized in Table 2-5 [Kojima and Lee, 2001].

Quantitative comparisons of some other technologies for the removal of heavy metals are summarized in Table 2-6 [Banerjee, 2000]. Table 2-7 provides rough estimates to the treatment costs for the different technologies. [Banerjee, 2000].

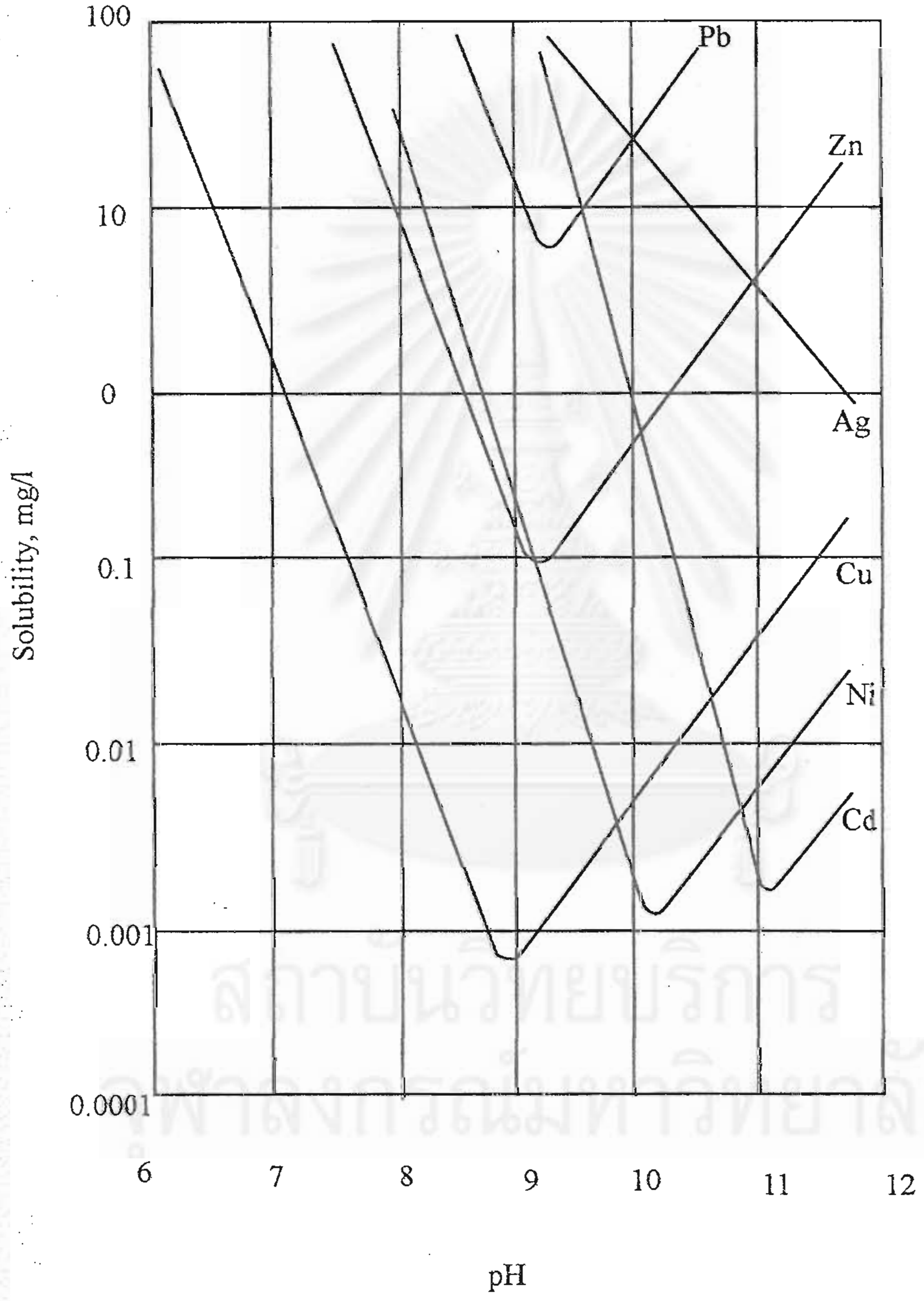

Figure 2.1: Solubility of metal hydroxides

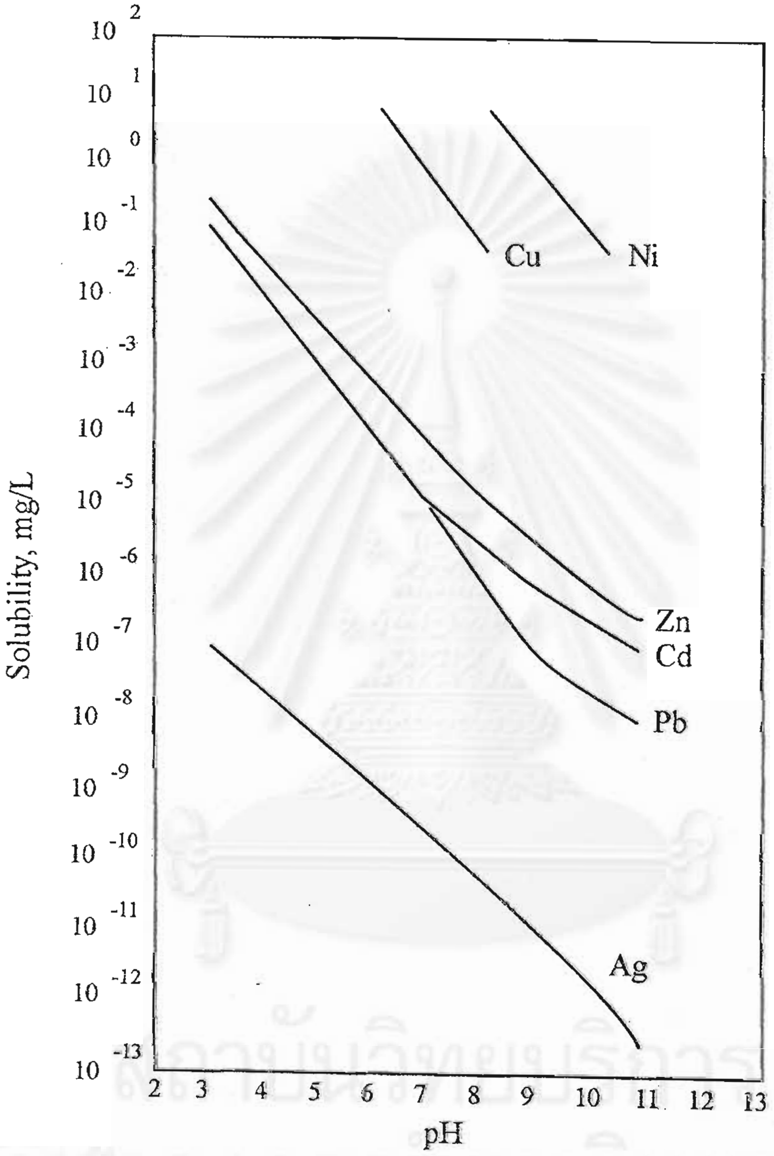

Figure 2.2 Solubility of metal sulfides

Table 2-1 Metals involved in various types of industry [Kaewsarn, 2000]

Industry	Metals								
	Ag	Al	As	Cd	Cr	Cu	Ni	Pb	Zn
Electroplating	●			●	●	●		●	●
Smelting and Refining	●	●		●		●		●	●
Paint, ink and associated products		●			●	●		●	
Petroleum refining					●			●	
Iron and steel manufacturer				●	●	●	●	●	●
Photographic industry	●								
Leather, tanning and fishing					●				
Wood preserving			●		●	●		●	
Battery manufactory	●			●			●	●	

Table 2-2 Heavy metals, their threshold limiting values (TLV) and poisoning effect [Friberg et al., 1979].

Heavy metals	TLV (mg/l)	Effect of poisoning
Pb	0.05	Toxicity to fetus, reduces fertility in women, altered spermatogenesis for men, impaired kidney function
Cd	0.005	Bronchitis, pneumonitis, nausea, gastroenteritis, intense pain in bones, cancer, damage to kidneys, lungs and liver
Cu	1.0	Damage to liver, vomiting, nausea
Ni	No TLV	Lung cancer
Cr	0.5	Irritant, vomiting, nausea, carcinogenic
Hg	0.1	Damage to nerve system, liver and kidney
Zn	No TLV	Vomiting, nausea

Table 2-3 Summary of some possible technologies for metal removal [Patterson, 1981]

Metals	Treatment technology
As	- Arsenic oxidation - Lime precipitation or iron or alum coprecipitation - Gravity clarification
Ba	- Sulphate precipitation - Coagulation - Gravity clarification
Cd	- High pH precipitation - Gravity clarification for lime or filtration for caustic
Cr (VI)	- Acidic reduction to trivalent Cr or ion exchange at pH below 6.0
Cu	- Precipitation - Gravity clarification
Fe	- Oxidation at neutral pH to ferrous or ferric iron - Precipitation - Gravity clarification or filtration
Pb	- High pH precipitation - Gravity clarification for lime or filtration for caustic
Hg	- Ion exchange or coagulation plus filtration
Ni	- High pH precipitation - Gravity clarification and/or filtration
Ag	- Ion exchange for ferric chloride coprecipitation plus filtration
Zn	- Precipitation at optimised pH - Gravity clarification and filtration

Table 2-4 Comparison of metal uptake capacities of various biosorbents [Kaewsarn, 2000]

Species	Capacity (mmol/g)
Bacterial biomass	0.20-0.48
Fungi and yeast	0.03-0.90
Fresh water algae	0.50-1.20
Marine algae	0.68-1.81
Other biological materials	0.17-0.67

Table 2-5 Removal characteristics of treatment technologies for heavy metals [Banerjee, 2000]

Properties	Technology					
	Biosorption	Precipitation	Ion Exchange	Activated Carbon	Membrane	Evaporation
Concentration Dependence	Ok	No	Ok	Ok	No	Ok
Effluent quality (mg/l)	<1	>5	<1	No*	1-5	1-5
pH variation	Ok	No	some	some	some	Ok
Selectivity	Ok	No**	Ok	No	No	No
Versatility	Ok	No	Ok	Ok	Ok	No
Organic tolerance	Ok	Ok	No	Ok	No	Ok
Regeneration	Ok	No	Ok	Ok	No	No
Cost	Ok	Ok	No	Ok	No	No

Notes: Ok -good
No* -No report on
No** -sulphide precipitation can be selective

Table 2-6 Comparative evaluation of five different separation technologies [Banerjee, 2000]

Evaluation	Reverse Osmosis	Ion Exchange	Electrolytic Cell	Evaporation	Precipitation
Water quantity	low	low-medium	low-medium	low-medium	substantial
Water recovery	high	high	Low	medium	low
Effluent TDS	20-35 ppm	~1-10 ppm	~100 ppm	~50 ppm	1,000-3,000ppm
Closed-loop potential	high	high	low	low-medium	low
Operation and maintenance	~$3K/y	~$3K/y	~$3-5K/y	~$3K/y	~$5-8K/y
Chemical usage	low	medium	low	zero-low	medium-high
Energy requirement	low	low	medium	medium-high	medium
Space requirement	medium	medium	medium-large	small-medium	medium
Labour requirement	medium	medium	medium-high	high	high
Overall	good	good	fair-good	fair-good	fair
Capital costs and capacity	$30-40K 20 l/min	$25-40K 20 l/min	$25-50K 400-800 l/min	$20-40K 200-400 l/hr	$40-60K 20-50 l/min

Note: Not found for biosorption technology

Table 2-7 Costs associated with treatment technologies [Banerjee, 2000]

Treatment technology	Capital cost of plant	Annual operating cost	Cost per kiloliter
Biosorption	\$332,224	\$56,937	\$0.49
Precipitation	\$370,189	\$159,634	\$1.08
Adsorption	\$239,327	\$150,805	\$0.96
Ion Exchange	\$332,224	\$110,026	\$0.78
Membrane	\$294,554	\$129,507	\$0.87

CHAPTER 3

LITERATURE REVIEW: BIOSORPTION

3.1 Microbial removal of heavy metals

3.1.1 Microorganisms vs. heavy metals removal

Algae have a high negative surface charge; therefore, they have an affinity for polyvalent cations with a strong positive charge. This is an advantage of waste treatment but a disadvantage if the intent is to use waste-grown algae for animal feed [Borowitzka and Borowitzka, 1988].

Microorganisms can accumulate heavy metals and radionuclides from their external environment. Amount of accumulation can be large and a variety of physical, chemical and biological mechanisms including adsorption, precipitation, complexation and inter/intracellular transports, may involve. Living and dead cells, as well as products excreted by, or derived from microbial cells, such as cell wall constituents, pigments, polysaccharides and metal-binding proteins, are capable of metal or radionuclide removal from solution. The removal of radionuclide, metal or metalloid species, compounds and particulates from solution by biological material, particularly by non-directed physico-chemical interactions, is now termed “biosorption”. Biosorptions are important because the removal of potentially toxic and/or valuable metals and radionuclides from aqueous effluents can result in detoxification and therefore safe environmental discharge. Moreover, an appropriate treatment of loaded biomass can enable recovery of valuable elements for recycling or further containment.

The removal mechanisms employed by microorganisms can be quite diversified. Essential metals such as K, Ca, Mg, Cu, Zn, Fe, Co, Mn and those with no essential biological functions such as Cs, Cd, Pb, Al, Sn and Hg can be accumulated by microorganism. Precipitation, complexation, and crystallization of heavy metals and radionuclide exterior to cells can result in detoxification. Moreover, metal ions may be compartmentalized and/or converted to less toxic forms by microorganisms. Most living cells systems exploited to date have been used for decontamination of effluents containing metals at concentration below toxic levels. These systems may employ a mixture of microorganisms as well as higher plants for the same purpose.

For instance, algal and cyanobacterial blooms was used to adsorb Cu, Cd, Zn, Hg and Fe in mining effluents [Fry *et al.*, 1992].

Microorganisms regulate and influence trace element uptake, accumulation, transformation and release, which are direct and indirect mechanisms in natural aquatic systems. Many inorganic compounds are involved in the oxidative and reductive reactions that comprise cellular metabolism. Thus participation of toxic substances in biological process can lead to chemical transformation of a compound into a form quite different from the origin. Many cationic elements (Ag, Cd, Hg, and Zn) are rapidly sorbed. There are differences in accumulation between different phytoplankton species; however, that may be driven by physiological or biochemical differences between cells [Langston and Bebianno, 1998].

The use of macro algal biomass as a biosorbent was first reported by Kuyucak and Volesky in 1988. Their studies revealed a high capacity of marine algae, *Sargassum natans* in taking up gold from acidic aurochloride solutions. The gold uptake capacity of dried biomass was 420 mg/g, which was higher than those of ion exchange resin IRA-400 and activated carbon [Matheickal, 1998].

There are some other examples for microbial adsorption of heavy metals and radioactivity as shown in Tables 3-1 to 3-4.

3.2 *Caulerpa sp.*

Caulerpa is a macroalga that classified in Division *Chlorophyta* (green algae), Class *Caulerpaceae*, Order *Caulerpales*, Family *Caulerpaceae*. It grows in various shades of bright green colors, as well as different forms and shapes - some growing tall, others growing as mats. *Caulerpa* spreads almost entirely by asexual growth by fragmentation. A spore producing *Caulerpa* can often release such large numbers of spores that it turns the aquarium water into a milkly-green color. Most species grow well under brilliant lighting in a nutrient rich tank environment. There are over a hundred species of *Caulerpa* found worldwide [HDL-Technical Section, 2001]. Some of the species of *Caulerpa* are edible and are traditionally utilized as food in the form of fresh vegetable or salad. *Caulerpa lentillifera* is one of the favorite edible species due to its soft and succulent texture.

3.3 Mechanisms of biosorption

The kinetics of metal uptake by green algae is a two step mechanism.

1. The first step is the passive transport mechanism, which is fast (less than 5-10 minutes), reversible and metabolism-independent surface reaction called “biosorption” such as physical sorption or ion exchange of the adsorbate species at the cell surface of cells.
2. The second step is the active transport mechanism, which involves uptake of the heavy metals into the living or dead cells, slow metal binding process (up to 600 hours in some algae), irreversible and metabolism-dependent called “bioaccumulation” [Kojima and Lee, 2001].

Bacteria, cyanobacteria, algae, fungi and yeasts are able to remove metal ions from their surrounding environment by both mechanisms. Metabolism-independent adsorption of metal ions to cell wall which is polysaccharides or other materials occurs in living and non-viable cells and is generally rapid. Metabolism-dependent intracellular uptake or transport occurs in living cells and is usually a slower process than adsorption, although greater amounts of metal may be accumulated by this mechanism in some organisms. Although both living and non-viable cells are able to accumulate, there may be differences in the mechanisms involved in either case. The choice of living or non-viable biomass for metal ions removal depends on each particular case because both options have advantages and disadvantages. Changes of the cell wall may cause a variation of the number of functional groups or their states which affects the capacity of metal accumulation. Furthermore, when using non-viable biomass, mechanical or chemical treatments after cell growth may increase the maximum amount of metal ion removed by adsorption [Olguin *et al.*, 2000].

3.4 Equilibrium behavior and sorption isotherms

The constant-temperature equilibrium relationship between the quantity of adsorbate per unit of adsorbent, q, and the equilibrium concentration of adsorbate insolution is called the adsorption isotherm. The most widely used models to describe metal uptake in algae for systems at equilibrium are the well-known Langmuir and Freundlich sorption isotherms which can be expressed as follows:

Langmuir sorption isotherm:
$$q = \frac{x}{m} = \left\{ \frac{bC_e(\frac{x}{m})_{max}}{(1+bC_e)} \right\} \tag{3.1}$$

Freundlich sorption isotherms:
$$q = \frac{x}{m} = K_f\,(C_e)^{\frac{1}{n}} \tag{3.2}$$

and can be linearized as followed:

Langmuir sorption isotherm:
$$\frac{1}{q} = \frac{1}{q_{max}bC_e} + \frac{1}{q_{max}} \tag{3.3}$$

Or
$$\frac{C_e}{q} = \frac{1}{q_{max}b} + \frac{C_e}{q_{max}} \tag{3.4}$$

Freundlich sorption isotherms:
$$\log q = \log K_f + \frac{1}{n}\log C_e \tag{3.5}$$

where x/m is the solute (metal) concentration in the sorbent (algae), C_e, the equilibrium concentration of solute in the solution, K_f, a constant, $(x/m)_{max}$, the maximum solute concentration in the sorbent, and b and n are constants related to the energy of sorption.

The Langmuir isotherm was originally formulated based on the following theoretical assumptions:

1) the sorption reaction can be represented as a coordination reaction with 1:1 stoichiometry (i.e. monolayer sorption)
2) the activities of the surface sites are proportional to heavy metal concentration.
3) the number of sorption sites is fixed.

The Freundlich isotherm was based on sorption on heterogeneous surface, which has generally been considered an empirical relationship and has been used widely to fit experimental data [Kojima and Lee, 2001].

3.5 Controlling factors for the removal of heavy metals

Metal accumulation by algae is influenced by a number of abiotic and biotic factors [Kojima and Lee, 2001, Yang and Volesky, 1999] as shown in Table 3-5.

3.5.1 pH

pH is one of the most important parameters influencing biosorption processes. It has been known that the uptake increases when the pH of the metal solutions increases from 2.5 to 6 [Volesky, 1990]. A general trend, observed for different metals and biomass, is that metal uptake is negligible at low pH (pH 1 to 2). The metal uptake increases with an increase in pH (from pH 3 to 5) and there exists an optimum pH for a maximum metal uptake for each individual heavy metal species. The explanation given to such a phenomenon include: (1) high competition between metal cations and proton for the binding sites at lower pH values, (2) the unionized functional groups on the cell wall at low pH values, which thus makes the binding sites inaccessible to cations. As pH increases, more ligands with negative charge would be freed and become available, which enhances the attraction for positively charged metal ions. At high pH values, the metal precipitation in solution becomes an important factor, especially at pH greater than the optimum values for adsorption (pH 5 to 7) where the metal solubility has dropped dramatically. The optimum pH for bioremoval depends on the type of algae and other conditions as shown in Table 3-6 [Matheickal, 1998; Kaewsarn, 2000]. However, most of the heavy metals have low solubility and precipitate at higher pH > 5.5 and investigation on the adsorption of heavy metals should consider the effect of metal precipitation at high pH as it might lead to misinterpretation of the adsorption capability of the adsorbents.

3.5.2 Metal chemistry

A wide range of metals can be adsorbed onto the cell surface at different rates depending on their affinity to the surface. The chemical properties of metals can significantly contribute to the adsorption. For instance, metals such as Au and Hg are easily taken up due to the formation of covalent bonds between the metal and the biomass surface, while some other metals such as U can form hydroxides with base that can easily be adsorbed onto cell surfaces. The ability of metals to form complexes with ligands also influences the biosorption process to a large extent. However, the affinity of a metal to be adsorbed on the cell surface of microorganism will depend on the relative affinity of that metal to ligands in the solution. The metal binding also depends on the preferences that they have for certain donor atoms. For example, in the biological environment, Hg and Cd are sulfur seeking elements while Cu shows equal preference to nitrogen and oxygen [Matheickal, 1998].

3.5.3 Heavy metal concentration

The effect of heavy metal concentration depends on the type of algae and other factors. Adsorption capacity increases with increasing concentration of heavy metal until the binding sites of microorganism are saturated.

3.5.4 Size effect of algae

Literature showed that the effects of size of algae cannot be generalized as the adsorption depended significantly not only on cell size and shape, but also with cell wall structure which varied widely among the different algal species. Table 3-7 provides examples of the work carried out to investigate the effect of algal size on the biosorption [Kaewsarn, 2000].

3.5.5 Temperature

Temperature is not expected to have a significant effect on the metal biosorption by non-living biomass although living systems can be strongly affected by temperature. In most studies, temperature in the range 40 to 60°C did not have any effect . However, it was observed that native biomass lost their macroscopic structure at a very high temperature (> 100°C), resulting in a reduced metal uptake. In most metal biosorption studied with algae, temperature is usually maintained close to that employed in the laboratory (see Table 3-8 for detail) [Matheickal, 1998 and Kaewsarn, 2000].

3.5.6 Cations

Biosorption of metals is significantly affected by the presence of other cation species present in the system. As the metal binding sites on the biosorbent are limited, these cations can compete for the binding sites. Depending on the affinity of these ions for binding sites, the effect can vary considerably. Generally, it has been observed that competition from light metal ions is insignificant and cannot reduce the heavy metal adsorption capacity of the biomass. This is thought to be one of the positive aspects of using biosorbents in place of traditional resins since the performance of which are affected by the presence of other light metal ions. Studies using mixed metal solutions, where two or more heavy metal ions are present, have revealed that the two heavy metals systems are often subject to lower adsorption rate than that from single species solution, indicating the non-specific nature of cell wall functional groups. The extent of reduction is found to be dependent on the electronegativities of the other cations and also on the factors such as pH [Matheickal, 1998]. Examples are shown in Table 3-9.

3.5.7 Anions

The presence of a number of anionic compounds in the solution matrix is seen to influence the biosorption capacity. Generally, metal rich industrial effluents contain soluble organic or inorganic ligands to keep high metal concentration of ion solution. These ligands are capable of forming complexes with dissolved metal ions and the resulting complexes can markedly alter the chemical form of the metal in solution thereby altering the adsorption behavior. Metal adsorption sometimes increases and sometimes decreases, depending on the particular metal, ligand adsorbent and the range of pH being studies. The metal ligand complexes may be strongly adsorbed or weakly adsorbed. Another possibility is that the ligands themselves can interact with the charged cell surface directly so as to enhance or decrease the metal uptake. However, most of the biosorption studies reported that anions caused a wide degree of metal uptake inhibition with no evidence of enhancing metal uptake behavior (See Table 3-10 for detail).

3.5.8 Binary and multi-component biosorption

In practice, industrial wastewater rarely contains only one type of metal ion. The degree of removal of heavy metals from wastewater by biosorption depends on the multi-metal competitive interactions in solution with the biosorbent. The combined effects of two or more metal ions to non-living biomass depend on the number of metals competing for binding sites, metal combinations, levels of metal ion concentration, and residence time [Kaewsarn, 2000].

3.5.9 Regeneration and re-use of biosorption

In industrial applications of biosorption, the adsorbed metal ions need to be recovered from the adsorbent. Different processes have been proposed in literature and were summarized in articles by Matheickal, 1998 and Kaewsarn, 2000. In general, acidic conditions can repress biosorption which means that it might provide effective desorption environment. An important parameter for desorption is the ratio of loaded biomass (S) to volume of eluant (L), more commonly known as the S/L ratio. The S/L ratio needs to be maximized so that metals can be recovered in high concentrations. To increase this ratio, it is necessary to increase the eluent concentration. However, in the case of acids or chelating agents, the increased concentration can cause damage to the biomass.

Table 3-11 summarizes the study of adsorption of heavy metals conducted by various researchers.

Table 3-1 Metal biosorption by “living” microalgae suspended in metal solution [Wase and Foster, 1996]

Organism	Metal	Accumulation (%dry weight)
Chlorella vugaris	Cd	0.20
	Pb	8.50
	Zn	0.13
	Au	10.00
Chlorella regularis	U	0.39
	Cu	0.40
	Zn	2.80
	Co	0.19
	Mn	0.80
	Mo	1.32
Chorella salina	Cd	0.01
	Co	0.67
	Zn	0.02
	Mn	0.01
Chlorella homosphaera	Cd	0.55
	Zn	0.40
Chlorella sp.	Hg	0.01
	U	0.02
Scenedesmus obliguus	Cd	0.30
Scenedesmus sp.	Mo	2.30
	U	0.19
Chlamydomonas sp.	U	0.34
	Mo	2.10
	Cd	0.35
Dunaliella tertiolecta	U	0.01
Asterionella formosa	Cd	2.20
Fragilaria crotonensis	Cd	0.60
Ankiistrodesmus sp.	U	1.00
Selenastrum sp.	U	1.00

Table 3-1 (continued)

Organism	Metal	Accumulation (%dry weight)
Euglena sp.	Al	1.50
	Zn	0.01
	Mn	0.02
	Cu	0.01
	Pb	0.03
Thalassiosira rotula	Ni	0.06
	Cd	0.09
Cricosphaere elongata	Cu	0.07
	Cd	0.01
Hymenomonas elongata	Zn	0.07
Chlorella/*Scenedesmus*	Zn	0.13
(mixture)	Ni	0.02
	Mn	0.06
	Cr	0.02
	Cd	0.004
	Co	0.007

Table 3-2 Metal biosorption by “living” cyanobacteria suspended in metal solution [Wase and Foster, 1996]

Organism	Metal	Accumulation (%dry weight)
Anabaena cylindrica	Cd	0.250
Anacystis nidulans	Cd	0.170
	Ni	1.000
Aphanocapsa sp.	Cd	0.370
Synechocystis aquatilis	Cd	0.110
Spirulina platensis	Au	0.520
Synechococcus elongata	U	0.10
Caloxthrix crustacea	U	0.04
Oscillatoria woronicinii	Cd	0.009
	Zn	0.093
	Ag	0.001
	Hg	0.001
Oscillatoria sp.	Pb	0.058
Nostoc UAM 208	Cd	1.125
Nostoc sp.	Pb	0.024

Table 3-3 Biosorption by macroalgal biomass [Wase and Foster, 1996]

Organism	Metal	Accumulation (%dry weight)
Halimeda opuntia	Co	8.00
	Cd	5.20
Vaucheria sp.	Cu	3.20
Fucus vesiculosus	Cd	5.00
	Pb	17.40
Padina gymnospora	Pb	5.90
Codium taylori	Pb	13.00

Table 3-4 Examples of percentage uptake of heavy metals by microorganisms [Vymazal, 1995]

Organism	Element	Uptake (% dry weight)
Bacteria		
Streptomyces sp.	U	2-14
S.viridochromogenes	U	30
Thiobacillus ferrooxidans	Ag	25
Bacillus cereus	Cd	4-9
Zoogloea sp.	Co	25
	Cu	34
	Ni	13
	U	44
Citrobacter sp.	Pb	34-40
	Cd	40
	U	90
Algae		
Chlorella vulgaris	Au	10
Chlorella regularis	U	15
Fungi		
Phoma sp.	Ag	2
Penicillium sp.	U	8-17
Rhizopus arrluzus	Cu	1.6
	Cd	3
	Pb	10.4
	U	19.5
	Th	18.5

Table 3-4 (continued)

Organism	Metal	Accumulation (%dry weight)
Yeasts		
Saccharomyces cerevisiae	U	10-15
	Th	12

Table 3-5 Controlling factors for the removal of heavy metals [Kojima and Lee, 2001, Yang and Volesky, 1999]

Abiotic factors	Biotic factors
- Specific traits of metal (affinity of binding site, electronegativity)	- Species-specific characteristic (cell wall, mucilage, cellular composition)
- Metal concentration	- Algal biomass concentration
- Duration of exposure	- Extracellular products
- Concentration of other ions (e.g. Ca, Mg, P, other heavy metals)	- Stage of development
- pH	- Cellular activity
- Complexing and chelating agents	
- Redox conditions	
- Temperature	
- Light	
- Turbulance	

Table 3-6: Some examples indicating the effects of pH

Algae	Effects
Chroococcus sp. [Les *et al.*, 1984]	- Increasing adsorption rate of Cd, Cu and Zn with increasing pH
Chlorella vulgaris and *Scinedesmus quadricauda* [Harris and Ramelow, 1990]	- Rate of adsorption from high to low: Ag> Cu> Cd> Zn - No effect of pH on Ag adsorption - Optimal pH for best adsorption: Cu: 5 Cd: 7 Zn: 7
Biomass algae [Fry *et al.*, 1992]	- Strongly bound by algal biomass at pH 5 to 7 for Cu^{+2}, Cr^{+3}, Ni^{+2}, Pb^{+2}, Zn^{+2}, Cd^{+2} and Co^{+2}
Oocystis sp. and *Sargassum sp.* [Wase and Forster, 1996,]	- Decreasing adsorption rate of Cd and Cu with increasing pH by living *Oocystis sp.*

Table 3-7 Effect of size of algae on biosorption

Algae	Effects
Sargassum fluitans and *Ascophyllum nodosum* [Leusch *et.al.*, 1996]	- Studied 6 different sizes of dry algae. - larger sizes had more adsorb metals from high to low adsorption: Pb> Cd> Cu> Co> Zn> Ni - The stalk of *Sargassum fluitans* adsorbed more heavy metals than the leaf of algae
Sargassum fluitans [Yang, J. and Volesky, B., 1998]	- The particle size did not affect Cd removal rate.

Table 3-8: Effect of temperature on biosorption

Algae	Effect
[Failla et.al., 1976]	- No effect of temperature on Zn and Cu adsorption
Chlorella vulgaris [Aksu and Kutsal, 1991]	- Increasing Pb adsorption rate as temperature increased from 15 to 35°C
Chlorella sp. [Volesky, 1990]	- No effect on the biosorption of Mn and Mo between 0 and 30°C

Table 3-9: Effect of cation on biosorption

Algae	Effect
[Kuyucak and Volesky, 1989]	- Adsorption of Co decreased when other cations such as UO^{+2} and Pb^{+2} increased
A. nodosum [Voleskey, 1990	- Adsorption rate increased with increasing concentration of Pb in the presence of Cd
[Tsezos, 1983]	- Adsorption rate of U decreased with the presence of increasing the amount of Fe^{+2}

Table 3-10: Effect of anion on biosorption

Algae	Effects
Rhizopus arrihus [Tobin *et.al.*, 1987]	- Inhibition effect on the adsorption of La^{+3}, Cd^{+2}, Pb^{+2} and Ag^{+2} with the presence of EDTA - Effect of EDTA inhibition was largest for the uptake rate of Cd and Pb at equimolar concentration
[Kuyucak and Voleskey, 1989]	- Decreasing Co uptake with various anions such as PO_4^{-3}, SO_4^{-2} , CO_3^{-2} and NO_3^- - Strongest inhibition was NO_3^-

Table 3-11 Adsorption of heavy metals by bioadsorbents: Examples

Source	Algae	Heavy metal	Factor	Result
[Sakauchi *et al.*, 1979]	*Chlorella sp.*	Cd	Time	The adsorption was rapid during the first 30 minutes and continued at a slower rate.
			Temperature	Temperature did not affect the uptake of Cd.
			Desorption	Most of Cd adsorbed by *chlorella sp.* cell was easily released by EDTA.
[Aksu and Kutsal,1991]	*C.vulgaris*	Pb	Isotherm	The sorption phenomenon was expressed by the Freundlich adsorption isotherm.
[Kitjaharn, 1991]	*Aphanothece halophytica*	Pb	Time	Accumulation was rapid and became saturated at 90 µg/mg dry weight within 1 hour.
		Zn	Time	Accumulation increased at a slower rate of 7.8 µ g/h.mg dry weights after a higher rate in the first 10 minutes.
		Pb and Zn	pH	At pH 6.5, Pb and Zn accumulation by *A. halophytica* was the highest adsorption.
	Spirulina platensis	Pb	Time	Pb accumulated at a rapid rate in the first 10 minutes
		Zn	Time	Zinc accumulation rate was saturated within 5

				hours.
		Pb and Zn	pH	The Pb and Zn accumulation increased at a pH above 6.5 and 6.0 respectively.
	Aphanothece halophytica and *Spirulina platensis*	Pb and Zn	Algal mass	The total accumulation of lead and zinc increased with increasing cell density.
		Pb and Zn	Age of algae	The age of cell had only slight effect on Pb and Zn accumulations on both algae.
[Aksu *et. al*., 1992]	*C. vulagaris and Z. ramigera*	Cu	pH	The optimum pH was 4.0-4.5 for both algae.
			Isotherm	The adsorption process was determined by Freundlich model.
[Crist *et.al*., 1992]	*Rhizoclonium*	Pb	pH	Adsorption of Pb and Al under acidic conditions released H, Ca and Mg ions from algae while adsorption of Pb and Cd at pH 10 released hydroxides.
[Holan and Volesky, 1994]	-Brown algae (*A. nodosum, Fucua vesiculosus, S. natans, S. fluitns, S. vulgare and Padina gymnospora*), -Green algae	Pb	Ability of difference type of algae	Pb adsorption capacity, brown algae> green algae> red algae.
		Ni	Ability of difference type of algae	Ni adsorption capacity, brown algae> red algae> green algae
		Pb and Cd	pH	Adsorption ability increased with increasing

	(*Codium taylori*) and -Red algae (*Chondrus crispus, galaxura maginata and Palmaria palmata*).			pH.
[Ozer, *et.al*., 1994]	*Cladophora crispata*	Pb and Cr(VI)	pH	The optimum pH were 5.0 and 1.0, 25°C for Pb and Cr(VI) removal, respectively.
			Isotherm	The euqilibrium data fitted both Freundlich and Langmuir isotherms.
[Chong, 1995]	*Ascophyllum nodosum*	Cd, Cu and Zn	Binary and ternary system	Equilibrium batch sorptions were studied by using Cd+Cu, Cd+Zn, Cu+Zn and Cd+Cu+Zn. At pH 4.5, the inhibition dominance observed in the two and three metal system was Cu>Cd>Zn.
[Matheickal and Yu, 1996]	Brown marine algae, *Ecklonia radiata*	Pb, Cd and Cu	Isotherm	The equilibrium data fitted well to the Langmuir isotherm.
		Pb	Range of pH 4.5-5.5.	The uptake capacity of the algae for Pb was 1.36 mM/g (282 mg/l), which is much higher than those of powered activated carbon and natural zeolite.

		Pb	pH	Sorption of Pb increased as pH increased and reached an equilibration at pH 5.
		Pb	Time	The Pb uptake process was rapid, with 60% of the sorption completed within 10 minutes.
Sag and Kutsal, 1996]	*Rhizopus arrhizus*	pH	Fe and Cr (VI)	The optimum initial pH for the biosorption of Cr (VI) and Fe(III) ions was determined as 2.0
		Temperature	Fe and Cr(VI)	The biosorption rate increased when increasing temperature in the range 25-35°C and 25-45°C, respectively.
[Chu, *et.al*., 1997]	*Sargassum baccularia*	Cd	Desorption	It was found that HCl at pH 2 could desorb 80% and almost complete recovery by 3.24 mM EDTA solution.
[Kratochvil, 1997]	*Sagassum sp.*	Cu, Zn, Cd, Fe and Cr (III)	Mechanism	Confirmation that ion exchange was the principle mechanism of heavy metal biosorption.
[Inthorn, *et. al*., 1996; Nagase, *et. al*., 1997]	*Tolypothrix tenuis*	Cd	Light metals	Cd adsorption decreased when added Ca or Mg in solution.
			Pretreatment cells	Pretreatment cells with NaOH improved the efficiency of Cd removal.

Table 3-11 (continued)

Source	Algae	Heavy metal	Factor	Result
[Kapoor and Viraragharan, 1998]	*A. niger*	Pb, Cd and Cu	Pretrement algae with sodium hydroxide, detergent, formaldehyde and dimethyl sulphoxide	Adsorption of treatment cells increased when compared with living biomass.
		Ni	Pretrement algae with sodium hydroxide, detergent, formaldehyde and dimethyl sulphoxide	Adsorption decreased when compared with living biomass.
[Kratochvil and Voleskey, 1998]	*Sagassum biomass*	Cu	Binary adsorption	In the regions of high and negligible interference, Fe lowers the uptake of Cu by more than 40% and by the less than 10%, respectively as compared to only uptake Cu.
			pH	The optimum pH was in the range of pH 4-5.
[Matheickal , 1998]	*Durvillaea pototoruma*	Pb, Cu and Cd	Uptake capacity	Metal uptake capacity were found to be 1.6, 1.3, 1.2 mmol/g for Pb, Cu and Cd, respectively.

	Ecklonia radiata		Uptake capacity	Metal uptake capacity were found to be 1.3, 1.1, 1.0 mmol/g for Pb, Cu and Cd, respectively.
	Durvillaea pototoruma and *Ecklonia radiata*		Light metals (Ca, Mg, Na and K)	No effect significantly with binding of heavy metals.
[Srikrajib, 1998]	*Sargassum polycystum*	Cd	Temperature used to dry algae	The temperature tested at 80 and 100°C, which were used to prepare algae, did not affect sorption of Cd significantly.
			pH	Highest removal of Cd was at pH 4.0.
[Zhou *et.al*., 1998]	5 species of microalgae, 2 species of macroalgae; *Laminaria japonica* and *Sagassum kjellmanicnum*	Cu and Cd	pH	The optimum pH was found between 4 and 5 for macroalgae while for microalgae, optimum pH at 6.7.
			Isotherm	Isotherm followed the Freunlich equation.
			Desorption	HCl and EDTA solution was very effective (99.5%) for macroalgae of metal recovery.
[Sag *et.al*., 1998]	*C. vulagaris and Z. ramigera*	Cr(VI) and Fe (III)	Isotherm	In the single ion situation, the adsorption isotherm fitted to Freundlich and Langmuir isotherms. In the multi-component situation, the competitive Freundlich isotherm was satisfactory for data.

Table 3-11 (continued)

Source	Algae	Heavy metal	Factor	Result
[Yang and Volesky, 1998]	*Sargassum fluitans*	Cd	Particle size	The particle size did not affect the sorption rate.
[Aksu and Acikel, 1999]	*C. vulgaris*	Cu and Cr(VI)	pH	The pH values of 2.0 and 4.0 was chosen as the optimum biosorption for Cr(VI) and Cu, respectively.
			Isotherm	The sorption process was expressed by Freundlich adsorption isotherm.
[Donmez *et.al*., 1999]	*Chlorella vulgaris, Scenedesmus obliquua and Synechocystis sp.*	Cu, Ni and Cr (VI)	pH	Optimum pH of Cu, Ni and Cr(VI) were determined as 5.0, 4.5 and 2.0, respectively.
			Isotherm	Both the Freundlich and Langmuir isotherms were suitable for biosorption process.
[Matheickal and Yu, 1999]	*Duvillaea potatoru* and *Ecklonia radiata*	Pb and Cu	Capacity	Th maximum capacity for Pb and Cu were 1.6 and 1.3 mmol/g, respectively which was much higher than natural zeolites and powdered activated carbon.
		Pb and Cu	Time	The process was found complete at about 10 min

				with 90% removal.
		Pb and Cu	pH	The maximum adsorption was found at pH 4.5.
[Ozer *et. al.*, 1999]	*Schizomeris leibleinii*	Fe, Pb and Cd	pH	Optimum pH for Fe, Pb and Cd were 2.5, 4.5 and 5.0 at optimum temperature 30°C, respectively.
[Sanchez *et.al.*, 1999]	*Cymodocea nodosa*	Cu and Zn	pH Binary sorption	The optimum pH was 4.5. The three Langmuir models tested represent the data of two metal sorption system.
[Yang and Volesky, 1999]	*Sargassum* biomass	U	pH 4.0, 3.2, 2.6.	The maximum U uptake values were 560, 330 and 150 mg/g.
[Yu and Kaewsarn, 1999a]	*Durvillaea potatorum*	Cu and Cd	Time	The uptake process was very fast within 10 min for Cu and 30 min for Cd with 90% completed adsorption.
[Yu and Kaewsarn, 1999b]	*Durvillaea potatorum*	Ni, Cu and Cd	pH 1-6	The amount of Ni, Cu and Cd uptake as a function of solution pH. At pH<2, the amount of uptake was small. At pH increase, the amount of uptake increased.
[Yu *et.al.*, 1999]	*E. radiata* and *L. japonica*	Pb, Cu and Cd	Capacity	The range of capacity of Pb, Cu and Cd were 1.0-1.6, 1.0-1.2 and 0.8-1.2 mmol/g, respectively,

Table 3-11 (continued)

Source	Algae	Heavy metal	Factor	Result
[Figueira *et.al*., 2000]	*Durvillaea, Laminaria, Ecklonia and Homosira*	Cd	Pretreatment cells	The biosorption of pretreatment algae improved the efficiency of metal removal.
[Hamdy, 2000]	*Laurencia obtusa*	Cr, Co, Ni, Cu and Cd	Algal mass	The uptake of different metals was observed on increasing the algal mass from 5- 50 mg.
[Kaewsarn, 2000]	*Durvillaea pototoruma*	Pb, Cu and Cd	Binary and ternary adsorption	Adsorption rate decreased when compared with single metal adsorption.
		Cu	Desorption	HCl used an eluting agent that conserved the biosorption property after 5 times of desorption.
[Wong *et. al*., 2000]	*C. Vulgaris and C. Miniata*	Ni	Adsorption	The removal efficiency of *C. vulgaris* about 33-34% and *C. miniata* more than 99%.
			Isotherm	The sorption process was the Langmuir isotherm
[Yu and Kaewsarn, 2000]	*Durvillaea potatorum*	Ni	Adsorption capacity	The maximum capacity was obtained 1.13 mmol/g at pH 6.
			Time	The adsorption process was very fast (90%) within 25 min and equilibrium was reached at around 1 hour.

			Light ions	Ca, Mg Na and K did not significant interfere with the binding of Ni.
[Yu *et.al.*, 2000]	*Durvillaea potatorum*	Cu and Cd	Structure	The structural components of the alga had fiber-like cylinders. The internal structure was a highly connected network of cylinders with varying sizes. Methods of drying and pre-treatment of the biomass affected the structure. Heavy metal binding was confirmed by using an electron probe microanalyser.
[Dungkokkruad, 2001]	*Chlorella vulgaris*	Cd and Pb	% removal	*Chlorella vulgaris* removed 89% Cd and 88% Pb.
	Chrococcum sp.	Cd and Pb	% removal	*Chrococcum sp.* removed 94% Cd and 71% Pb.
	Nostoc paludosum	Cd and Pb	% removal	92% Pb and 92% Cd were removed.
	Phormidium angustissimum	Cd and Pb	% removal	77% Pb and 81% Cd were removed.
	All 4 algae	Cd and Pb	Isotherm	The removal of Cd and Pb could be explained by the Langmuir isotherm.

Table 3-11 (continued)

Source	Algae	Heavy metal	Factor	Result
[Kadukova *et al*., 2001]	*Chlorella kessleri*	Cu	Time	The whole process ran very quickly and concentration of metal dropped within first 30 minutes.
[Kaewsarn, 2002]	*Padina sp.*	Cu	Capacity	The maximum capacity was 0.80 mmol/g at pH 5.
			Kinetic	The kinetic was very fast with 90% within 15 min and equilibrium reached at 30 min.
			Light metal ions	No effect to Cu uptake.

CHAPTER 4

RESEARCH METHODOLOGY

4.1 Equipment and chemicals:

Equipment

- Rotary shaker
- Magnetic stirrer, Cimarec
- pH-meter, Hanna, HI 98240
- Oven, Memmert
- Analytical balance, Ohaus Corporation
- Dessicator
- Blender
- Filter paper No. 93, Whatman
- Atomic Absorption Spectrophotometer (AA), Varian, Spectr AA-300/400

  Wavelength (λ) for Cd: 228.8 nm

  Wavelength (λ) for Cu: 324.8 nm

  Wavelength (λ) for Pb: 213.9 nm

  Wavelength (λ) for Zn: 283.3 nm
- Fourier Transform Infrared Spectrometer (FTIR), Perkin Elmer, Model 1760X
- Laser Particle Size Analyzer, Malvern, Mastersizer-S long bed Ver 2.19
- Scanning Electron Microscopy (SEM), Jeol, JSM-5800LV

Glassware

- Erlenmeyer flasks
- Volumetric flasks
- Test tubes
- Beakers
- Pipettes
- Cylinders
- Funnel

- Vials

Chemical reagents:

- $Cu(NO_3)_2$. $2.5H_2O$, APS Finechem
- $Cu(SO_4)$. $5H_2O$, Merck
- $CuCl_2$. $2H_2O$, Merck
- $Zn(NO_3)_2$. $6H_2O$, APS Ajax Finechem
- $Zn(SO_4)$. $7H_2O$, Merck
- $Cd(NO_3)_2$.$4H_2O$, Merck
- $Pb(NO_3)_2$, Merck
- HNO_3 65%, Merck
- NaOH, Merck
- Deionized water
- pH 7.01 and 4.01 buffer solution, Hanna instruments
- EDTA (Ethylenediaminetetraacetic acid), Merck

4.2 Methodology

1. Algal collection

Caulerpa lentillifera was collected from Banjong Farm, Chachoengsao province.

2. Glassware preparation

2.1 Wash the glassware with water.

2.2 Immerse glassware in 20% HNO_3 overnight.

2.3 Wash the glassware with water to make sure that there is no acid deposited inside the glassware.

2.4 Rinse the glassware with deionized water.

2.5 Dry the glassware in 100ºC oven.

3. Preparation of algae

3.1 Wash the algae with deionized water.

3.2 Dry the algae at 80°C for 12 hours.

3.3 Store the algae in dessicator.

4. Preparation of synthetic wastewater:

4.1 Dissolve 500 mg of Cu in 1 l of deionized water as a stock solution.

4.2 Dilute the stock solution to 1, 10, 50 100,150 and 200 mg/l of Cu concentration.

4.3 Prepare standard solutions for Zn, Pb and Cd using procedure in 4.1-4.2

4.4 Store the stock solution in refrigerator at 4ºC.

5. Determinations of heavy metal removal by *Caulerpa lentillifera*.

5.1 Determination of equilibrium contact time

5.1.1 Mix 0.5g dry algae in 30 ml of solution with 10 mg/l of Cu.

5.1.2 Mix the solution slowly in a rotary shaker at 150 rpm.

5.1.3 Separate solid phase with filter paper No.93.

5.1.4 Measure heavy metal concentrations in the filtrate at 0, 1, 2, 5, 10, 15, 20, 30 and 60 minute by using Atomic Absorption Spectrophotometer (AA).

5.1.5 Repeat Steps 5.1.1-5.1.4 with Zn, Pb and Cd solutions.

5.2 Effect of algal concentration

5.2.1 Mix dry algae at various weights of 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 2000 mg in 30 ml solution, which contains 10 mg/l of Cu.

5.2.2 Mix the containers slowly using a rotary shaker at 150 rpm for 30 minutes.

5.2.3 Separate solid phase with filter paper No.93.

5.2.4 Measure Cu concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA).

5.2.5 Repeat Steps in 5.2.1-5.2.4 with Zn, Pb and Cd solutions.

5.3 Effect of particle size of algae

5.3.1 Grind dry algae into smaller sizes using blender.

5.3.2 Mix various ground algae 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 2000 mg in 30 ml solution, which contains 10 mg/l of Cu.

5.3.3 Mix the solution slowly in a rotary shaker at 150 rpm for 30 minutes.

5.3.4 Separate solid phase with filter paper No.93.

5.3.5 Measure Cu concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA).

5.3.6 Repeat Experiment in 5.3.1-5.3.5 with Zn, Pb and Cd solutions.

5.4 Effect of pH

5.4.1 Mix dry algae (with a quantity determined in Section 5.2) in 30 ml of solution with 10 mg/l of Cu at different pH (in a range of 1-7). Note that the effect of pH on the solubility of the heavy metals is also investigated in Experiment 5.6.

5.4.2 Mix the solution in a rotary shaker at 150 rpm at the contact time (as determined from Experiment in 5.1).

5.4.3 Separate solid phase with filter paper No.93.

5.4.4 Measure heavy metal concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA).

5.4.5 Repeat Steps 5.4.1-5.4.4 with Zn, Pb and Cd solutions.

5.5 Effect of heavy metal concentration

5.5.1 Mix dry algae in 30 ml solution of Cu at various concentrations: 1, 10, 50, 100, 150 and 200 mg/l, at pH 5

5.5.2 Mix the solution in a rotary shaker at 150 rpm

5.5.3 Separate solid phase with filter paper No.93

5.5.4 Measure heavy metal concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA)

5.5.5 Repeat Steps 5.5.1-5.5.4 with Pb, Zn and Cd solutions

5.6 Effect of pH on the solubility of the heavy metal

5.6.1 Prepare eight Cu solutions at a concentration of 100 mg/l

5.6.2 Adjust the pH in each solution using 0.03 N of nitric acid and sodium hydroxide, and vary the pH in the range of 1-8

5.6.3 Shake the solution in a rotary shaker at 150 rpm at optimum contact time found in Experiment 5.1.

5.6.4 Measure heavy metal concentrations before and after Experiment 5.6.2 in the filtrate by Atomic Absorption Spectrophotometer (AA).

5.6.5 Compare the concentrations of Cu before and after Experiment 5.6.2. Heavy metal concentrations were measured in the filtrate by Atomic Absorption Spectrophotometer (AA) before and after Experiment 5.6.2 with no algae to measure the amount of precipitation of heavy metals.

5.6.6 Repeat Steps 5.6.1- 5.6.5 with Pb, Zn and Cd solutions.

5.7 Desorption tests

5.7.1 Mix dry algae in 30 ml solution of Cu at concentration 100 mg/l at pH 5.

5.7.2 Mix the solution in a rotary shaker at 150 rpm

5.7.3 Separate solid phase with filter paper No.93

5.7.4 Measure heavy metal concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA)

5.7.5 Dry the Cu-loaded algae at 80°C for 12 hours

5.7.6 Mix the Cu-loaded algae with 0.1 M EDTA for 24 hours in a rotary shaker at a rate of 150 rpm

5.7.7 Separate solid phase with filter paper No.93

5.7.8 Measure heavy metal concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA)

5.7.9 Determine the amount of desorption from the amount of adsorption

5.7.10 Repeat Steps 5.7.1 - 5.7.9 with hot water (60°C)

5.7.11 Repeat Steps 5.7.1 - 5.7.10 with with Pb, Zn and Cd solutions

5.8 Effect of heavy metal compounds

5.8.1 Mix various dry algae 50, 100, 200, 300, 400, 500 and 600 mg in 30 ml solution, which contains 10 mg/l of $CuSO_4$.

5.8.2 Mix the containers slowly using a rotary shaker at 150 rpm

5.8.3 Separate solid phase with filter paper No.93.

5.8.4 Measure Cu concentrations in the filtrate by Atomic Absorption Spectrophotometer (AA).

5.8.5 Repeat Steps in 5.8.1-5.8.4 with $CuCl_2$, $CuNO_3$.

5.8.6 Repeat Steps in 5.8.1-5.8.4 with $ZnSO_4$ and $ZnNO_3$.

Experiments were repeated at least three times to ensure the accuracy of the experimental results. In the case that the results are not clear, further repetition was carried out.

4.3 Calculation

Cu, Zn, Pb and Cd adsorptions in solution before and after experiment with biomass is calculated from:

$$\% \text{ Removal} = \left(\frac{C_i - C_f}{C_i} \right) \text{x } 100 \qquad (4.1)$$

$$q = \frac{V\left(C_i - C_f\right)}{W} \qquad (4.2)$$

where	q	is Cu adsorption (mg/g dry wt)
	C_i	is the initial heavy metal concentrations (mg/l)
	C_f	is the equilibrium (final) heavy metal concentrations (mg/l)
	V	is the volume of the heavy metal concentration (ml)
	W	is the dry weight of the biomass-added (g)

4.4 Analytical experiment

4.4.1 Determination of heavy metal concentration

The quantity of heavy metal in the solution was determined using the Atomic Absorption Spectrophotometer (AA) where the quantity of heavy metals accumulated in the algae was subsequently calculated from a simple mass balance equation of the heavy metal based on the fact that the heavy metal can neither be created nor destroyed.

4.4.2 Determination of functional groups of cell wall of algae

The functional groups of algae was determined using FT-IR (Fourier Transform Infrared Spectrometer) at the Scientific and Technological Research Equipment Center, Chulalongkorn University to discuss how the algae adsorb the heavy metals. FTIR spectra were obtained for the biomass samples. Samples of 100 mg KBr disks containing 1% finely ground power (<20 μm) of each biomass sample were prepared for this study.

4.4.3 Analysis of heavy metal in algae

The presence of heavy metal binding in the biomass could be detected using SEM (Scanning Electron Microscopy) with EDX (Electron dispersive X-ray spectroscopy) at the Scientific and Technological Research Equipment Center, Chulalongkorn University. The analysis was applied to the algae before and after the adsorption of heavy metals.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Solubility tests

The solubility of metals at high pH is important as metal precipitation might lead to a misperception of the adsorption process. The solubility of metal compounds is related with various pH and complex forms such as hydroxide, carbonate, sulfite. To date, there was no experimental data on the solubility of metal nitrate. The objective of this part was to find the relation between pH and solubility of metal nitrates. Note that for all experiments in this section the initial metal concentration is approximately 100 mg/l. Results in Figures 5.1.1-5.1.4 demonstrate that all solubility curves exhibited a Z-curve character with details summarized in the following tabulation.

Heavy metal	Upper end		Lower end	
	Solubility range (mg/l)	pH range	Solubility range (mg/l)	pH range
Pb	100-85	1-5	35-10	6-8
Zn	100-80	1-6	10-5	7-8
Cu	90-85	1-5	5-1	6-8
Cd	90-85	1-6	65-5	7-8

Subsequent experiments were conducted at heavy metal concentration of 10 mg/l and, hence, the ranges of pH suitable for these experiments with lead, zinc, copper and cadmium were 1-5, 1-6, 1-5 and 1-7, respectively.

5.2 Adsorption of heavy metals by *Caulerpa lentillifera*

5.2.1 Effect of different compounds

Metal ions can form complexes with various anions, e.g. sulfate, nitrate, chloride, etc. The objective of this section was to investigate the efficiency of the adsorption of different metal compounds by *Caulerpa* biomass. Figure 5.2.1.a shows the comparison between the removal efficiency of $ZnSO_4$ and $ZnNO_3$ by *Caulerpa lentillifera* biomass, where X-axis is the amount of algal mass and Y-axis the % removal of zinc ions in $ZnSO_4$ and $ZnNO_3$ forms. As can be seen from this figure, only slight difference in the adsorption capacity of algae for $ZnSO_4$ and $ZnNO_3$ was observed with a slightly better removal efficiency for $ZnSO_4$. The highest percent removal of $ZnSO_4$ was found to be about 80% at the amount of algal mass of 0.4 g (for an initial $ZnSO_4$ concentration of 10 mg/l). After that point, the removal efficiency of *Caulerpa lentillifera* became leveled off. The highest removal of $ZnNO_3$ was about 70% at 0.5 g of algal mass, afterwhich the removal efficiency of *Caulerpa lentillifera*, reached its plateau.

An illustration in Figure 5.2.1.b is the comparison between the removal efficiency of $CuCl_2$, $CuSO_4$ and $CuNO_3$ by *Caulerpa lentillifera* It was observed that the removal efficiency of the three heavy metal compounds was more or less at the same level, which was at approximately 70%. Hence, any form of these metal complexes could be selected for the subsequent experiment. Although the metal chloride at low concentration can be safely detected using AA, high doses might present some technical constraints and therefore metal chlorides were not used in this study. Although the adsorption of sulfate compounds presents a slightly better removal efficiency, these compounds usually have low solubility, which narrows down our experimental range. Hence, metal nitrate compounds were selected as a model study for this work.

5.2.2 Identification of functional groups in algae

In the study of adsorption, the determination of the nature of functional group(s) is of considerable importance. The functional groups of *Caulerpa lentillifera* can be interpreted by the FT-IR. Figures 5.2.2 is the results from this test, which shows that *Caulerpa lentillifera* consisted of functional groups as shown in tabulation:

Functional group	Standard Wavenumber (cm^{-1}) [Skoog and Leary, 1992]	Wavenumber (cm^{-1}) from the results	Relative quantity*
Hydroxyl; O-H**	3250-3700	3200-3600	1
Carboxyl; COOH**	2400-3300	3200-3600	1
Amine; NH_2**	3300-3500	3200-3600	1
C-O***	1050-1300	1000-1200	2
Sulfonyl; S=O***	1040-1200	1000-1200	2
Carbonyl; C=O	1670-1780	1600-1750	3
S-O	550-650	500	4
Alkyl; C-H	Carboxylic acid: 2500-3100 Alcohol: 3400-3640	2900-3000	5

* The quantity in the order from large to small (1 is the most abundant and 4 is the least).

, * Overlapped transmittance.

From the results, the amounts of hydroxyl, carboxyl or amine groups were higher than C-O or sulfonyl, carbonyl, S-O and alkyl, respectively. The interactions of the various functional groups might also complicate the discussion here as hydroxyl might interact with carbonyl group resulting in carboxyl group. Similarly, amine might interact with carboxyl resulting in amino group, whilst sulfonyl might interact with S-O resulting in sulfonate (SO_3^{2-}). Also the adsorption at a wave number of 1000-1200 cm^{-1} might also represent the carboxyl group in addition to the C-O. Moreover, the adsorption band at 3200-3600 cm^{-1}, which was indicated as O-H group might in fact overlay the adsorption band of carboxyl and amine groups. Also, C-O stretching may have overlapped with sulfonyl group. Therefore it was highly possible that there existed carboxyl, sulfoxyl, sulfonate and amino groups (and perhaps other groups too) within the cell wall matrix of *Caulerpa lentillifera* Hence, the heavy metals could, hence, form a variety of complexes with these functional groups depending on their physical/chemical affinity of the pairs. Literature has revealed some important observations, which will help facilitate the description of the adsorption mechanisms on *Caulerpa lentillifera* Some heavy metals were reported to be adsorbed onto some specific functional groups and these are summarized as follows.

Algae	Functional group	Metal	Reference
Scenedesmus obliquus	**Carboxyl groups**	**Cd**	[Crist *et al*., 1981]
Sargassum natans,	Amino, carboxyl, sulfonate group	Au	[Crist *et al*., 1981; Mameri, 1999; Sar *et al*., 1999; Figueira *et al*., 2000]
Cladophora, Rhizoclonium and Polysiphonia	**Amino, carboxyl, sulfonate group**	**Pb**, Al, **Cd**	[Crist *et al*., 1990; Crist *et al*., 1992]

It is therefore possible that the carboxyl, amino and sulfonate group in *Caulerpa lentillifera* were responsible for the adsorption of Pb and Cd in our experiment.

To further verify this point, also consider the chemistry of the heavy metals. Pearson, 1976 classified metallic ions according to a hardness scale defined by their binding strength with F^- and I^- and identified as hard or soft by the thermodynamic stability of the complex forms. Hard ions are usually required as nutrients by microorganisms whereas the borderline and soft ions are toxic heavy metals. The hard ions form very strong bonds with hard cations, also the soft ions form strong bond with soft anions as shown in Table below [Voleskey, 1990; Shriver and Atkins, 1999]:

	I. Hard Ions	II. Borderline	III. Soft Ions
Lewis Acids	H^+, Li^+, Na^+, K^+, Be^{2+}, Mg^{2+}, Ca^{2+}, Cr^{2+}, Cr^{3+}, Al^{3+}, SO_3, BF_3	Fe^{2+}, Co^{2+}, Ni^{2+}, $\mathbf{Cu^{2+}}$, $\mathbf{Zn^{2+}}$, $\mathbf{Pb^{2+}}$, SO_2, BBr_3	Cu^+, Ag^+, Au^+, Tl^+, Hg^+, Pd^{2+}, $\mathbf{Cd^{2+}}$, Pt^{2+}, Hg^{2+}, BH_3
Lewis Bases	F^-, O^{2-}, $\mathbf{OH^-}$, H_2O, CO_3^{2-}, SO_4^{2-}, $ROSO_3^-$, NO_3^-, HPO_4^{2-}, PO_4^{3-}, ROH, $\mathbf{RCOO^-}$, ROR, $\mathbf{CO^{2-}}$	Cl^-, Br^-, N_3^-, NO_2^-, $\mathbf{SO_3^{2-}}$, NH_3, N_2, $\mathbf{RNH_2}$, $\mathbf{R_2NH}$, R_3N, =N-, -CO-N-R, O_2, O_2^-, O_2^{2-}	H^-, I^-, R^-, CN^-, **CO**, S^{2-}, RS^-, R_2S, R_3As

Nierboer and Richardson, 1980, proposed a classification based on the atomic properties and the solution chemistry of the metal ions. They also classified metal ions into three classes [Voleskey, 1990] and two dominant interactions between hard acid cations and soft acid cations as shown below [Shriver and Atkins, 1999].

	I. Hard Ions	II. Borderline	III. Soft Ions
Class	Oxygen seeking	Intermediate	Nitrogen seeking and Sulfur seeking
Bonding within group	Coulombic interactions or Electrostatic		Covalent bonding

From this information, the four selected heavy metals (Cd, Cu, Pb, Zn) are among the soft and borderline ions where most of the functional groups in the algae are found to be in a soft ion or Lewis bases group. The metals that belong to the intermediate group, i.e. Cu, Pb, and Zn, could form bonds with anions from all three classes while Cd should theoretically form a covalent bond with anion in the soft group. From the reason given above, the various possible couplings between the heavy metals and functional groups in the algae could be formulated as follows:

Heavy metal	Class	Possible coupling functional group	Associated bonding type
Pb	Intermediate	Carboxyl, Amine, Amino, Carbonyl, Hydroxyl, C-O, Sulfonyl, S-O, Sulfonate, Alkyl	Electrostatic and/or Covalent
Zn	Intermediate	Carboxyl, Amine, Amino, Carbonyl, Hydroxyl, C-O, Sulfonyl, S-O, Sulfonate, Alkyl	Electrostatic and/or Covalent
Cu	Intermediate	Carboxyl, Amine, Amino, Carbonyl, Hydroxyl, C-O, Sulfonyl, S-O, Sulfonate, Alkyl	Electrostatic and/or Covalent
Cd	Soft cation	C-O	Covalent

According to the finding of Crist *et al.*, 1981, it is possible that in this work, Cd formed a covalent bond with the C-O stretching in carboxyl functional group. Pb, Cu, and Zn, on the other hand, could also formed a covalent bond with the soft C-O, or they could form an electrostatic or ionic bond with a hard carboxyl (COO^-), carbonyl ($C=O$) group or form covalent or ionic bonds with the intermediate groups; amine (NH^{2-}), amino (NH_2^-), sulfonate (SO_3^{2-}) group.

5.2.3 Confirmation of heavy metals adsorption

The presence of heavy metal binding in the biomass was confirmed by using a SEM with EDX before and after adsorption and the results are illustrated in Figures 5.2.3.a-5.2.3.f. The results of this technique show the finger print of each element on the cell wall of *Caulerpa lentillifera* The tests were performed for the adsorption experiments with Cu, Cd, Zn, Pb and the mixture of these 4 heavy metals. Figure 5.2.3.a shows the elements of algae structure before the adsorption process, which composed of C, O, Fe, Na, Mg, Al, Si, S, Cl, K and Ca. The elements may have come from the seawater and the intra-cellular composition of algae. The results in Figures 5.2.3.b-5.2.3.f, which is from the samples after the adsorption, demonstrate the spectrums of lead, zinc, copper and cadmium ions in the algae samples. This confirmed the adsorbing capability for the selected metals of the algae. However, the speciation forms and the quantity of heavy metals could not be identified due to the limitation of the measurement technique. Moreover, an identification of metal ion location in relation to the inner structure of algae was not possible.

5.2.4 Desorption tests

The purpose of this section was to examine the desorption characteristics of the metals from *Caulerpa lentillifera* biomass. The desorption can also help identify the type of adsorption process taken place by *Caulerpa lentillifera* The initial concentration of heavy metals was 100 ppm to ensure a detectable level of eluted heavy metals. The heavy metal-loaded biomass was eluted by 2 elutants; EDTA (0.1 M) and hot water (60°C). The filtrates were sampled to measure the quantity of eluted metals after 24 hours of elution and the results of the desorption test were illustrated in Figure 5.2.4. The results are summarized in a tabulation form here:

Heavy metal	% Desorption by EDTA	% Desorption by hot water
Pb	93%	1%
Zn	99%	5%
Cu	82%	4%
Cd	90%	7%

It is clear that all of the heavy metals employed in this work could not be eluted effectively by hot water where only a maximum of 7% could be obtained with Cd adsorbed biomass, and among all, Pb presented the lowest desorption character with only as much as 1% recovery. EDTA, on the other hand, was found to be a much better eluting agent. This finding implies that:

- The adsorption on the surface of *Caulerpa lentillifera* should not involve Van Der Waals’s forces at the cellulose network of the cell wall. This is because Van Der Waals is a weak force and adsorption due to this kind of force should be readily eluted using weak eluting agents such as hot water.

- The bonding between heavy metals and algae must have involved the formation of strong molecular bonds such as ionic or covalent bonds. This confirms the discussion in Section 5.2.2. However, this experiment could not identify the type of bonding taken place in this adsorption process.

5.3 Contact time

The time-profiles of the adsorption of Cu, Cd, Zn and Pb ions by *Caulerpa lentillifera* are given in Figures 5.3.1-5.3.4. In these experiments, the amount of algae was 0.5 g and the initial heavy metal concentrations were controlled at 10 mg/l. The results can be summarized as follows.

Heavy metal	Optimum contact time (min)	% removal
Pb	20	90%
Zn	20	85%
Cu	15	85%
Cd	20	90%

In all cases, the metals uptake was rapid with the system reaching 80-90% of the equilibrium loading within 20 minutes. After the optimal time, the removal efficiencies became quite independent with adsorption time with a very slow increasing rate. These results confirmed the findings in the literature that the adsorption process could be explained as comprising 2 mechanisms:

1. A passive transport mechanism where the adsorption took place at the cell wall of the algae. This is a fast, reversible and metabolism-independent process, which means that it can take place even with a non-viable cell. This rapid kinetics has significant practical importance, as it facilitates smaller adsorption volumes ensuring economy and efficiency. Similar rapid uptake has been reported for the adsorption of metal ions by other macroalgal species e.g. Crist *et al.*, 1992; Matheickal and Yu, 1996; Holan and Volesky, 1994; Kaewsarn, 2002.

2. An intracellular adsorption, which is a diffusion of the heavy metal to the cell of algae. This process is retarded by the diffusional mass transfer resistance and, hence, is more time consuming than the first mechanism and it can take several hours to complete the process.

5.4 Adsorption capacity of algal mass

The results from Figure 5.4 demonstrate that the effect of algal mass on the adsorption ability of lead, zinc, copper and cadmium ions, which can be concluded as follows:

Heavy metal	Initial conc.		At equilibrium		
	mg/l	mmol/l	minimal algal mass, g	% removal	minimal algal mass per unit mass of heavy metal, g/mg
Pb	10.16	0.049	0.8-2.5	90%	0.079
Zn	9.54	0.146	0.7-2.5	75%	0.073
Cu	9.62	0.151	0.4-2.5	70%	0.042
Cd	9.21	0.082	0.4-2.5	65%	0.043

The minimal algal mass was considered as the minimum amount of algae that gave a constant removal efficiency. This means that an increase in the algal mass beyond this minimal would not enhance the removal efficiency. The minimal algal mass per unit mass of heavy metal were found to be 0.079, 0.073, 0.043 and 0.042 g/mg for lead, zinc, cadmium and copper, respectively. Below the minimal algal mass, the results indicated that the removal efficiency increased with an increase in algal mass. In particular, at very low algal mass, the relationship between algal mass and removal efficiency was linear. This is because, at small algal mass, the number of adsorbing sites were not adequate for the heavy metals. In this region, an increase in algal mass results in a larger number of adsorbing sites available for the heavy metals. Therefore, a first order relationship between the algal mass and removal efficiency was observed. At large quantity of algal mass, the number of adsorbing sites were greater than required by heavy metals. Hence, increasing quantity of algal mass or adsorbing sites did not affect the removal efficiency, i.e. zero order relationship between the algal mass and removal efficiency.

5.5 Effect of algal grinding

In order to investigate the effect of particle size on the adsorption by *Caulerpa lentillifera*, experiments with two different sizes of algae: (i) whole thallus and (ii) ground thallus (particle diameter at about 800-1000 μm by using the Laser Particle Size Analyzer). Experiments were carried out with an initial metal concentration of 10 mg/l and the adsorption was performed for 30 min. The results are displayed in Figures 5.5.1-5.5.4 and can be summarized as follows:

Heavy metal	Whole thallus of algae	Ground thallus
	% removal	% removal
Pb	90%	90%
Zn	75%	80%
Cu	70%	70%
Cd	80%	80%

Most of the data indicated that the percent removals of heavy metals were independent of particle size. It is interesting to note that particle size seemed to have effect on Zn adsorption at high algal mass. This may be because the number of adsorption sites for Zn increased when the algae were ground. In addition, this implies that the adsorption site for Zn might not be the same as those for other heavy metals.

However, the experiment with ground algae revealed that the adsorption could take place at a more rapid rate than the experiment with whole cell algae. Figure 5.5.5 shows a comparative adsorption time-profile of Pb between whole cell and ground algae. The maximum adsorption capacities for both cases were found to be similar but the adsorption with ground algae occurred at a significantly faster rate. This might be because the ground algae provided a higher cell surface area per unit weight (as stated by Fisher, 1985) which facilitated the adsorption process.

5.6 Effect of pH

According to the preliminary experiment on the solubility of the heavy metals in Section 5.1, the investigation on the effect of pH was confined to the pH ranges of 1-7, 1-6, 1-5 and 1-7 for Pb, Zn, Cu and Cd adsorption, respectively. Figures 5.6.1-5.6.4 illustrate the influence of pH on the removal efficiency of various metals. In most cases, the removal efficiency increased steadily with pH. However, the adsorption of Pb presented a different character where the removal efficiency increased with pH only at very low pH range afterwhich the removal reached a maximum at around 80-90%. The removal efficiency was then found to decrease when pH was higher than 5.5.

To explain these phenomena, it is recommended that the discussion be divided into 2 sections:

- Adsorption of Zn, Cd, and Cu: The adsorption at low pH range usually took place with a low removal efficiency. This is because there was a high concentration of proton in the solution and this proton competed with metal ions in forming a bond with the active site on the functional group on the surface of the algae. The active site, which was bonded with proton became unionized and was inaccessible to other cations. These results are in good agreement with the findings of Matheickal, 1998, Yin, 1999, Kapoor *et al.*, 1999, and Kaewsarn, 2000. An increase in pH means a lower quantity of proton, which decreases a competition between proton and heavy metals. Hence, an increase in the adsorption capacity (or removal efficiency) could be observed.

  The effect of pH could also be explained using the discussion by Greene and Darnall, 1990 [cited in Wase and Forster, 1996]. According to their work, all of the heavy metals investigated here (Cu, Pb, Cd, Zn) were categorized (based on the metals' pH dependence of biosorption to algae) into the metal class I. This metal class was tightly bound to the algae at pH > 5 and can be desorped at pH < 2. This group's pH profile was explained as being consistent with metal cations being bound to cell wall ligands, but as the pH was lowered the overall charge on the cell wall became positive, which inhibited the approach of positive ions. Protons also competed for the same active binding sites on the algal cell wall as the metals, thus reducing the amount of metal biosorbed at high proton concentration (low pH).

- Adsorption of Pb: The results from the experiment with the adsorption of Pb indicated that at low pH, there were a high competition between proton and Pb ions to active sites on the algae. The competition decreased with an increase in pH resulting a better adsorption characteristic. However, at higher pH (pH 6-7), the adsorption efficiency decreased with pH. This might be resulted from the formation of a more stable metal complex between Pb and hydroxide ion in the solution, and this prevented the adsorption of Pb on the algae biomass.

5.7 Adsorption isotherms of *Caulerpa sp.*

Figures 5.7.1.a, 5.7.2.a, 5.7.3.a and 5.7.4.a are the plots between the removal efficiency and the concentration of heavy metals in the solution. This information was then subsequently used to construct an isotherm plot. All experiments in this section were performed for 30 min, at pH = 5: the pH where no heavy metals could not precipitation was observed (in Section 5.1), and with the biomass concentration of 0.5 g/l. The concentrations of heavy metals are 1, 10, 50, 100, 150 and 200 mg/l. Figures 5.7.1.b, 5.7.2.b, 5.7.3.b and 5.7.4.b are the results from the rearrangement of the experimental results according to Langmuir isotherm expression, whereas Figures 5.7.1.c, 5.7.2.c, 5.7.3.c and 5.7.4.c are the plots with respect to the Freundlich adsorption isotherm. The corresponding values for the parameters in each isotherm could be summarized as follows:

Heavy metals	Langmuir isotherm	Freundlich isotherm
Pb	q_{max} = 11.36 mg/g* (or 0.05 mmol/g**)	K_f = 5.483 mg/g* (or 0.026 mmol/g**)
	b = 8.15 l/mg (or 1689 l/mmol)	$1/n$ = 5.855
	R^2 = 0.9989	R^2 = 0.9015
Zn	q_{max} = 6.14 mg/g* (or 0.09 mmol/g**)	K_f = 3.816 mg/g* (or 0.058 mmol/g**)
	b = 5.8597 l/mg (or 383 l/mmol)	$1/n$ = 4.486
	R^2 = 0.9587	R^2 = 0.9512
Cu	q_{max} = 8.92 mg/g* (or 0.14 mmol/g**)	K_f = 3.798 mg/g* (or 0.060 mmol/g**)
	b = 4.0616 l/mg (or 258 l/mmol)	$1/n$ = 3.15
	R^2 = 0.9985	R^2 = 0.871
Cd	q_{max} = 56.18 mg/g* (or 0.50 mmol/g**)	K_f = 3.339 mg/g* (0.030 mmol/g**)
	b = 0.1775 l/mg (or 21.7 l/mmol)	$1/n$ = 2.594
	R^2 = 0.9814	R^2 = 0.926

* mg-heavy metal/ g-algae, ** mmol-heavy metal/g-algae

- In the linear regression of the experimental results to Langmuir isotherm, R^2 of close to 1 for all metals indicated that the adsorption isotherms for all heavy metals followed the Langmuir type.
- For Langmuir isotherm, a good adsorbent is the one with a high q_{max} and a steep initial sorption isotherm slope (high b value). The experiment reveals that adsorption capacity (q_{max}) was highest with the adsorption of Cd and lowest with Pb whilst adsorptions of Cu and Zn were in a similar range with a slightly higher capacity for Cu than Zn. The b value increased in order from cadmium, copper, zinc and lead, respectively. The high b value indicated a high affinity of the heavy metal onto the binding site of the algae. This means that the binding site in the algae was most active with the adsorption of Pb and least with the adsorption of Cd.
- Linear regression of the experimental data to the Freundlich isotherms demonstrated that the R^2 was not as close to unity as those obtained in the Langmuir isotherm. However, the value of R^2 was relatively close to 1 and it could be concluded that the isotherms also followed the Freundlich type.
- For the Freundlich isotherm, a good adsorbent is the one with a high sorption capacity (K_f) and a generally low sorption intensity indicator, ($1/n$). K_f was in the order from low to high as follows: lead, cadmium, zinc and copper, respectively. $1/n$ decreased in order from cadmium, copper, zinc and lead, respectively.

In short, the isotherms for the adsorption of all metals, i.e. Pb, Zn, Cu, Cd, were found to fit Langmuir more than Freundlich isotherm expressions because

Langmuir isotherm is the ideal for monolayer adsorption but Freundlich isotherm is ideal for heterogeneous adsorption. From the aforementioned discussion, it was quite clear that the adsorption of heavy metals on *Caulerpa lentillifera* took place at the functional group binding sites on the surface of the algae which should be regarded as a monolayer adsorption and hence, the isotherm should theoretically follow Langmuir type. This agreed well with the experimental finding in this section.

To further illustrate the adsorption capacity of *Caulerpa lentillifera*, the results from this work were compared with the adsorption of the same heavy metals using other types of bioadsorbents. Tables 5.1-5.8 summarize the adsorption characteristics of *Caulerpa lentillifera* with their associated Langmuir (Tables 5.1-5.4) and Freundlich (Tables 5.5-5.8) isotherm parameters along with data from other investigations (available in literature). Note that there was no reported investigation on Zn adsorption with Freundlich isotherm at the time the work was carried out. It can be observed from these tables that the adsorption capacity of *Caulerpa lentillifera* for lead and zinc were the lowest among the various types of bioadsorbents, whereas the adsorption capacity for Cu was in the same range with others. Interestingly, *Caulerpa lentillifera* demonstrated an outstandingly high adsorption capacity for Cd as can be seen from q_{max} in Langmuir isotherm.

5.8 Concluding remarks

There are several implications that could be extracted from the findings in this work and these are delineated as follows:

1. The desorption test indicates that the adsorption between the heavy metals investigated in this work and *Caulerpa lentillifera* did not take place due to a weak Van Der Waals force because this force should have been suppressed easily with hot water. Stronger forces due to covalent, hydrogen, or electrostatic bonds should actually be developed between the heavy metals and the functional groups on the surface of the algae.
2. The examination of particle size in Section 5.4 shows that Zn adsorption increased when the algae was ground (see more detail explanation in Section 5.4). This suggests that the target functional group for Zn might not be the same as those for other heavy metals. The target functional group for Zn seemed to increase when the algae was ground to smaller size where the others did not.

3. Results from the isotherm experiment imply two possible mechanisms for the adsorption of the heavy metals by *Caulerpa lentillifera* Firstly, the target functional group for Cd is not the same as the targets for Cu, Zn, and Pb as otherwise the algae should have exhibited similar adsorption characteristics. Similar conclusion is proposed for the Pb adsorption as the removal efficiency was found to be extraordinary low. Secondly, the target functional group might be the same for all algae but it has different affinity for the different metals, with the highest for Cd and the lowest for Pb. The mixture of these two mechanisms is also possible.
4. *Caulerpa lentillifera* is ideal for the adsorption of Cd.

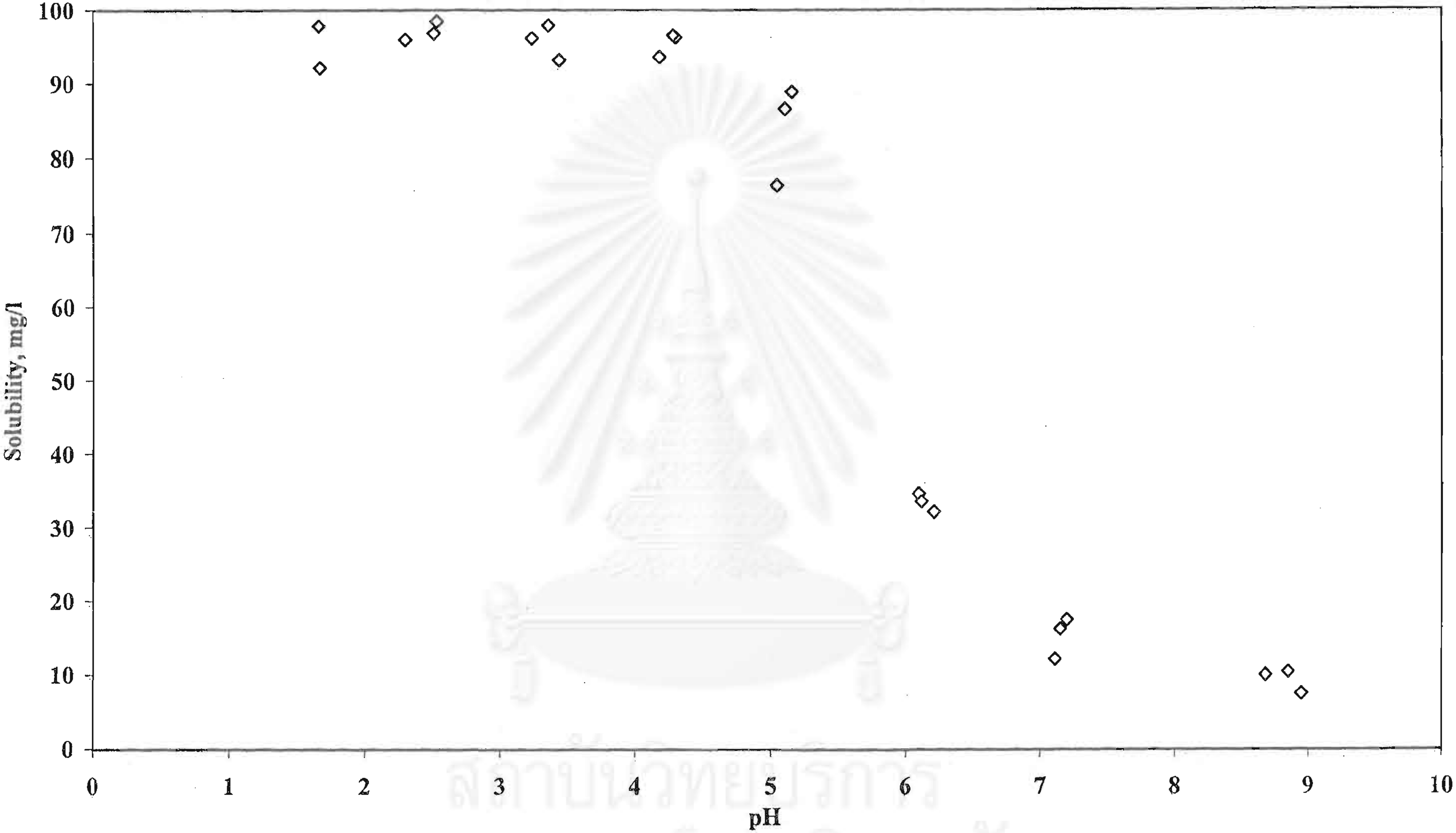

Figure 5.1.1 Solubility of lead nitrate

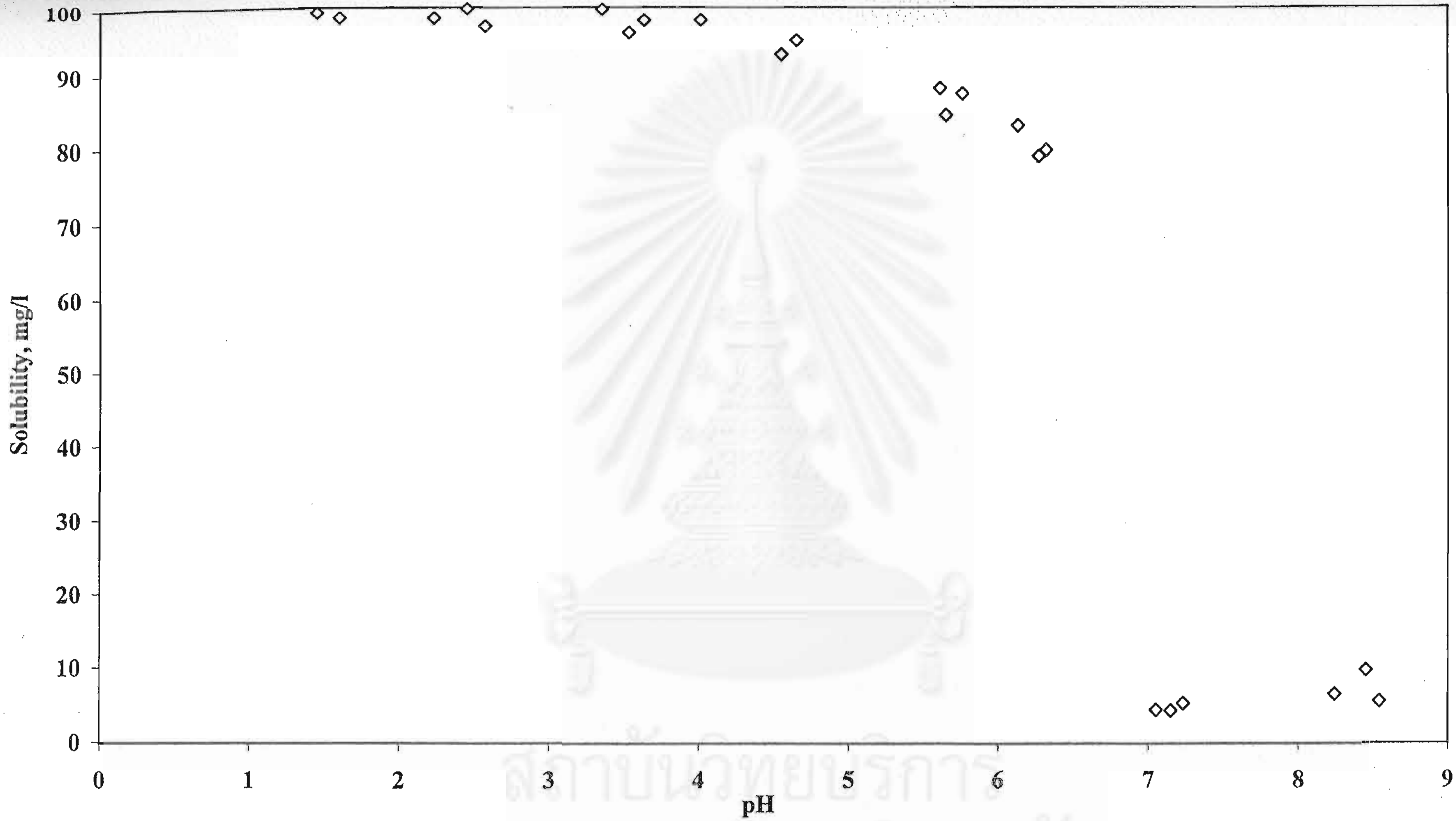

Figure 5.1.2 Solubility of zinc nitrate

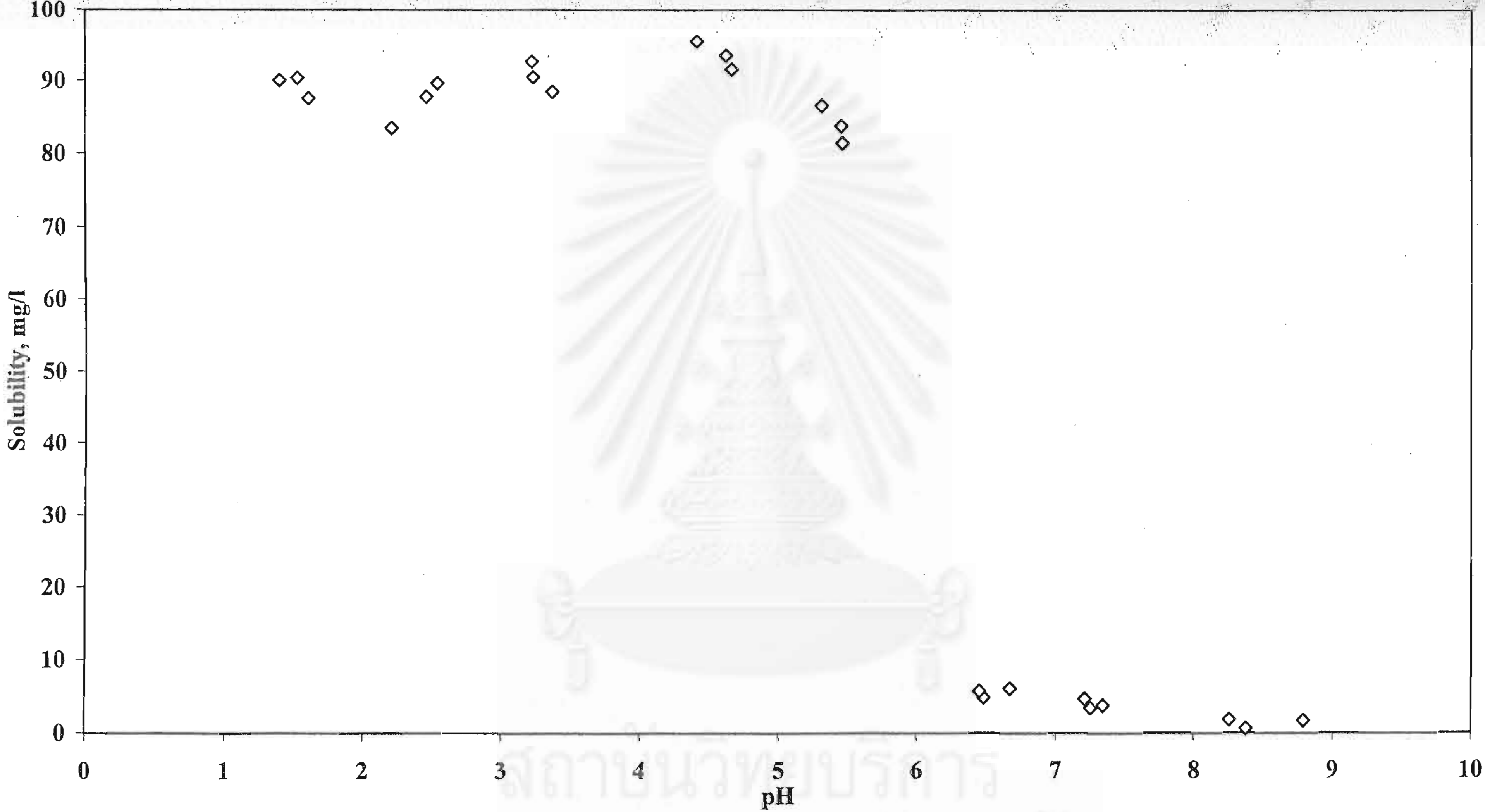

Figure 5.1.3 Solubility of copper nitrate

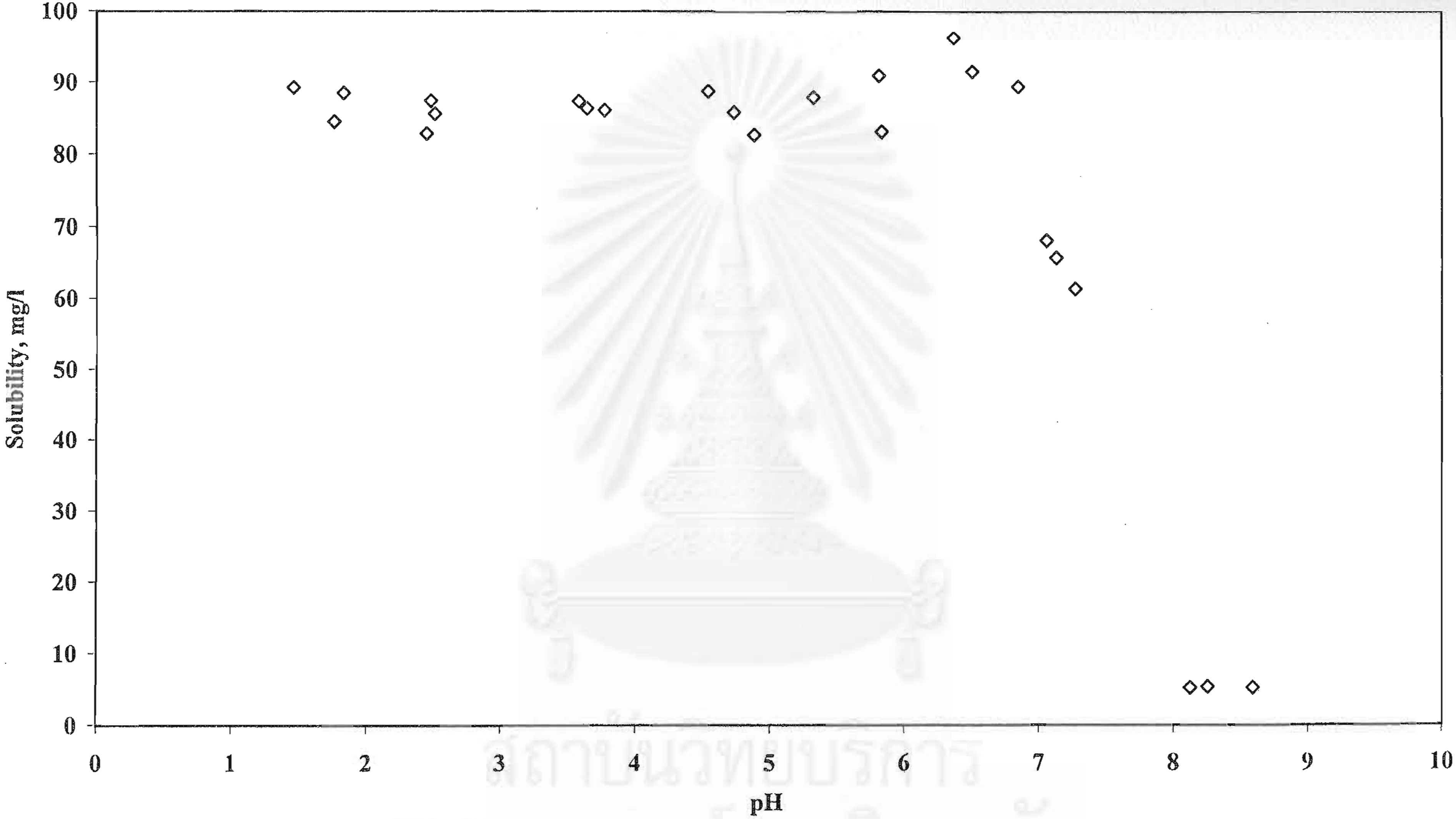

Figure 5.1.4 Solubility of cadmium nitrate

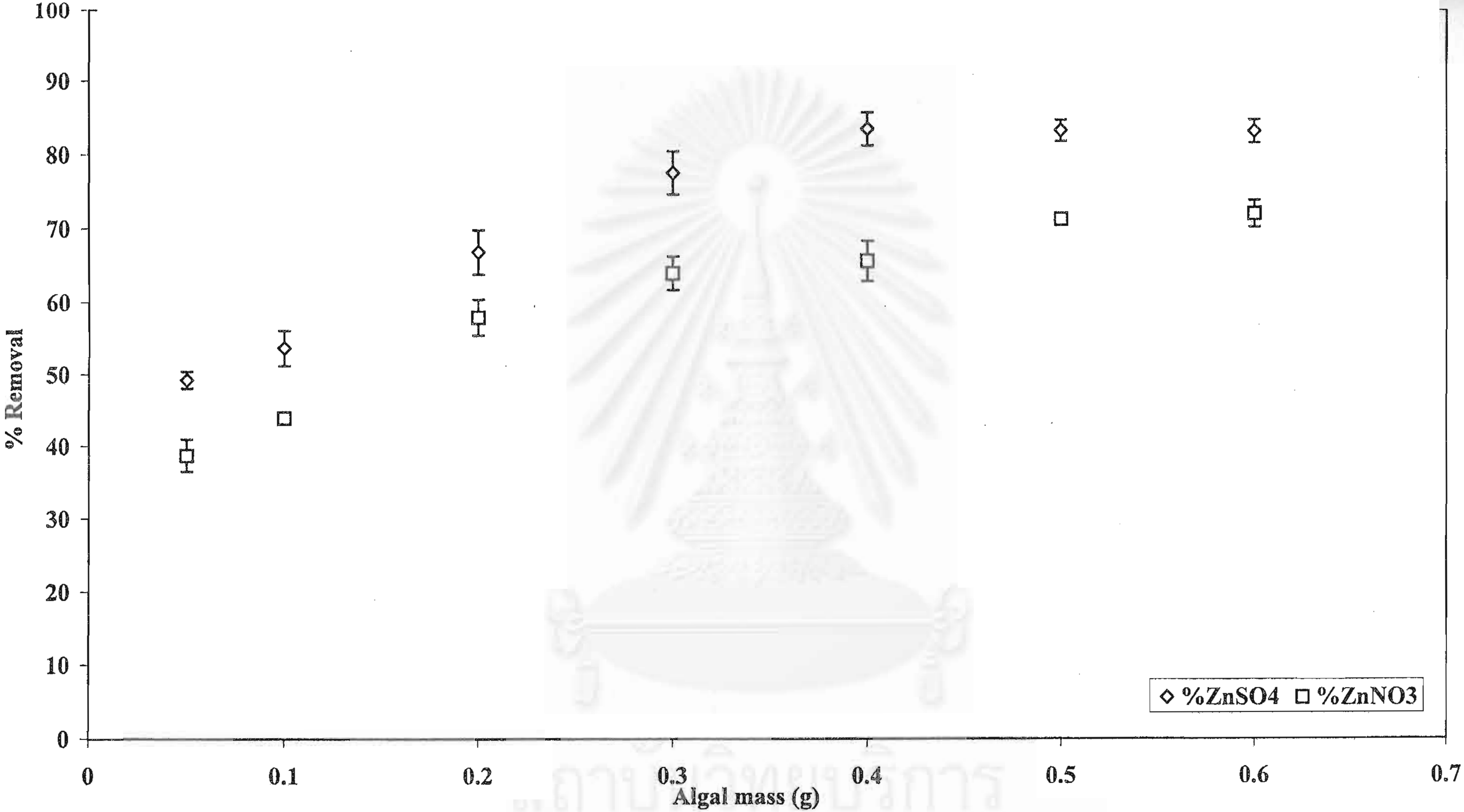

Figure 5.2.1.a Adsorption of ZnSO4 and ZnNO3 by *C. lentillifera*
(contact time 30 min, initial conc.= 10 mg Zn/l)

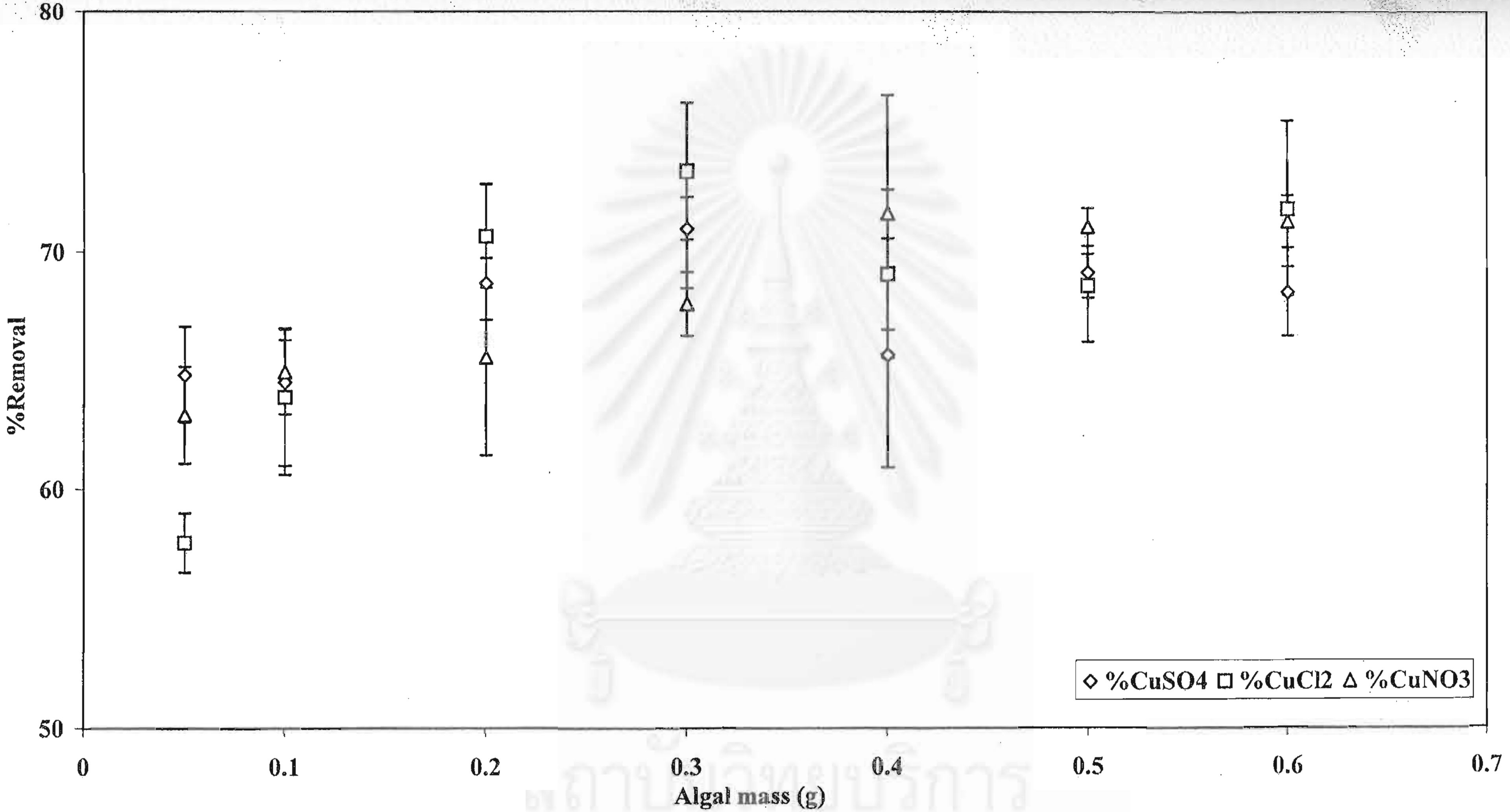

Figure 5.2.1.b Adsorption of CuSO4 , CuCl2 and CuNO3 by *C. lentillifera*
(contact time 30 min, initial conc.= 10 mg Cu/l)

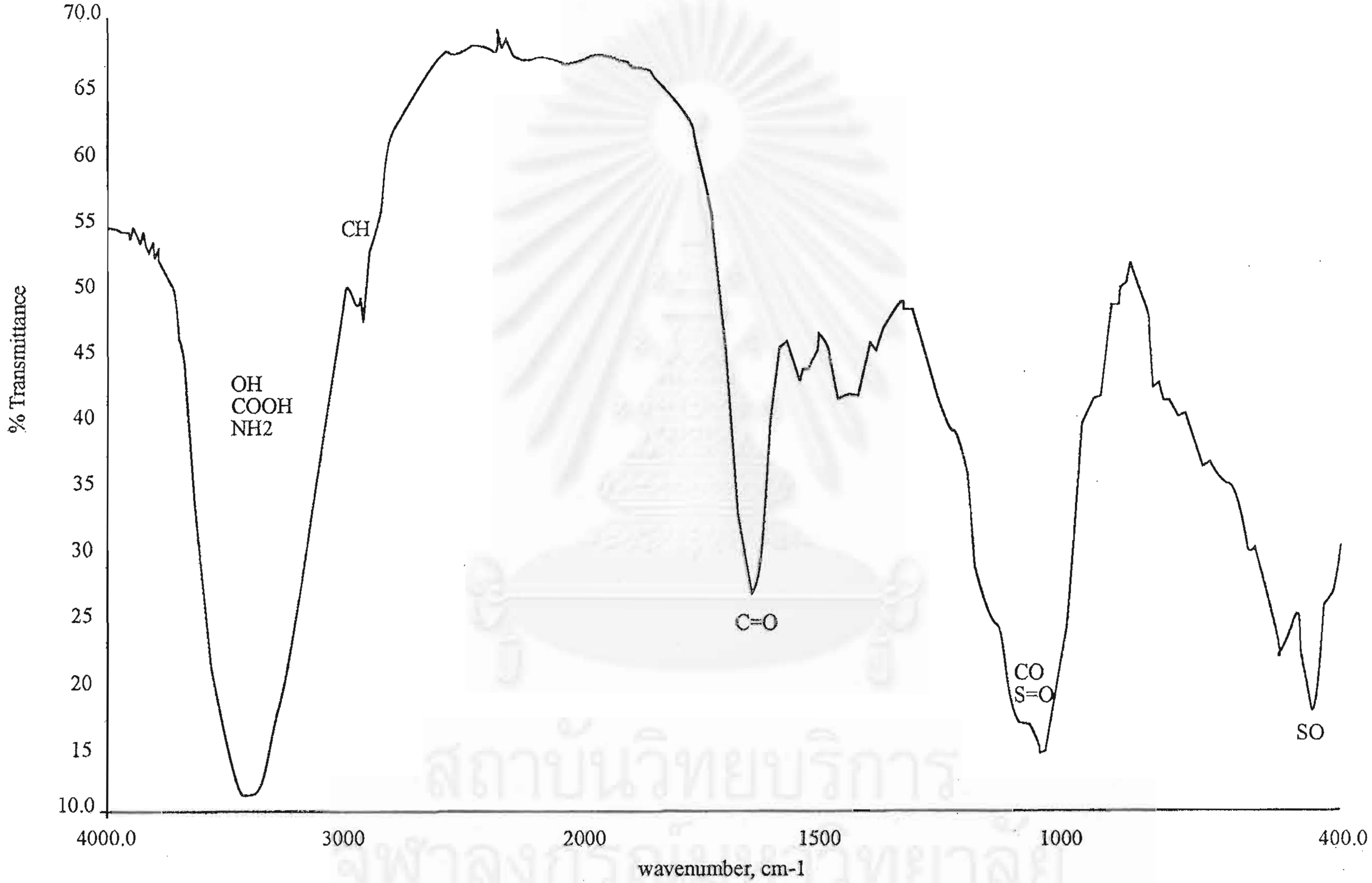

Figure 5.2.2 FTIR results: fresh *C.lentillifera*

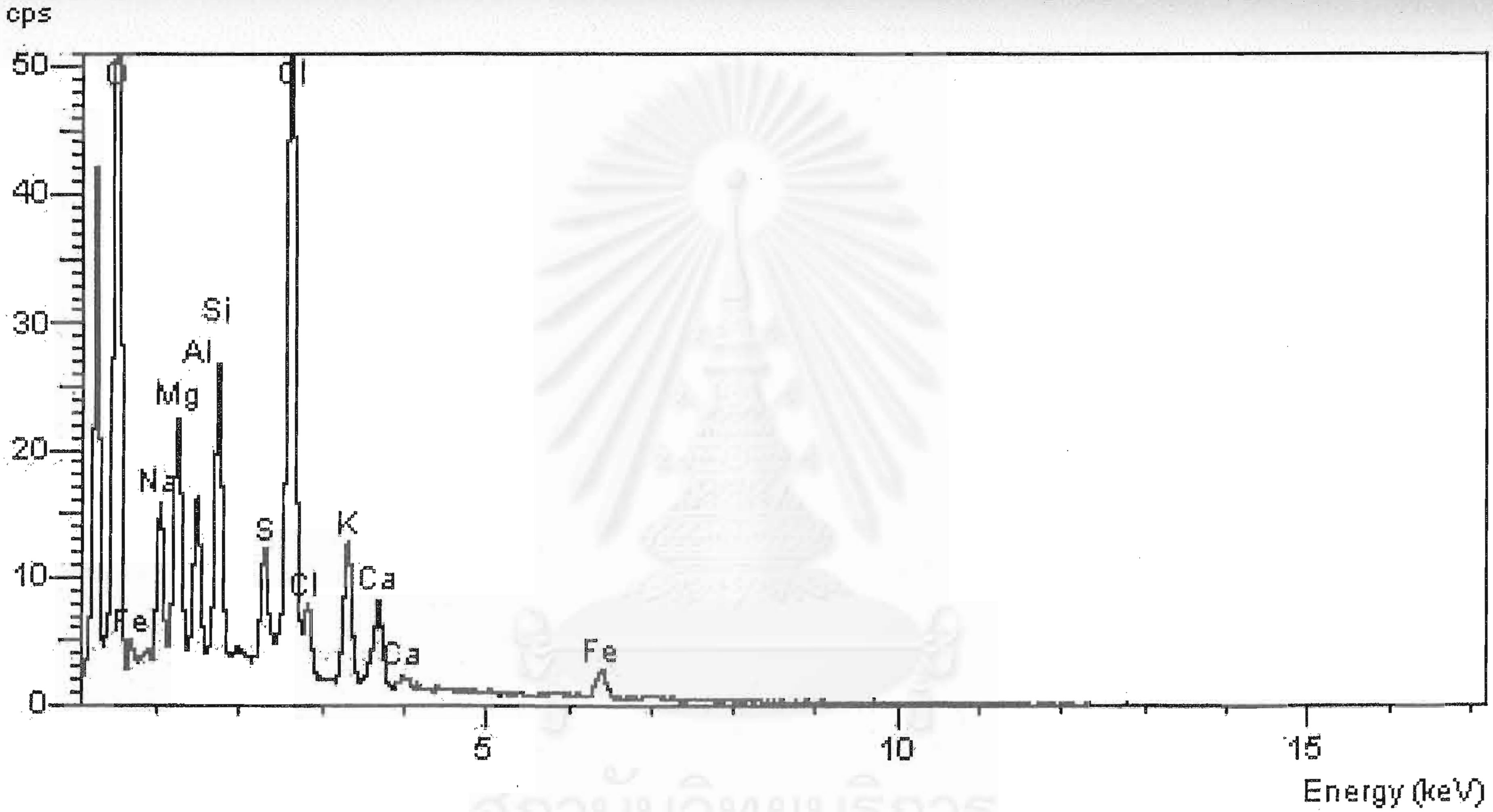

Figure 5.2.3.a: SEM-EDX results: *C. lentillifera* before heavy metal adsorption

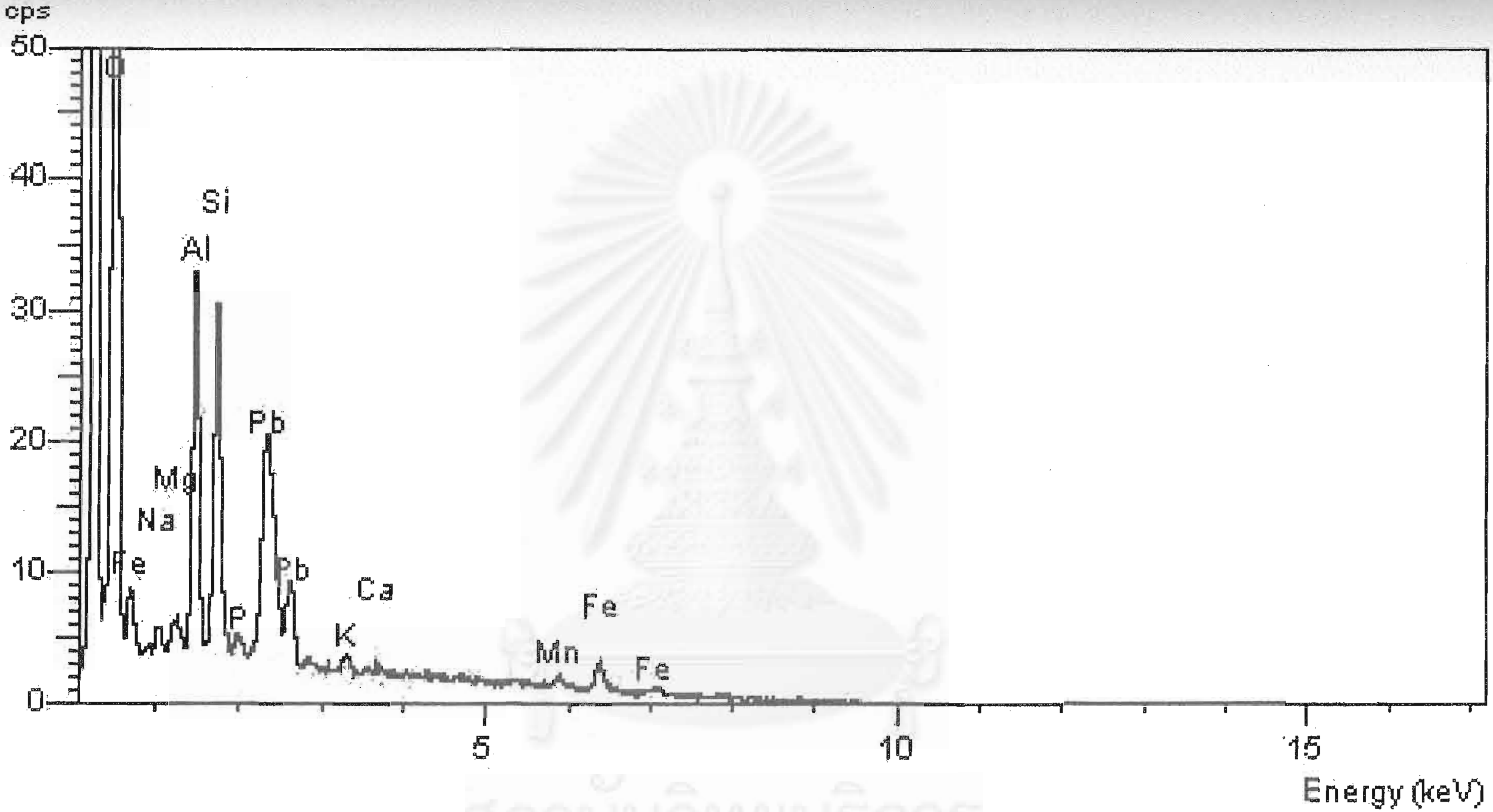

Figure 5.2.3.b: SEM-EDX results: *C. lentillifera* after Pb adsorption

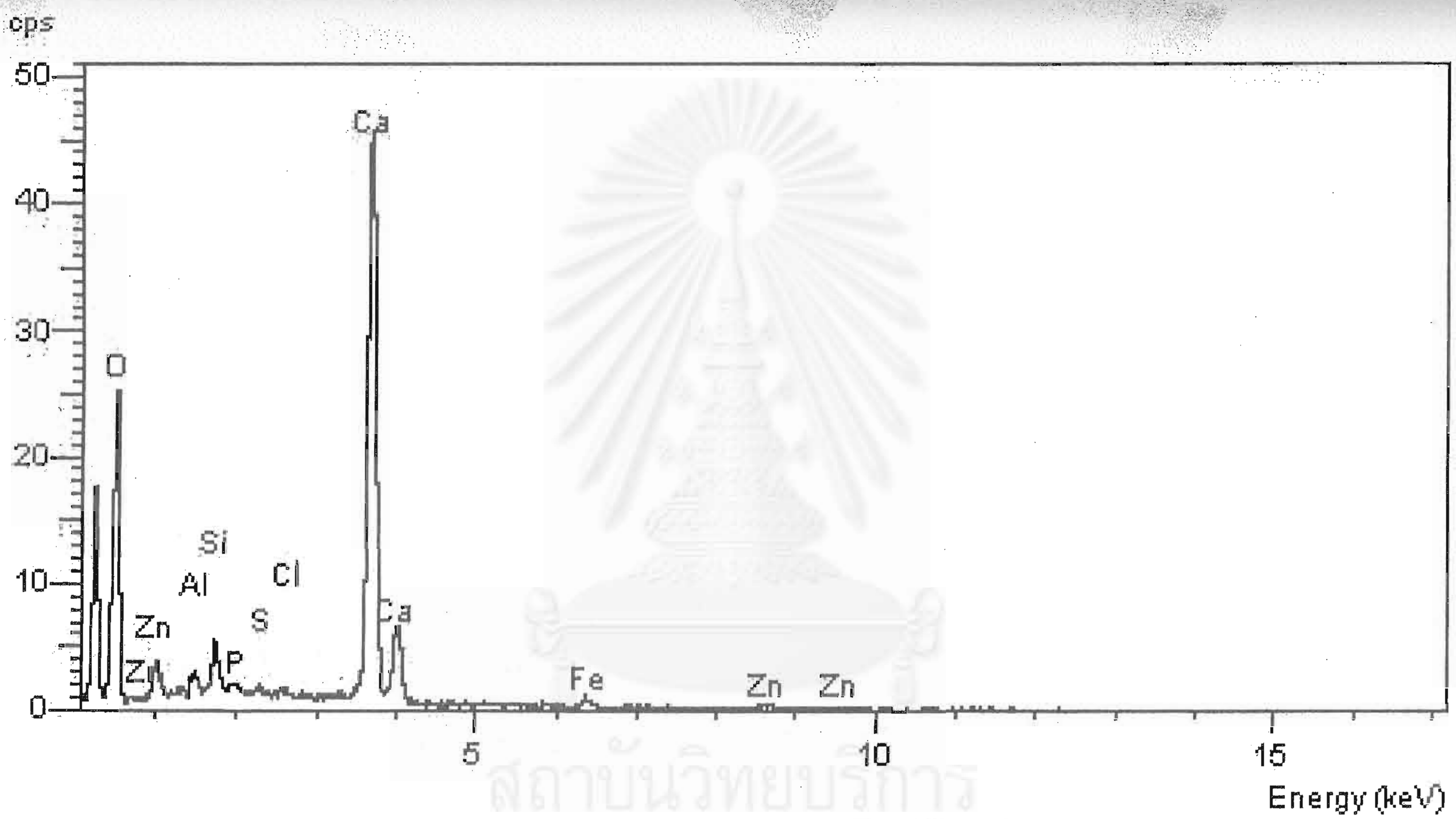

Figure 5.2.3.c: SEM-EDX results: *C. lentillifera* after Zn adsorption

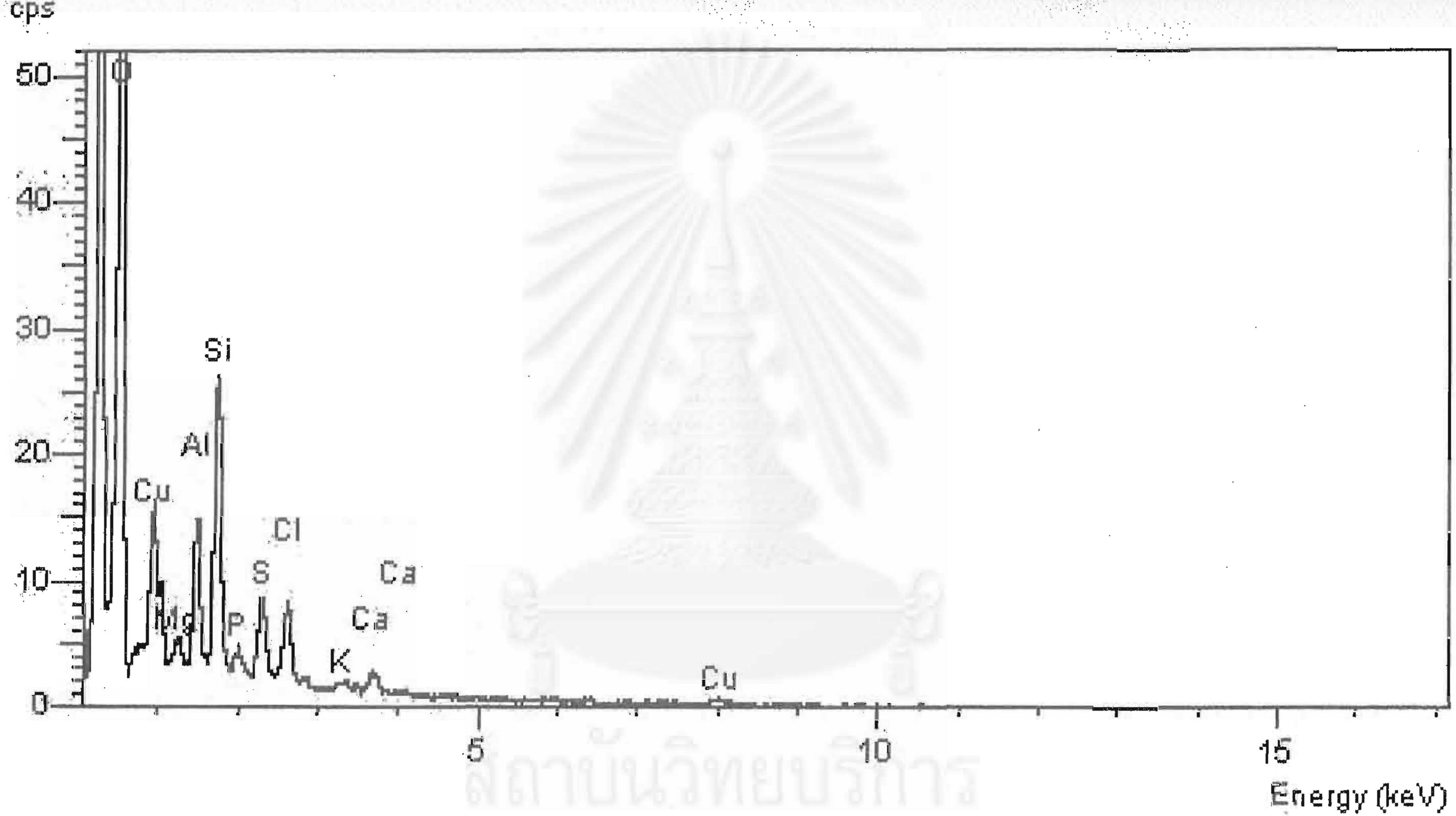

Figure 5.2.3.d: SEM-EDX results: *C. lentillifera* after Cu adsorption

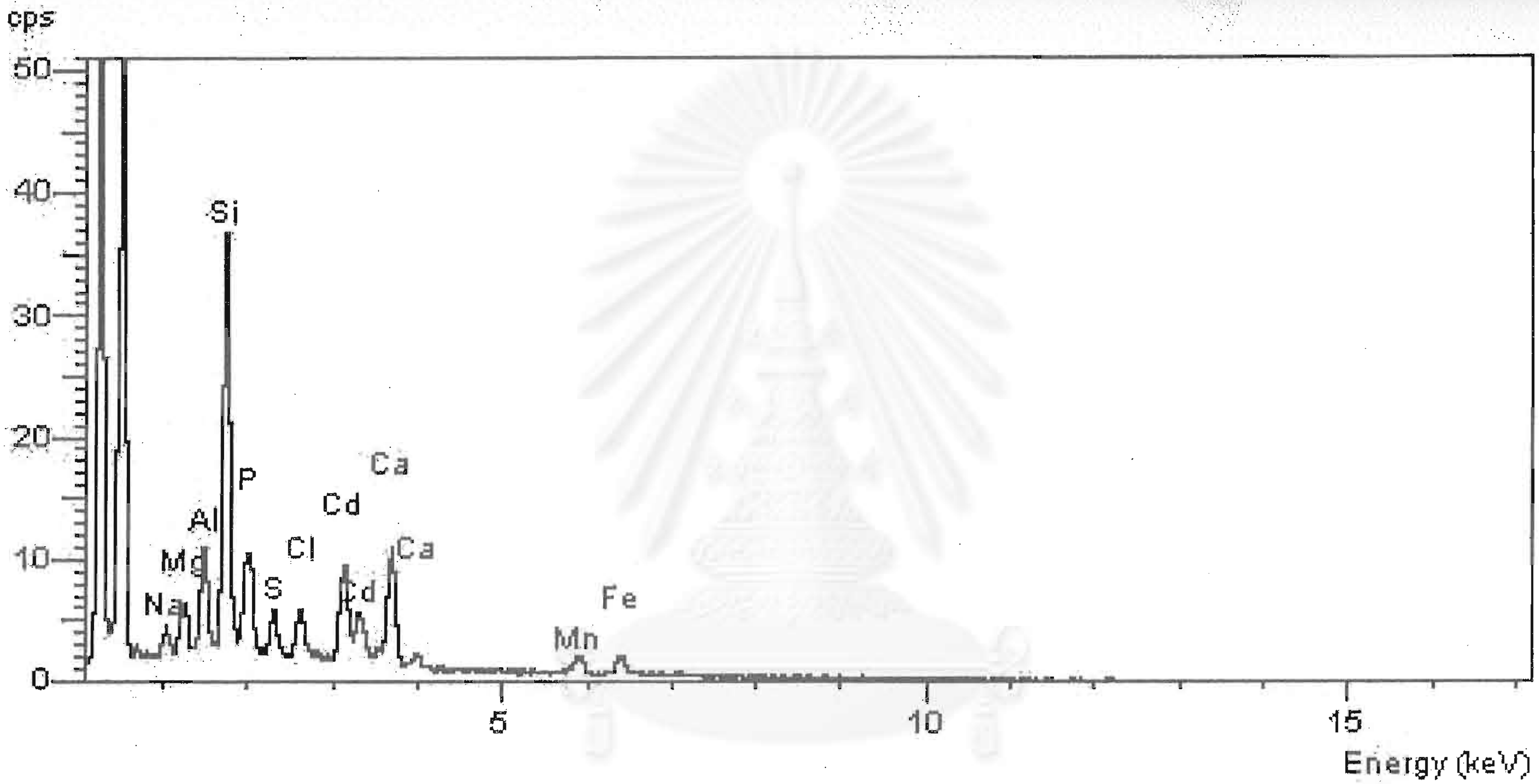

Figure 5.2.3.e: SEM-EDX results: *C. lentillifera* after Cd adsorption

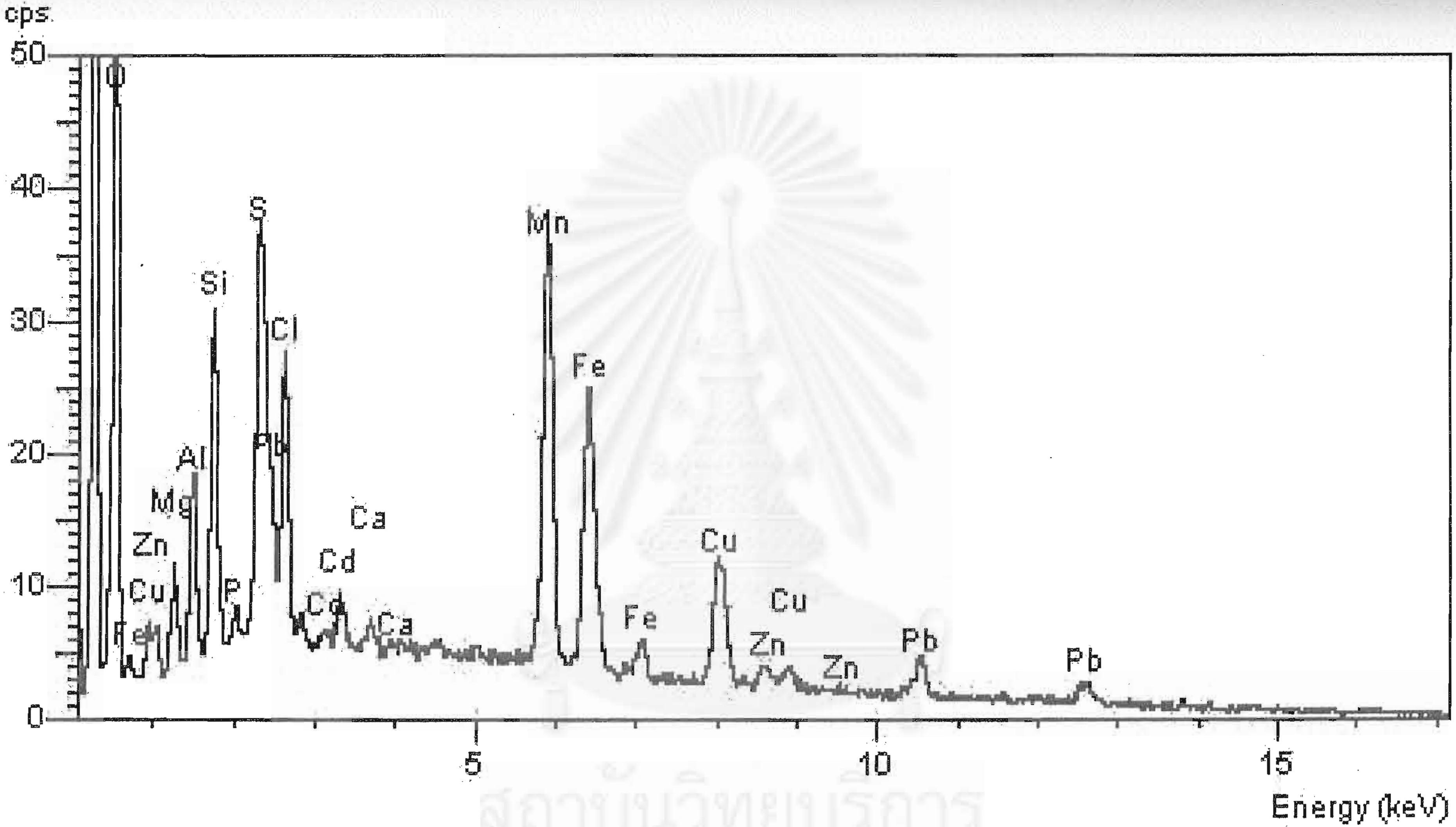

Figure 5.2.3.f: SEM-EDX results: *C. lentillifera* after adsorption of mixture of heavy metals

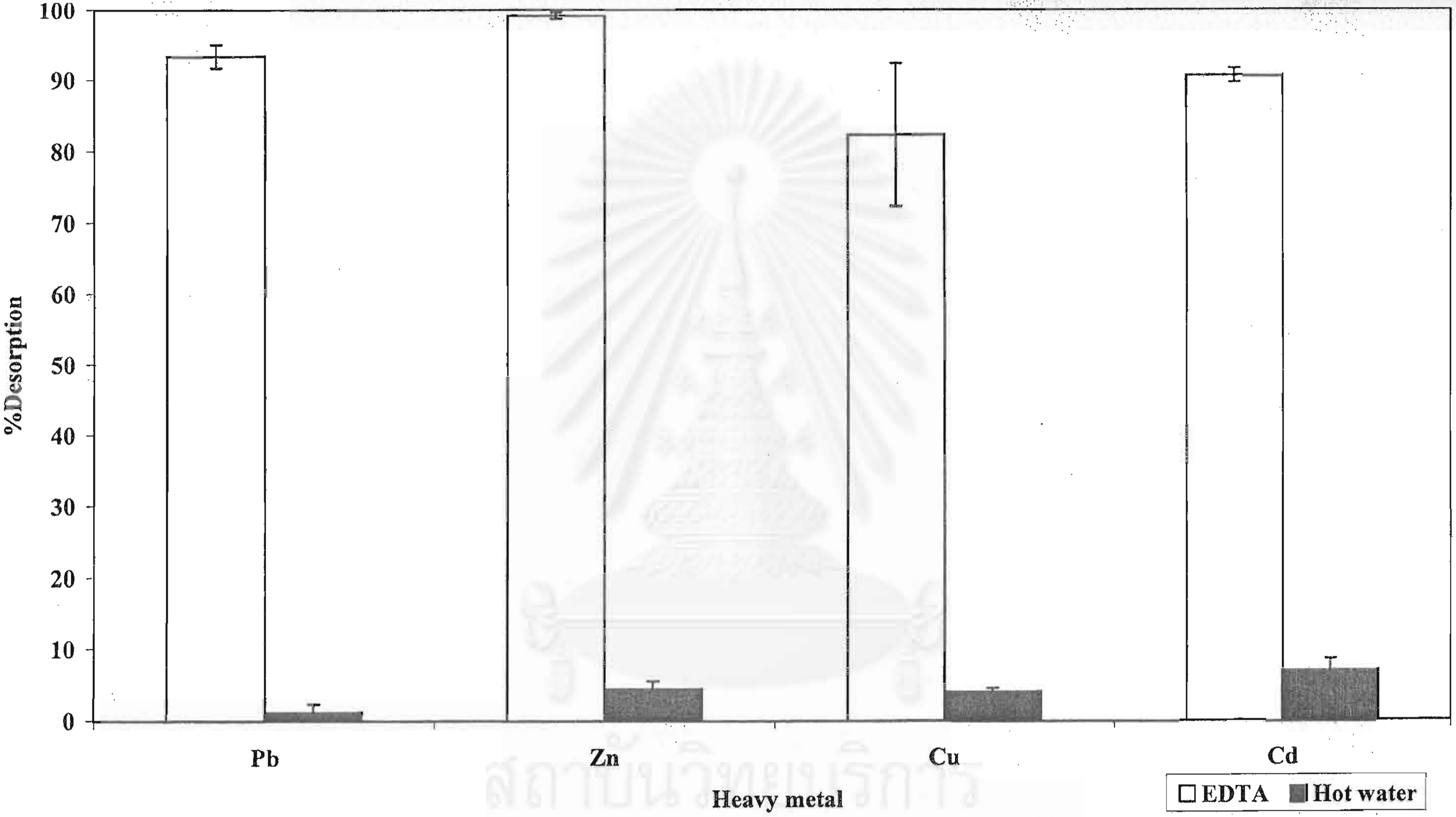

Figure 5.2.4 Desorption of Pb, Zn, Cu and Cd

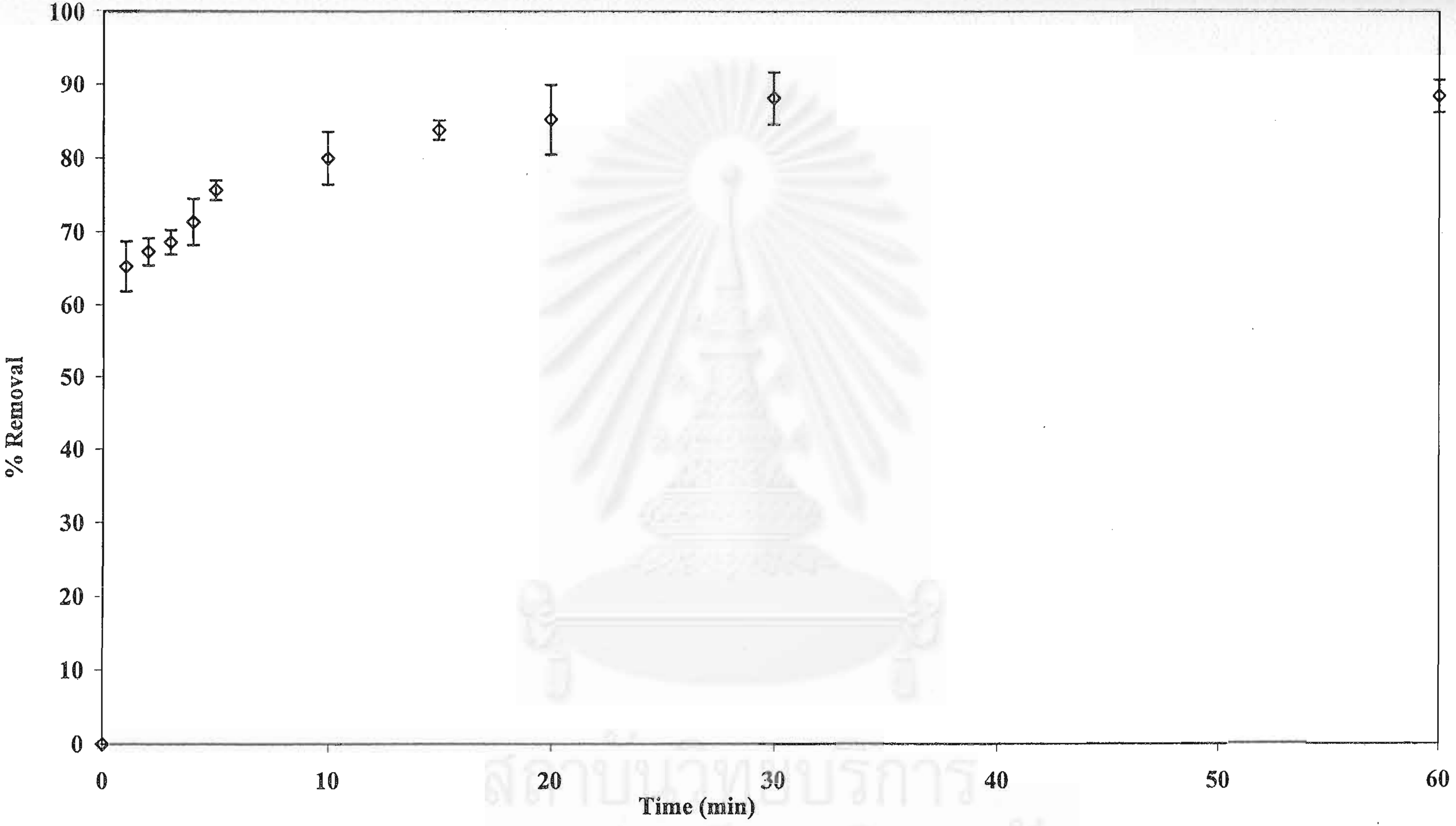

Figure 5.3.1 Effect of contact time on Pb removal

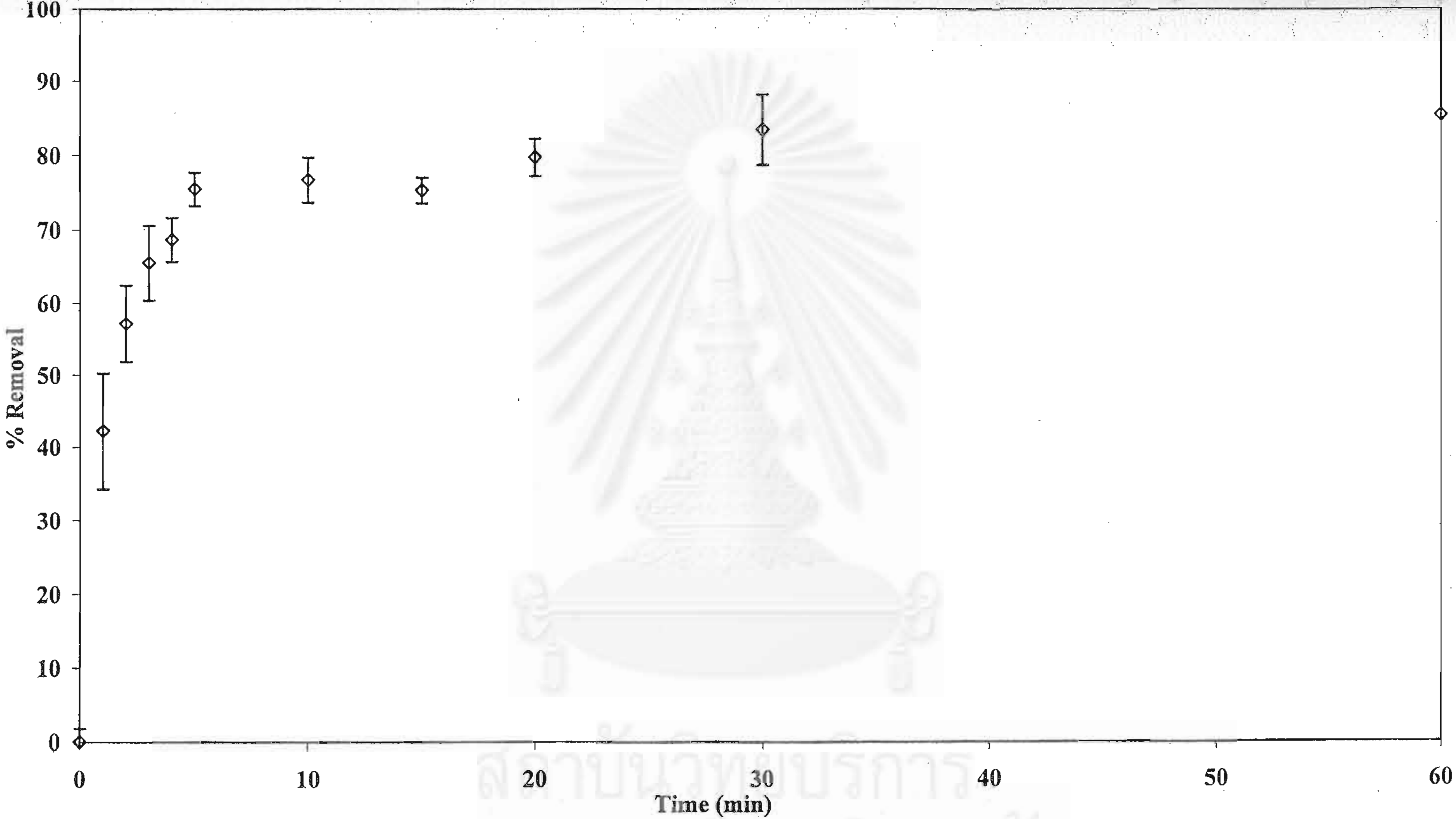

Figure 5.3.2 Effect of contact time on Zn removal

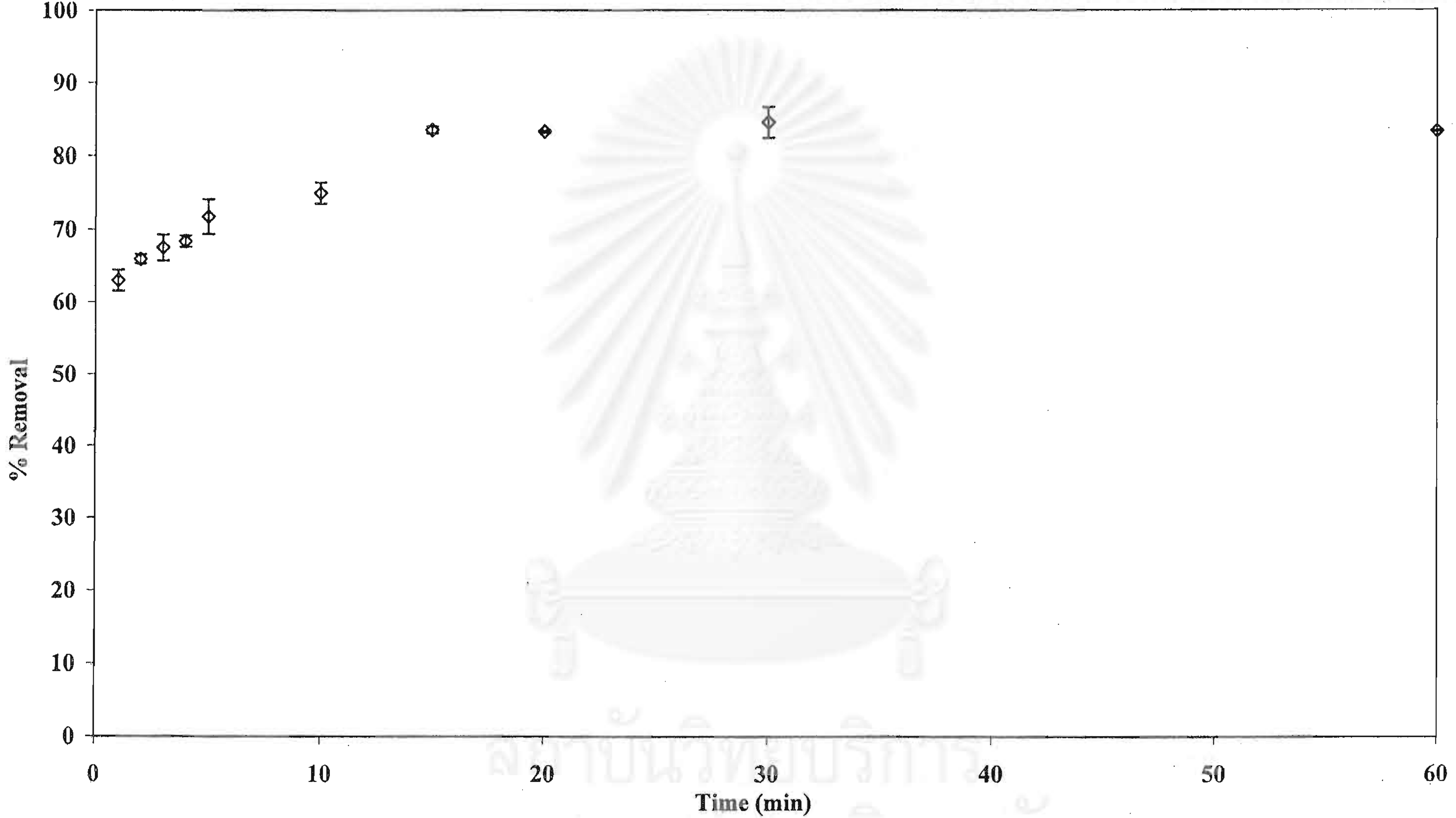

Figure 5.3.3 Effect of contact time on Cu removal

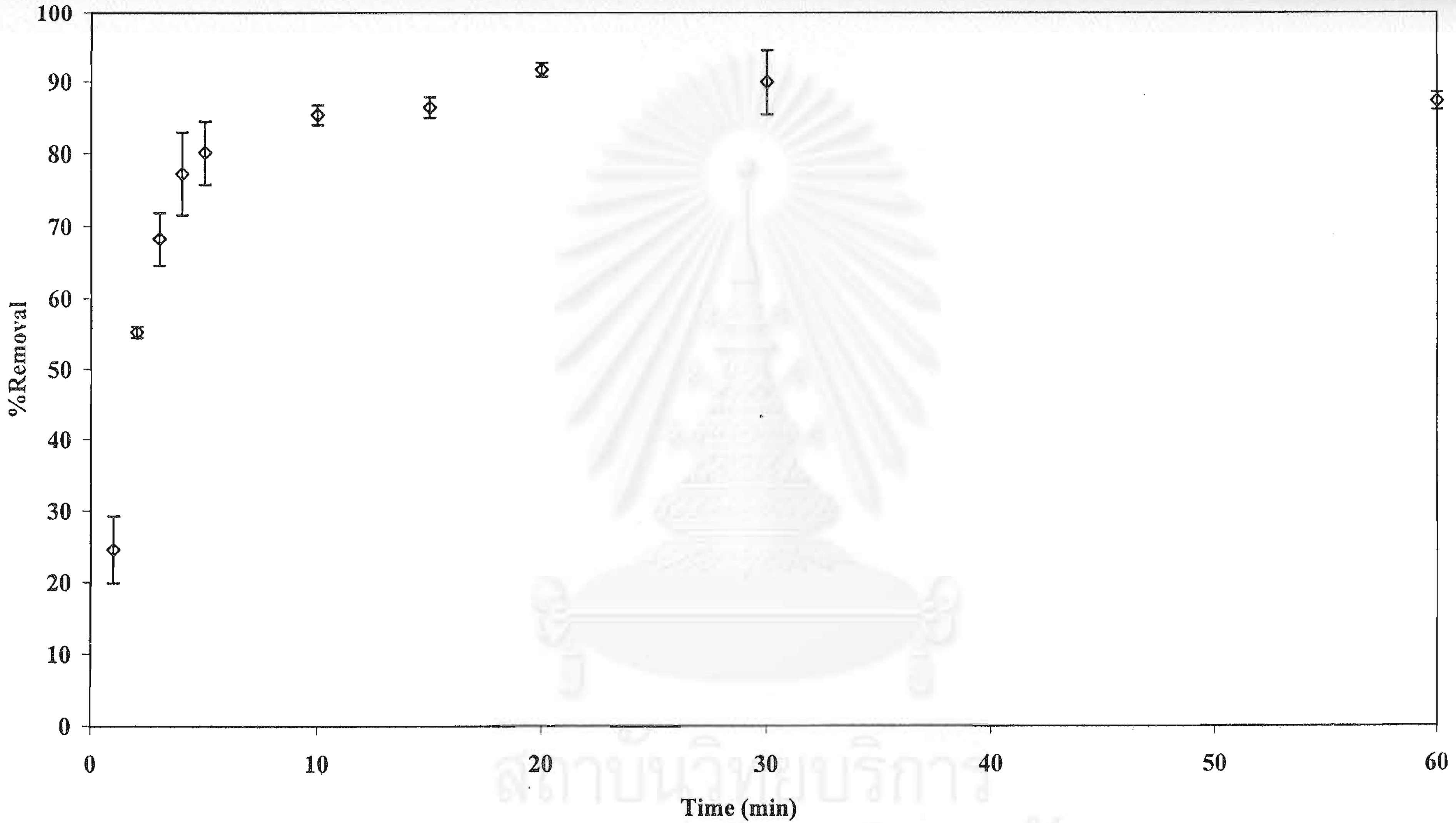

Figure 5.3.4 Effect of contact time on Cd removal

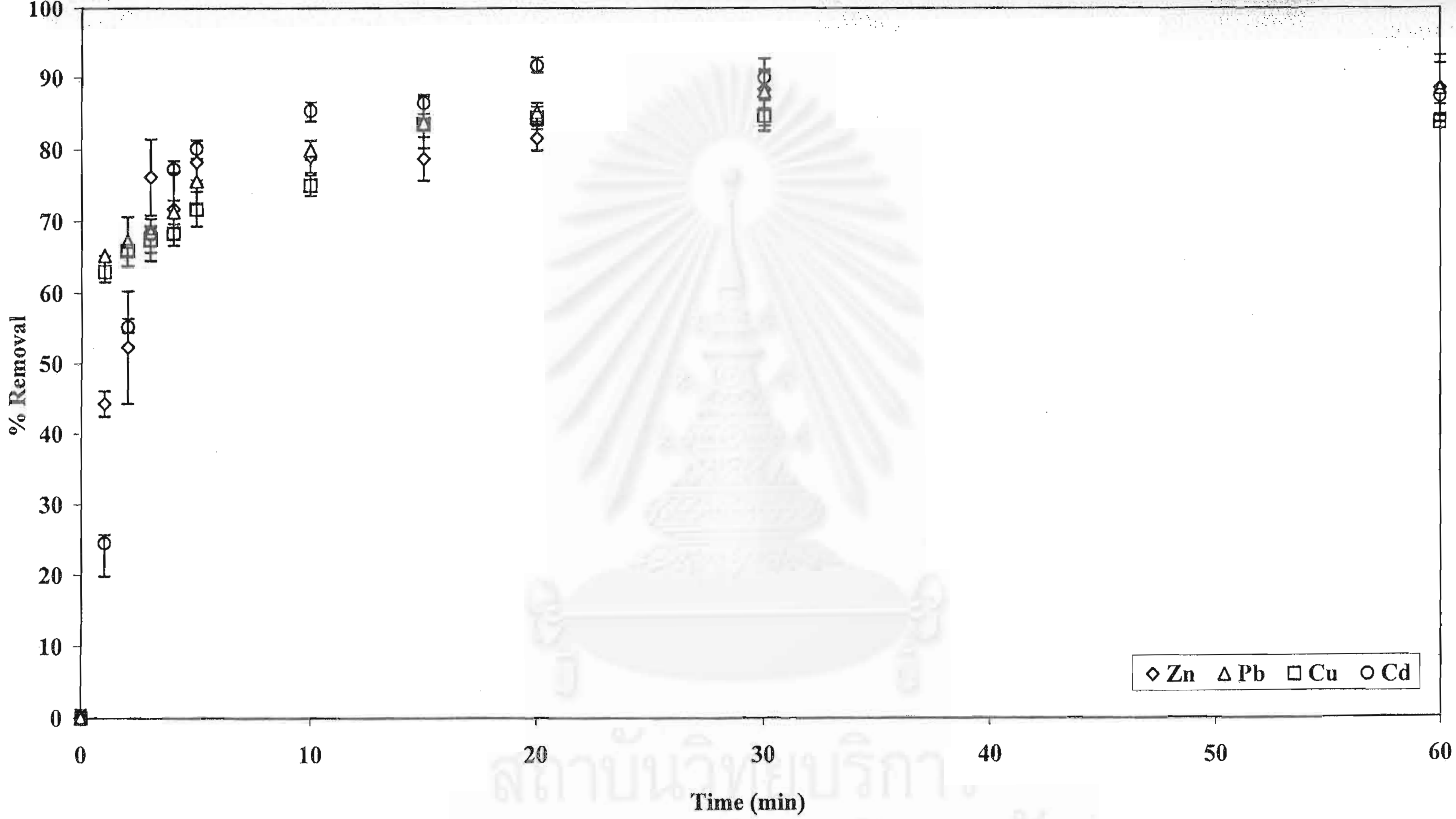

Figure 5.3.5 Comparison between the time-profile of the adsorption of heavy metals

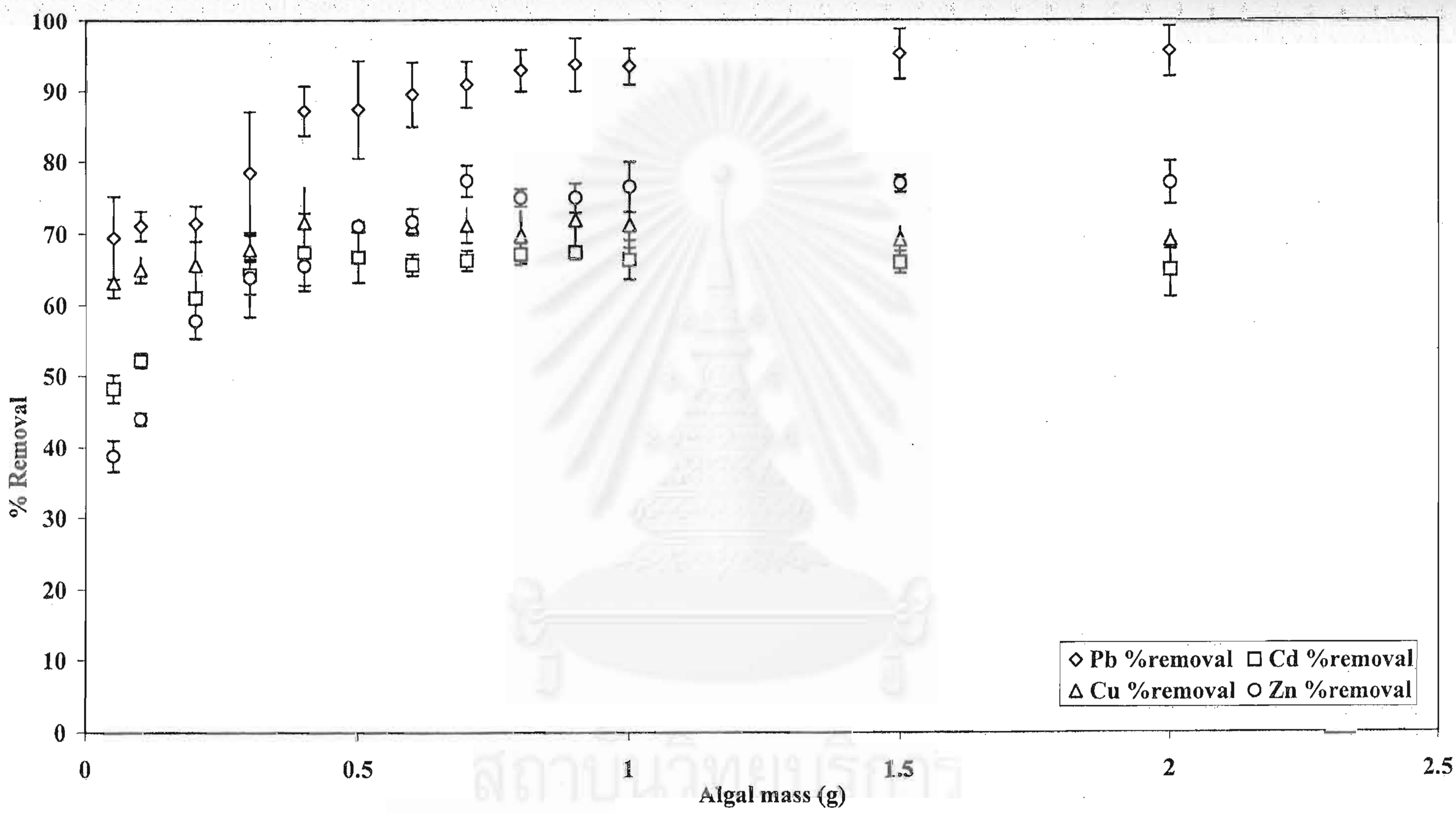

Figure 5.4 Effect of algal mass on removal efficiency of Pb, Cd, Cu and Zn (initial conc.=10mg heavy metal/l, contact time 30 min)

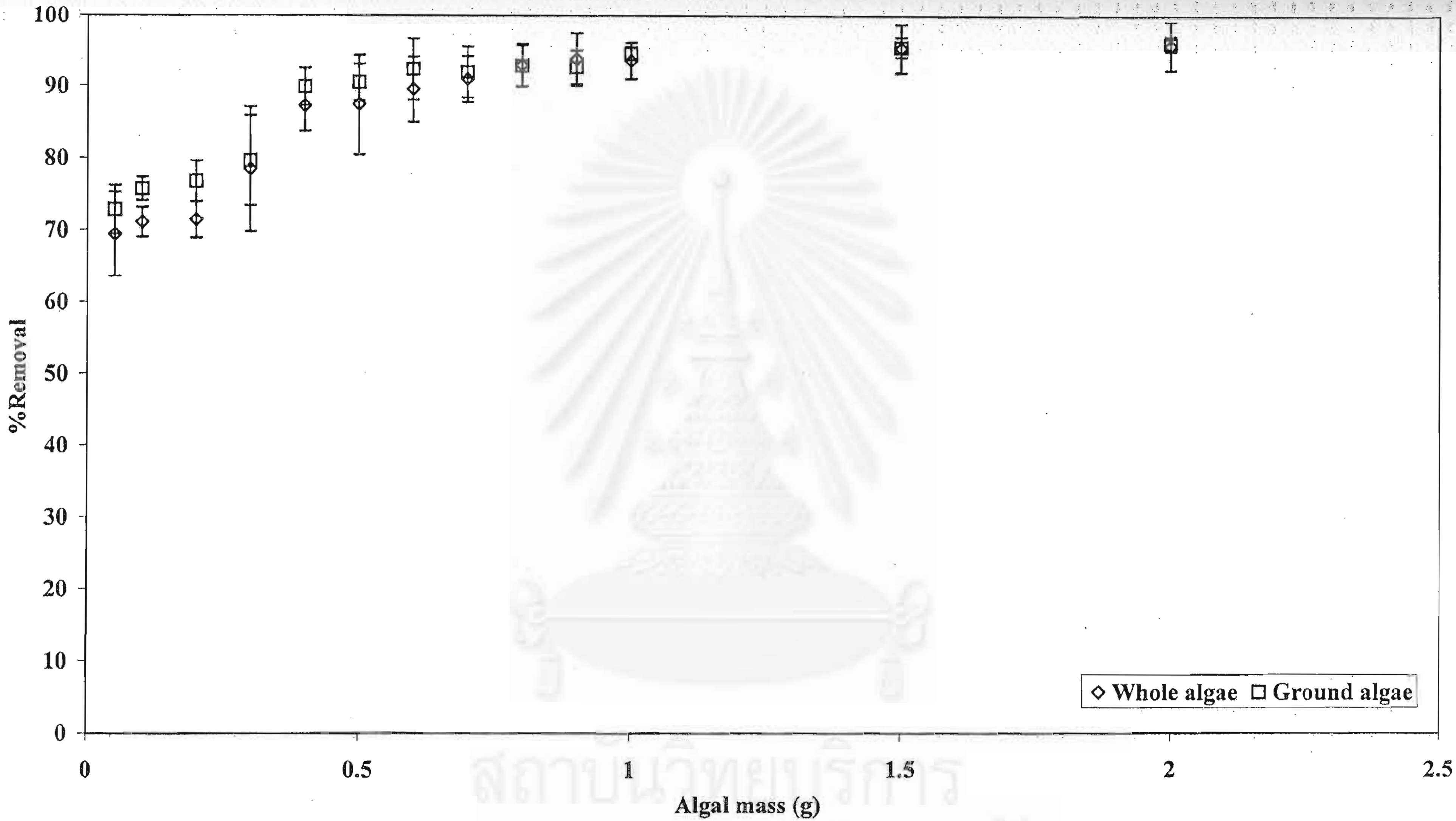

Figure 5.5.1 Effect of particle size on Pb removal

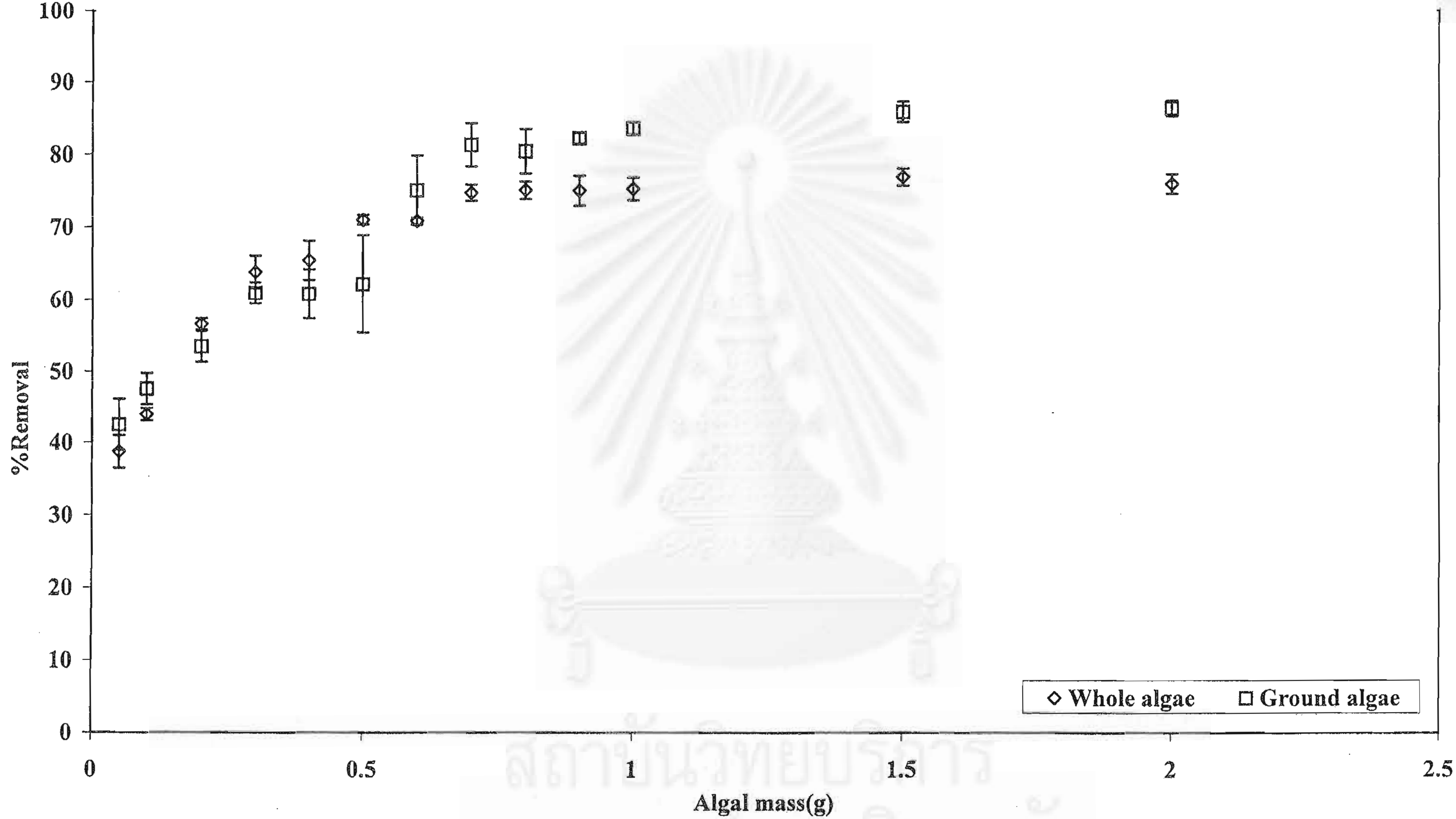

Figure 5.5.2 Effect of particle size on Zn removal

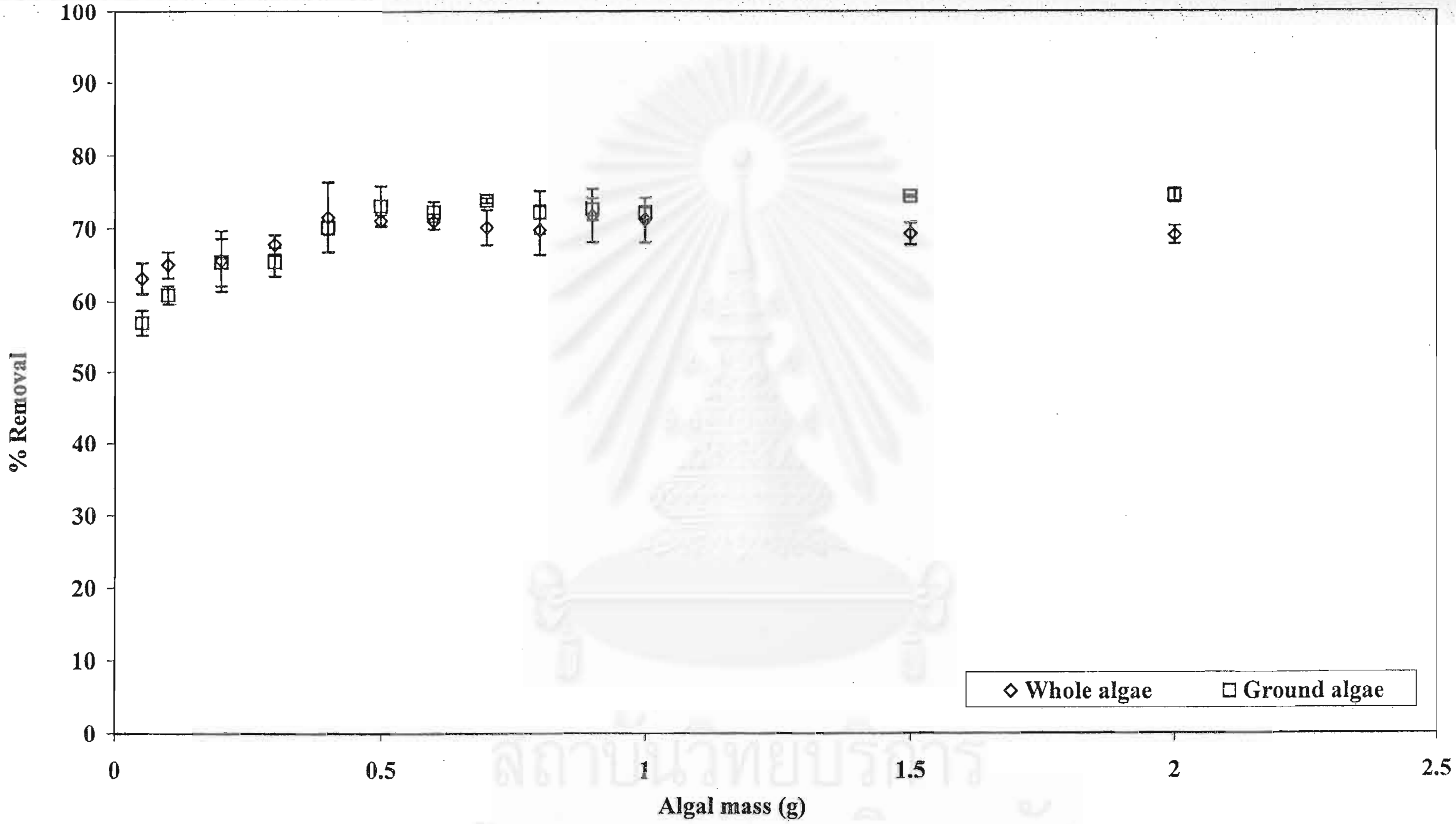

Figure 5.5.3 Effect of particle size on Cu removal

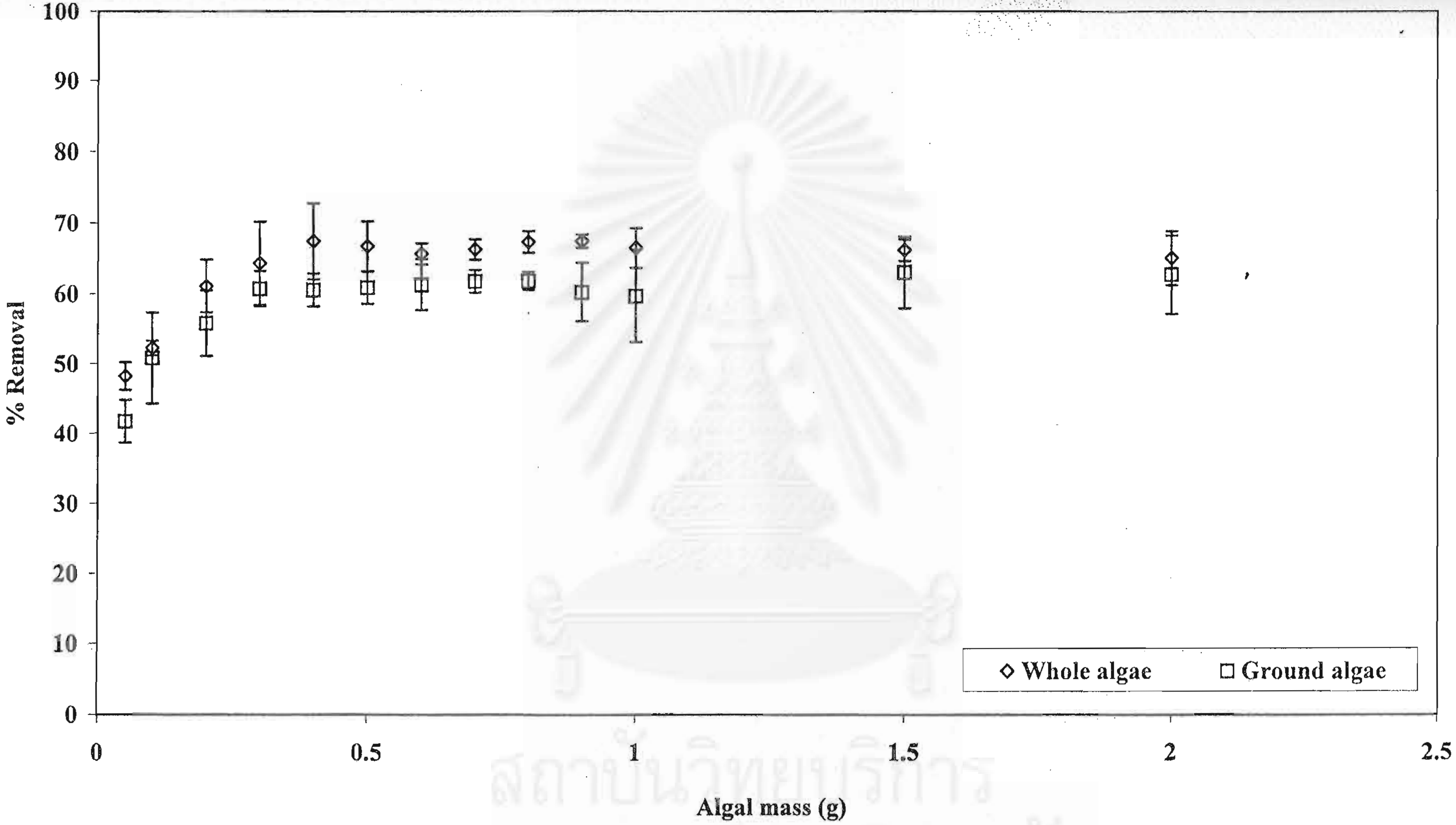

Figure 5.5.4 Effect of particle size on Cd removal

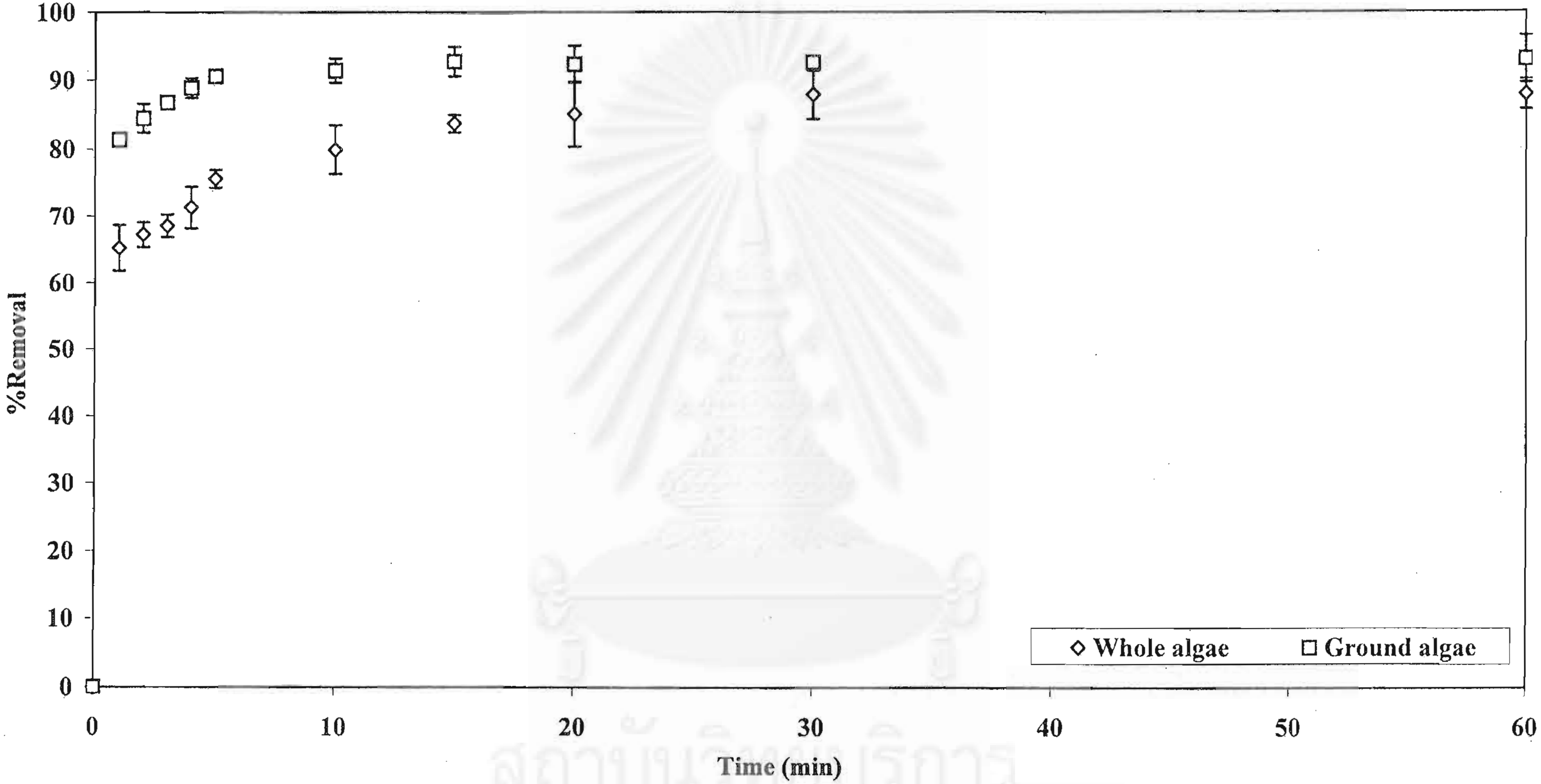

Figure 5.5.5 Effect of contact time on Pb removal

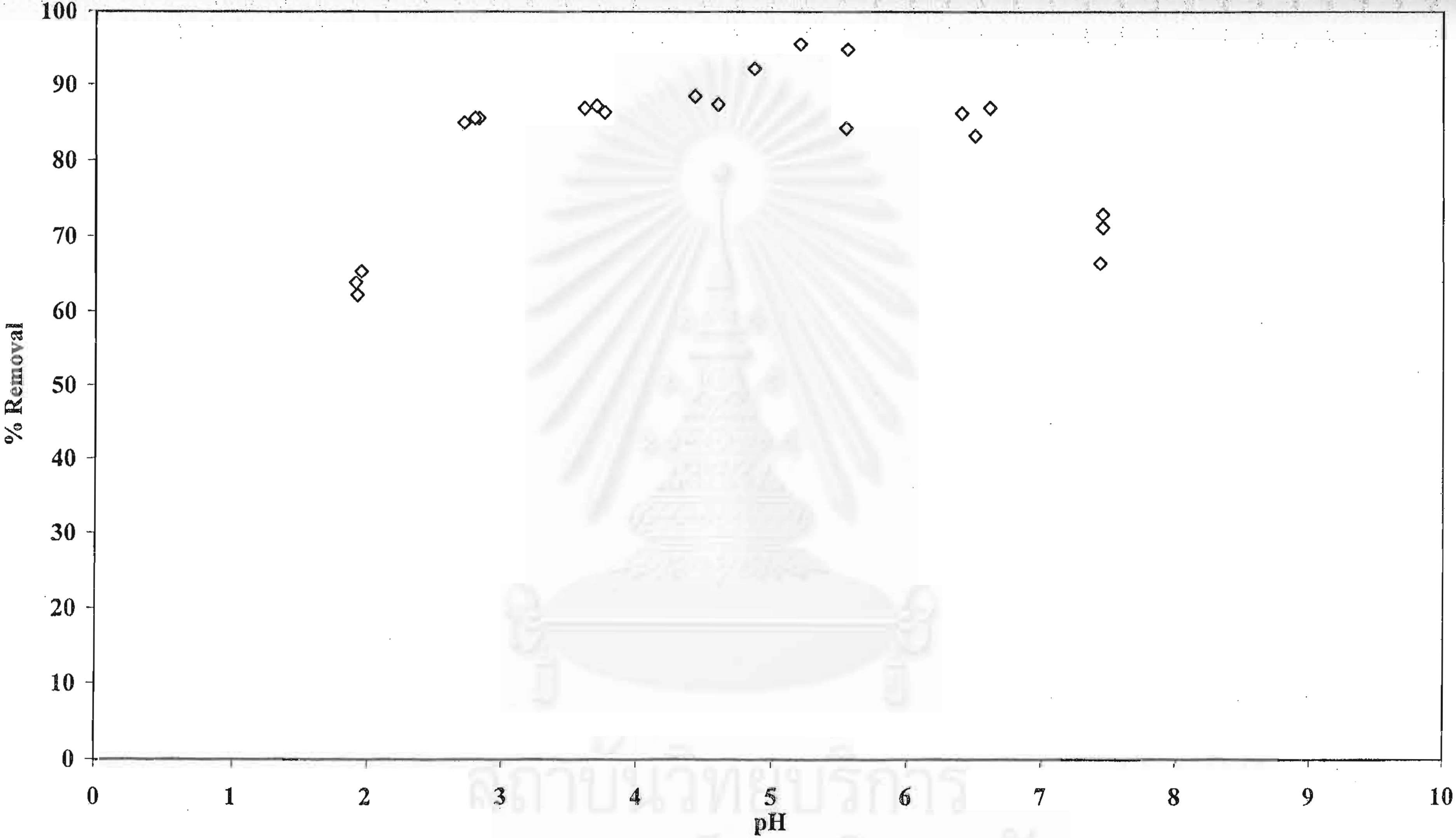

Figure 5.6.1 Effect of pH on Pb removal

Figure 5.6.2 Effect of pH on Zn removal

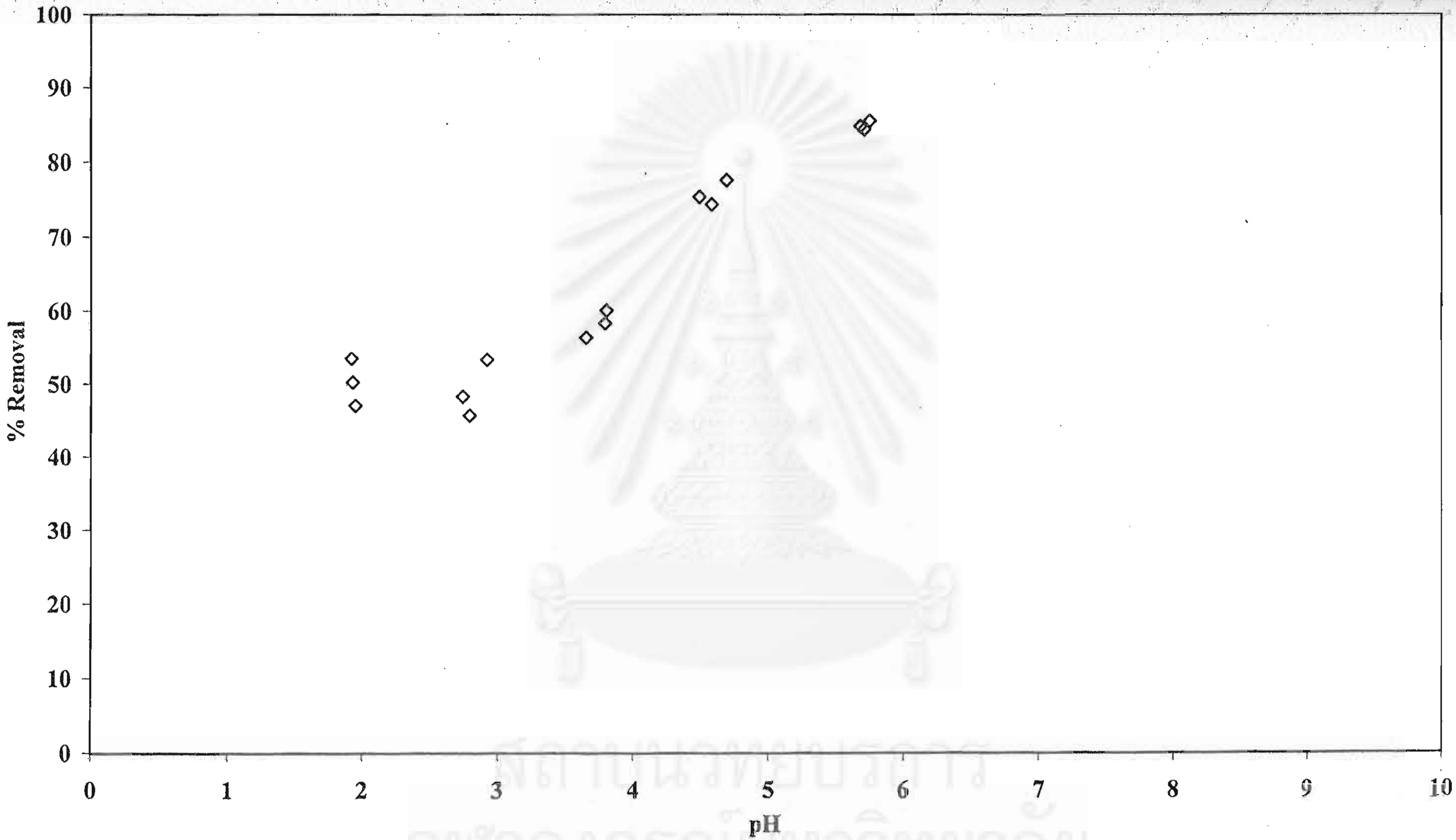

Figure 5.6.3 Effect of pH on Cu removal

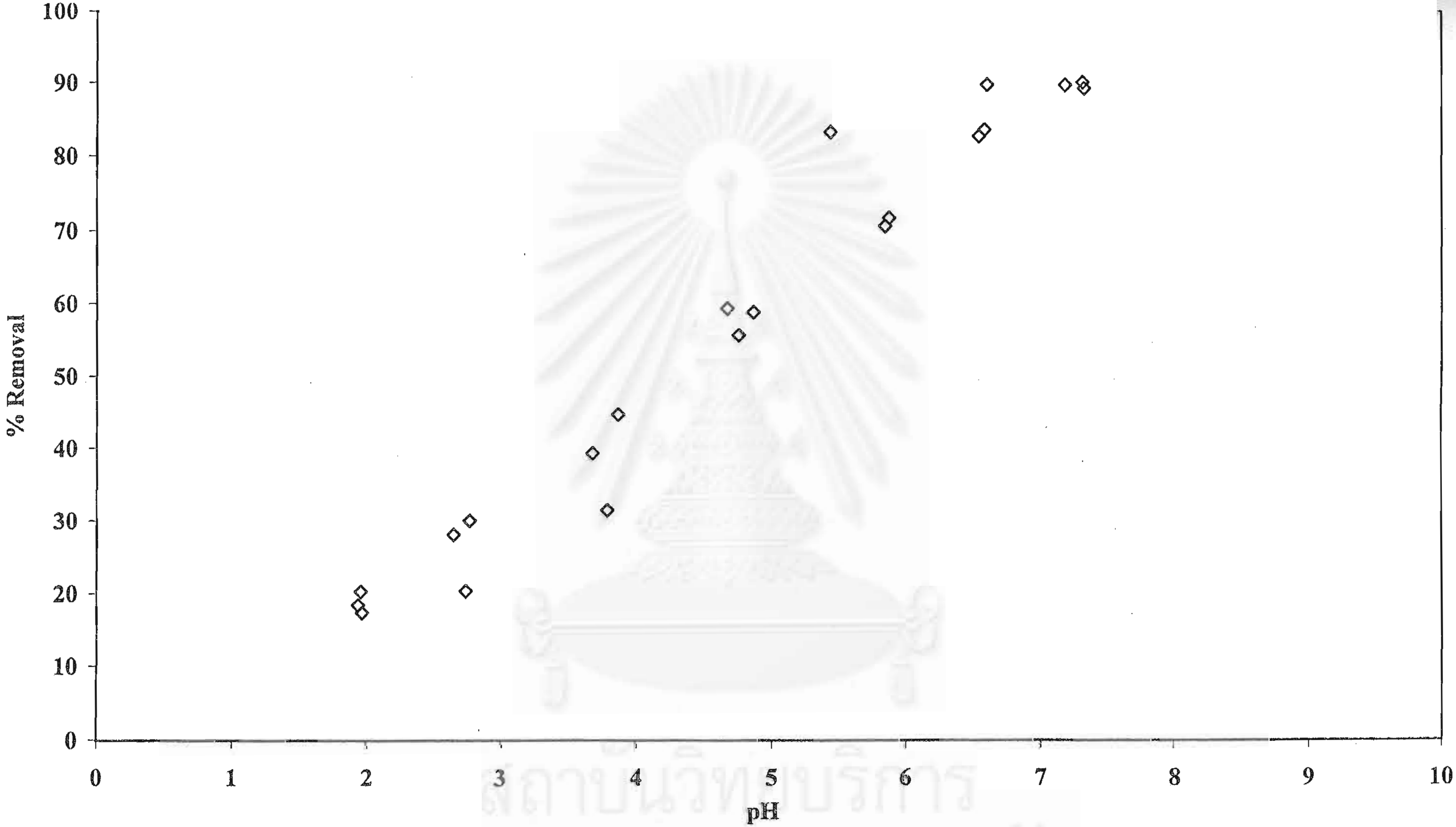

Figure 5.6.4 Effect of pH on Cd removal

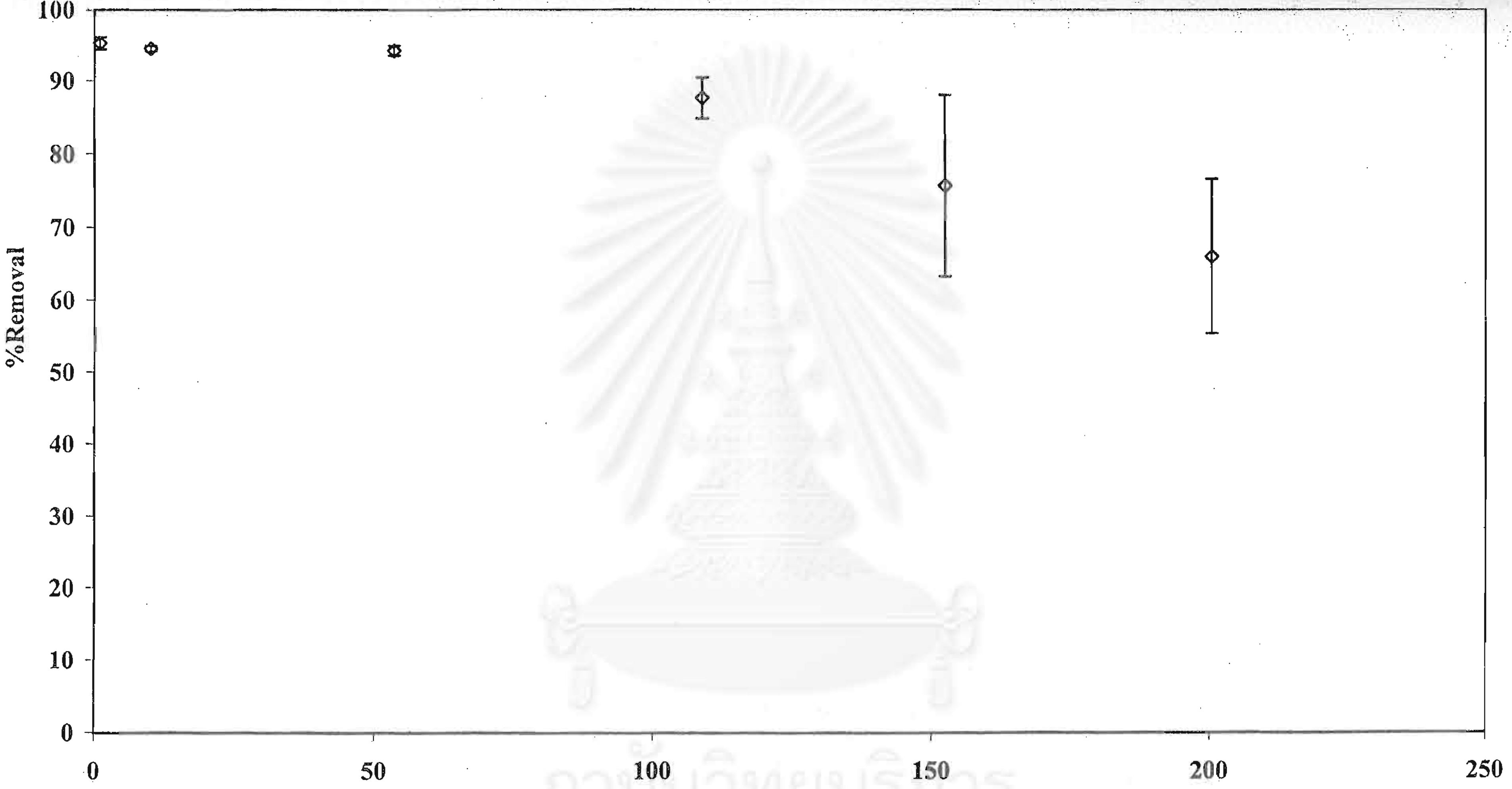

Figure 5.7.1.a Adsorption isotherm for Pb
(algal mass = 0.5 g, initial conc.= 10 mg Pb/l, contact time 30 min, pH =5)

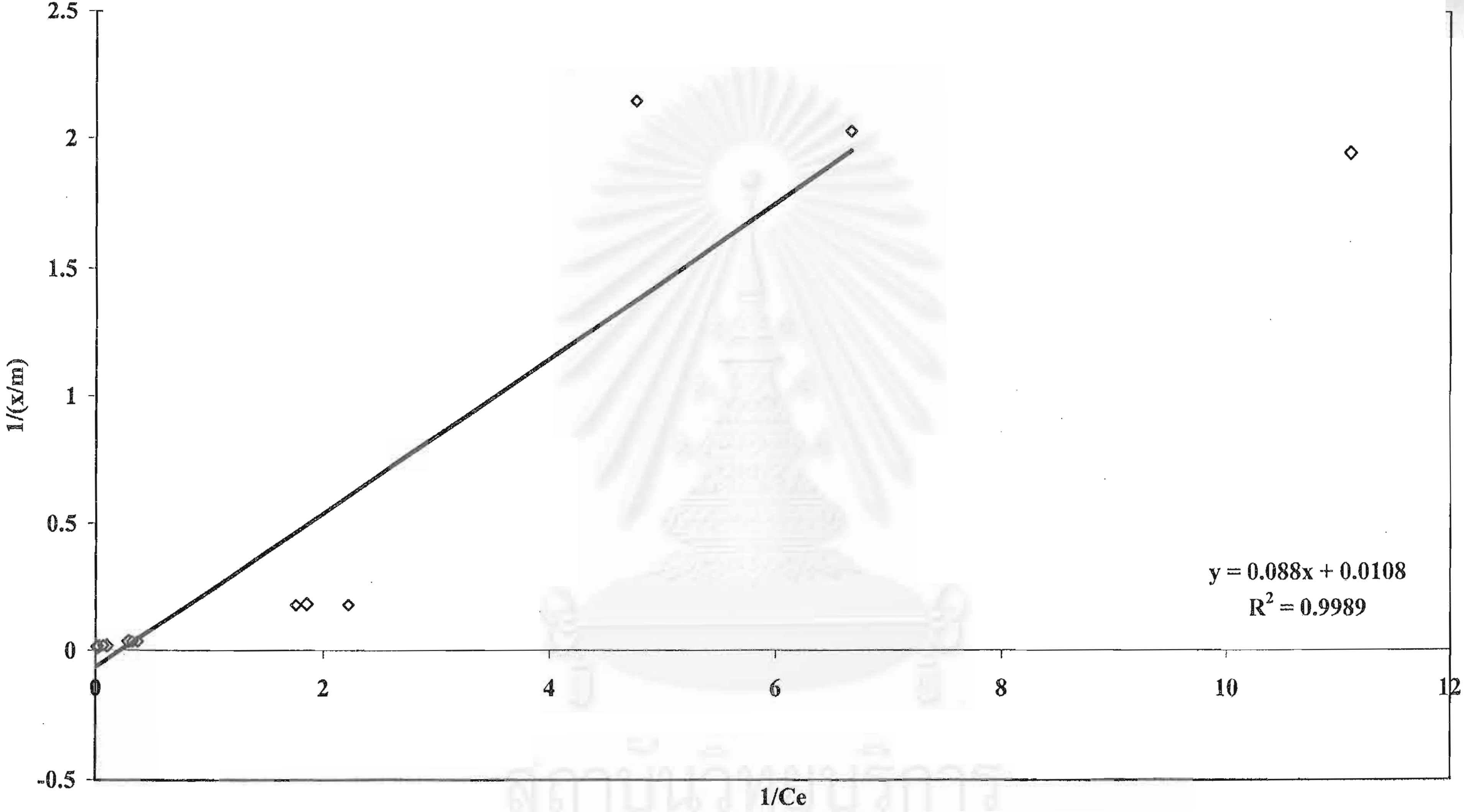

Figure 5.7.1.b Langmuir isotherm of Pb
(algal mass = 0.5 g, initial conc.= 10 mg Pb/l, contact time 30 min, pH =5)

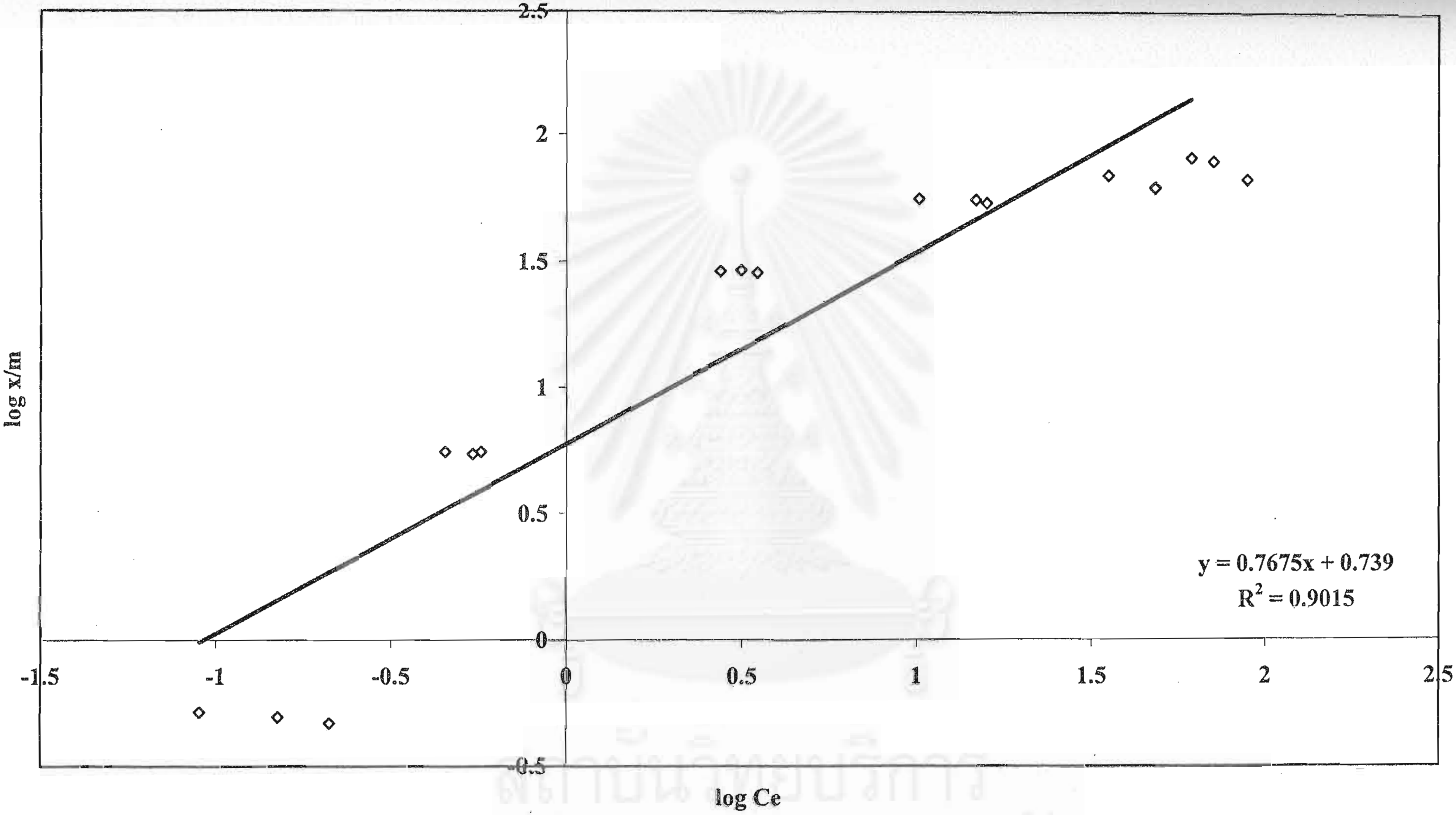

Figure 5.7.1.c Freundlich isotherm of Pb
(algal mass = 0.5 g, initial conc.= 10 mg Pb/l, contact time 30 min, pH =5)

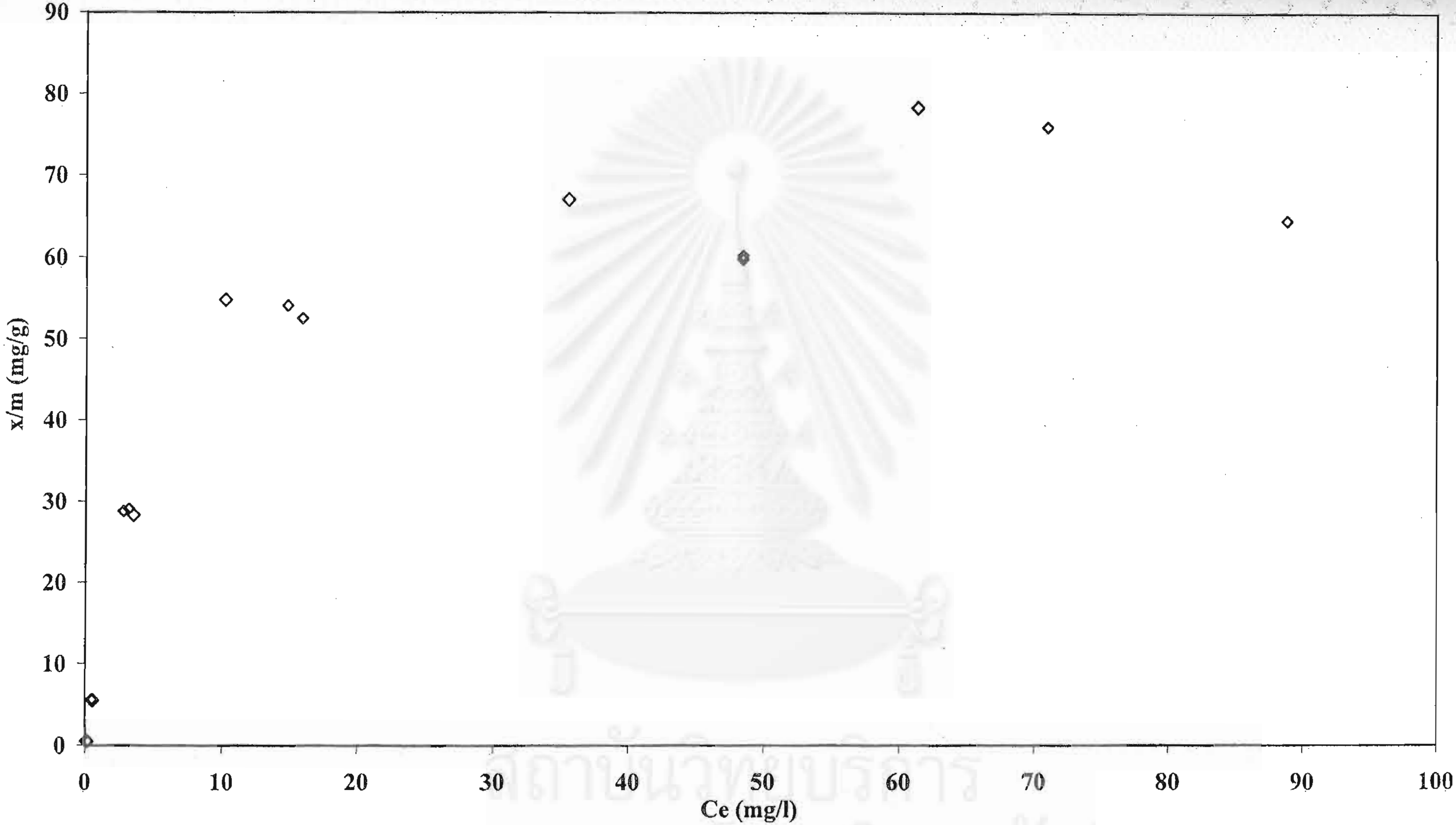

Figure 5.7.1.d Isotherm of Pb
(algal mass = 0.5 g, initial conc.= 10 mg Pb/l, contact time 30 min, pH =5)

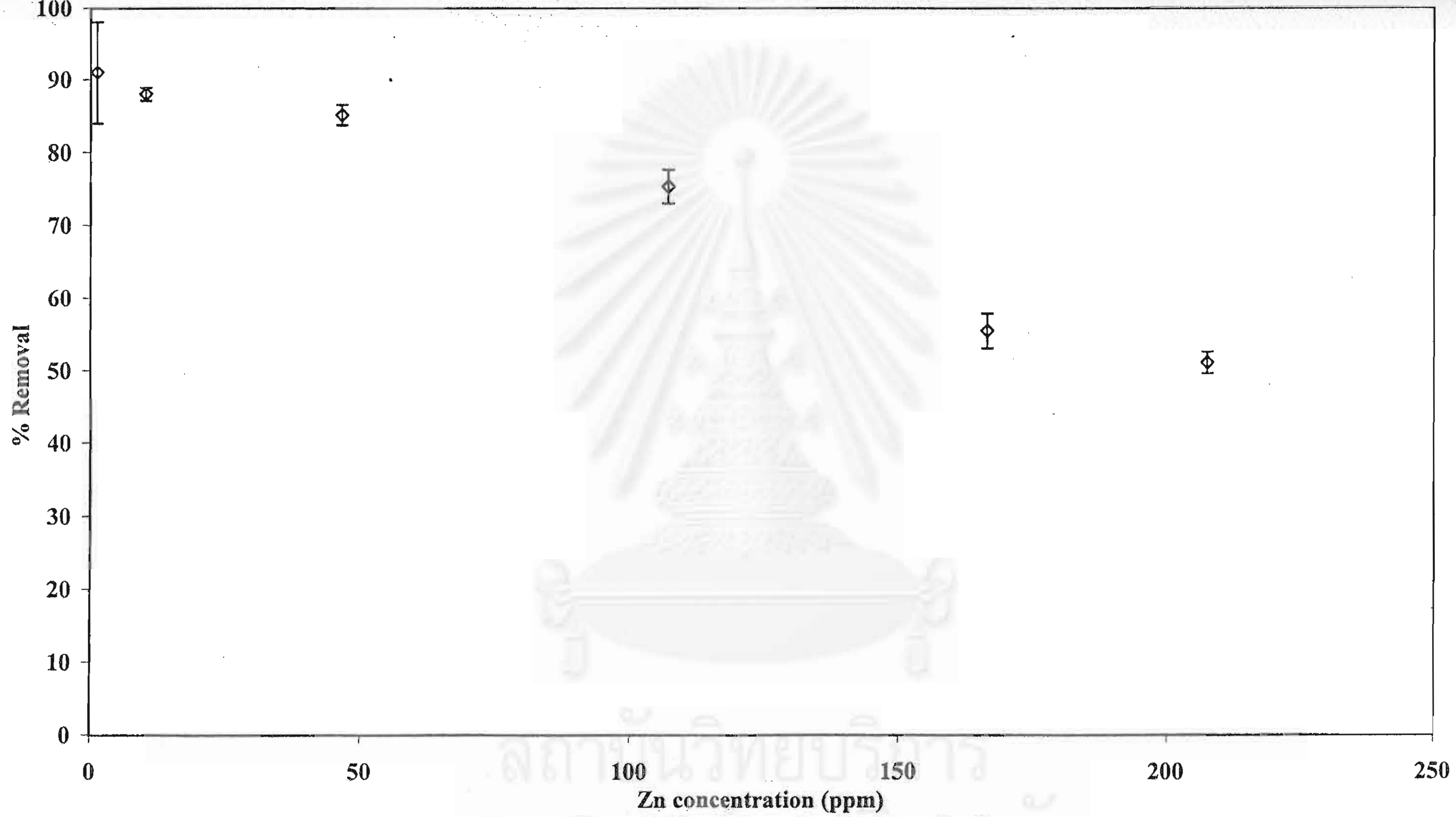

Figure 5.7.2.a Adsorption isotherm for Zn
(algal mass = 0.5 g, initial conc.= 10 mg Zn/l, contact time 30 min, pH =5)

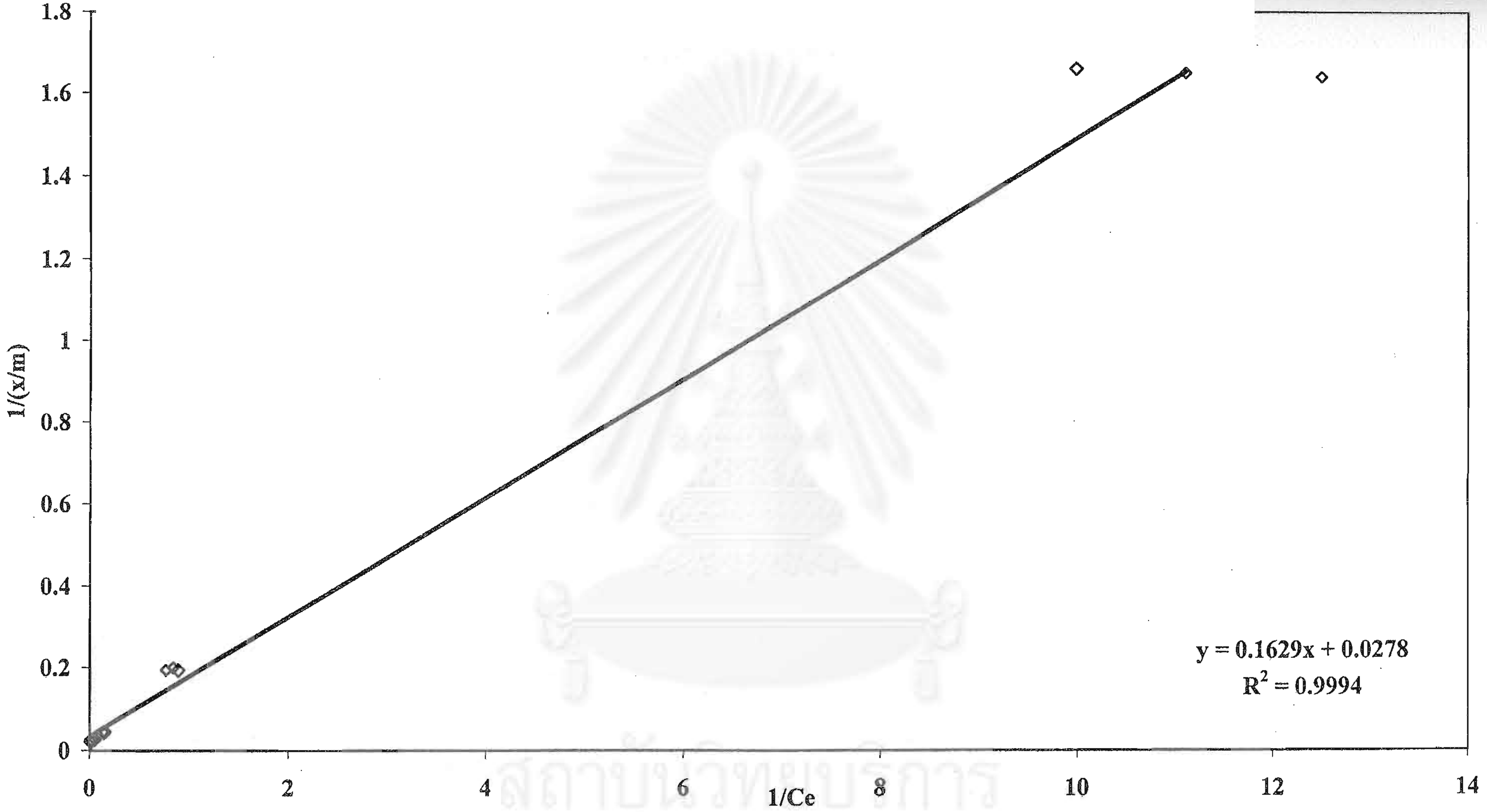

Figure 5.7.2.b Langmuir isotherm of Zn
(algal mass = 0.5 g, initial conc.= 10 mg Zn/l, contact time 30 min, pH =5)

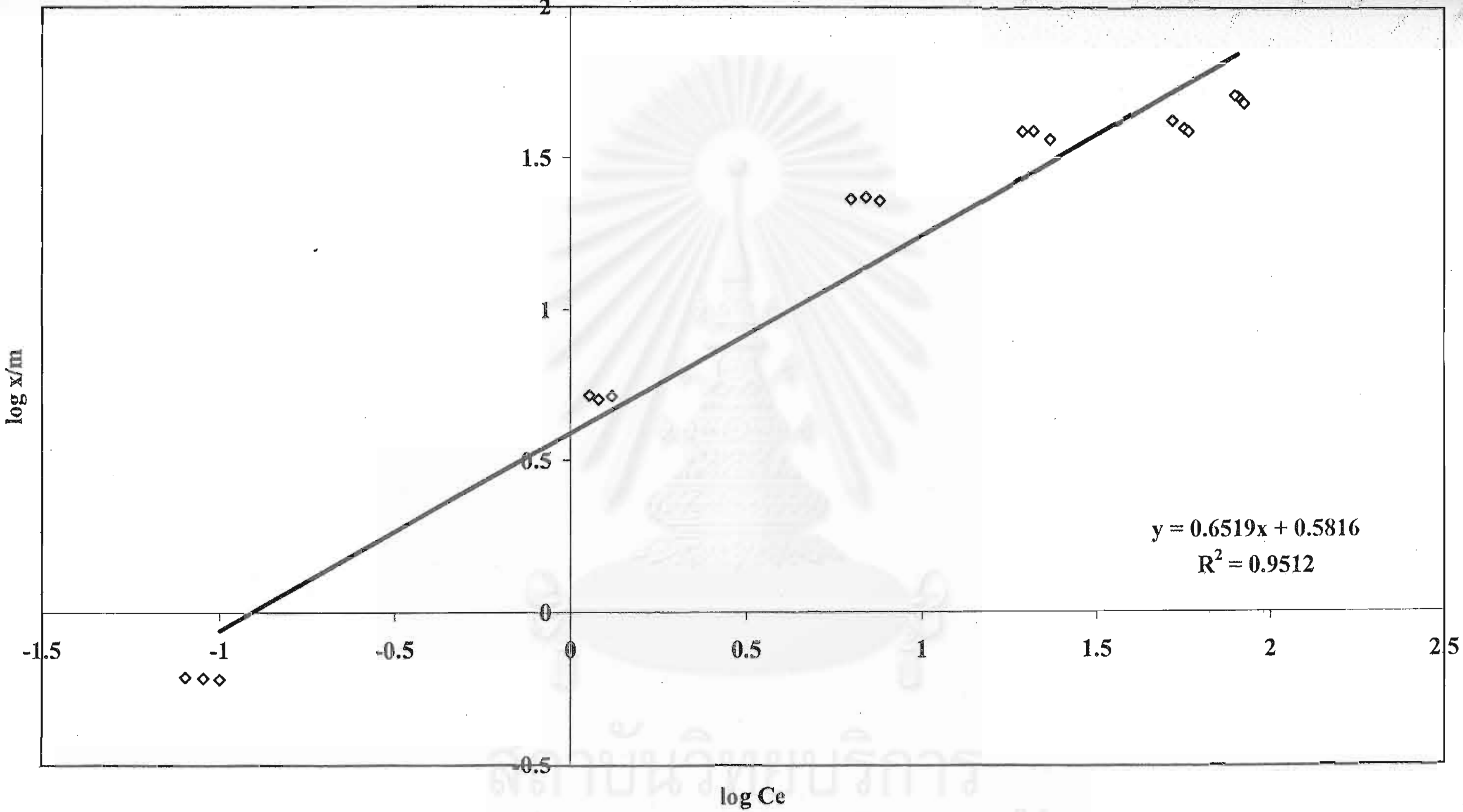

Figure 5.7.2.c Freundlich isotherm of Zn
(algal mass = 0.5 g, initial conc.= 10 mg Zn/l, contact time 30 min, pH =5)

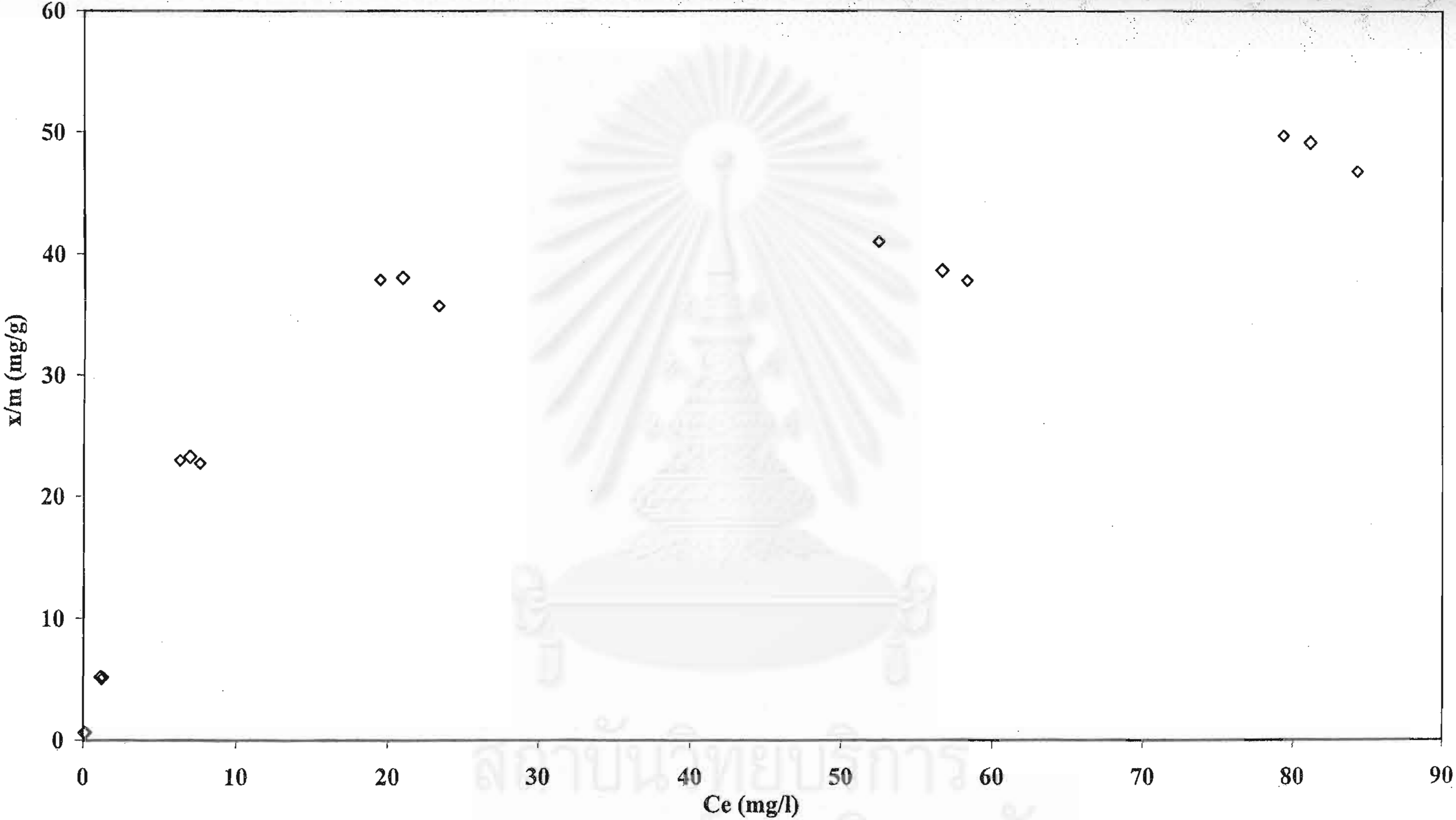

Figure 5.7.2.d Isotherm of Zn
(algal mass = 0.5 g, initial conc.= 10 mg Zn/l, contact time 30 min, pH =5)

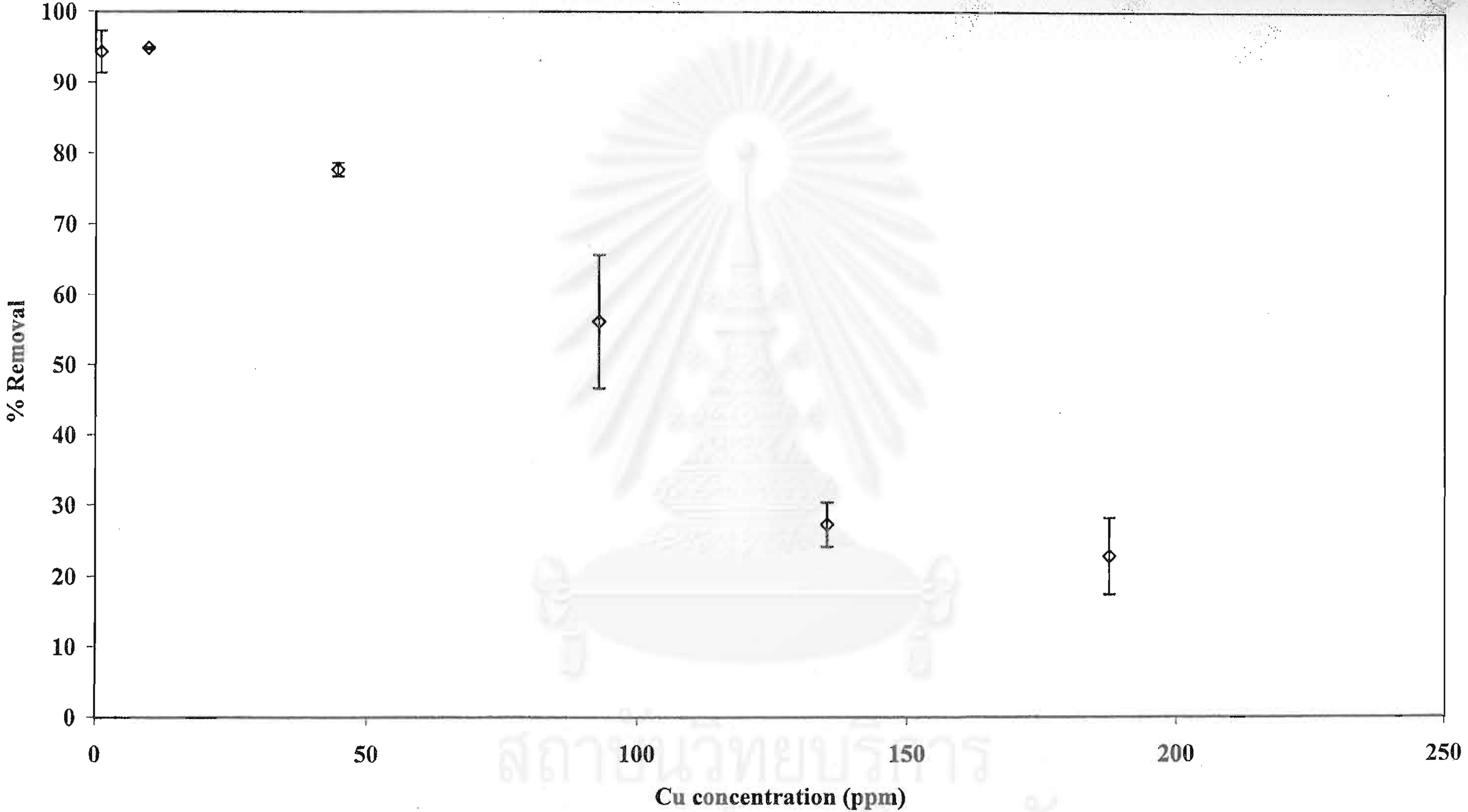

Figure 5.7.3.a Adsorption isotherm for Cu
(algal mass = 0.5 g, initial conc.= 10 mg Cu/l, contact time 30 min, pH =5)

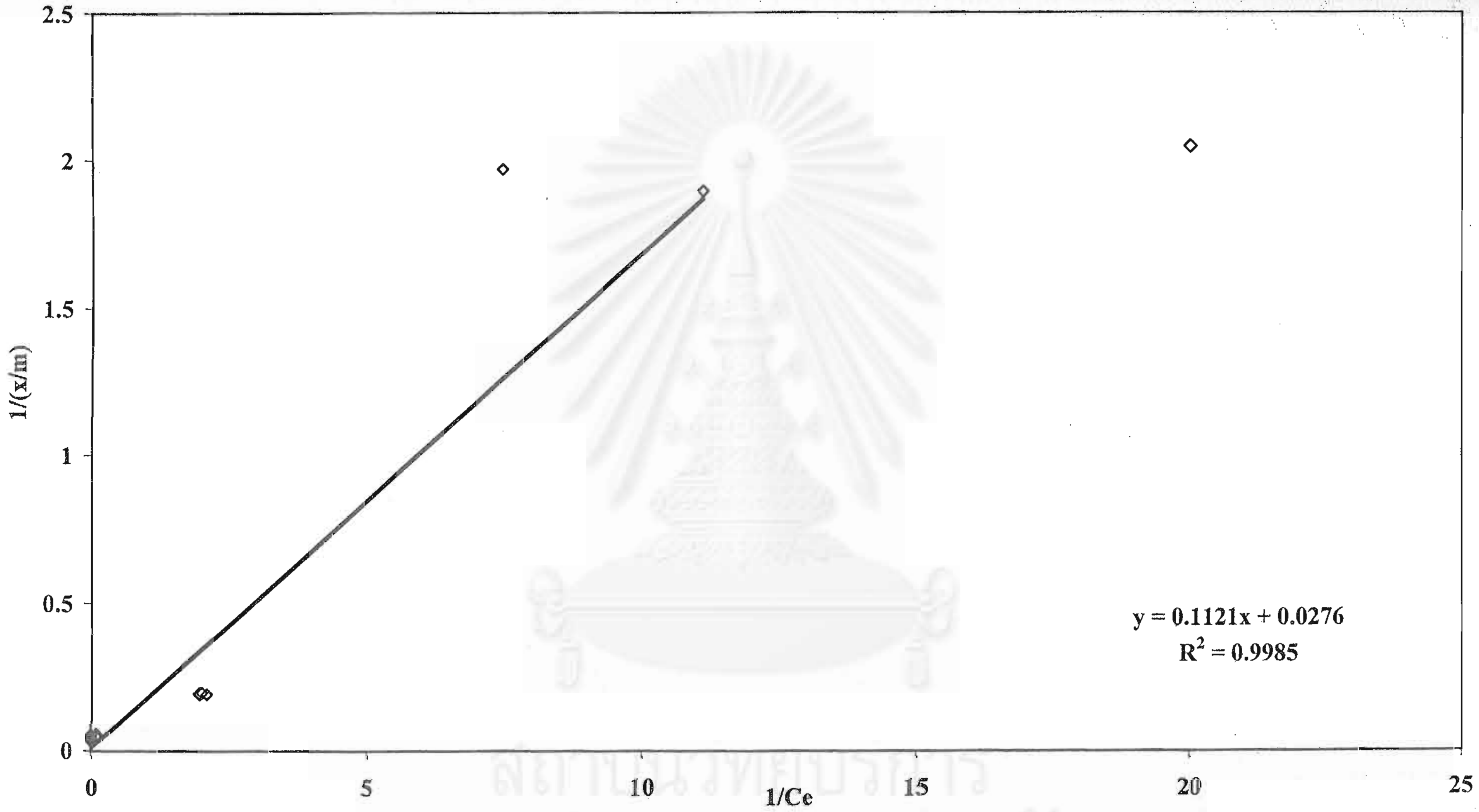

Figure 5.7.3.b Langmuir isotherm of Cu
(algal mass = 0.5 g, initial conc.= 10 mg Cu/l, contact time 30 min, pH =5)

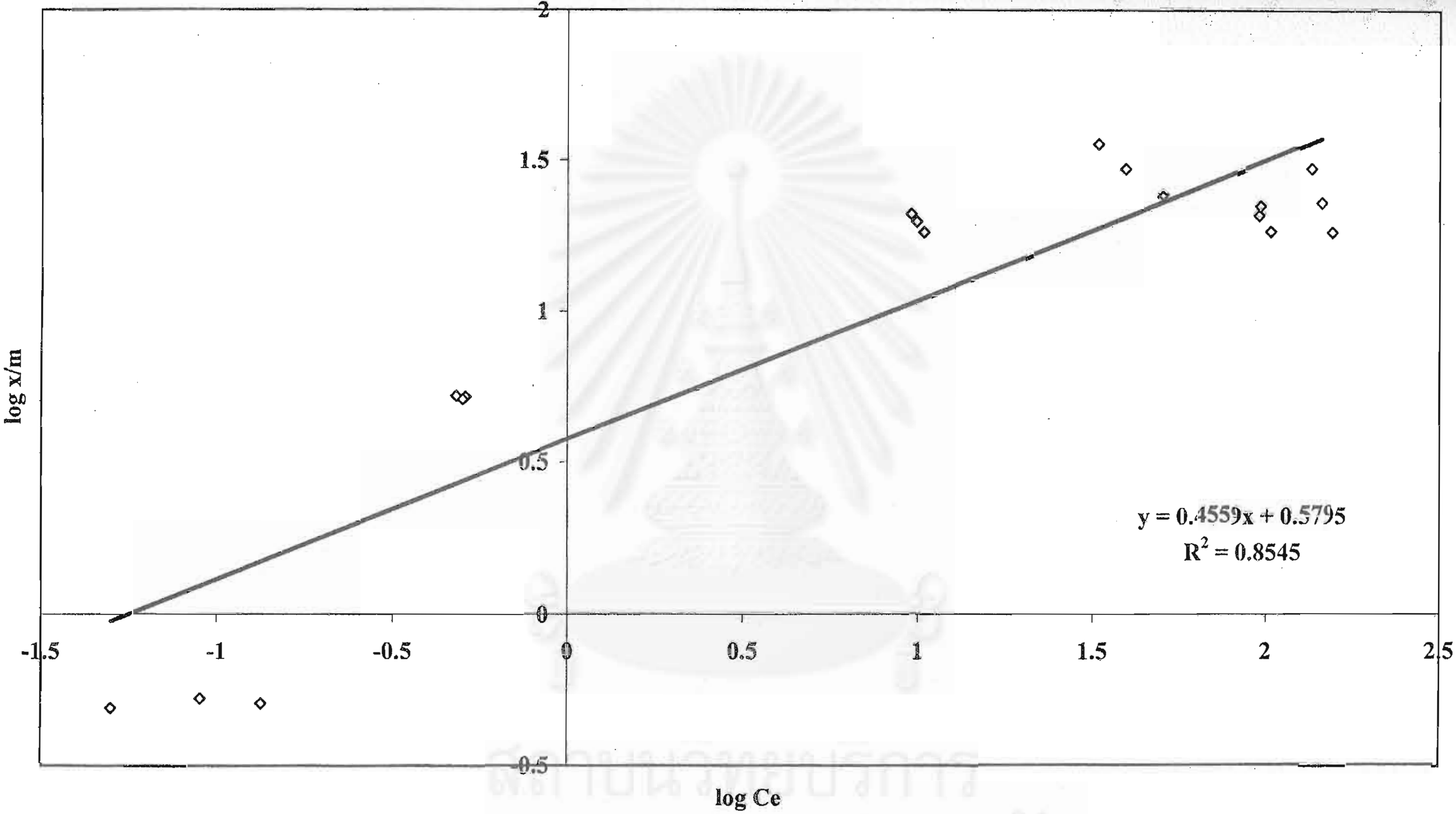

Figure 5.7.3.c Freundlich isotherm of Cu
(algal mass = 0.5 g, initial conc.= 10 mg Cu/l, contact time 30 min, pH =5)

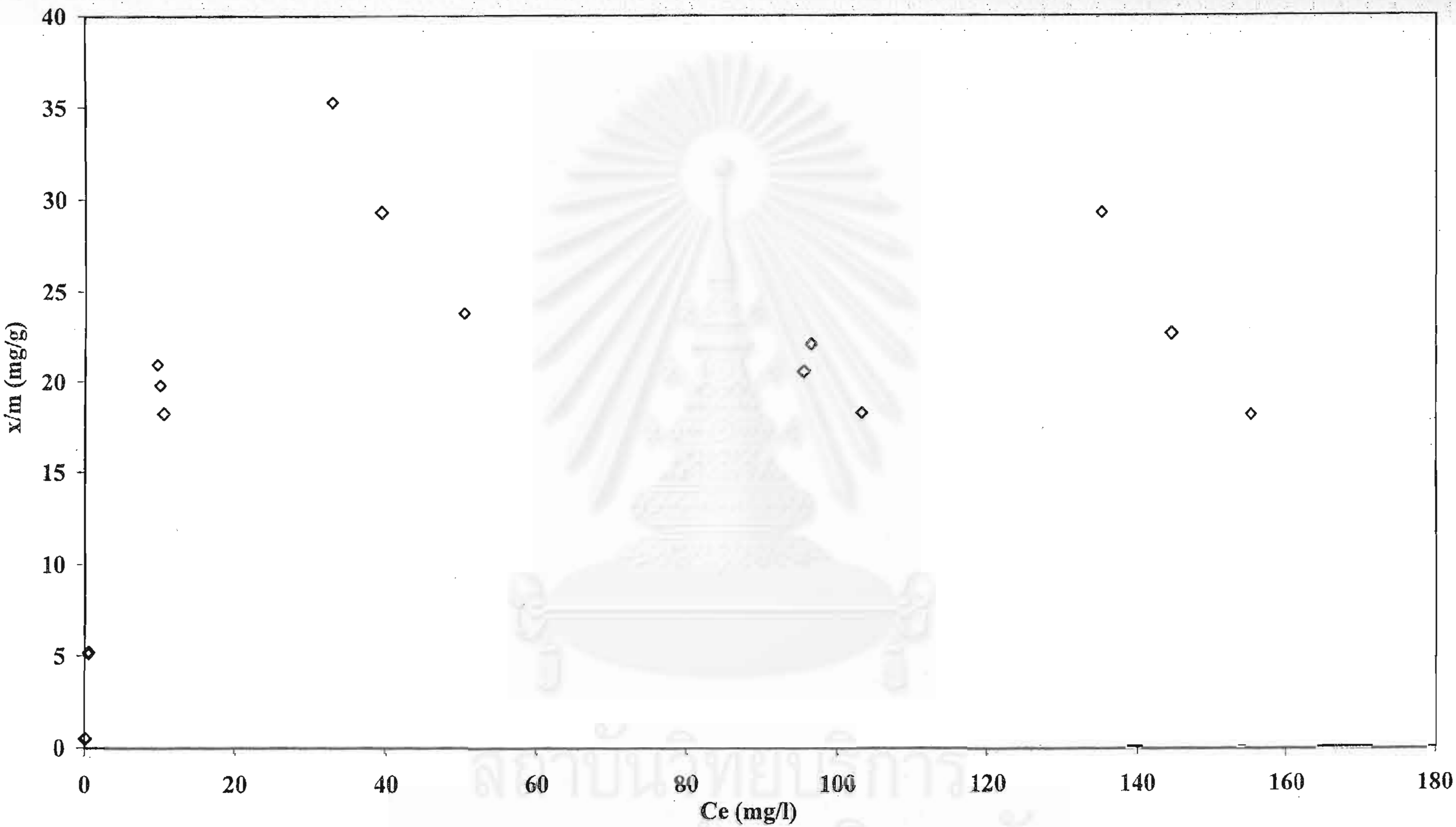

Figure 5.7.3.d Isotherm of Cu
(algal mass = 0.5 g, initial conc.= 10 mg Cu/l, contact time 30 min, pH =5)

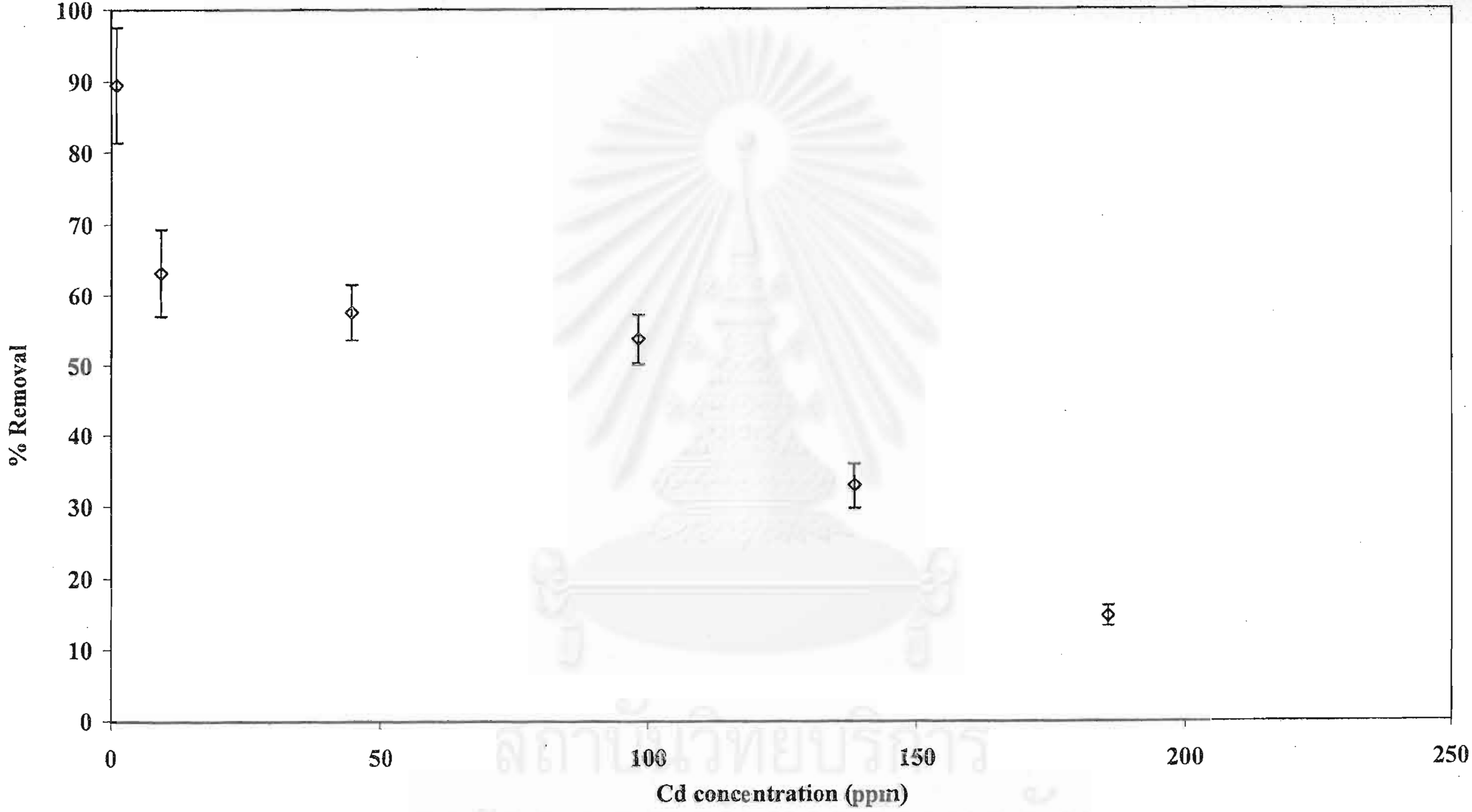

Figure 5.7.4.a Adsorption isotherm for Cd
(algal mass = 0.5 g, initial conc.= 10 mg Cd/l, contact time 30 min, pH =5)

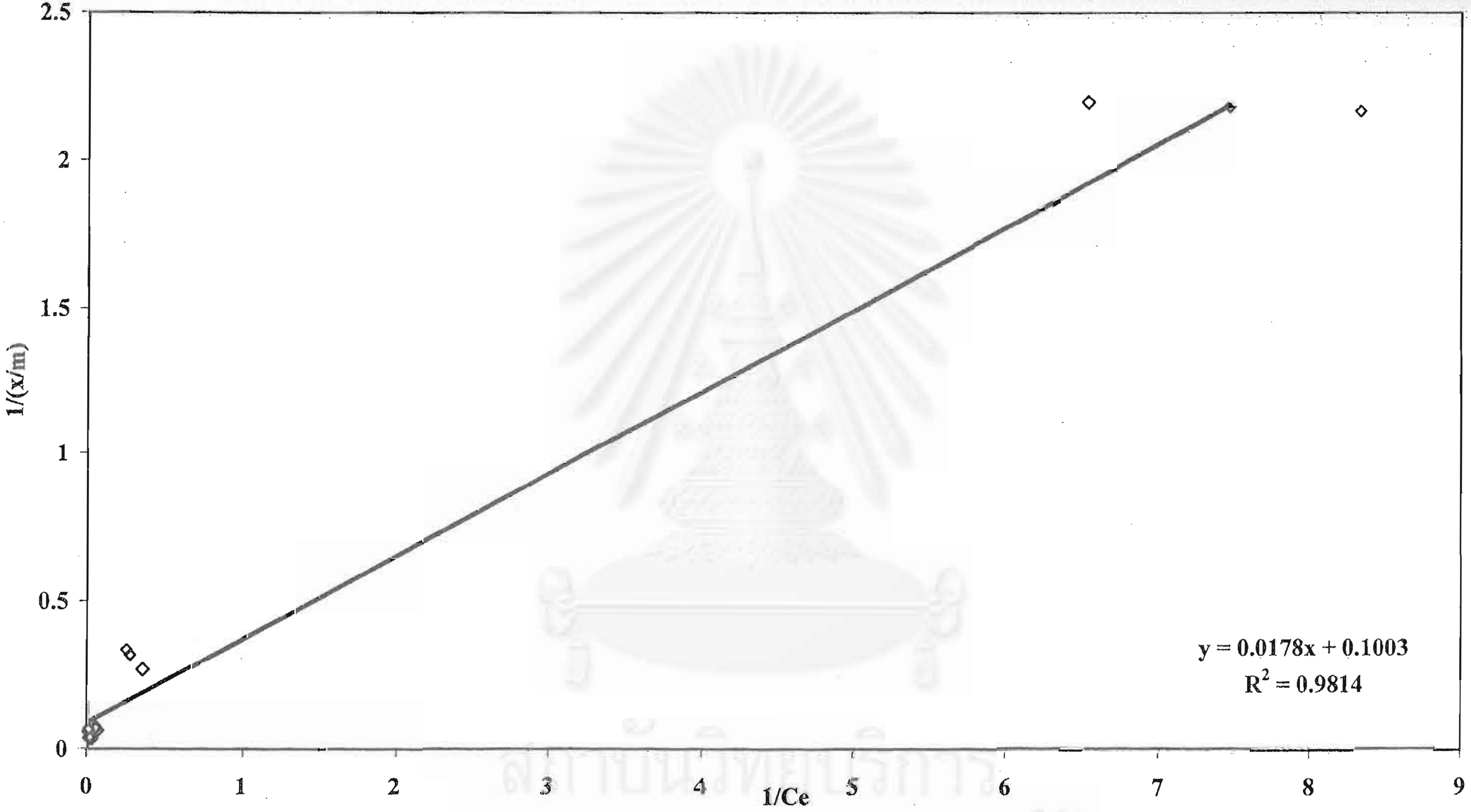

Figure 5.7.4.b Langmuir isotherm of Cd
(algal mass = 0.5 g, initial conc.= 10 mg Cd/l, contact time 30 min, pH =5)

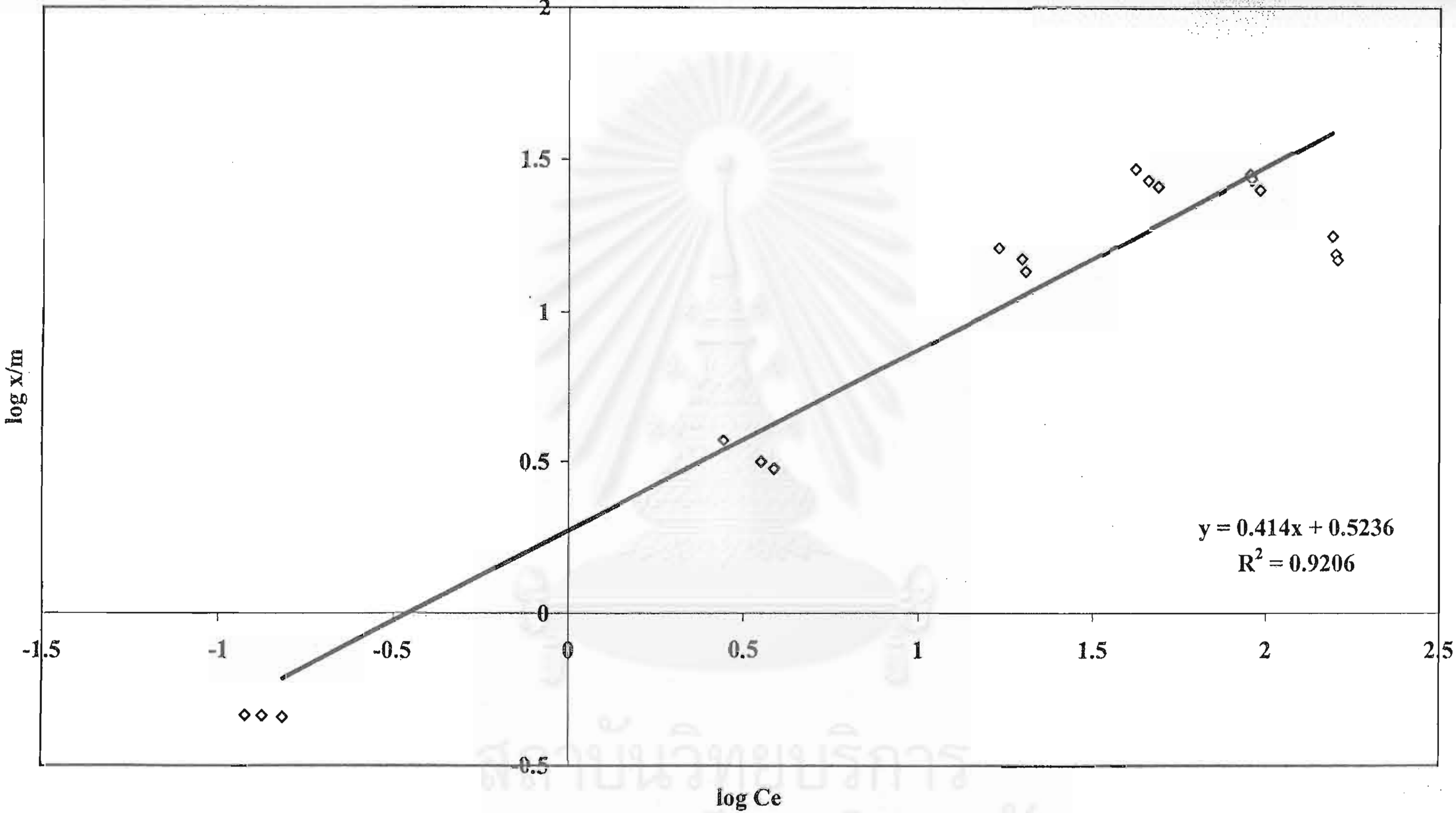

Figure 5.7.4.c Freundlich isotherm of Cd
(algal mass = 0.5 g, initial conc.= 10 mg Cd/l, contact time 30 min, pH =5)

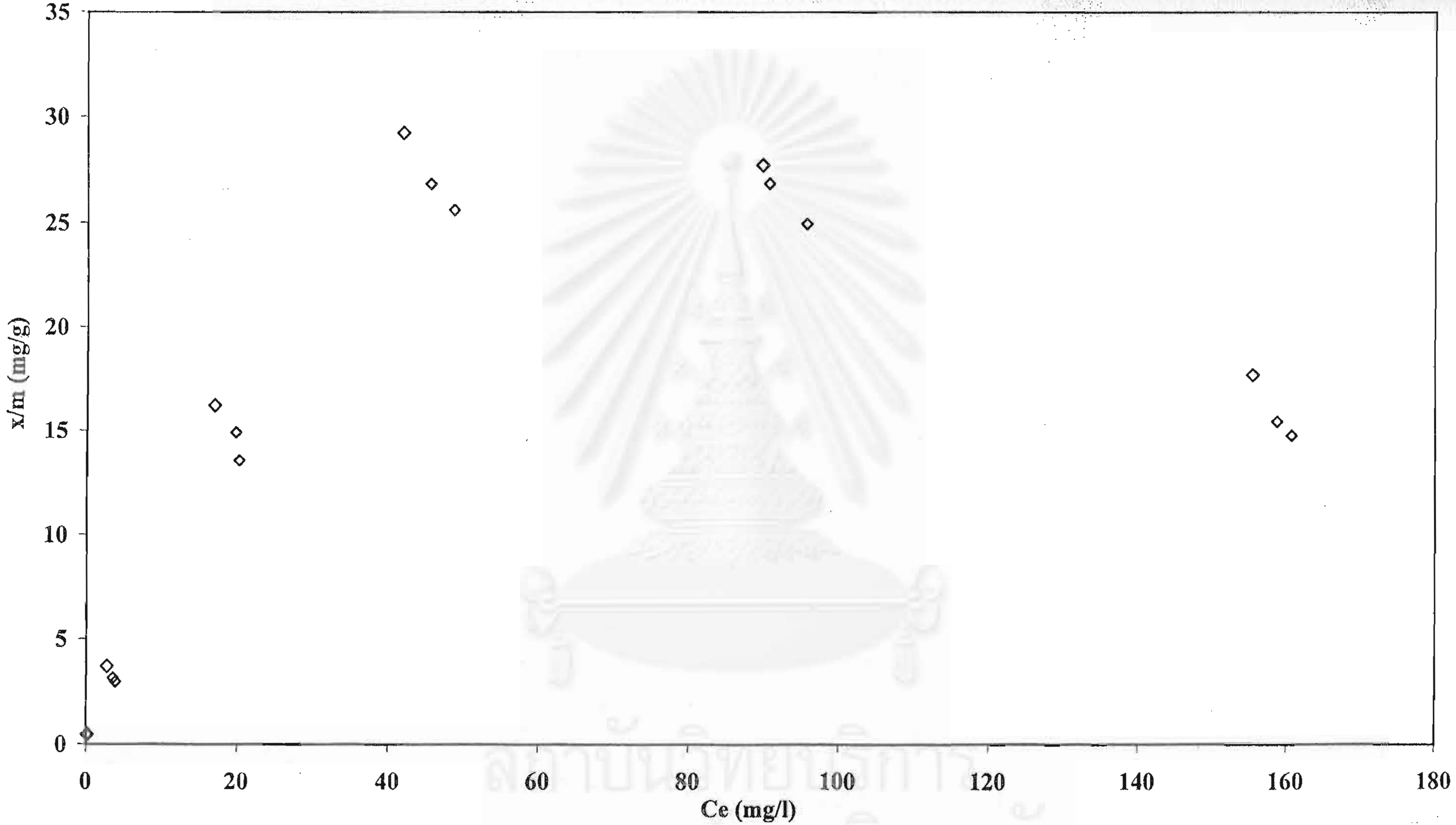

Figure 5.7.4.d Isotherm of Cd
(algal mass = 0.5 g, initial conc.= 10 mg Cd/l, contact time 30 min, pH =5)

Table 5.1 Comparison on adsorption capacity (q_{max}) and *b* on Langmuir isotherm for the removal of Pb

Adsorbent	q_{max}, mmol/g	*b*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
-*U. lactuca* (marine alge)	0.61	4.78	-	4.5	30	200	2	Jalali, *et al*, 2002
-*C. glomerata*	0.35	10.42	-	4.5	30	200	2	
-*G. corticata*	0.26	7.63	-	4.5	30	200	2	
-*G. canaliculata*	0.20	11.20	-	4.5	30	200	2	
-*S. hystrix*	1.38	2.08	-	4.5	30	200	2	
-*S. natans*	1.15	2.70	-	4.5	30	200	2	
-*P. violacea*	0.49	63.71	-	4.5	30	200	2	
-*P. pavonia*	1.05	3.47	-	4.5	30	200	2	
-*Durvillaea potatorum* (marine algae)	0.02	0.00	0.2 M $CaCl_2$	1	21	100	2	Matheical and Yu, 1999
	0.76	2.62		2	21	100	2	
	1.29	90.50		3	21	100	2	
	1.47	247.2		4	21	100	2	
	1.55	**496.6**		**5**	**21**	**100**	**2**	
-*Ecklonia radiata* (marine algae)	0.05	0.89		1	21	100	2	
	0.42	9.25		2	21	100	2	
	0.99	25.97		3	21	100	2	
	1.17	35.89		4	21	100	2	
	1.26	**38.88**		**5**	**21**	**100**	**2**	
-*Aspergillus niger* (bacteria)	0.049	7.8	NaOH	5		75	0.2	Kupoor *et al.*, 1999
-*Caulerpa sp.* (marine algae)	**0.05**	**8.15**	**-**	**5**	**30**	**30**	**0.5**	**This study**

Table 5.2 Comparison on adsorption capacity (q_{max}) and *b* on Langmuir isotherm for the removal of Zn

Adsorbent	q_{max}, mmol/g	*b*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
-*Cymodocea nodosa*	0.68	0.10	-	6.5		200	3	Sanchez *et al.*, 1999
	0.71	**0.12**		**5.5**		**200**	**3**	
	0.69	0.11		4.5		200	3	
	0.56	0.36		3.5		200	3	
-*Caulerpa sp.* (marine algae)	**0.09**	**5.86**	**-**	**5**	**30**	**30**	**0.5**	**This study**

Table 5.3 Comparison on adsorption capacity (q_{max}) and *b* on Langmuir isotherm for the removal of Cu

Adsorbent	q_{max}, mmol/g	*b*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
-*Cymodocea nodosa*	0.67	0.05	-	5.3		200	3	Sanchez *et al.*, 1999
	0.83	**0.07**		**4.5**		**200**	**3**	
	0.70	0.07		4		200	3	
	0.52	0.06		3		200	3	
-*Durvillaea potatorum (marine algae)*	0.04	0.00	0.2 M $CaCl_2$	1	21	100	2	Matheical and Yu, 1999
	0.18	0.02		2	21	100	2	
	0.99	7.28		3	21	100	2	
	1.21	11.11		4	21	100	2	
	1.30	**16.68**		**5**	**21**	**100**	**2**	
-*Ecklonia radiata*	0.07	0.89		1	21	100	2	
	0.45	9.25		2	21	100	2	
	0.95	25.97		3	21	100	2	
	1.06	35.89		4	21	100	2	
	1.11	**38.88**		**5**	**21**	**100**	**2**	
- *Padina sp.*	0.17	6.23	0.2 M $CaCl_2$	2	25	100	1	Kaewsarn, 2002
	0.46	7.14		4	25	100	1	
	0.80	**7.98**		**5**	**25**	**100**	**1**	
	0.79	7.95		6	25	100	1	
- *Aspergillus niger* (bacteria)	**0.10**	**0.19**	NaOH	**6**		**75**	**0.2**	Kupoor *et al.*, 1999
	0.073	0.30		5		75	0.2	
-*Caulerpa* *sp.* (marine algae)	**0.14**	**4.06**	**-**	**5**	**30**	**30**	**0.5**	**This study**

Table 5.4 Comparison on adsorption capacity (q_{max}) and *b* on Langmuir isotherm for the removal of Cd

Adsorbent	q_{max}, mmol/g	*b*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
- *Aspergillus niger* (bacteria)	0.035	1.1	NaOH	5		75	0.2	Kupoor *et al.*, 1999
	0.039	**1.13**		**6**		**75**	**0.2**	
-*Caulerpa* *sp.* (marine algae)	**0.50**	**0.18**	**-**	**5**	**30**	**30**	**0.5**	**This study**

Table 5.5 Comparison on K_f and *1/n* on Freundlich isotherm for the removal of Pb

Adsorbent	K_f, mmol/g	*1/n*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
Cladophora crispata	0.015	0.595	-	3.5	25	100	0.25	Ozer *et al*., 1994
	0.032	0.481	-	4.0	25	100	0.25	
	0.075	**0.488**	-	**5.0**	**25**	**100**	**0.25**	
	0.059	0.535	-	5.0	25	100	0.25	
	0.050	0.446	-	4.0	25	100	0.25	
Chlorella vulgaris (microalgae)	0.082		-	2	25	90	10 ml	Aksu and Kutsal, 1991
	0.036			3	25	90	10 ml	
	0.068			4	25	90	10 ml	
	0.083			**5**	**25**	**90**	**10 ml**	
	0.057			5	15	90	10 ml	
	0.107			5	35	90	10 ml	
-*U. lactuca* (marine alge)	0.205	0.19	-	4.5	30	200	2	Jalali, *et al*, 2002
-*C. glomerata*	0.181	0.12	-	4.5	30	200	2	
-*G. corticata*	0.120	0.13	-	4.5	30	200	2	
-*G. canaliculata*	0.103	0.11	-	4.5	30	200	2	
-*S. hystrix*	0.191	0.35	-	4.5	30	200	2	
-*S. natans*	0.229	0.29	-	4.5	30	200	2	
-*P. violacea*	0.433	0.02	-	4.5	30	200	2	
-*P. pavonia*	0.233	0.27	-	4.5	30	200	2	
- *Aspergillus niger* (bacteria)	0.040	0.14	NaOH	5		75	0.2	Kupoor *et al*., 1999
-*Caulerpa sp.* (marine algae)	**0.026**	**5.86**	-	**5**	**30**	**30**	**0.5**	**This study**

Table 5.6 Comparison on K_f and *1/n* on Freundlich isotherm for the removal of Zn

Adsorbent	K_f, mmol/g	*1/n*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
-*Caulerpa sp.* (marine algae)	**0.058**	**4.49**	**-**	**5**	**30**	**30**	**0.5**	**This study**

Table 5.7 Comparison on K_f and *1/n* on Freundlich isotherm for the removal of Cu

Adsorbent	K_f, mmol/g	*1/n*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
-*Chlorella vulgaris* (microalgae)	0.003	0.5	-	2	25		1	Aksu *et al.*, 1992
	0.042	**0.4**		**3**	**25**		**1**	
-*Zoogloea ramigera* (microalgae)	**0.161**	**0.3**	-	**4**	**25**		**1**	
	0.130	0.3		5	25		1	
-*Chlorella vulgaris* (microalgae)	0.008	0.59	-	2	25	90	10 ml	Aksu and Acikel, 1999
	0.081	**0.32**		**4**	**25**	**90**	**10 ml**	
-*Laminaria japonica* (macroalgae)	0.006	0.22	-	5	19	100	1	Zhou *et al.*, 1998
-*Sargassum kjellmanianum* (macroalgae)	0.006	0.22	-	5	19	100	1	
- *Aspergillus niger* (bacteria)	**0.023**	**0.35**	NaOH	**6**		**75**	**0.2**	Kupoor *et al.*, 1999
	0.023	1.27		5		75	0.2	
-*Caulerpa sp.* (marine algae)	**0.060**	**3.15**	**-**	**5**	**30**	**30**	**0.5**	**This study**

Table 5.8 Comparison on K_f and *1/n* on Freundlich isotherm for the removal of Cd

Adsorbent	$K_{f,}$ mmol/g	*1/n*	Conditions					Reference
			Pretreated algae	pH	Temp (°C)	Volume (ml)	Bio-mass (g)	
-*Laminaria japonica* (macroalgae)	0.305	0.19	-	5	19	100	1	Zhou *et al.*,1998
-*Sargassum kjellmanianum* (macroalgae)	0.276	0.22	-	5	19	100	1	
-*Spirulina platensis* (microalgae)	0.028	0.88	-	5	19	100	1	
-*Nannochloropsi soculata* (microalgae)	0.018	0.96	-	5	19	100	1	
-*Phaeodactylum tricornutum* (microalgae)	0.013	0.86	-	5	19	100	1	
- *Platymonas cordifolia* (microalgae)	0.002	1.31	-	5	19	100	1	
-*Chaetoceros minutissimus* (microalgae)	0.003	1.06	-	5	19	100	1	
-*Spirulina platensis (microalgae)*	0.001	1.22	-	2.0	19	100	1	
-*Spirulina platensis (microalgae)*	0.011	0.65	-	3.5	19	100	1	
-*Spirulina platensis (microalgae)*	0.039	0.62	-	5.7	19	100	1	
- *Spirulina platensis (microalgae)*	0.039	0.85	-	6.7	19	100	1	
- *Spirulina platensis (microalgae)*	0.020	1.08	-	8.6	19	100	1	
- *Aspergillus niger* (bacteria)	0.023	6.6	NaOH	5		75	0.2	Kupoor *et al.*, 1999
	0.025	**5.99**		**6**		**75**	**0.2**	
-*Caulerpa sp.* (marine algae)	**0.030**	**2.59**	**-**	**5**	**30**	**30**	**0.5**	**This study**

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results of biosorption of heavy metals on *Caulerpa lentillifera* biomass can be summarized as follows.

1. The ranges of pH suitable for these experiments with lead, zinc, copper and cadmium were 1-7, 1-6, 1-5 and 1-7, respectively.
2. *Caulerpa lentillifera* biomass had similar adsorption capacities for metals of different forms, e.g. the removal efficiencies for $CuCl_2$, $CuSO_4$ and $CuNO_3$ were approximately within the same range of 70%.
3. The algae consisted of functional groups in order from large to small quantity as follows: hydroxyl (or carboxyl or amine) groups > C-O (or sulfonyl) > carbonyl > S-O > alkyl. From the results, it is possible that Cd forms a covalent bond with the C-O stretching in carboxyl functional group. On the other hand, Pb, Zn and Cu could also form a covalent bond with the soft C-O, or they can form an electrostatic or ionic bond with a hard carboxyl (COO^-), sulfoxyl (OSO_3^-) and amino (NH_2^-) groups.
4. The eluting agent, EDTA was found to be more efficient in stripping the adsorbed heavy metal from the biomass than was the hot water. The results implied that the adsorption bonding should not be Van Der Waals, but should involve stronger forces such as ionic or covalent bondings.
5. The optimal algal mass per unit mass of heavy metal were found to be 0.079, 0.073, 0.043 and 0.042 g/mg for lead, zinc, cadmium and copper, respectively. Most of equilibrium adsorption efficiencies were independent of particle sizes except for the adsorption of Zn where the removal efficiency was dependent on the particle sizes.
6. The kinetics of adsorption by the biomass was rapid with 80-90% removal within 20 minutes. The adsorption was believed to follow a passive transport mechanism.
7. The optimum pH of Pb, Zn, Cu and Cd were found to be 5, 6, 5 and 7, respectively.

8. The adsorption capacities fitted better to Langmuir than Freundlich isotherms. Adsorption capacity of Cd was found to be rather high and Pb was rather low compared with the reported values in literature.

6.2 Contribution of this work

This work has shown that *Caulerpa lentillifera* which is the fast-growing seawater algae could be used as a supplement adsorbent for the removal of heavy metals from the low strength wastewater. This algal biomass was found to be particularly suitable for the adsorption of Cd. Details on various adsorption characteristics e.g. effect of pH, particle sizes, etc. including the adsorption isotherms were provided in this report. This contributes greatly to the research in this field as there was so far no reports regarding the adsorption aspect of *Caulerpa lentillifera*

Although this work still could not identify exactly the adsorption mechanism taking place during the adsorption process, it proposes a few possible alternatives that might have occurred and this will be useful as a starting point for further investigation in this area. This information will be essential for the future development of this adsorption process.

6.3 Recommendations

Although large research areas were covered in this work, there are still some points that could not be fully examined. In order to ensure the completeness of this study, further work should be carried out. Some recommendations are inherited during the course of this work and these are summarized as follows:

1. Detailed investigation on the functional groups including both quantitative and qualitative analyses of *Caulerpa lentillifera* should be carried out to further identify the target functional groups for each of the metals.
2. Some indispensable issues are needed to be examined before real application of this system could be established. These include:
    - the adsorption efficiency of the treated *Caulerpa lentillifera.*
    - the study on the characteristics of the adsorption column such as the breakthrough point, the mass transfer zone, etc.
    - the interaction between heavy metals in a multi-metal adsorption system.
3. The optimal condition and chemical reagents needed for the desorption of the spent algae should be thoroughly understood.

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APPENDICES

APPENDIX A

Properties of heavy metals

Copper (Cu)

Atomic number : 29

Atomic weight : 63.546

Periodic Table : group IB

Valences : 1, 2; two stable isotopes

Oxidation states : +2, +4

Properties : Distinctive reddish color, specific gravity 8.96, melting point 1083° C, boiling point 2595° C, dissolves in nitric acid and hot concentrated sulfuric acid, dissolve slowly in hydrochloric and dilute sulfuric acid but only when exposed to the atmosphere.

Hazard : The toxicity of metallic copper is very low. However, inhalation of dusts, fumes, mists or salt can cause adverse health effects. Many copper (II) salts are toxic.

Uses : Copper is used in electric cables and wires, switches, plumbing, heating; roofing and building construction; chemical and pharmaceutical machinery; alloys (brass, bronze, and a new alloy with 3% beryllium that is particularly vibration resistant); alloy castings; electroplated protective coatings and undercoats for nickel, chromium, zinc, etc.. cooking utensils.

Exposure Limits : TLV-TWA 1 mg (Cu)/m^3 (dust and mists) (ACGIH and MSHA); 0.2 mg/m^3 (fumes) (ACGIH).

Method : The atomic absorption spectrometric, the inductively coupled plasma and the neuocuproine method are recommended because of their freedom from interference.

Sampling and storage : Copper ion tends to be adsorbed on the surface of sample containers. Therefore, analyze samples as soon as possible after collection. To store sample, use 0.5 mL 1+1 HCl/100 mL sample to prevent this adsorption.

Cadmium (Cd)

Atomic number : 48

Atomic weight : 112.4

Periodic Table : group IIB

Valences : 2

Oxidation states : +2

Properties : Soft, blue-white, malleable metal or grayish-white powder. Tarnishes in moist air; corrosion resistance poor in industrial atmospheres. Becomes brittle at 80ºC. Resistant to alkalies; high neutron absorber. Specific gravity 8.642, melting point 320.9° C, boiling point 767° C, soluble in acids, especially nitric and ammonium nitrate solutions

Hazard : Highly toxic, especially by inhalation of dust or fumes. It is a known carcinogen (OSHA).

Uses : Electrodeposites and dipped coating on metals; bearing and low-melting alloys; brazing alloys; fire-protection systems; nickel-cadmium storage batteries; power transmission wire; TV phosphors; basis of pigments used in ceramic glazes.

Exposure Limits : TLV-TWA 0.15 mg/m^3 (ACGIH and MSHA); 0.05 mg/m^3 (OSHA); 10hr TWA 0.1 mg(inorganic lead)/m^3 (NIOSH) .

Method : The atomic absorption spectrometric, the inductively coupled plasma and the neuocuproine method are recommended because of their freedom from interference.

Sampling and storage : Cadmium ion tends to be adsorbed on the surface of sample containers. Therefore, analyze samples as soon a possible after collection. To storage sample, use 0.5 mL 1+1 HCl/100 mL sample to prevent this adsorption.

Lead (Pb)

Atomic number : 82

Atomic weight : 207.2

Periodic Table : group IVA

Valences : 2,4

Oxidation states : +2

Properties : Heavy, ductile, soft gray solid. Specific gravity 11.35, melting point 327.4° C, boiling point 1755° C, soluble in dilute nitric acid; insoluble in water but dissolves slowly in water containing a weak acid; resists corrosion.

Hazard : Toxic by ingestion and inhalation of dust or fumes.

Uses : Storage batteries; tetraethyllead (gasoline additive); radiation shielding; cable covering; ammunition; chemical reaction equipment (piping, tank linings, etc.); solder and fusible alloys; type metal; vibration damping in heavy metal construction; foil; and other bearing alloys.

Exposure Limits : TLV-TWA 0.15 mg/m^3 (ACGIH and MSHA); 0.05 mg/m^3 (OSHA); 10hr TWA 0.1 mg(inorganic lead)/m^3 (NIOSH) .

Method : The atomic absorption spectrometric, the inductively coupled plasma and the neuocuproine method are recommended because of their freedom from interference.

Sampling and storage : Lead ion tends to be adsorbed on the surface of sample containers. Therefore, analyze samples as soon a possible after collection. To storage sample, use 0.5 mL 1+1 HCl/100 mL sample to prevent this adsorption.

Zinc (Zn)

Atomic number : 30

Atomic weight : 65.37

Periodic Table : group IIB

Valences : 2

Oxidation states : +2

Properties : Shining white metal with bluish gray luster (called spelter). Not found native. It is soluble in acids and alkalies but insoluble in water. Specific gravity 7.14, melting point 419° C, boiling point 907° C, soluble in acids, especially nitric and ammonium nitrate solutions

Hazard : Low toxicity, zinc dust is flammable, dangerous fire and explosion risk.

Uses : Alloy (brass, bronze and die-casting alloys); galvanizing iron and other metals; electroplating; metal spraying; automotive parts; electrical fuses, storage and dry cell batteries.

Method : The atomic absorption spectrometric, the inductively coupled plasma and the neuocuproine method are recommended because of their freedom from interference.

Sampling and storage : Zinc ion tends to be adsorbed on the surface of sample containers. Therefore, analyze samples as soon a possible after collection. To storage sample, use 0.5 mL 1+1 HCl/100 mL sample to prevent this adsorption.

Source: [Hawley, 1981; Clesceri, 1989; Patnaik, 1999]

APPENDIX B

Table B.1 The industrial effluent standard of heavy metal

Heavy metals	**Criteria**
Zinc	Not more than 5.0 mg/L
Copper	Not more than 2.0 mg/L
Lead	Not more than 0.2 mg/L
Mercury	Not more than 0.005 mg/L
Chromium (hexavalent)	Not more than 0.25 mg/L
Chromium (trivalent)	Not more than 0.75 mg/L
Cadmium	Not more than 0.03 mg/L
Barium	Not more than 1.0 mg/L
Nickel	Not more than 1.0 mg/L
Manganese	Not more than 5.0 mg/L
Arsenic	Not more than 0.25 mg/L
Selenium	Not more than 0.02 mg/L

Source: Notification the Ministry of Science, Technology and Environment, No.3, B.E. 2539 (1996) issued under the Enhancement and Conservation of the National Environmental Quality Act B.E. 2535 (1992), published in the Royal Government Gazette, Vol. 113 Part 13 D, dated February 13, B.E. 2539 (1996).

BIOGRAPHY

Miss Vimonrat Sungkhum was born on 22nd September, 1976 in Bangkok. She finished her higher secondary course from Satriwitthaya School, Bangkok in March, 1994. After that, she studied in the major of Chemistry in Faculty of Science at Kasetsart University. She continued her further study for Master’s degree in International Environmental Management Science at Chulalongkorn University and achieved her Master’s degree in April, 2003.