



**REMOVAL OF MERCURY, CADMIUM AND LEAD BY  
THE USE OF SELECTED MICROALGAL STRAINS**



**NALIN SIDTITOON**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT  
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THE USE OF SELECTED MICROALGAL STRAINS**



.....

Miss Nalin Sidtitoon  
Candidate



.....

Asst. Prof. Duangrat Inthorn, Ph.D.  
Major-Advisor



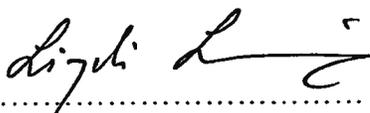
.....

Prof. Amaret Bhumiratana, Ph.D  
Co- Advisor



.....

Assoc. Prof. Suthop Silapanuntakul, Ph.D.  
Co- Advisor



.....

Prof. Liangchai Limlomwongse, Ph.D.  
Dean  
Faculty of Graduate studies  
Mahidol University



.....

Assoc. Prof. Udom Kompayak, M.PH.  
Chairman  
Master of science Programme  
in Environmental sanitation  
Faculty of Public Health

Thesis

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on

September 14, 2000

*Nalin Sidtitoon*

Miss Nalin Sidtitoon  
Candidate

*Duangrat Inthorn*

Asst. Prof. Duangrat Inthorn, Ph.D.  
Chairman

*Aran Incharoensakdi*

Assoc. Prof. Aran Incharoensakdi,  
Ph.D.  
Member

*Amaret Bhumiratana*

Prof. Amaret Bhumiratana, Ph.D.  
Member

*Udom Kompayak*

Assoc. Prof. Udom Kompayak, M.PH  
Member

*S. Silapanuntakul*

Assoc. Prof. Suthep Silapanuntakul, Ph.D.  
Member

*Liangchai Limlomwongse*

Prof. Liangchai Limlomwongse, Ph.D.  
Dean  
Faculty of Graduate studies.  
Mahidol University

*Kanda Vathanophas*

Assoc. Prof. Kanda Vathanophas, M. D.,  
M. Sc. in Hygiene ( P. H. Microbiology)  
Dean  
Faculty of Public Health  
Mahidol University

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Thirty-five strains of microalgae were screened on plates with various concentration of heavy metal in order to determine the high Mercury (Hg), Cadmium (Cd) and Lead (Pb) tolerance. Heavy metals removal in aqueous solution showed that *Scenedesmus acutus* had the highest Hg removal capacity at 85.18 %, T5 had the highest Cd removal capacity at 93.85 % whereas *Calothrix parietina* had the highest Pb removal capacity at 90.59 %. The results showed that 14 strains exhibited a high tolerance and the relative toxicity in increasing order was Hg > Cd > Pb. *Scenedesmus acutus* had highest concentration factor at 3412, 4591 and 4078 for Hg, Cd and Pb, respectively. Additionally, the adsorption experiment revealed that *Tolypothrix tenuis* and *Calothrix parietina* could remove Hg, Cd and Pb rapidly within 5-10 min and become equilibrium while *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* could remove Hg, Cd and Pb rapidly within 10 mins and could remove these metals slowly afterwards. The adsorption capacity values of Hg were 15.60-26.86 mg Hg/g dry wt. and *Tolypothrix tenuis* had the highest maximum adsorption capacity of 26.86 mg Hg/g dry wt. at a minimum concentration of 1.04 ppm. The adsorption capacity values of Cd were 61.68-109.57 mg Cd/g dry wt. *Scenedesmus acutus* had the highest maximum adsorption capacity of 109.57 mg Cd/g dry wt. at a minimum concentration of 109 ppm. The adsorption capacity values of Pb were 31.48-126.66 mg Pb/g dry wt. and *Chlorella vulgaris* had the highest maximum adsorption capacity of 126.66 mg Pb/g dry wt. at a minimum concentration of 129.5 ppm.

The results of this study suggests that filamentous cyanobacteria, *Tolypothrix tenuis* and *Calothrix parietina* have feasibility to apply to be used in real wastewater treatment system because of high removal capacity, high growth rate and easy separation of the biomass from treated wastewater by simple filtration method.

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ศึกษาความทนทานของสาหร่าย 35 สายพันธุ์ต่อปรอท แคดเมียมและตะกั่วในงานเพาะเชื้อที่ความเข้มข้นของโลหะหนักต่างๆ กัน พบว่ามีสาหร่าย 14 สายพันธุ์ที่มีความทนทานต่อโลหะหนักได้สูงและความเป็นพิษต่อสาหร่ายของโลหะหนักทั้งสามชนิดคือ ปรอท แคดเมียม และตะกั่ว จากมากไปน้อยตามลำดับ จากการศึกษาความสามารถในการกำจัดปรอท แคดเมียมและตะกั่วของสาหร่ายในสารละลายโลหะหนัก พบว่า *Scenedesmus acutus* สามารถกำจัดปรอทได้สูงสุด คือ ร้อยละ 85.18 ส่วน T5 สามารถกำจัดแคดเมียมสูงสุดคือร้อยละ 93.85 และ *Calothrix parietina* สามารถในการกำจัดตะกั่วได้สูงสุดคือ ร้อยละ 90.59 ความสามารถในการกำจัดโลหะหนักจากค่า Concentration factor พบว่า *Scenedesmus acutus* มีค่า Concentration factor สูงที่สุดในปรอท แคดเมียม และ ตะกั่ว คือ 3,412, 4,591 และ 4,078 ตามลำดับและจากการทำการศึกษาดูดซับในสารละลายโลหะหนักภายใน 30 นาที พบว่า *Tolypothrix tenuis* และ *Calothrix parietina* สามารถดูดซับปรอท แคดเมียมและตะกั่วได้อย่างรวดเร็วภายใน 5-10 นาทีและเข้าสู่สมดุล ในขณะที่ *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) และ *Scenedesmus acutus* สามารถดูดซับปรอท แคดเมียมและตะกั่วภายใน 10 นาทีแรก หลังจากนั้นการดูดซับจะลดลงแต่ยังคงดำเนินต่อไป เมื่อทำการทดลองหาความสามารถสูงสุดของสาหร่ายทั้ง 5 ชนิดในการดูดซับโลหะหนักที่ความเข้มข้นต่างๆ พบว่า ในสาหร่ายทั้ง 5 ชนิดสามารถดูดซับปรอทได้สูงสุดอยู่ในช่วง 15.60-26.86 mg Hg/g dry wt. *Tolypothrix tenuis* สามารถดูดซับปรอทได้สูงสุด คือ 26.86 mg Hg/g dry wt. ที่ความเข้มข้นต่ำสุด 1.04 ppm ส่วนในแคดเมียม สาหร่ายทั้ง 5 ชนิดสามารถดูดซับแคดเมียมได้สูงสุดอยู่ในช่วง 61.68 -109.57 mg Cd/g dry wt. *Scenedesmus acutus* สามารถดูดซับแคดเมียมได้สูงสุด คือ 109.57 mg Cd/g dry wt. ที่ความเข้มข้นต่ำสุด 109 ppm และในตะกั่ว สาหร่ายทั้ง 5 ชนิดสามารถดูดซับตะกั่วได้สูงสุดอยู่ในช่วง 31.48-126.66 mg Pb/g dry wt. *Chlorella vulgaris* สามารถดูดซับตะกั่วได้สูงสุด คือ 126.66 mg Pb/g dry wt. ที่ความเข้มข้นต่ำสุด 129.5 ppm จากผลการทดลองพบว่า *Tolypothrix tenuis* และ *Calothrix parietina* ซึ่งเป็นสาหร่ายสีเขียวแกมน้ำเงินมีความเป็นไปได้ในการนำไปใช้กำจัดโลหะหนักในน้ำเสีย เนื่องจากสามารถกำจัดโลหะหนักได้ดี เจริญเติบโตเร็วและลักษณะของเซลล์เป็นเส้นสายทำให้สามารถแยกสาหร่ายออกจากร่างน้ำเสียได้ง่ายโดยการกรอง

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## ABBREVIATION

Cd	Cadmium
CF	Concentration factor
Hg	Mercury
l	litre
mg	milligram ( $10^{-3}$ gram)
min	minute
ml	millilitre ( $10^{-3}$ litre)
mM	millimolar ( $10^{-3}$ molar)
Pb	Lead
ppm	part per million (mg/l)
wt	weight

## **CHAPTER I**

### **INTRODUCTION**

#### **1.1 Background**

Toxicity of heavy metal contamination in the environment affected the human health and ecosystem. Mercury, cadmium and lead are the severely to human health. Both Itai-Itai and Minamata disease caused by cadmium and mercury were the well-known diseases occurred in Japan (1). Therefore, the removal of heavy metals in the industrial wastewater before discharge into the environment is indeed necessary. In Thailand, the Department of Industrial of Works, Ministry of Industry has set a standard for allowable effluent mercury, cadmium and lead concentration at 0.005, 0.03 and 0.2 mg/l, respectively (2). Traditional removal of heavy metals to the low concentration such as ion exchange, precipitation and reverse osmosis are complicated and expensive (3). At present, a biological method is quite promising to solve this problem. The use of various microorganisms such as bacteria (4), yeasts (5), fungus (6), algae (7, 8, 9) and plants tissue (10) have been reported to remove heavy metals from aqueous solution. There are many advantages of using biological method such as using of naturally biomaterials that can be produced cheaply, ability to treat large volume of wastewater due to rapid kinetics and high selectivity in terms of removal and recovery of specific heavy metals (7).

Microalgae have been reported to remove heavy metals from aqueous solution. The advantages of using microalgae such as growing in expose and open air for wastewater treatment, rapid kinetic for heavy metal removal and easy disposal. Mechanism of heavy metal removal follow two phases; first is a rapid metabolism-independent phase with binding or adsorption to cell walls and external surface usually complete 5-10 minutes and the second is slower metabolism dependent phase with transport across the membrane slower lasting hours or day (11). Some microalgae able to accumulate heavy metals by rapid adsorption on cell surfaces and transportation into the cells. *Chlorella vulgaris* (12), green algae, have rapid cadmium adsorption during the first 30 minutes and then continued to be absorbed more slowly. *Tolypothrix tenuis* (13), cyanobacteria, have cadmium adsorption equilibrated within 30 minutes by adsorption of Cd onto the cell surface. *Spirulina platensis* (14), cyanobacteria, was able to accumulate lead at a rapid rate in first 10 minutes and slow down. *Nostoc calcicola* (15), cyanobacteria, had Hg uptake pattern consisted of two phases: rapid binding on cell surface in 10 minutes and its subsequent metabolism-dependent intracellular import at least up to 40 minutes.

This study has focused on screening of microalgal strains, which have high tolerance and high efficiency for mercury (Hg), cadmium (Cd), and lead (Pb) removal. Plate screening method was used for selecting tolerance microalgae strains. Efficiency of removal capacity, the maximum adsorption capacity ( $q_{max}$ ) in aqueous solution and the binding constant (k) were also study.

## 1.2 Objectives

### 1.2.1 General Objective

Screening for microalgal strains which have high efficiency for mercury (Hg), cadmium (Cd) and lead (Pb) removal.

### 1.2.2 Specific Objectives

1.2.2.1 To screen for Hg, Cd and Pb tolerant microalgal strains

1.2.2.2 To study the Hg, Cd and Pb removal capacity (%) and concentration factor (CF) by microalgal strains

1.2.2.3 To study the maximum adsorption capacity ( $q_{max}$ ) and the binding constant (k) of Hg, Cd and Pb removal by selected microalgal strains

## 1.3 Variables of the study

### 1.3.1 Screening for Hg, Cd and Pb tolerant microalgae strains on plate.

Independent Variable	- Microalgal strains
Dependent Variable	- Tolerant of microalgal strains
Control Variable	- Lights

### 1.3.2 Hg, Cd and Pb removal in aqueous solution.

Independent Variable	- Microalgal strains
Dependent Variables	- Hg, Cd and Pb removal capacity (%) - Concentration factor (CF)
Control Variables	- Concentration of Hg, Cd and Pb aqueous solution - pH

### 1.3.3 Hg, Cd and Pb removal in various heavy metal concentrations

Independent Variable	- Concentration of Hg, Cd and Pb aqueous solution
Dependent Variables	- The maximum adsorption capacity ( $q_{max}$ ) - The binding constant (k)
Control Variable	- pH

## 1.4 Research definitions

**Microalgal strains** : the microalgal strains were obtained from the Microbiological Research Center (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), Bangkok and from Gottingen University's cultures collection, Gottingen, Germany. Twelve additionally collected microalgae in Thailand along Chao Praya River, Bangkok and Nonthaburi including industrial area in Samutprakarn.

**Heavy metals (Hg, Cd and Pb)** : Mercury, cadmium and lead were added in solution as  $\text{HgCl}_2$ ,  $\text{Cd}(\text{NO}_3)_2$  and  $\text{Pb}(\text{NO}_3)_2$ , respectively.

**Tolerance of microalgae** : Microalgae can growth on 1.5% agar plate medium with various concentrations of heavy metal.

**Heavy metals (Hg, Cd and Pb) removal capacity (%)** : Heavy metals removal are defined as  $(C_i - C_f) / C_i \times 100$  (%), where  $C_i$  is the initial heavy metal concentration (mg/l) and  $C_f$  is the final heavy metal concentration (mg/l).

**Concentration factor (CF)** is calculated as follow

$$CF = \frac{\text{mg metal removed/g dry wt.}}{\text{mg metal in solution/ml solution}}$$

**Maximum adsorption capacity ( $q_{max}$ ) and the binding constant (k)** are calculated as follow the Langmuir adsorption equation. The Langmuir adsorption equation is calculated by  $q = q_{max} C / (C+k)$  Where  $q$  is the heavy metal adsorbed to the solid phase (mg/g dry wt.), and  $C$  is the equilibrium concentration of heavy metal in solution (mg/l).

## 1.5 Scope of study

**1.5.1 Screening for microalgal strains from Chao Praya River, Bangkok, Nonthaburi and Bang-pu Industrial Estate area in Samutprakarn**

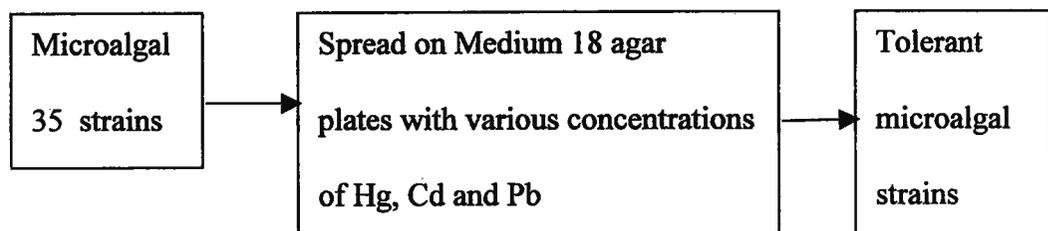
**1.5.2 Screening for microalgal strains that tolerant to Hg, Cd and Pb on plate**

**1.5.3 Removal of Hg, Cd and Pb in aqueous solution**

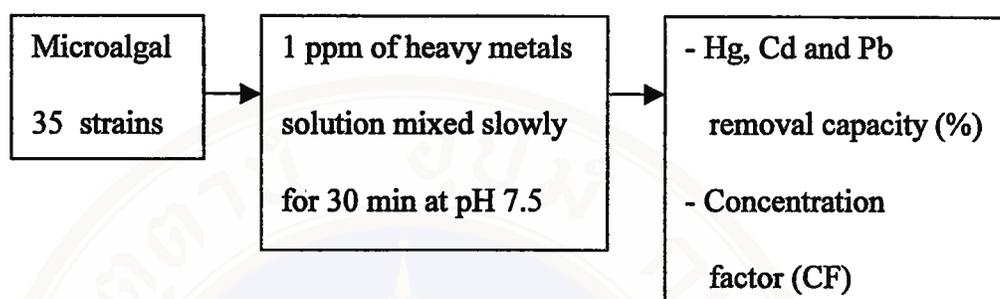
**1.5.4 Study of the maximum adsorption capacity ( $q_{max}$ ) and the binding constant (k) of selected microalgae**

## 1.6 Conceptual Framework

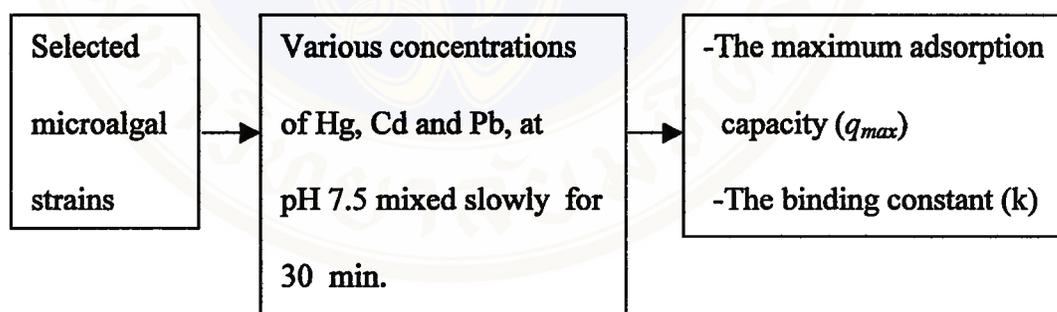
**1.6.1 Screening on plate for Hg, Cd and Pb tolerant microalgal strains**



### 1.6.2 Hg, Cd and Pb removal in aqueous solution



### 1.6.3 Hg, Cd and Pb removal in various heavy metals concentrations



## **CHAPTER II**

### **LITERATURE REVIEW**

#### **2.1 Heavy metals**

Heavy metals is a general collective term applying to the group of metals and metalloids with an atomic density greater than 6 g/cm. Although it is only a loosely defined term it is widely recognized and usually apply to the elements such as Cadmium (Cd), Mercury (Hg), Lead (Pb), Chromium (Cr), Copper (Cu), Nickel (Ni) and Zinc (Zn) which are commonly associated with pollution and toxicity problem. Mercury (Hg) cadmium (Cd) and lead (Pb) are found in wastewaters from a number of industrial and agricultural activities.

Mercury generates concern of any of the heavy-metal pollutants. Mercury is found as a trace component of many minerals, atomic weight 200.59, boiling point 356.9 °C, freezing point -38.87 °C, and specific gravity 13.545. Mercury enters the environment from a large number of miscellaneous sources related to human use of the element. Sewage effluent sometime contains up to 10 times the level of mercury found in typical natural waters. The toxicity of mercury was tragically illustrated in the Minamata Bay area of Japan during the period 1953-1960 have high concentrations reported values up to 600 ng/l of local contamination (1, 3, 16).

Cadmium is the one of the most dangerous elements to human health with atomic weight 112.4, melting point 320.9 °C, boiling point 767 °C and specific gravity 8.65. Cadmium in water arises from industrial discharges and mining wastes.

The pattern of cadmium consumption has changed in recent years with significant decreases in electroplating and increases in batteries and specialized electronic uses. The effects of acute cadmium poisoning in human are very serious. Environmental exposure to cadmium was the case of a disease was first report in 1955 as occurring in Japan. It was called itai-itai disease meaning it hurts, due to the pain caused by the deformed bones (1, 3, 17).

Lead is a malleable metal, atomic weight 207.2, melting point 327.502 °C, valency 0, 2, 4 and specific gravity 11.35. The major sources of lead in the environment arise from the industrial and other technological uses of lead are significance for human health. Man's exposure to lead through water is generally low in comparison with exposure through air and food. Lead from leaded gasoline used to be a major source of atmospheric and terrestrial lead, much of which entered natural water systems. In addition to pollutant sources, lead bearing limestone and galena (PbS) contribute lead to natural waters in some locations. Acute lead poisoning in human causes severe dysfunction in the kidneys, reproductive system, liver, brain and central nervous system. Lead poisoning from environmental exposure is thought to have caused mental retardation in many children. Mild lead poisoning causes anemia. The victim may have headache and sore muscles and may feel irritable (1, 3, 18).

## **2.2 Heavy metals treatment process**

### **2.2.1 Physical-chemical treatment process**

Various physical-chemical treatment processes effectively remove heavy metals from wastewater (3).

Lime treatment removes heavy metals as insoluble hydroxides, basic salts, or coprecipitated with calcium carbonate or ferric hydroxide. Lime precipitation does not normally permit recovery of heavy metals and is sometimes undesirable from the economic viewpoint.

Electrolysis is reduction of metal ions to metal by electrons at an electrode. Electrolysis is process in which one species in solution is reduced by electrons at the cathode and another gives up electrons to the anode and is oxidized there. In hazardous waste applications electrolysis is most widely used in the recovery of cadmium, copper, gold, lead, silver, and zinc.

Reverse osmosis uses high pressures to force permeate through the membrane. It operates on a different principle in that the membrane is selectively permeable to water and excludes ionic solutes.

Ion exchange is a means of removing cations or anions from solution onto a solid resin, which can be regenerated by treatment with acids, bases and salts. The greatest use of ion exchange in hazardous waste treatment is for the removal of low levels of heavy metal ions from wastewater.

Activated carbon adsorption effectively removes some metals from water at the part per million level. Sometimes a chelating agent is sorbed to the charcoal to increase metal removal.

In the past, removal of heavy metals has been largely benefit of wastewater treatment process. Currently, however, more consideration is being given to design and operating parameters that specifically enhance heavy metals removal as part of wastewater treatment.

### 2.2.2 Biosorption treatment process

Many microorganisms are known to be remove metal ion from dilute aqueous solution and accumulating them with in the structure of the microorganism in Table 1 (19). The removal of metal from solution by biological material is now frequently termed "biosorption" (20). Biosorption are importance because the removal of toxic and valuable metals from aqueous effluents can result in detoxification and safe environmental discharge. Bioremoval is better than precipitation in terms of ability to adjust to changes in pH and heavy metal concentrations, and better than ion exchange and reverse osmosis in terms of sensitivity to the presence of suspended solids, organics, and the presence of other heavy metals. The limited data comparing bioremoval with conventional heavy metal removal methods indicated that several advantages are possible with bioremoval precesses including (21)

1. Use of naturally abundant biomaterials that can be cheaply produced
2. Treat large volumes of wastewater due to rapid kinetics
3. High selectivity removal and recovery of specific heavy metals
4. Handle multiple heavy metals and mixed waste (22, 23, 24)
5. Reducing metals to below 1 ppb in many cases
6. Less need for additional expensive process reagents which typically cause disposal and space problems
7. Operation over a wide range of conditions was including temperature, pH and presence of other ions including  $\text{Ca}^{2+}$  and  $\text{Mg}^{+2}$ (13).
8. Low capital investment and low operational costs
9. Reduced volume of hazardous waste produced.

**Table 1** Some example of microbial heavy metal accumulation (16)

<b>Organism</b>	<b>Element</b>	<b>Uptake (% dry weight)</b>
<b>Bacteria</b>		
<i>Thiobacillus ferrooxidans</i>	Silver	25
<i>Bacillus cereus</i>	Cadmium	4-9
<i>Citrobacter sp.</i>	Lead	34-40
	Cadmium	40
<i>Bacillus sp.</i>	Lead	60.1
	Copper	15.2
	Cadmium	21.4
<b>Algae</b>		
<i>Chlorella vulgaris</i>	Gold	10
<i>Chlorella regularis</i>	Manganese	0.8
<b>Fungi</b>		
<i>Phomu sp.</i>	Silver	2
<i>Rhizopus arrhious</i>	Copper	1.6
	Cadmium	3
	Lead	10.4
	Silver	5.4
<b>Yeasts</b>		
<i>Saccharomyces cerevisiae</i>	Zinc	0.5

### 2.3 Algae

The algae are a highly varied group of photoautotrophic organism classified into six major and several minor phylum as showed in Table 2 (25). All algae use CO<sub>2</sub> as a carbon source and light as an energy source (11).

Table 2 Characteristics of major phylum of algae (25)

Phylum	Usual Habitat	Principal Pigments*	Storage Products	Cell Walls	Mode of Motility	Modes of Reproduction
Chlorophyta	Fresh and salt water; soil; treebark.	Chlorophyll-b ; carotenes; Xanthophylls	Starch ( $\alpha$ -1,4-glucan)	Cellulose and pectin	Mostly nonmotile(except one order), but some reproductive elements may be flagellated	Asexual, multiple fission;spore or sexual
Cyanophyta	Fresh water; soil	Carotenes Phycocerythrin Phycocyanin	Glycogen; floridean-starch	Peptidoglycan lipopolysaccharide	Motile cells	Fission;spore or sexual
Euglenophyta	Fresh water	Chlorophyll-b ; carotenes;	Fats; starchlike Carbohydrates	Lacking, but have elastic particle	One to three anterior flagella	Asexual only, by binary fission
Heterokontophyta	Fresh and salt water; soil; higher plant	Xanthophylls Carotenes	Starchlike Carbohydrates ( $\beta$ -1,3-glucan); oils	Pectin, often impregnated with silica or calcium	Unique diatom motility; one; two or more unequal flagella	Asexual or sexual
Dinophyta	Mostly salt water but common in fresh water	Carotenes; Xanthophylls	Starch ; oils	Cellulose and pectin	Two unequal lateral flagella in different planes	Asexual; rarely sexual
Rhodophyta	Mostly salt water but several genera in fresh water	Phycocerythrin and other phycobilins; carotenes; xanthophylls	Starchlike carbohydrates	Cellulose and pectin; agar; carrageenan	Nonmotile	Sexual, ametes; asexual spores

\* In addition to chlorophyll-a, which is present in algae of all phylum

Heavy metals exert their harmful effects to algae in photosynthetic electron transport and photosynthetic carbon fixation resulting in decreased photosynthesis. They have effect to formation of stable precipitates or chelate with essential metabolites such as Fe with ATP and antimetabolic behavior of some anions such as selenate and arsenate where as inhibition of nutrient uptake process, protein synthesis, enzyme activities, respiratory, O<sub>2</sub> consumption and essential metal ions displacement. Impairment of motility and abnormal morphological development obtain effect from heavy metals.

### **2.3.1 Mechanism of heavy metals uptake**

#### **2.3.1.1 Metabolism-independent uptake**

The metabolism-independent phase is a rapid phase with binding or adsorption to cell walls and external surfaces: This is also called biosorption and involves the accumulation of inorganic chemical to cell wall components. Biosorption of heavy metals is often rapid, reversible, and usually complete in 5-10 min in algae. Biosorption is not influenced by light, temperature or the presence of metabolic inhibitors. Most metal accumulated this way is easily removed by washing algae with distilled water or with a chelator such as EDTA (11). For example, *Chlorella vulgaris* and *Chlorella regularis* had rapid cadmium adsorption and approached equilibrium within 10 min and 30 min, respectively (26). Hg<sup>+2</sup> uptake in the cyanobacterium *Nostoc calcicola* involves rapid binding of the cation to the negative charged cell surface at the first 10 min (15). The removal of Cd by the filamentous cyanobacterium *Tolypothrix tenuis* was equilibrated within 30 min and was caused by adsorption of Cd onto the cell surface (13). *Aphanothece halophytica* was able to accumulate lead

rapidly and became saturated within 1 hour. For zinc accumulation, it still increased at a slower rate after a rapid rate in the first 10 min. *Spirulina platensis* was able to accumulate lead at a rapid rate in 10 minutes and slowed down (14).

### 2.3.1.2 Metabolism-dependent uptake

The metabolism-dependent phase is often slow (lasting hours or days) and is inhibited by low temperature, absence of energy source, metabolic inhibitors and uncouplers and influenced by the health of the cells and the characteristic of the growth medium. An important route for intracellular uptake is passive diffusion owing to increased permeability of cell membranes from stress (11).

For example, uptake of Cd by *Chlorella vulgaris* adsorbed at the slow rate during next 72 hours (12). Hg<sup>+2</sup> slow rate uptake in the cyanobacterium *Nostoc calcicola* least up to 40 minutes (15).

### 2.3.2 Heavy metal removal ability

The concentration factor (CF) is the ratio concentration of an element in dry biomass and in water that calculated as follow

$$CF = \frac{\text{mg metal removed/g dry wt.}}{\text{mg metal in solution/ml solution}}$$

This concentration factor is often used to compare heavy metal removal ability among various algae as shown in Table 3.

**Table 3** Heavy metal uptake by algae (7)

Concentration Factor	Metal(s)	Type of Algae
Ca. 4,000	Zn, Cu, Cd	<i>Chroococcus parisi</i>
2,000,000	Cd	<i>Chlorella pyrenoidosa</i>
< 4,000	Cd	<i>Chlorella pyrenoidosa</i>
1,000-100,000	Cd, Pb, Hg	various spp.
500-30,000	Various	various spp.
2,327	Cd	<i>Chlorella</i>
570-31,000	Pb	various spp.
2,000-25,000	Pb	<i>Cladophora glomerata</i>
16,000-20,000	Pb	<i>Cladophora glomerata</i>
620-7,700	Pb, Cd, Zn, Cr	Green algae

### 2.3.3 Factors affecting the biosorption

#### 2.3.3.1 pH

The relationship between pH and toxicity of heavy metals to aquatic biota can be divided into 2 groups. The first group is decreasing in pH results in a decreased biological response and the second is the effect of low pH is to increase metals availability. Optimal pH for bioremoval is unpredictable and dependent on the type of algae used and other conditions. For example, the accumulation of lead and zinc by the blue green algae, *Aphanothece halophylica* increased at pH above 6.5 and 6.0, respectively (14). Cadmium uptake capacity by the marine algal *Sargassum polycystum* dried at 80 °C had highest cadmium uptake at pH 4.0 (27).

A summary of the published literature reporting the optimal pH for the uptake of toxic metals appears in Table 4.

**Table 4** Optimal pH for binding of selected metals by algal (7)

<b>Metal ion</b>	<b>Optimal pH</b>	<b>Algal species</b>
Cd (II)	> 5.0	<i>Chlorella vulgaris</i>
Cd (II)	8.0	<i>Navicula pelliculosa</i>
Cd (II)	6.0	<i>Chlamydomonas sp.</i>
Cd (II)	7.0	<i>Chlorella pyrenoidosa</i>
Cd (II)	7.0	<i>Scenedesmus obliquus</i>
Cd (II)	5.0	<i>Ulothrix fimbriata</i>
Cd (II)	8.0	<i>Nostoc sp.</i>
Cd (II)	7.0	<i>Oscillatoria sp.</i>
Cd (II)	7.0	<i>Stichococcus bacillaris</i>
Hg (II)	3-7	<i>Chlorella vulgaris</i>
Hg (II)	6.0	<i>Navicula pelliculosa</i>
Hg (II)	4.0	<i>Chlamydomonas sp.</i>
Hg (II)	8.0	<i>Chlorella pyrenoidosa</i>
Hg (II)	6.0	<i>Scenedesmus obliquus</i>
Hg (II)	10.0	<i>Ulothrix fimbriata</i>
Hg (II)	4.0	<i>Nostoc sp.</i>
Hg (II)	5.0	<i>Oscillatoria sp.</i>
Hg (II)	9.0	<i>Schizothrix calcicola</i>
Pb (II)	> 5.0	<i>Chlorella vulgaris</i>
Pb (II)	9.0	<i>Chlamydomonas sp.</i>
Pb (II)	9.0	<i>Mougeotia sp.</i>
Pb (II)	9.0	<i>Ulothrix fimbriata</i>
Pb (II)	9.0	<i>Nostoc muscorum</i>
Pb (II)	9.0	<i>Oscillatoria sp.</i>
Pb (II)	10.0	<i>Schizothrix calcicola</i>

### 2.3.3.2 Temperature

Temperature significantly influences metal bioremoval. The binding of most metals to algae by biosorption is enhanced as temperature is increased. For example, the reduction of Cr (VI) to Cr (III) is increased as temperature is increased

from 25 °C to 55 °C (7). Some research suggests that cooler temperatures decrease metal stress, which may be a consequence of inhibited metabolism (11).

#### 2.3.3.3 Salinity

There been a reported of salinity inhibition on growth of *Chlorella ellipsoidea* when the cells were grown in media containing 2.5 ppm of cadmium. *Chlorella salina* in 5 ppm of cadmium with high salt concentration could still survive and grow well (28).

#### 2.3.3.4 Density of cell

The total lead accumulation of blue green algae, *Aphanothese halophylotica* and *Spirulina platensis* increased with increasing density of cells. The efficiency of accumulation was unchanged when the density of cell was up to 4 mg dry wt./ml for *A. halophylotica* and *S. platensis*, the efficiency of lead accumulation was reduced when the density of cell increased up to 1.6 mg dry wt./ ml (14).

#### 2.3.3.5 Aging effect

Aging of microalgae cell could affect the heavy metals accumulation. For example, the ability of lead accumulation by *Aphanothese halophylotica* was reduced when the age of cell increased up to 14 days. The accumulation of lead by *Spirulina platensis* was reduced when the age of cell increased up to 8 days (14).

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Research design

This research study was designed as screening of microalgal strains from Chao Praya River, Bangkok and Nonthaburi and Bang-pu Industrial Estate area in Samutprakarn. The experiment was performed in laboratory.

#### 3.2 The place of experiment

The experiment was performed at the laboratory of the Department of Environmental Health Sciences, Faculty of Public Health, Mahidol University.

#### 3.3 Microalgae

##### Cyanobacteria

Twenty one microalgal strains: *Anabaena siamensis* TISTR, *Anabaena torulosa* TISTR, *Anabaena variabilis* TISTR M-2, *Aurosisa fertilissima* TISTR M-126, *Calothrix parietina* TISTR 8093, *Calothrix sp.* TISTR 8113, *Calothrix sp.* TISTR 8130, *Nostoc muscorum* TISTR M-14, *Nostoc muscorum* TISTR 8164, *Nostoc sp.* TISTR AMN, *Scytonema schmidlei* TISTR 8207 and *Tolypothrix tenuis* TISTR 8063 were obtained from the Microbiological Resources Center (MIRCEN), Thailand Institute of Scientific

and Technological Research (TISTR), Bangkok. *Spirulina platensis* IRFD No.9 was obtained from Institute of Research and Food Product Development, Kasetsart University, Bangkok. T2, T3, T6, T7, T9, T11 and T12 strains were isolated from Chao Praya River, Bangkok and Bang-pu Industrial Estate, Sumutprakarn and Osaka strain was isolated from Yodo River in Osaka, Japan.

### **Green algae**

Fourteen microalgal strains: *Chlorella saccharophilla* CCAP211/1A, *Chlorella vulgaris* CCAP211/11B and *Scenedesmus vacuolatus* CCAP211 were obtained from Gottingen University's culture collection, Gottingen, Germany. *Chlorella sp.* TISTR and *Kirchneriella sp.* TISTR were obtained from Thailand Institute of Scientific and Technological Research (TISTR). *Chlorella ellipsoidea* IFRPD1162 *Chlorella vulgaris* IFRPD1118, *Scenedesmus acutus* IFRPD1020 and *Scenedesmus pertoratus* IFRPD1010 were obtained from Institute of Research and Food Product Development, Kasetsart University, Bangkok. T1, T4, T5, T8 and T10 were isolated from Chao Praya River, Bangkok and Bang-pu Industrial Estate, Sumutprakarn.

## **3.4 Equipment and chemical reagents**

### **Equipment**

1. Autoclave HA-240M, Hirayama, Japan
2. Magnetic stirrer SR 306, Advantec, Japan
3. Dry thermo bath serial 02344 Boekel, U.S.A.

4. Hot air oven Memmert, Germany
5. Centrifuge Hetich universal II, D-7200, Japan
6. Vortex mixer K-550-GE, U.S.A.
7. Lamina air flow RFC 120000, Australia
8. Micropipette Gilson 20, 250, 1000 M, France
9. pH-meter Eutech Cybernetics pH scan I, Singapore
10. Atomic absorption spectrophotometer Z-8200 Polarized, Zeeman, Hitachi, Japan
11. Atomic absorption spectrophotometer Varian, Model Spectr AA600, Australia
12. Balance meter AT-201, Switzerland
13. Desiccator, Auto-C series, Sanplatec, Japan
14. Milli-Q UF Plas, Millipore, France

#### **Glasses and others**

1. Petridish
2. Erlenmeyer flask size 125 and 250 ml
3. Volumetric flask 50 and 100 ml
4. Cylinder size 25 and 100 ml
5. Spreader
6. Test tubes

**Chemical reagents:**  $\text{Cd}(\text{NO}_3)_2$  and  $\text{Pb}(\text{NO}_3)_2$  were from Unilab, Australia.  $\text{HgCl}_2$ ,  $\text{HNO}_3$  90% and  $\text{NaNO}_3$  were from Carlo erba, Italy.  $\text{HNO}_3$  69%,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  were from BDH Laboratory Supplies, England.  $\text{H}_2\text{O}_2$ ,  $\text{NaOH}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,

$K_2HPO_4$  and  $CaCl_2 \cdot 2H_2O$  were from Merck, Germany.  $Fe(SO_4)_3 \cdot nH_2O$ ,  $NaMoO_4 \cdot 2H_2O$ ,  $H_3BO_3$ ,  $MnSO_4 \cdot 4H_2O$  and  $CuSO_4 \cdot 5H_2O$  were from May and Baker Ltd. Dagenham, England. Agar power Pearl mermaid (commercial grade), Thailand. Methyl alcohol was from the government pharmaceutical organization, Thailand. NaCl was from Riedel-de HaenAG Seeize-Hannover, Germany.

## **3.5 Experiment methods**

### **3.5.1 Screening and isolation of microalgae**

#### **3.5.1.1 Isolation**

Water at the surface of the canal and river were sampled in 500 ml of sampling bottles and microalgae were sampled by using nylon sieve (50  $\mu$ ) across the surface of the water and added in the same sampling bottle at the same sampling place. Some filamentous strains were picked up from the rock or soil along the sampling site. Temperature and pH were measured at the surface of the water. Two ml of sampled water were added to a test tube with 8 ml of Medium18 and incubated at 28 °C under illumination of white fluorescent light 4,000 lux for light 12 hours and 12 hours dark for 2 weeks. The samples were streaked on Medium18 agar plate with 1.5% agar and incubated at 28 °C under illumination of white fluorescent light 4,000 lux for light 12 hours and 12 hours dark. After the appearance of grown cells within 2 weeks, each single colony was picked up and re-streaked on Medium 18 agar plates many times to obtain an

unialgal culture. Single colonies were incubated into 2 ml of Medium 18 at the same condition for 1 week as stock culture.

### 3.5.1.2 Cultivation system

Fifteen ml of stock culture were incubated in 150 ml of Medium 18 (see Appendix) in 200 ml test tubes. Microalgae were cultivated under illumination with white fluorescent lamp of 4000 lux under the light 12 hours and 12 hours dark interval in medium 18 for 7- 10 days before using as a stock culture in this experiment. Mixed air bubbles provided aeration system with 1% CO<sub>2</sub> supply.

## 3.5.2 Screening on plate for Hg, Cd and Pb tolerant strains

### 3.5.2.1 Preparation of Hg, Cd and Pb solution

Hg, Cd and Pb were added in stock solution as HgCl<sub>2</sub>, Cd (NO<sub>3</sub>)<sub>2</sub> and Pb(NO<sub>3</sub>)<sub>2</sub>, respectively. Stock solution of Hg, Cd and Pb at 100 mM were prepared by adding 2.715 g of HgCl<sub>2</sub>, 3.085 g of Cd (NO<sub>3</sub>)<sub>2</sub> and 3.31 g of Pb(NO<sub>3</sub>)<sub>2</sub> to 100 ml of milli-Q water (Milli-Q UF Plus, Millipore, France) and filtered with millipore paper. The stock solution was kept in refrigerator. The calculation of heavy metals concentration was done by using an equation;  $M_1V_1 = M_2V_2$  where as  $M_1$  is stock solution concentration;  $M_2$  is require concentration;  $V_1$  is volume of stock solution;  $V_2$  is volume of solution at required concentration.

### 3.5.2.2 Screening on plates

Microalgae strains 0.25 ml was spread on Medium 18 plates containing heavy metal at various concentrations; Hg at concentration of  $1.5 \times 10^{-3}$ -  $5 \times 10^{-3}$  mM (0.25-1 ppm), Cd at concentration of 0.05-0.4 mM (5-45 ppm) and Pb at concentration of

0.05-1.25 mM (10-256 ppm). Spread plates were incubated under illumination with white fluorescent of 4000 lux for light 12 hours and 12 hours dark interval. After 2 weeks or growth appeared and growth was observed in comparison with control plates without heavy metal.

### **3.5.3 Hg, Cd and Pb removal in aqueous solution**

#### **3.5.3.1 Hg, Cd and Pb removal ability**

Microalgal strains 0.5 g wet wt. was used. The cells were centrifuged and washed twice in deionized water. The cells were then suspended in 50 ml of 1 mg/l of Hg, Cd and Pb solution pH 7.5, respectively. The suspensions were mixed on magnetic stirrer slowly (200 rpm) and the cell-free solution was sampled at 30 min. The heavy metals removal ability was calculated from  $(C_i - C_f) / C_i \times 100$  (%), where  $C_i$  is the initial concentration (mg/l) and  $C_f$  is the final concentration (mg/l). Samples of each heavy metal were measured by using atomic absorption spectrophotometer.

#### **3.5.3.2 Time course of Hg, Cd and Pb removal by selected microalgae**

Microalgal strains 0.5 g wet wt. was used. The cells were centrifuged and washed twice in deionized water. The cells were then suspended in 50 ml of 1 mg/l of Hg, Cd and Pb solution, respectively. The suspension were mixed on magnetic stirrer slowly (200 rpm) and 1 ml of the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min, respectively. Samples of each heavy metal were measured by using atomic absorption spectrophotometer.

### 3.5.3.3 Calculation of concentration factor

The concentration factor (CF) is used to compare heavy metal removal ability in different strains of microalgae. The concentration factor is calculated as follow:

$$CF = \frac{\text{mg metal removed/g dry wt.}}{\text{mg metal in solution/ml solution}}$$

### 3.5.4 Hg, Cd and Pb removal with various heavy metal concentrations

The selected microalgal strains (0.5 g wet wt.) were used. The cells were centrifuged and washed twice in deionized water. The cells were then suspended in 50 ml of various heavy metal solution; Hg concentration of 0.5, 1, 2, 5, 10, 20, 40, 80 and 150 mg/l; Cd concentration of 5, 10, 20, 40, 80, 100, 150 and 200 mg/l and Pb concentration of 5, 10, 20, 40, 80, 160, 200, 400 and 600 mg/l. Each sample was mixed slowly for 30 min. Heavy metal concentration was measured by using atomic absorption spectrophotometer. The adsorption of Hg, Cd and Pb,  $q_{max}$  (mg/g dry wt.), was determined as described previously.  $q_{max}$  and  $k$  were calculated by using Langmuir equation as

$$q = \frac{q_{max} C}{C + k}$$

Where  $q$  is the heavy metal adsorbed to the solid phase (mg/g dry wt.)

$q_{max}$  is the maximum adsorption capacity (mg/g dry wt.)

$k$  is the binding constant (mg/l)

$C$  is the equilibrium concentration of heavy metal in solution (mg/l)

### **3.5.5 Dry weight determination**

Microalgal cells were separated from aqueous solution; blue green algae were filtered with nylon sieve and green algae were centrifuged at 4000 rpm for 10 min, respectively. The cells were washed 3 times with deionized water and dried in an oven at 100°C for 24 hours or until dry weight became constant. This was followed by cooling down in desiccator for 30 min and dry weight was measured.

### **3.5.6 Digestion of microalgal cells**

The dried sample was digested with 1 ml of conc.  $\text{HNO}_3$  in a dry thermo bath until the solution was dried. After cooling, 1 ml of 30%  $\text{H}_2\text{O}_2$  was added. The sample was digested for 1 hour or until the solution was evaporated to dryness. This step was repeated 2 times until the white ash was obtained. Five ml of 0.5 N  $\text{HNO}_3$  was added to the digested sample and heavy metal concentration was measured by an atomic absorption spectrophotometer.

### **3.5.7 Atomic absorption analysis**

#### **3.5.7.1 Hg analysis**

For Hg analysis, the samples were diluted by milli-Q water before measuring in the range of 25-40 ppb by using graphite system wavelength 253.7 nm. Sample of Hg solution was measured by atomic absorption spectrophotometer (Varian, Model Spectr AA600, Australia).

### 3.5.7.2 Cd analysis

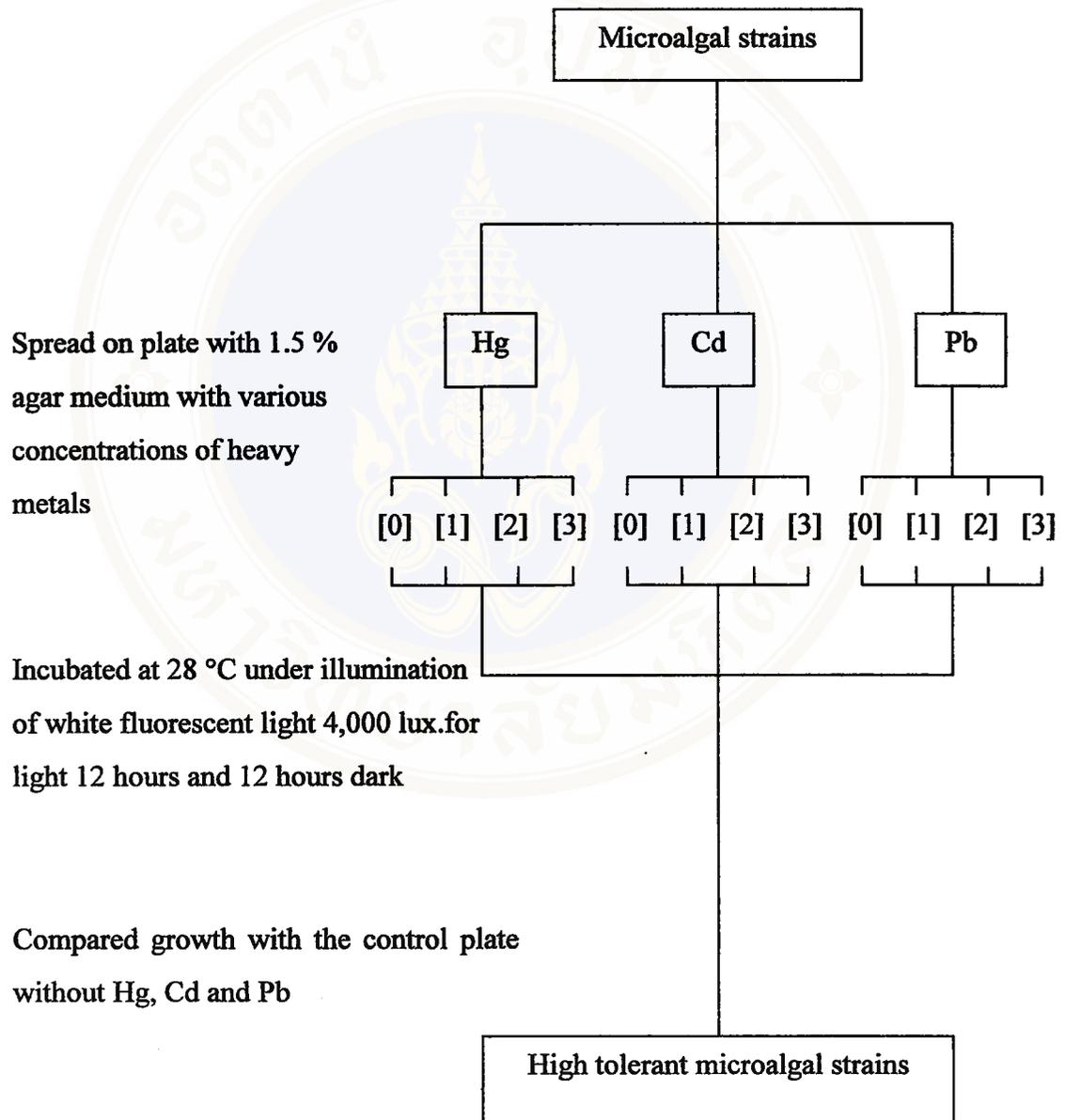
The sample of Cd solution was measured by atomic absorption spectrophotometer (Z-8200 Polarized Zeeman, Hitachi, Japan) by using graphite system wavelength 228.8 nm. The sample solution was fixed by using 0.05 HNO<sub>3</sub>.

### 3.5.7.3 Pb analysis

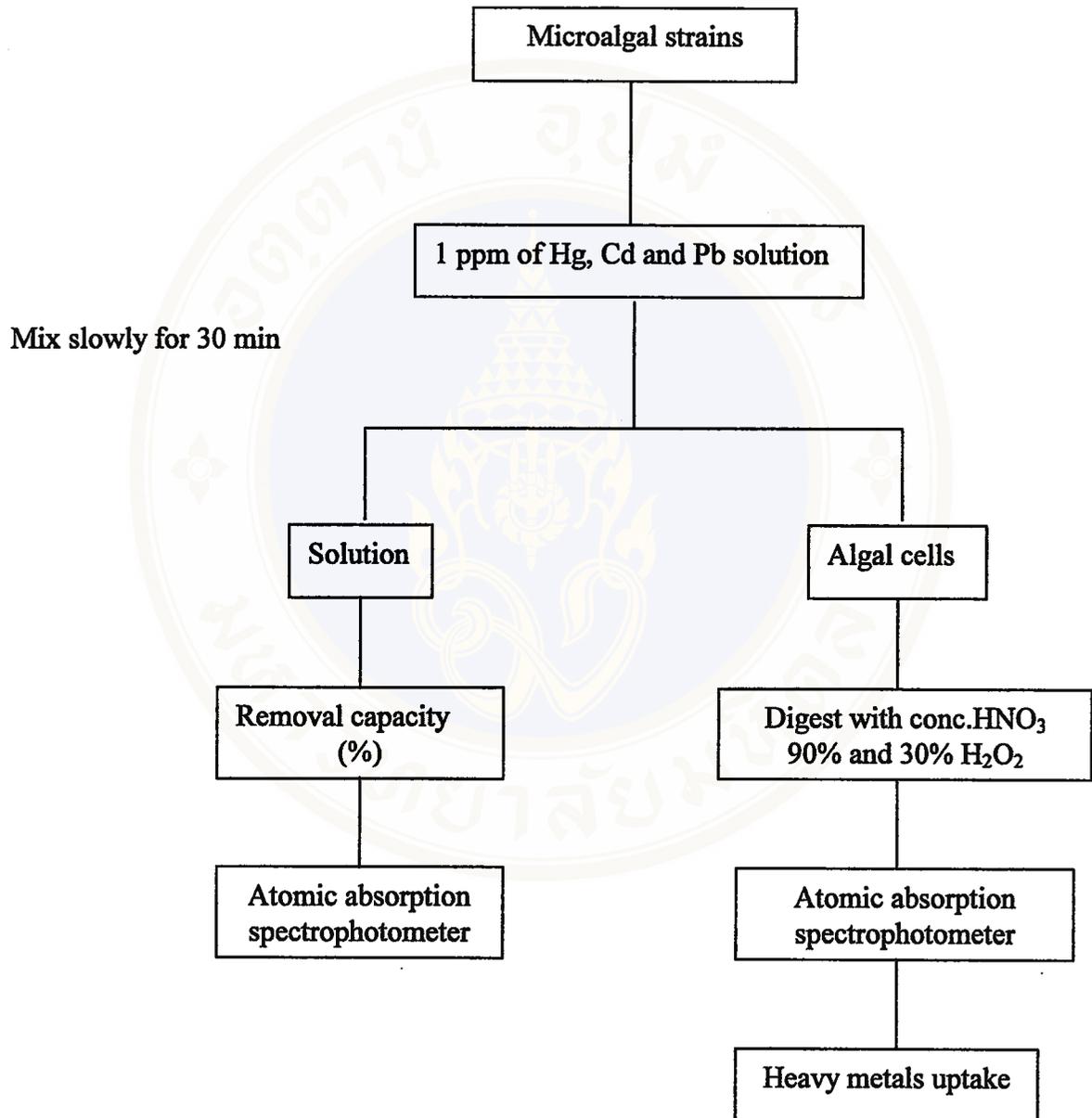
The sample of Pb solution was measured by atomic absorption spectrophotometer (Z-8200 Polarized Zeeman, Hitachi, Japan) by using graphite system wavelength 283.3 nm. The sample solution was fixed by using 0.05 HNO<sub>3</sub>.

### 3.6 Research diagram

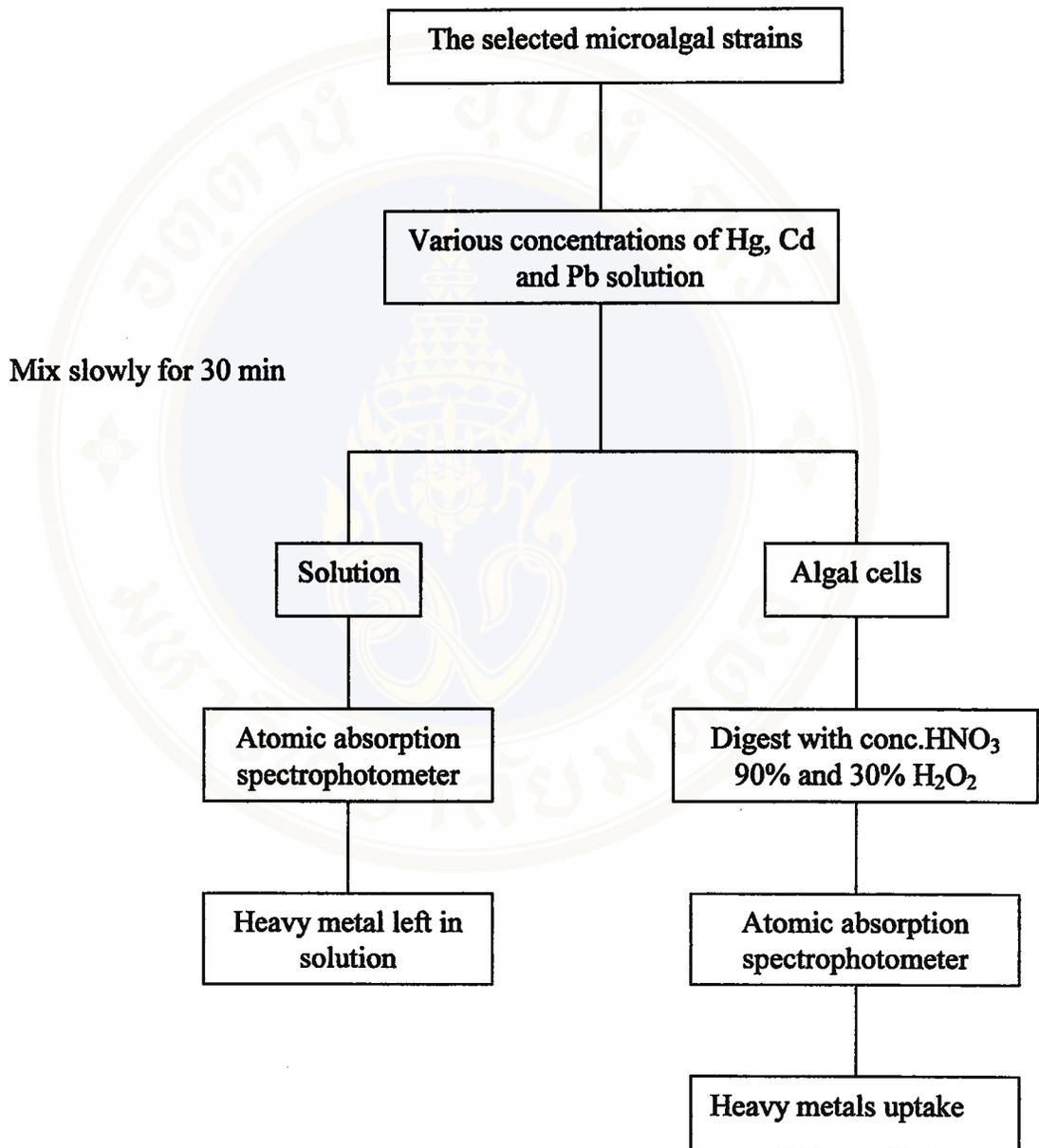
#### 3.6.1 Screening on plate for Hg, Cd and Pb tolerant microalgal strains



### 3.6.2 Removal of Hg, Cd and Pb in aqueous solution



### 3.6.3 Hg, Cd and Pb removal with various heavy metal concentrations



## CHAPTER IV

### RESULTS

#### 4.1 Screening and isolation of microalgae

Microalgae were screened from Chao Praya River, Bangkok and Nonthaburi and Bang-pu Industrial Estate area in Samutprakarn. At the sampling point, microalgae were sampled by using nylon sieve (50  $\mu$ ) across the surface of water as shown in Fig. 1. Twelve unialgal strains as shown in Table 5 were obtained by re-streak on plates.



**Fig. 1** Sampling of microalgae from Chao Praya River and Bang-pu Industrial Estate area

**Table 5** Microalgae isolated from Chao Praya River and Bang-pu Industrial Estate area in Samutprakarn

<b>Microalgae</b>	<b>Sampling point</b>	<b>Type</b>
T1	Rajathiwas Temple, Bangkok	green algae
T2	Thepnaree Temple's Ferry, Bangkok	cyanobacteria
T3	Pak-Kret, Nonthaburi	cyanobacteria
T4	Thai Containers Industry Co.Ltd. Bang-pu Industrial Estate, Samutprakarn	green algae
T5	Thai Containers Industry Co.Ltd. Bang-pu Industrial Estate, Samutprakarn	green algae
T6	Thai Containers Industry Co.Ltd. Bang-pu Industrial Estate, Samutprakarn	cyanobacteria
T7	Pak-Kret, Nonthaburi	cyanobacteria
T8	HWA Fong Rubbur Co.Ltd. Bang-pu Industrial Estate Soi 6, Samutprakarn	green algae
T9	Tawanna Containers Co.Ltd. Bang-pu Industrial Estate Soi 14, Samutprakarn	cyanobacteria
T10	Thai Pattana iron Industry Co.Ltd. Bang-pu Industrial Estate Soi 14, Samutprakarn	green algae
T11	Sam mongkut Industry Co.Ltd Bang-pu Industrial Estate Soi 10, Samutprakarn	cyanobacteria
T12	Vac Vute Co.Ltd. Bang-pu Industrial Estate Soi 7, Samutprakarn	cyanobacteria

#### 4.2 Screening on plate for Hg, Cd and Pb tolerant microalgal strains

Microalgae (0.25 ml) were spread on Medium 18 plates (1.5% agar) with various concentrations of Hg, Cd and Pb. Plates were incubated with light illumination of 4000 lux. Growth rates were classified into three level with respect to the control as shown in Fig. 2 and 3. Table 6 showed growth of microalgae in agar plates which contained Hg at concentrations of  $1.5 \times 10^{-3}$  mM (0.25 ppm),  $2.4 \times 10^{-3}$  mM (0.5 ppm) and  $5 \times 10^{-3}$  mM (1 ppm). At concentrations of  $1.5 \times 10^{-3}$  mM (0.25 ppm) and  $2.4 \times 10^{-3}$  mM (0.5 ppm) many strains could grow but *Calothrix sp.*, *Nostoc sp.*, *Nostoc muscorum*, *Scytonema schmidlei*, *Tolypothrix tenuis*, T2, T3, T6, T7 and T12 could not grow. At the highest concentration tested,  $5 \times 10^{-3}$  mM (1 ppm) of Hg, green algae could grow, except *Kirchneriella sp.*, T1, T4 and T5. For cyanobacteria, *Anabaena siamensis*, *Anabaena torulosa*, *Aurosisa fertisissima*, *Calothrix sp.*, *Calothrix parietina* TISTR 8113, *Nostoc muscorum* TISTR 523 and T11 could grow but the other strains could not grow.

In Table 7, microalgae were spreaded on agar plates containing Cd at concentrations of 0.05 mM (5.7 ppm), 0.2 mM (22.8 ppm) and 0.4 mM (45.6 ppm). Cadmium started to inhibit growth in some strains at 0.05 mM (5.7 ppm). At 0.2 mM (22.8 ppm) Cd started to inhibit growth in many strains. Green algae; *Chlorella sp.*, *Chlorella eillipsoidea*, *Chlorella vulgaris*, *Scenedesmus acutus*, *Scenedesmus pertoratus* and T1 could grow and for cyanobacteria only *Tolypothrix tenuis* and *Spirulina platensis* could grow. At the highest concentration tested of 0.4 mM (45.6 ppm), no growth was observed in all strains.



In plates containing Pb at concentrations of 0.05 mM (10.4 ppm) and 0.5 mM (104 ppm) all strains could grow except *Anabaena variabilis*, *Aurosisia fertisissima* and *Spirulina platensis*. Lead started to inhibit growth in a few strains at 0.5 mM (104 ppm). At the highest concentration of 1.25 mM (256 ppm), green algae; *Chlorella sp.*, *Chlorella eillipsoidea*, *Chlorella vulgaris*, *Scenedesmus acutus*, *Scenedesmus pertoratus* and T5 could grow. For cyanobacteria only *Nostoc sp.* and *Scytonema schmidlei* could grow. The result from this study indicated that relative toxicity in decreasing order was Hg > Cd > Pb.

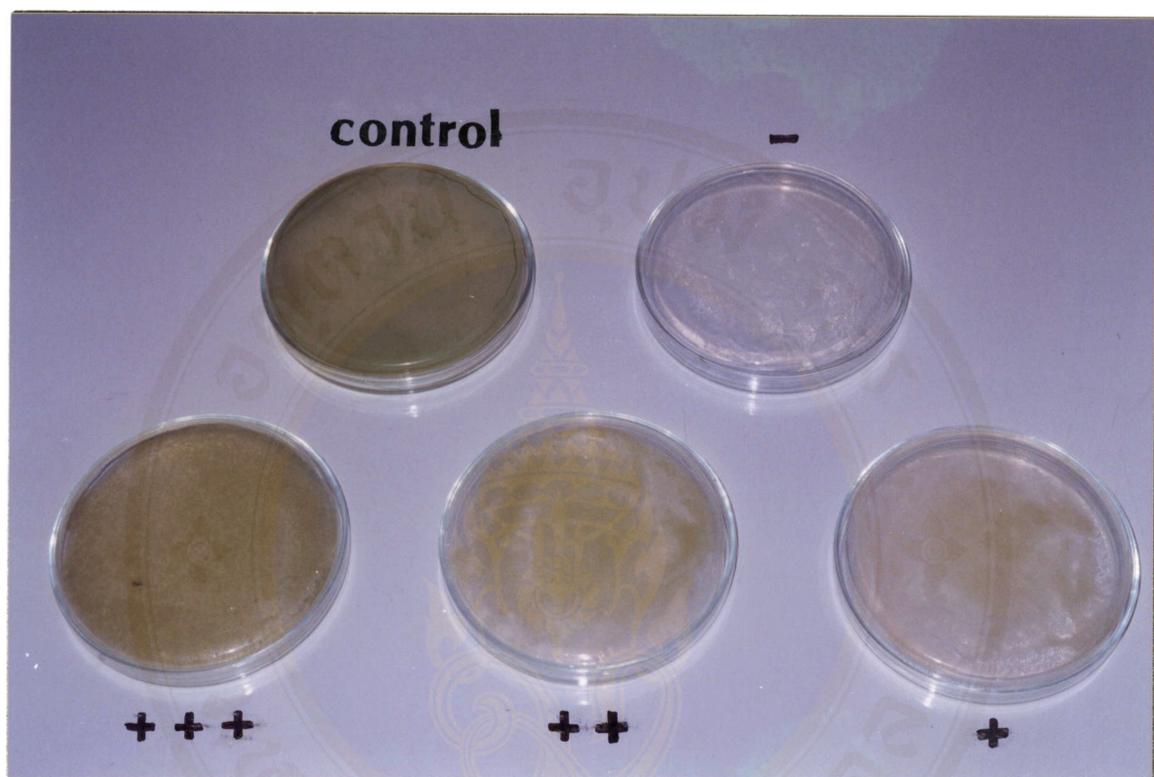
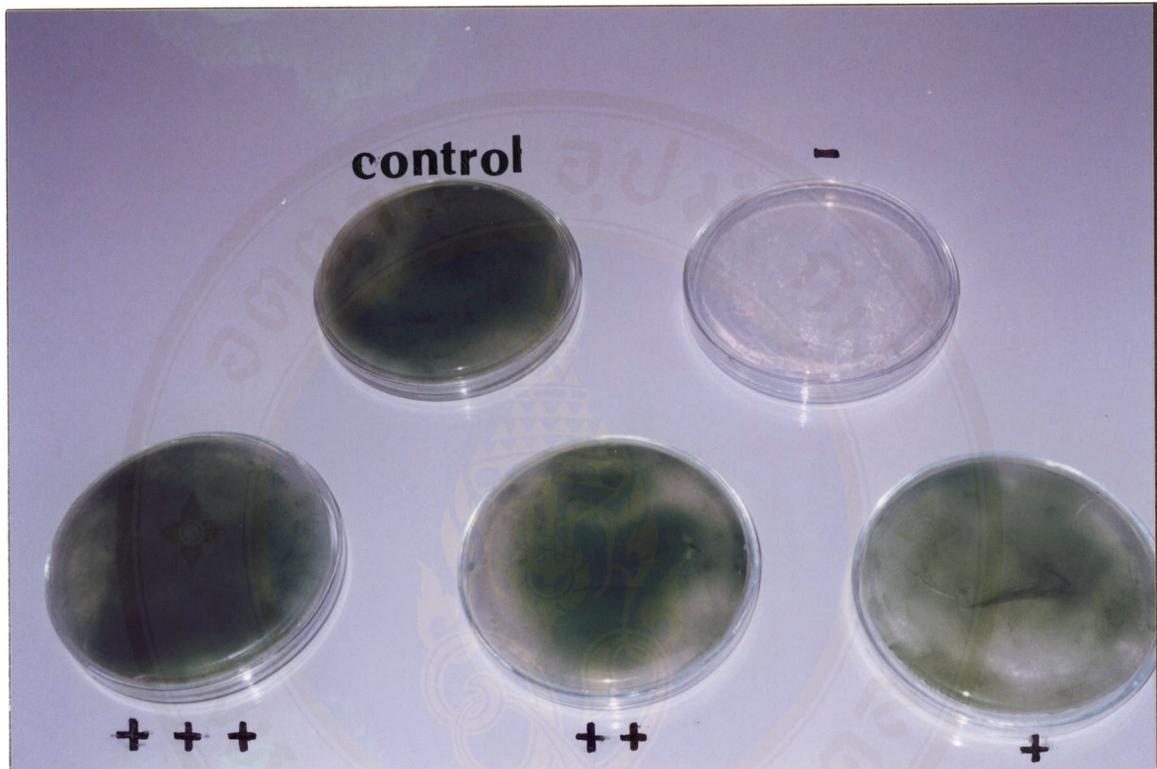


Fig. 2 Screening of green algae on plate

Microalgae were spreaded on Medium 18 plates (1.5% agar) with various concentrations of Hg, Cd and Pb. Plates were incubated with light illumination of 4000 lux. Growth rates were defined as follow; +++: High growth (as control), ++: medium growth, +: Low growth, -: No growth



**Fig. 3** Screening of cyanobacteria on plate

Microalgae were spreaded on Medium 18 plates (1.5% agar) with various concentrations of Hg, Cd and Pb. Plates were incubated with light illumination of 4000 lux. Growth rates were defined as follow; +++: High growth (as control), ++: medium growth, +: Low growth, -: No growth

**Table 6** Growth of microalgae in the presence of Hg

Microalgae	Concentration of Hg			
	Control	0.25 ppm $1.5 \times 10^{-3}$ mM	0.5 ppm $2.4 \times 10^{-3}$ mM	1 ppm $5 \times 10^{-3}$ mM
<b>Green algae</b>				
<i>Chlorella sp.</i>	+++	+++	++	++
<i>Chlorella ellipsoidea</i>	+++	++	++	+
<i>Chlorella saccharophilla</i>	+++	++	+	+
<i>Chlorella vulgaris</i>	+++	++	+	+
<i>Chlorella vulgaris</i> (CCAP 211/11B)	+++	+++	++	++
<i>Kirchneriella sp.</i>	+++	+++	++	-
<i>Scenedesmus acutus</i>	+++	++	++	+
<i>Scenedesmus pertoratus</i>	+++	+++	++	+
<i>Scenedesmus vacuolatus</i>	+++	++	+	+
T1	+++	++	+	-
T4	+++	++	+	-
T5	+++	++	+	-
T8	+++	++	++	-
T10	+++	++	+	+
<b>Cyanobacteria</b>				
<i>Anabaena siamensis</i>	+++	+++	+++	++
<i>Anabaena torulosa</i>	+++	+++	+++	++
<i>Anabaena variabilis</i>	+++	++	++	-
<i>Aurosisa fertississima</i>	+++	++	+	+
<i>Calothrix sp.</i> TISTR 8130	+++	+	-	-
<i>Calothrix sp.</i> TISTR 8113	+++	+++	++	+
<i>Calothrix parietina</i>	+++	+++	+++	+
<i>Nostoc sp.</i>	+++	++	-	-
<i>Nostoc muscorum</i> TISTR 8164	+++	++	++	+
<i>Nostoc muscorum</i> TISTR M-14	+++	-	-	-
<i>Osaka</i>	+++	++	+	-
<i>Spirulina platensis</i>	+++	++	+	-
<i>Scytonema schmidlei</i>	+++	+	-	-
<i>Tolypothrix tenuis</i>	+++	-	-	-
T2	+++	+	-	-
T3	+++	+	-	-
T6	+++	++	-	-
T7	+++	+	-	-
T9	+++	++	+	-
T11	+++	++	++	+
T12	+++	-	-	-

Microalgae were spread on Medium 18 plates (1.5% agar) with various concentrations of Hg. Plates were incubated with light illumination of 4000 lux. Growth rates were defined as follow; +++: High growth (as control), ++: medium growth, +: Low growth, -: No growth

**Table 7** Growth of microalgae in the presence of Cd

Microalgae	Concentration of Cd			
	Control	5.7 ppm 0.05 mM	22.8 ppm 0.2 mM	45.6 ppm 0.4 mM
<b>Green algae</b>				
<i>Chlorella sp.</i>	+++	++	+	-
<i>Chlorella ellipsoidea</i>	+++	+++	++	-
<i>Chlorella saccharophilla</i>	+++	++	-	-
<i>Chlorella vulgaris</i>	+++	++	+	-
<i>Chlorella vulgaris</i> (CCAP 211/11B)	+++	++	-	-
<i>Kirchneriella sp.</i>	+++	++	-	-
<i>Scenedesmus acutus</i>	+++	++	+	-
<i>Scenedesmus pertoratus</i>	+++	++	+	-
<i>Scenedesmus vacuolatus</i>	+++	++	-	-
T1	+++	++	+	-
T4	+++	+	-	-
T5	+++	+	-	-
T8	+++	+	-	-
T10	+++	-	-	-
<b>Cyanobacteria</b>				
<i>Anabaena siamensis</i>	+++	-	-	-
<i>Anabaena torulosa</i>	+++	-	-	-
<i>Anabaena variabilis</i>	+++	-	-	-
<i>Aurosisia fertisissima</i>	+++	+	-	-
<i>Calothrix sp.</i> TISTR 8130	+++	-	-	-
<i>Calothrix sp.</i> TISTR 8113	+++	-	-	-
<i>Calothrix parietina</i>	+++	-	-	-
<i>Nostoc sp.</i>	+++	-	-	-
<i>Nostoc muscorum</i> TISTR 8164	+++	-	-	-
<i>Nostoc muscorum</i> TISTR M-14	+++	-	-	-
<i>Osaka</i>	+++	-	-	-
<i>Spirulina platensis</i>	+++	++	+	-
<i>Scytonema schmidlei</i>	+++	+	-	-
<i>Tolypothrix tenuis</i>	+++	++	+	-
T2	+++	+	-	-
T3	+++	+	-	-
T6	+++	-	-	-
T7	+++	-	-	-
T9	+++	-	-	-
T11	+++	-	-	-
T12	+++	-	-	-

Microalgae were spread on Medium 18 plates (1.5% agar) with various concentrations of Cd. Plates were incubated with light illumination of 4000 lux. Growth rates were defined as follow; +++: High growth (as control), ++: medium growth, +: Low growth, -: No growth

**Table 8** Growth of microalgae in the presence of Pb

Microalgae	Concentration of Pb			
	Control	10.4 ppm 0.05 mM	104 ppm 0.5 mM	256 ppm 1.25 mM
<b>Green algae</b>				
<i>Chlorella sp.</i>	+++	+++	+++	+
<i>Chlorella ellipsoidea</i>	+++	+++	+++	+
<i>Chlorella saccharophilla</i>	+++	+++	+++	-
<i>Chlorella vulgaris</i>	+++	+++	+++	+
<i>Chlorella vulgaris</i> (CCAP 211/11B)	+++	+++	+	-
<i>Kirchneriella sp.</i>	+++	+++	+++	-
<i>Scenedesmus acutus</i>	+++	+++	+++	+
<i>Scenedesmus pertoratus</i>	+++	+++	+++	+
<i>Scenedesmus vacuolatus</i>	+++	+++	+	-
T1	+++	+++	+++	-
T4	+++	+++	+++	-
T5	+++	+++	+++	+
T8	+++	+++	++	-
T10	+++	+++	++	-
<b>Cyanobacteria</b>				
<i>Anabaena siamensis</i>	+++	+++	+	-
<i>Anabaena torulosa</i>	+++	+++	+	-
<i>Anabaena variabilis</i>	+++	+++	-	-
<i>Aurosisa fertilissima</i>	+++	+++	-	-
<i>Calothrix sp.</i> TISTR 8130	+++	+++	+	-
<i>Calothrix sp.</i> TISTR 8113	+++	+++	++	-
<i>Calothrix parietina</i>	+++	+++	++	-
<i>Nostoc sp.</i>	+++	+++	+	+
<i>Nostoc muscorum</i> TISTR 8164	+++	+++	++	-
<i>Nostoc muscorum</i> TISTR M-14	+++	+++	+	-
<i>Osaka</i>	+++	+++	++	-
<i>Spirulina platensis</i>	+++	+++	-	-
<i>Scytonema schmidlei</i>	+++	+++	++	+
<i>Tolypothrix tenuis</i>	+++	+++	+++	-
T2	+++	+++	+	-
T3	+++	+++	+	-
T6	+++	+++	+	-
T7	+++	+++	++	-
T9	+++	+++	++	-
T11	+++	+++	++	-
T12	+++	+++	++	-

Microalgae were spread on Medium 18 plates (1.5% agar) with various concentrations of Pb. Plates were incubated with light illumination of 4000 lux. Growth rates were defined as follow; +++: High growth (as control), ++: medium growth, +: Low growth, -: No growth

### 4.3 Hg, Cd and Pb removal in aqueous solution

#### 4.3.1 Hg, Cd and Pb removal ability

The trend in this result showed that Hg, Cd and Pb removal capacity by green algae was higher than cyanobacteria such as *Scenedesmus acutus* had high Hg, Cd and Pb removal capacity 85.18%, 87.89% and 88.89%, respectively. *Chlorella sp.*, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus pertoratus* also have high removal capacity and *Chlorella eillipsoidea* had quite high Hg and Cd removal capacity 72.60% and 77.73%, respectively but had very low Pb removal capacity 27.08%. In cyanobacteria *Tolypothrix tenuis* had Hg, Cd and Pb removal capacity 81.42%, 87.77% and 87.68% and *Calothrix parietina* had Hg, Cd and Pb removal capacity 50.26%, 87.89% and 90.59%, respectively. In some cyanobacteria; *Anabaena siamensis*, *Spirulina platensis*, *Scytonema schmidlei* and T6 had high Cd removal capacity 71.96%, 76.54%, 70.36% and 76.76%, respectively as shown in Table 9.

#### 4.3.2 Concentration factor of Hg, Cd and Pb removal

Heavy metals removal by microalgae as concentration factor (CF) was shown in Table 10. Concentration factor of green algae; *Scenedesmus acutus* and *Scenedesmus pertoratus* had higher value than other green algae. *Scenedesmus acutus* had CF of Hg, Cd and Pb at 3412, 4591 and 4078, respectively and *Scenedesmus pertoratus* had CF of Hg, Cd and Pb at 2711, 3730 and 3083, respectively. For cyanobacteria, *Calothrix parietina*, *Spirulina platensis* T2 and T3 had higher concentration factor for three heavy metals than other cyanobacteria. *Tolypothrix tenuis* had highest concentration factor at 3028 and 2963 in Cd and Pb, respectively.

**Table 9** Heavy metal removal in aqueous solution by microalgae

Microalgae	Heavy metal removal (%)		
	Hg	Cd	Pb
<b>Green algae</b>			
<i>Chlorella sp.</i>	71.44*	87.93*	77.02*
<i>Chlorella eilipsoidea</i>	72.60	77.73*	27.08*
<i>Chlorella saccharophilla</i>	70.31	80.98	78.82
<i>Chlorella vulgaris</i>	74.34*	85.80*	84.68
<i>Chlorella vulgaris</i> (CCAP 211/11B)	75.27*	83.41*	81.18*
<i>Kirchneriella sp.</i>	72.08	80.18	80.82
<i>Scenedesmus acutus</i>	85.18	87.89*	88.89*
<i>Scenedesmus pertoratus</i>	78.58	83.14	79.54*
<i>Scenedesmus vacuolatus</i>	81.30	72.71	85.15
T1	68.21	83.50*	71.78
T4	72.10	76.40	68.03
T5	73.22	93.85	74.86*
T8	69.05	77.98	70.91
T10	70.15	81.07	73.69
<b>Cyanobacteria</b>			
<i>Anabaena siamensis</i>	32.10*	71.96	21.24
<i>Anabaena torulosa</i>	28.97*	40.33	48.81
<i>Anabaena variabilis</i>	40.93	53.16	35.04
<i>Aurosisa fertisissima</i>	14.00	66.04	22.36
<i>Calothrix sp.</i> TISTR 8130	40.07	81.73	85.67
<i>Calothrix sp.</i> TISTR 8113	37.16	84.44	87.98
<i>Calothrix parietina</i>	50.26*	87.89	90.59
<i>Nostoc sp.</i>	12.00	16.77	25.97
<i>Nostoc muscorum</i> TISTR 8164	22.25	35.90	33.50
<i>Nostoc muscorum</i> TISTR M-14	28.17	40.57	32.37
<i>Osaka</i>	45.12	76.14	43.29
<i>Spirulina platensis</i>	36.47	76.54*	24.33
<i>Scytonema schmidlei</i>	35.67	70.36	28.90*
<i>Tolypothrix tenuis</i>	81.42	87.77*	87.68
T2	75.83	68.87	36.31
T3	46.86	26.14	26.71
T6	45.83	76.76	25.18
T7	20.37	66.43	22.33
T9	36.44	47.31	26.43
T11	42.42	66.10	36.61
T12	38.92	72.28	41.27

Remark \* high tolerance strains which could grow on plate containing with heavy metal; Hg concentration of 1 ppm ( $1.5 \times 10^{-3}$  mM), Cd 22.8 ppm (0.05 mM) and Pb 256 ppm (1.25 mM)

**Table 10** Heavy metal removal in aqueous solution by microalgae expressed as concentration factor (CF)

Microalgae	Concentration Factor (CF)		
	Hg	Cd	Pb
<b>Green algae</b>			
<i>Chlorella sp.</i>	857	1184	1167
<i>Chlorella eillipsoidea</i>	1745	2018	953
<i>Chlorella saccharophilla</i>	869	1196	895
<i>Chlorella vulgaris</i>	1785	2601	2293
<i>Chlorella vulgaris</i> (CCAP 211/11B)	488	980	863
<i>Kirchneriella sp.</i>	1066	1372	1066
<i>Scenedesmus acutus</i>	3412	4591	4078
<i>Scenedesmus pertoratus</i>	2711	3730	3083
<i>Scenedesmus vacuolatus</i>	1274	1351	1358
T1	1060	1537	793
T4	1226	1463	1069
T5	327	774	586
T8	1200	1452	1149
T10	1460	1878	1442
<b>Cyanobacteria</b>			
<i>Anabaena siamensis</i>	803	1492	437
<i>Anabaena torulosa</i>	606	1022	1284
<i>Anabaena variabilis</i>	839	1256	678
<i>Aurosisa fertisissima</i>	1122	1776	839
<i>Calothrix sp.</i> TISTR 8130	825	1722	1478
<i>Calothrix sp.</i> TISTR 8113	725	1749	1404
<i>Calothrix parietina</i>	1096	1292	1784
<i>Nostoc muscorum</i> TISTR 8164	504	872	658
<i>Nostoc muscorum</i> TISTR M-14	609	940	608
<i>Nostoc sp.</i>	234	343	586
<i>Osaka</i>	870	1778	907
<i>Spirulina platensis</i>	1228	2096	1305
<i>Scytonema schmidlei</i>	648	2242	438
<i>Tolythrix tenuis</i>	919	3028	2963
T2	1951	2392	1748
T3	2313	2375	1640
T6	1271	1732	672
T7	353	1640	549
T9	1203	1113	791
T11	1024	1520	751
T 12	1044	1776	860

Remark \* Concentration factor is calculated from

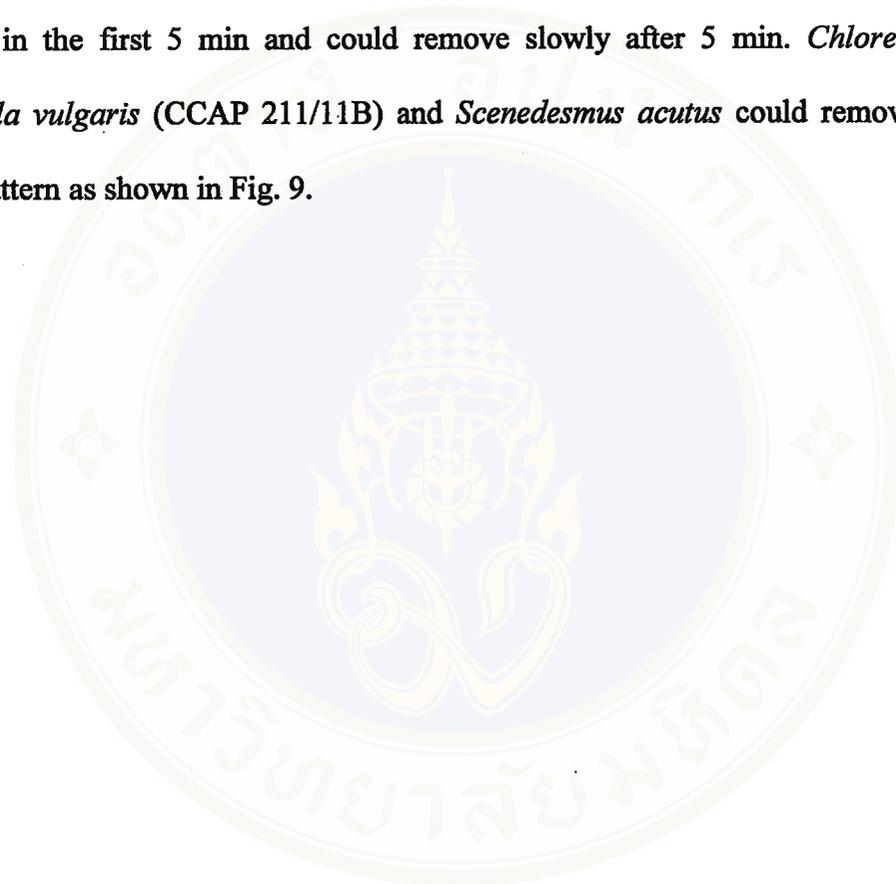
$$CF = \frac{\text{mg heavy metal removal/g dry wt.}}{\text{mg heavy metal in solution/ml solution}}$$

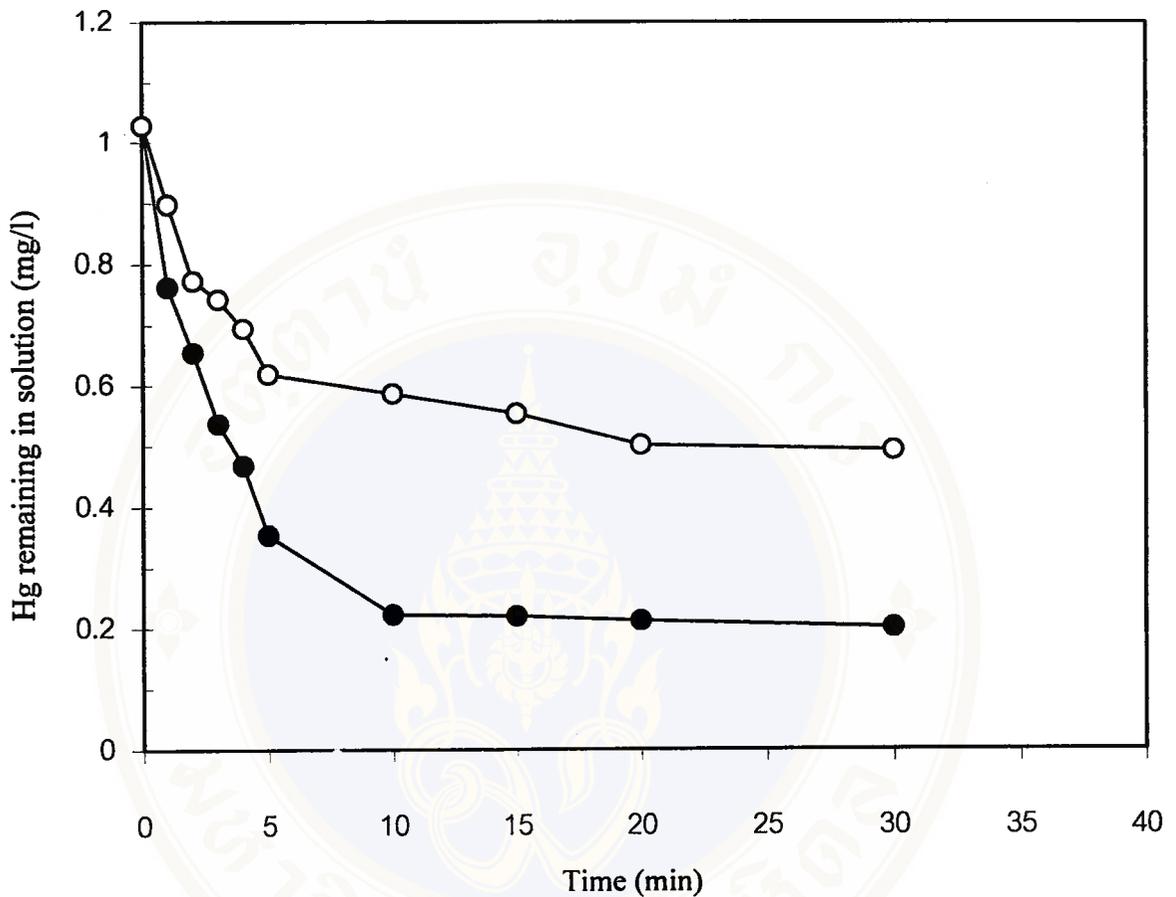
From the results of removal ability and concentration factor, 3 strains of green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* were selected for the further experiment. Because of their high tolerance on plate, high removal capacity and high concentration factor. Two strains of cyanobacteria *Tolypothrix tenuis* and *Calothrix parietina* were selected for the further study because they had high removal capacity and easily to separate by filtration because of filamentous morphology which is amenable for application in out door condition.

#### 4.3.3 Time courses of Hg, Cd and Pb removal by selected microalgae

Figure 4 showed Hg remaining in solution in 2 cyanobacteria *Tolypothrix tenuis* and *Calothrix parietina* could remove Hg in solution rapidly with in 10 min and 20 min, respectively and became equilibrium. *Tolypothrix tenuis* could remove Hg 2-fold better than *Calothrix parietina* 2 times. For green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus*, the rate of Hg removal was rapid in the first 5 min and slow down until equilibrium at 20 min but in *Scenedesmus acutus* it still decreased as shown in Fig. 5. For cyanobacteria, *Tolypothrix tenuis* and *Calothrix parietina* could remove Cd from solution rapidly in the first 5 min, slow down and became equilibrium at 15 min *Tolypothrix tenuis* could remove Cd as same pattern as *Calothrix parietina* (Fig. 6). In green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* could remove Cd rapidly in the first 5 min and could removed slowly after 5 min. *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* could remove Cd with the same pattern as shown in Fig. 7. As shown in Fig. 8, *Tolypothrix tenuis* and *Calothrix parietina* could remove Pb

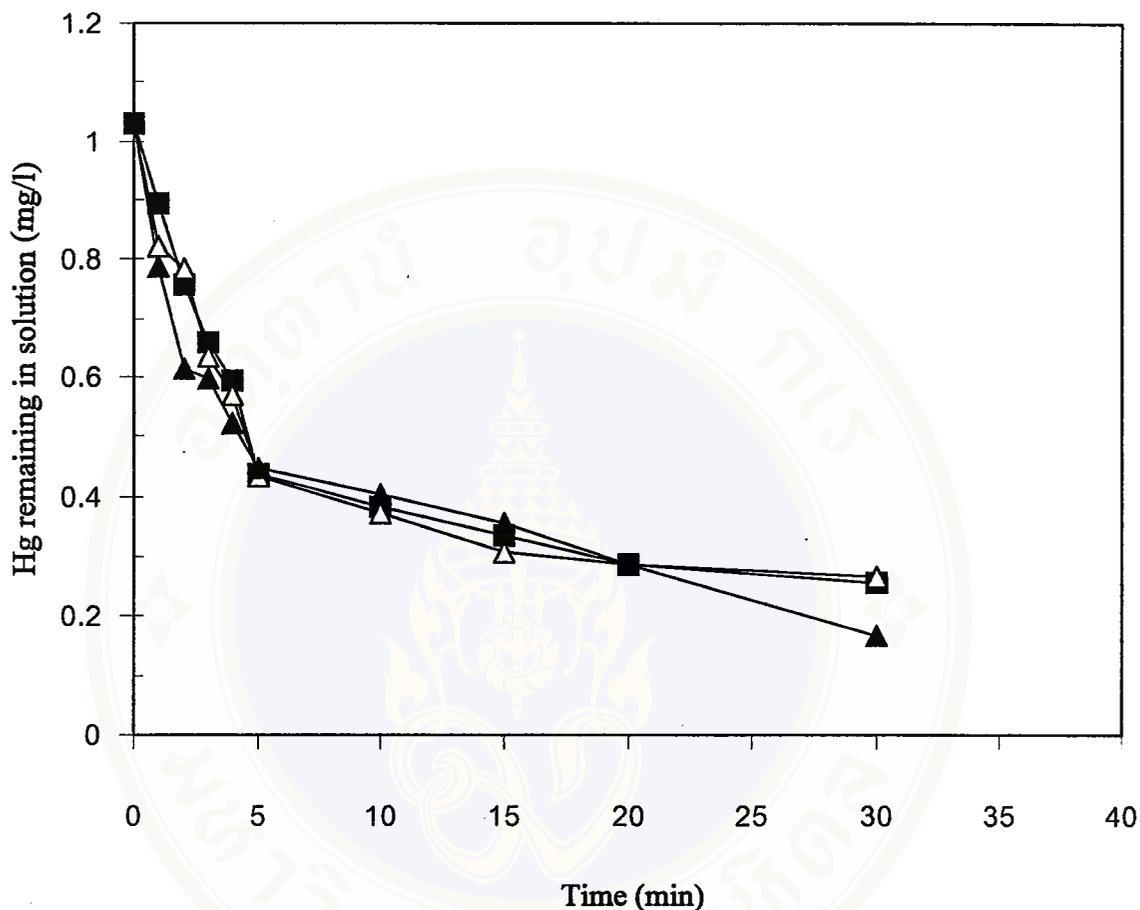
from solution rapidly within 10 min and were equilibrium after that. *Tolypothrix tenuis* could remove Pb at the same level as *Calothrix parietina*. In green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* could remove Pb rapidly in the first 5 min and could remove slowly after 5 min. *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* could remove Cd in the same pattern as shown in Fig. 9.





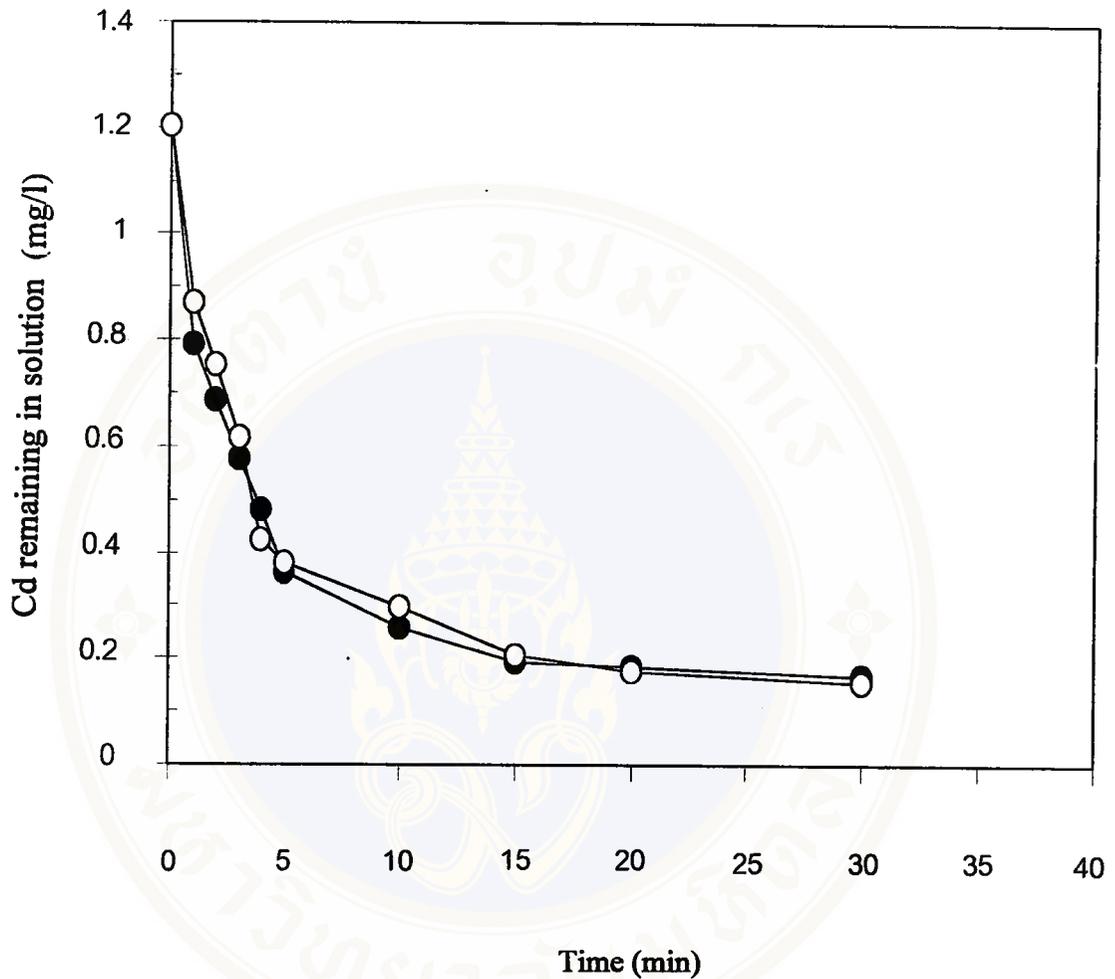
**Fig. 4** Time courses of Hg concentration in aqueous solution

Microalgal cells (0.5 g wet wt.) were suspended in 50 ml of 1 ppm of Hg solution. The suspensions were mixed on magnetic stirrer slowly and the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. Symbols: ●, *Tolypothrix tenuis*; ○, *Calothrix parietina*



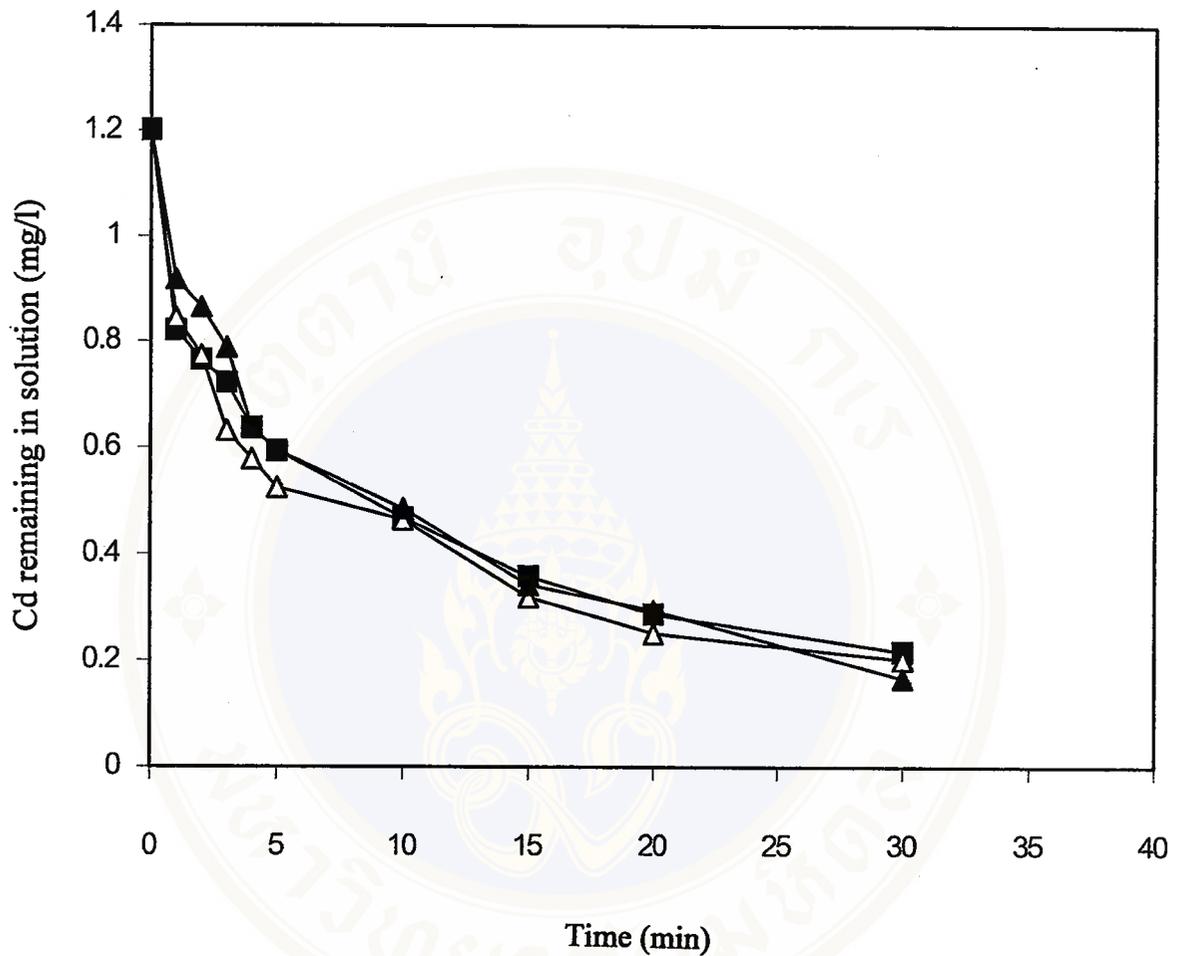
**Fig. 5** Time courses of Hg concentration in aqueous solution

Microalgal cells (0.5 g wet wt.) were suspended in 50 ml of 1 mg/l of Hg solution. The suspensions were mixed on magnetic stirrer slowly and the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. Symbols; ■, *Chlorella vulgaris*; △, *Chlorella vulgaris* (CCAP 211/11B); ▲, *Scenedesmus acutus*



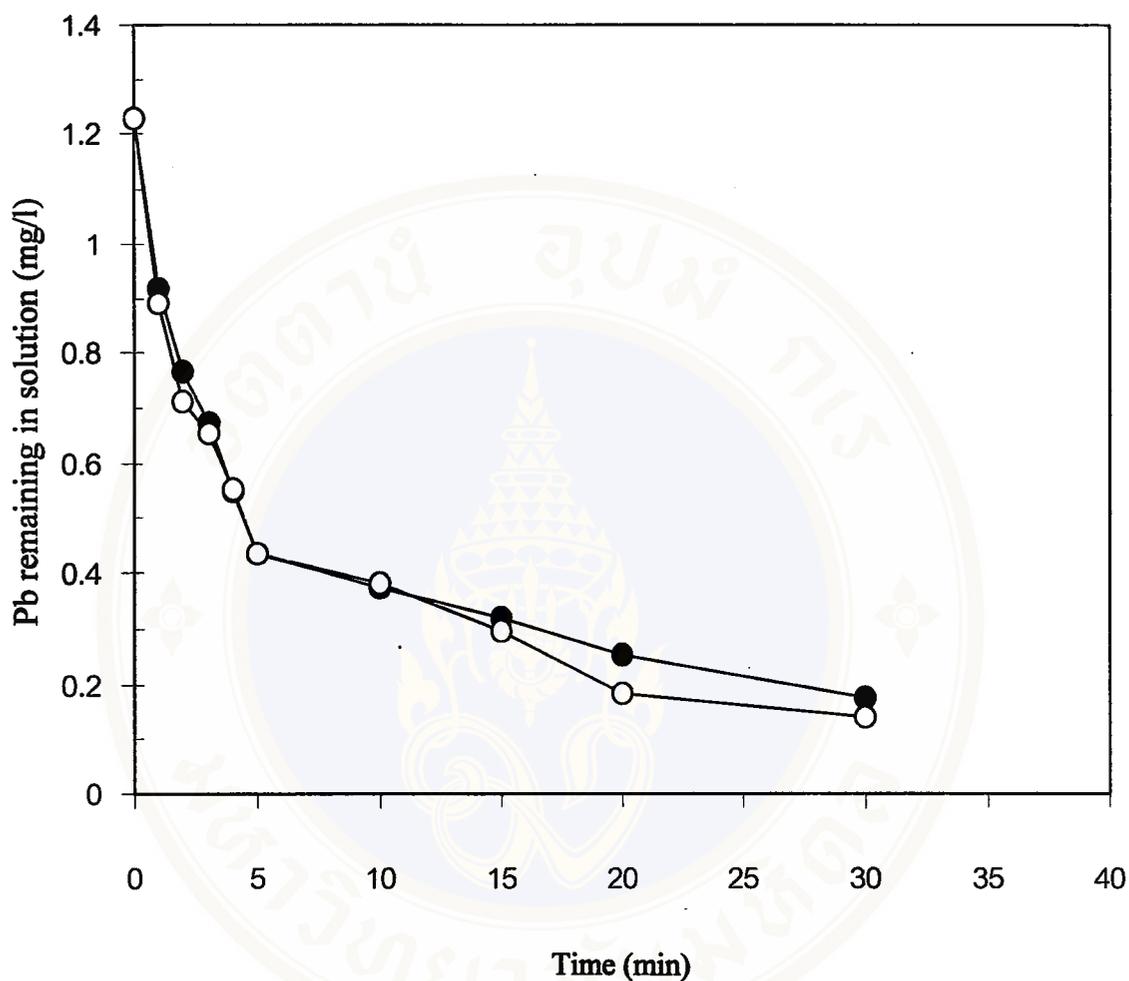
**Fig. 6** Time courses of Cd concentration in aqueous solution

Microalgal cells (0.5 g wet wt.) were suspended in 50 ml of 1 ppm of Cd solution. The suspensions were mixed on magnetic stirrer slowly and the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. Symbols: ●, *Tolypothrix tenuis*; ○, *Calothrix parietina*



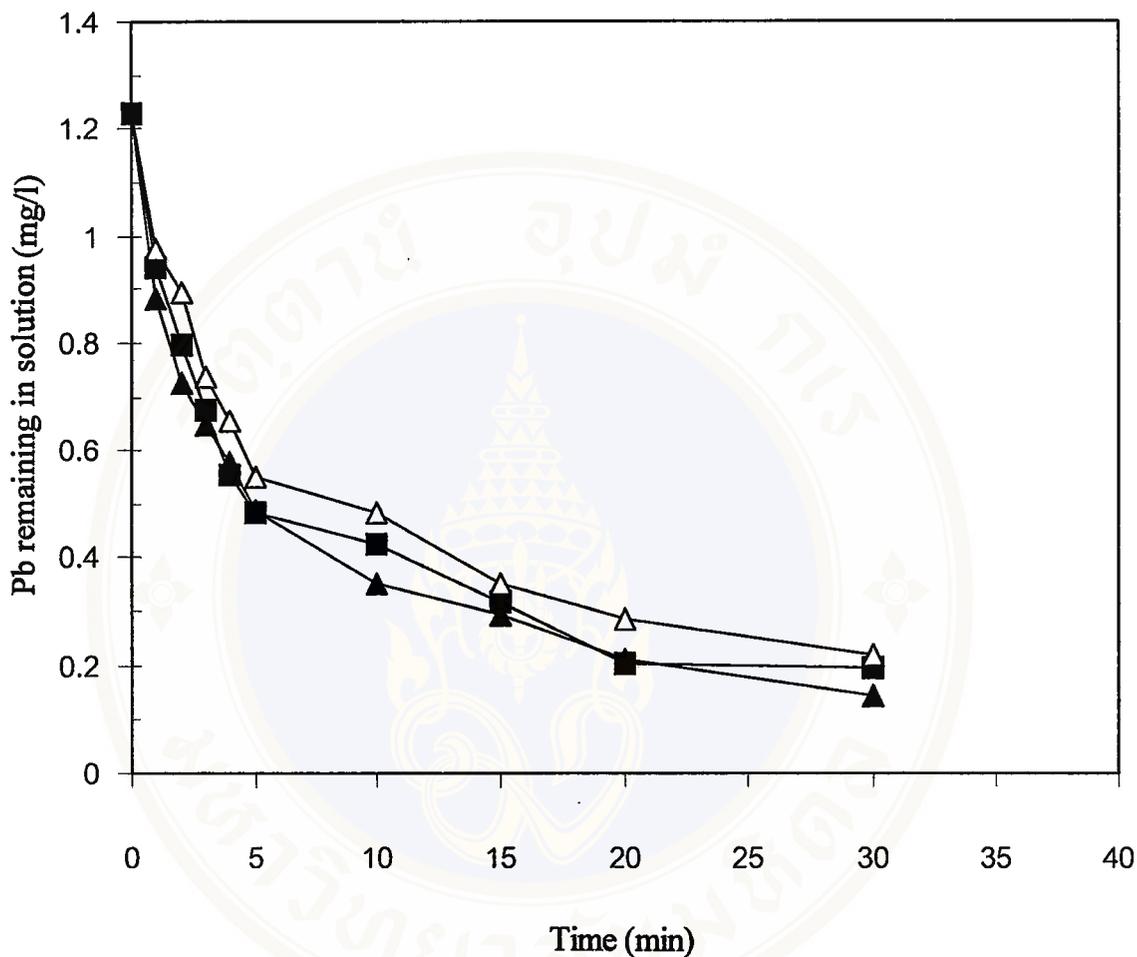
**Fig. 7** Time courses of Cd concentration in aqueous solution

Microalgal cells (0.5 g wet wt.) were suspended in 50 ml of 1 mg/l of Cd solution. The suspensions were mixed on magnetic stirrer slowly and the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. Symbols; ■, *Chlorella vulgaris*; Δ, *Chlorella vulgaris* (CCAP 211/11B); ▲, *Scenedesmus acutus*



**Fig. 8** Time courses of Pb concentration in aqueous solution

Microalgal cells (0.5 g wet wt.) were suspended in 50 ml of 1 mg/l of Pb solution. The suspensions were mixed on magnetic stirrer slowly and the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. Symbols: ●, *Tolypothrix tenuis*; ○, *Calothrix parietina*



**Fig. 9** Time courses of Pb concentration in aqueous solution

Microalgal cells (0.5 g wet wt.) were suspended in 50 ml of 1 mg/l of Pb solution. The suspensions were mixed on magnetic stirrer slowly and the cell-free solution was sampled for 1 ml at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 30 min. Symbols: ■, *Chlorella vulgaris*; △, *Chlorella vulgaris* (CCAP 211/11B); ▲, *Scenedesmus acutus*

#### 4.4 Hg, Cd and Pb removal with various heavy metal concentrations

Three strains of green algae, *Chlorella vulgaris* (IFRDP1118), *Chlorella vulgaris* (CCAP211/11B) and *Scenedesmus acutus* were selected for the further study because of their high tolerance on plate, high removal capacity and high concentrations factor. Two strains of cyanobacteria *Tolypothrix tenuis* and *Calothrix parietina* were selected for this experiment because they had high removal capacity and as filamentous strains they are easy to separate and can be applied to use in outdoor application.

The Langmuir equation used to describe in this experiment. The Langmuir equation is given by

$$q = \frac{q_{max} C}{C+k}$$

Where  $q$  is the heavy metal adsorbed to the solid phase (mg/g dry wt.),  $q_{max}$  is the maximum adsorption capacity (mg/g dry wt.),  $k$  is the binding constant (mg/l), and  $C$  is the equilibrium concentration of heavy metal in solution (mg/l). The maximum adsorption capacity ( $q_{max}$ ) and the binding constant ( $k$ ) is shown in Table 11.

The maximum adsorption capacity ( $q_{max}$ ) of Hg for two cyanobacteria, *Tolypothrix tenuis* and *Calothrix parietina* were 26.86 and 19.01 mg Hg/g dry wt., respectively. The  $q_{max}$  for 3 strains of green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* were 17.87, 15.60 and 19.64 mg Hg/g dry wt., respectively. This result indicated that the cyanobacteria, *Tolypothrix tenuis* could adsorb Hg more than *Calothrix parietina* and also more than 3 green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus*. In case of Cd, green algae,

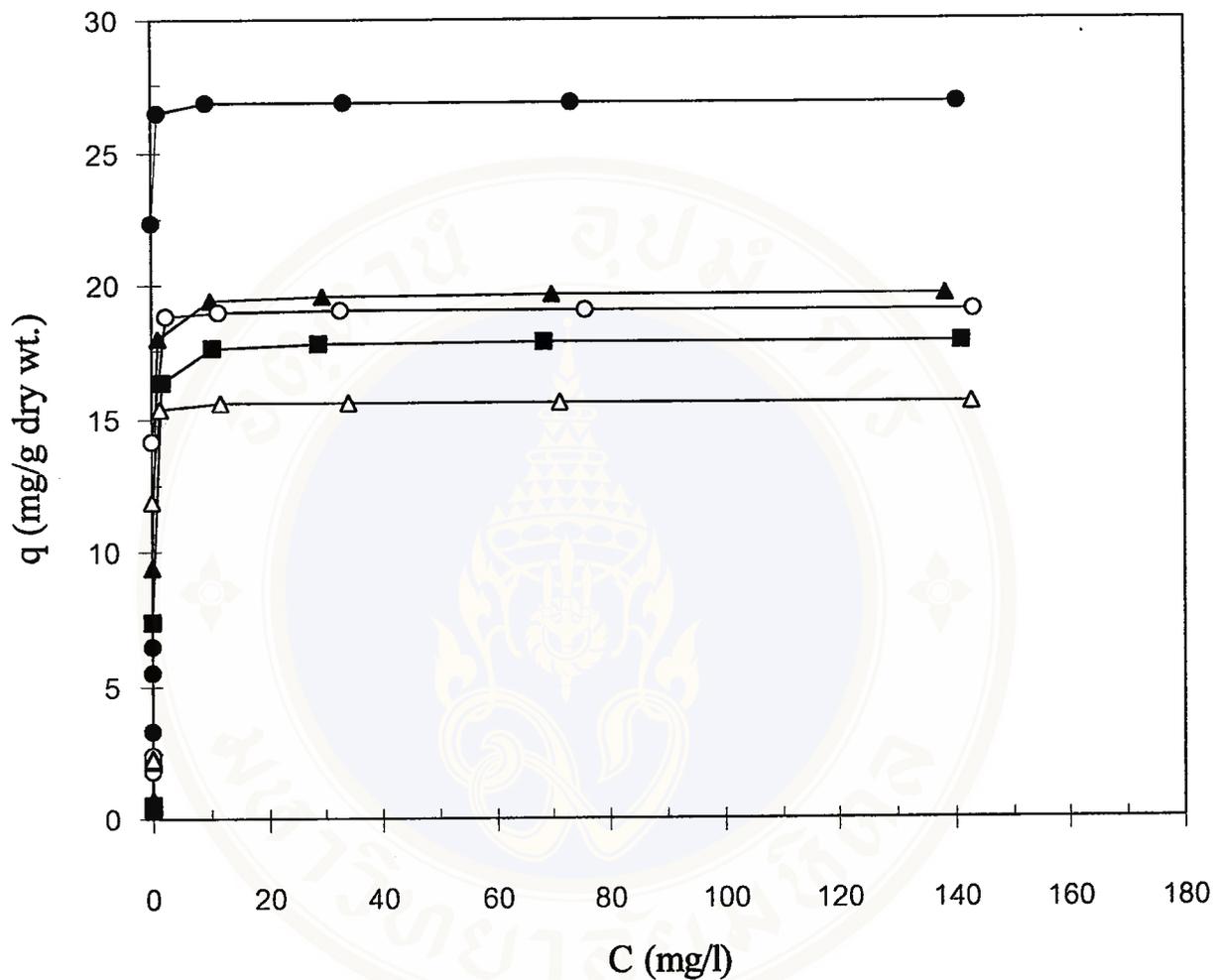
*Scenedesmus acutus* could adsorb Cd better than others strains at 109.59 mg Cd/g dry wt. Two cyanobacteria, *Tolypothrix tenuis* and *Calothrix parietina* could adsorb Cd more than *Chlorella vulgaris* and *Chlorella vulgaris* (CCAP 211/11B). For Pb adsorption, green algae except *Chlorella vulgaris* (CCAP 211/11B) could adsorb Pb better than cyanobacteria, *Tolypothrix tenuis* and *Calothrix parietina*.

**Table 11** The maximum adsorption capacity ( $q_{max}$ ) and the binding constant (k)

Microalgae	Heavy Metal					
	Hg		Cd		Pb	
	k	$q_{max}$ (mg/g dry wt.)	k	$q_{max}$ (mg/g dry wt.)	k	$q_{max}$ (mg/g dry wt.)
<b>Cyanobacteria</b>						
<i>Tolypothrix tenuis</i>	0.01	26.86	0.62	90.01	7.83	31.48
<i>Calothrix parietina</i>	0.03	19.01	0.92	79.32	6.87	44.74
<b>Green algae</b>						
<i>Chlorella vulgaris</i>	0.15	17.87	3.83	76.20	3.06	126.66
<i>Chlorella vulgaris</i> (CCAP211/11B)	0.03	15.60	1.60	61.68	3.06	38.68
<i>Scenedesmus acutus</i>	0.11	19.64	1.57	109.57	9.45	90.15

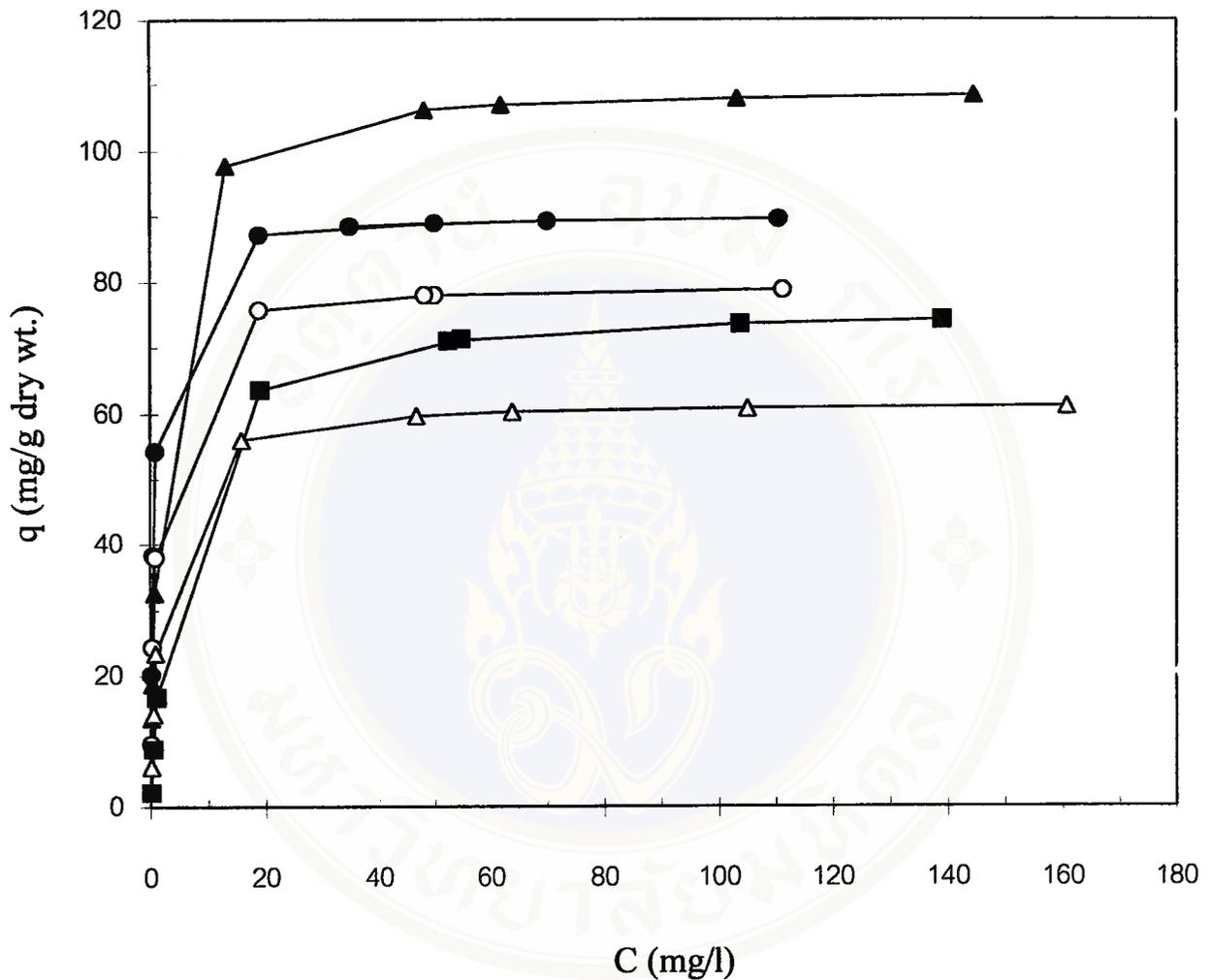
The binding constant value ( $k$ ) of microalgal strains represented the affinity to heavy metal. For Hg, *Tolypothrix tenuis* had  $k$  value of 0.01, which was lower than other microalgal cells, suggesting that *Tolypothrix tenuis* cells had higher affinity to Hg than other strains. In the case of Cd, *Tolypothrix tenuis* and *Calothrix parietina* had higher affinity ( $k = 0.62$  and  $0.92$ ) than *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus*. *Chlorella vulgaris* and *Chlorella vulgaris* (CCAP 211/11B) ( $k = 3.06$  and  $3.06$ ) had higher affinity to Pb than *Tolypothrix tenuis* and *Calothrix parietina* as shown in Table 12.

Figure 10 showed the equilibrium isotherm of Hg adsorption by microalgae, *Tolypothrix tenuis* could adsorb Hg at 26.86 mg Hg/g dry wt. at minimum concentration of 1.04 ppm, which was more than other strains. Equilibrium isotherm of Cd adsorption showed that *Scenedesmus acutus* could adsorb Cd at 109 mg Cd/ g dry wt. at minimum concentration of 48 ppm which was more than other strains (Fig. 11). In Fig. 12, the equilibrium isotherm of Pb adsorption showed that *Chlorella vulgaris* could adsorb Pb 109 mg Pb/ g dry wt. at minimum concentration of 129.5 ppm which was more than other strains.



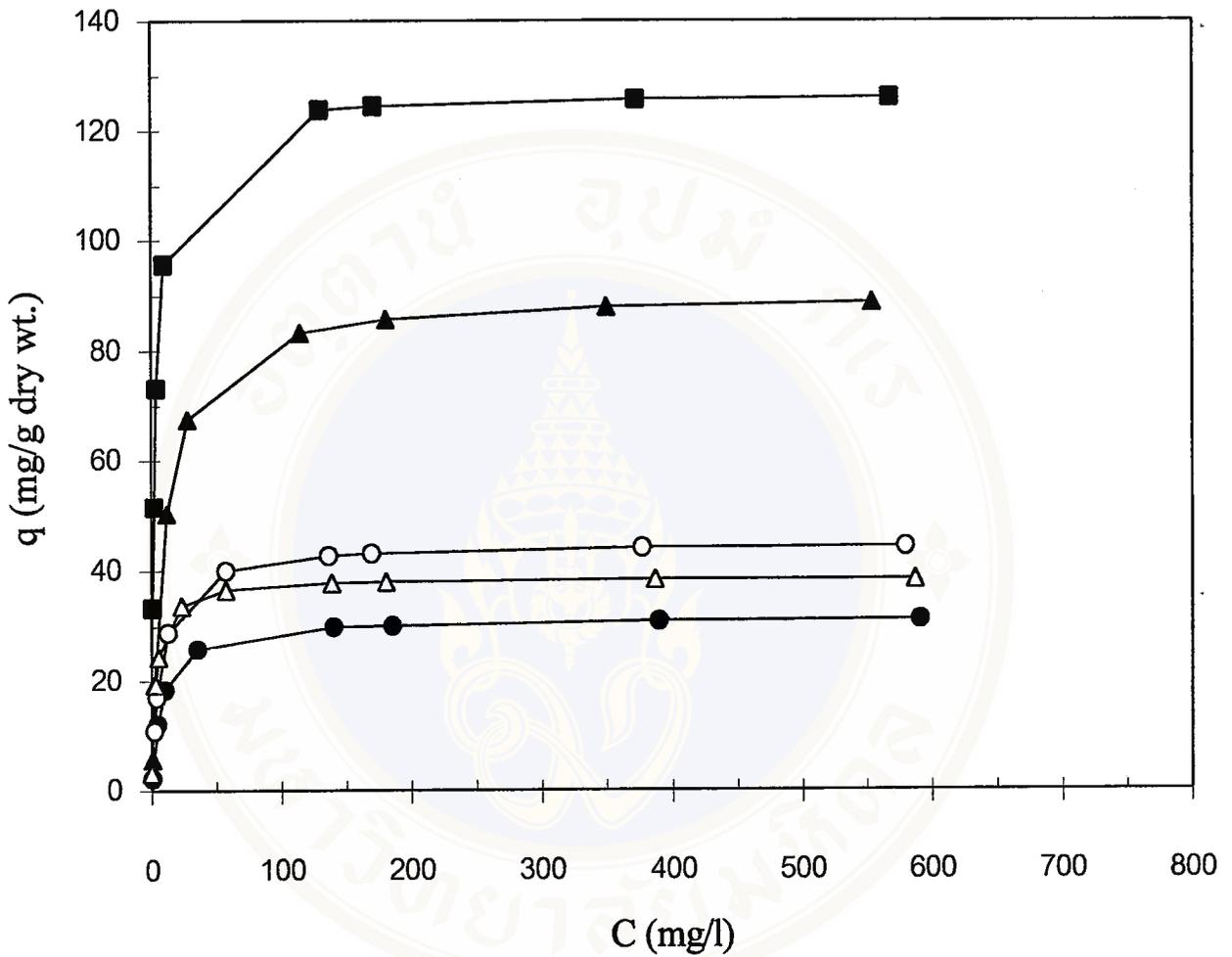
**Fig. 10** Adsorption of Hg by microalgae at various concentrations

Microalgal cells (0.5 g dry wt.) were suspended in 50 ml with various concentration of Hg solution for 30 min. Symbols: ●, *Tolypothrix tenuis*; ○, *Calothrix parietina*; ■, *Chlorella vulgaris*; △, *Chlorella vulgaris* (CCAP 211/11B); ▲, *Scenedesmus acutus*



**Fig. 11** Adsorptions of Cd by microalgae at various concentrations

Microalgal cells (0.5 g dry wt.) were suspended in 50 ml with various concentration of Hg solution for 30 min. Symbols: ●, *Tolypothrix tenuis*; ○, *Calothrix parietina*; ■, *Chlorella vulgaris*; △, *Chlorella vulgaris* (CCAP 211/11B); ▲, *Scenedesmus acutus*



**Fig. 12** Adsorption of Pb by microalgae at various concentrations

Microalgal cells (0.5 g dry wt.) were suspended in 50 ml with various concentration of Hg solution for 30 min. Symbols: ●, *Tolypothrix tenuis*; ○, *Calothrix parietina*; ■, *Chlorella vulgaris*; △, *Chlorella vulgaris* (CCAP 211/11B); ▲, *Scenedesmus acutus*

## CHAPTER V

### DISCUSSION

#### 5.1 Screening and isolation of microalgae

Microalgal strains were collected by using a nylon sieve (50  $\mu$ ) across the surface of the water. Known strains of microalgal were obtained from the Microbiological Resources Center (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), Kasetsart University, Bangkok and from Gottingen University's culture collection, Gottingen, Germany, all of them were asexual culture which have no contamination of other microorganisms. All of screened microalgae used in this experiment were unialgal strains, which were obtained by re-streak on agar plate.

The sampling water from Chao Praya River and Industrial Estate area were also measured for pH, temperature and some heavy metals (Fe, Zn, Cr, Mn, Cu, Cd and Pb). Temperature was between 27-30 °C and pH was between 7.2-7.5. From heavy metal measurement found that the sampling place had low contamination of heavy metal (lower than 0.01 mg/l) except the sampling from Thai containers Industry Co.Ltd. which contain Fe, Zn, Mn and Cu at 0.126, 0.104, 0.046 and 0.142 mg/l, respectively. In some sampling place at Bang-pu Industrial Estate area, no heavy metal contamination was found because the sampling places are outside the industries and wastewater from the industries were treated by central wastewater treatment plant.

## 5.2 Screening on plate for Hg, Cd and Pb tolerant strains

The ability of algae to reproduce and survive in metal polluted habitats depends on genetics adaptation, genetic change or change physiology resulting from metal exposure. "Tolerance" and "resistance" are terms often used interchangeably. Tolerance may rely on intrinsic properties of the organism or the physical and chemical nature of the environment whereas, "resistance" is sometimes used in connection with a direct response to metal exposure (29).

From the result microalgae could grow in different concentration of Hg, Cd and Pb. Green algae appeared to be more tolerant than cyanobacteria because cell wall structure of green algae are composed of cellulose and pectin which is stronger than cell wall of cyanobacteria which contain peptidoglycan layer and lipopolysaccharide layer (25). Heavy metal could inhibit growth of microalgae and it was found that Cu, Cd and Pb could inhibit growth of green algae, *Ankistrodesmus braunii* and the cyanobacteria, *Anabaena* strain 7120 (30). The study on the toxicological responses of the cyanobacterium, *Anabaena flosaquae*, showed that concentration of cadmium at 0.013 ppm could inhibit the growth of *Anabaena flosaquae* in a similar manner to this experiment showing cyanobacteria (*Anabaena variabilis*, *Anabaena siamensis* and *Anabaena torulosa*) could not grow on plate containing 5.7 ppm of cadmium concentration which was the lowest concentration (31). In the case of green algae, previous study showed that *Chlorella regularis* was affected by 0 to 20 ppm of cadmium ion (12) this was closed to the previous study. For example, green algae *Chlorella ellipsoidea*, *Chlorella sp.* and *Chlorella vulgaris* could grow on plate containing Cd

22.8 ppm but *Chlorella vulgaris* (CCAP211/11B) and *Chlorella saccharphilla* could grow on plate containing cadmium 5.7 ppm. The study on removal of cadmium ions by the marine diatom *Phaeodactylum tricornutum* with regard to accumulation and long term kinetics of uptake showed that growth rate of *Phaeodactylum tricornutum* decreased as cadmium concentration increased and at cadmium concentration of 10 ppm the growth rate of *Phaeodactylum tricornutum* became constant (32). In this study *Chlorella ellipsoidea*, *Chlorella sp.* and *Chlorella vulgaris* could grow on plate which contained higher concentration of Cd than *Chlorella vulgaris* (CCAP211/11B) and *Chlorella saccharphilla*. This might be caused by the detoxification of microalgae. Heavy metal detoxification is one mechanism that microalgae could tolerate high heavy metal concentrations as reported by Hart BA. and Bertram PE for the study of cadmium binding protein in a cadmium tolerant strain of the green algae, *Chlorella pyrenoidosa*. They found that *Chlorella pyrenoidosa* could accumulate and tolerate Cd and this was related to its ability in synthesis of cadmium binding protein under cadmium exposure condition (33). Metal binding proteins have been reported for different microalgae such as *Chlorella ellipsoidea*, *Chlorella pyrenoidosa*, *Dunaliella bioculata*, *Synechococcus sp.*, *Euglena gracilis*, *Scenedesmus acutiformis* and *Scenedesmus quadricauda* (34). Some microalgae could change their morphology and adapt genetic to survive and reproduce in metal polluted habitat (11).

Toxicity of heavy metals inhibited photosynthesis of microalgae and caused decreasing growth rate of microalgae such as Cu, Cd and Zn. Heavy metals also affected the photosynthesis of freshwater benthic algae. The sensitivity depended on heavy metal

ion and strains of fresh benthic algae strains (35). From the study by Stratton GW. and Corke C.T., the concentration of Cd at 1.0-2.0 ppm affected to photosynthesis of cyanobacteria, *Anabaena inaequalis* (36).

The relative toxicity of heavy metals to algae has been studied and showed that  $Hg > Cd > Pb$ . Related study of response to copper, cadmium, and lead by cyanobacteria, *Anabaena sp.* showed that the relative toxicity of heavy metals was  $Cu > Cd > Pb$  (30). The relative toxicity of heavy metals to algae has been summarized in several reviews (37, 38). The most common overall toxicity in decreasing order is  $Hg > Cu > Cd > Ag > Pb > Zn$  (29, 37). From the result found that all of heavy metals were effected on growth of microalgae. The relative toxicity from both reports had the same direction that toxicity of Cd more than the toxicity of Pb.

### **5.3 Hg, Cd and Pb removal in aqueous solution**

#### **5.3.1 Hg, Cd and Pb removal ability**

The adsorption of Hg, Cd and Pb were determined by comparing the Hg, Cd and Pb concentration in the aqueous solution before and after contacting with the microalgal cells. From this experiment green algae had higher removal capacity than cyanobacteria for example, *Scenedesmus acutus* had removal capacity for Hg, Cd and Pb at 85.18, 87.89 and 88.89%, respectively and *Chlorella vulgaris* had removal capacity for Hg, Cd and Pb at 74.34, 85.80 and 84.68%, respectively. For cyanobacteria, only *Tolypothrix tenuis* had high removal capacity for Hg, Cd and Pb at 81.42, 87.77 and 87.68%, respectively. From the results of this experiment it was found that some microalgae that had high

tolerance from screening on plate could remove heavy metal less than some non-tolerant. For example cyanobacteria, *Tolypothrix tenuis* could remove 81.42% of Hg while tolerant strains such as *Anabaena siamensis*, *Anabaena torulosa* and *Calothrix parietina* could remove only 32.10, 28.97 and 50.26 % of Hg. Cyanobacteria *Calothrix parietina* could remove 87.89 % of Cd which was higher than some tolerant strains of green algae *Chlorella ellipsoidea*, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP211/11B) and T1. In the case of Pb removal cyanobacteria *Tolypothrix tenuis* and *Calothrix parietina* could remove 87.89 and 90.59 %, respectively, which was higher than tolerant strain *Scytonema schmidlei* which could remove only 28.90%.

*Chlorella vulgaris* is one of the green algae usually used to study the adsorption of heavy metal (9, 23, 26, 35) but in this study *Scenedesmus acutus* had higher removal capacity than *Chlorella vulgaris*. Cyanobacteria had removal capacity less than green algae but in some studies, such as Kitjaharn P. reported cyanobacteria *Aphanothece halophytica* and *Spirulina platensis* could remove lead only 22% and 35% from the Battery Organization wastewater respectively (14). We selected cyanobacteria to use in heavy metals adsorption because cyanobacteria had high growth rate and easy to separate from solution by simple filtration.

### 5.3.2 Concentration factor of Hg, Cd and Pb removal

The concentration factor (CF) is the ratio of the metal concentration to metal in aqueous solution. CF is the parameter used to compare heavy metal removal ability between different strains. Hassate JM. used microplate technique to determine accumulation of metal by algae in order to investigate effect of algae species, culture age,

metal (mercury, lead, cadmium and zinc) and pH found that green algae and cyanobacteria appeared to accumulate metals  $Hg > Cd > Pb > Zn$  and green algae had better cadmium accumulation than cyanobacteria and found that concentration factors for heavy metal in green algae *Scenedesmus obliquus* in 11 days old cultured, 3 hours, 22°C in the dark were 5,040 and 3,600 for Hg and Cd, respectively (40) which was closed to the present experiment. Green algae *Scenedesmus acutus* that had higher CF of Hg and Cd than other microalgae showed the value of 3,412 and 4,591, respectively. In the case of cyanobacteria, *Nostoc sp.*, it was found that concentration factor of Hg and Pb were 1,950 and 1,920, respectively but in this present experiment concentration factor of Hg and Pb by *Nostoc sp.* were only 234 and 343, respectively. The difference between the value of concentration factor depended on the variation between biomass sources, and experimental condition such as microalgal strains, culture age, pH and time of exposure.

### 5.3.3 Time courses of Hg, Cd and Pb removal by selected microalgae

Time dependent adsorption experiment showed that the metal adsorption on microalgae involved two stages; rapid metabolism independent surface adsorption and subsequent slow accumulation. The kinetics of heavy metal uptake for cyanobacteria were more rapid than green algae. Kitjahn P. studied the use of cyanobacteria for lead (Pb) removal from wastewater and found that *Aphanathece halophytica* was able to accumulate Pb at first 10 min and became saturated within 1 hour and could be leached by EDTA (ethylene diamine tetraacetic acid) washing because lead accumulated at the cell surface. *Spirulina platensis* was able to accumulate Pb at first 10 min and about 50-70% of the accumulated lead could not be washed out by EDTA because lead

was likely to be transported into the cell (14). *Tolypothrix tenuis* could remove 90 % of Cd in solution within 10 minutes onto the cell surface and was saturated within 30 min and easily released by EDTA (13). *Nostoc calcicola* binded with Hg on cell surface within first 10 min and was saturated at least up to 40 min (15). From the present experiment of short-term uptake within 30 min it was found that blue green algae, *Tolypothrix tenuis* and *Calothrix parietina* were able to accumulate Hg, Cd and Pb very rapidly within 5-10 min and were saturated within 30 min. *Tolypothrix tenuis* and *Calothrix parietina* might adsorb Hg, Cd and Pb onto the cell surface similar to other studies. Cho DY. studied in green algae and found that approximately 43% of the Cd adsorption by *Chlorella vulgaris* took place within 30 min and the remainder was adsorbed during next 72 hours at the slow rate (26). The adsorption experiment in short time (30 min) showed that green algae, *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP211/11B) and *Scenedesmus acutus* were able to adsorb Hg, Cd and Pb rapidly at first 10 min. After 30 min 3 strains of green algae *Chlorella vulgaris*, *Chlorella vulgaris* (CCAP211/11B) and *Scenedesmus acutus*, could remove Hg, Cd and Pb at slow rate similar to other studies which used many hours or days for uptake.

#### **5.4 Hg, Cd and Pb removal with various heavy metal concentrations**

In order to know the maximum adsorption capacity, the langmuir equation was used for this experiment. Srikrajib S. reported a value for the maximum adsorption capacity ( $q_{max}$ ) of 103.357 and 95.491 mg Cd /g dry wt. obtained from isotherm of Cd to *Sargassum polycystum* dried at 80 °C and 100 °C, respectively (27). From the present



experiment the maximum adsorption capacity ( $q_{max}$ ) of cadmium by *Scenedesmus acutus*, which was higher than other strains was calculated to be 109.57 mg Cd/g dry wt. and in other strains the values of  $q_{max}$  were 61.68 - 90.01 mg Cd/g dry wt. Both algae, *Sargassum polycystum* and *Scenedesmus acutus* had similar maximum adsorption capacity ( $q_{max}$ ) while *Sargassum polycystum* was dead cells and *Scenedesmus acutus* was living cells. Zumriye Aksu and Tulin Kutsal studied the adsorption of lead from waste water by using green algae *Chlorella vulgaris* in a single staged batch reactor and reported a value for the maximum adsorption capacity ( $q_{max}$ ) of 82.9 mg Pb /g dry wt..They also found that the adsorption of lead by *Chlorella. vulgaris* was increased with the in increased pH and temperature (39). From the present experiment the maximum adsorption capacity ( $q_{max}$ ) of lead by *Chlorella. vulgaris*, which was higher than others strain was calculated to be 126.66 mg Pb/g dry wt. and in other strains the values of  $q_{max}$  were 31.48 - 90.15 mg Pb/g dry wt. Both experiment were used *Chlorella. vulgaris* but the value of  $q_{max}$  were different because the condition of each experiment was different.

## CHAPTER VI

### CONCLUSION AND SUGGESTION

#### 6.1 Conclusion

1. Unknown strains T1-T12 were screened and isolated from Chao Praya River, Bangkok, Nonthaburi and Bang-pu Industrial Estate area in Samutprakarn.

2. From plate screening method it was found that tolerant microalgal strains could grow on plate containing Hg concentration of 1 ppm ( $1.5 \times 10^{-3}$  mM), Cd 22.8 ppm (0.05 mM) and Pb 256 ppm (1.25 mM) and found that the relative toxicity in decreasing order was Hg > Cd > Pb.

3. Percentage of Hg removal was 12.00-81.42 in cyanobacteria and 68.21-85.18 in green algae. Cadmium removal was 16.77-87.89 % in cyanobacteria and 72.71-93.85 % in green algae. Cyanobacteria could remove Pb 21.24-90.59 % and in green algae could remove Pb 27.08-88.89 %. *Scenedesmus acutus*, T5 and *Calothrix parietina* had highest Hg Cd and Pb removal capacity respectively.

4. Three strains of green algae, *Chlorella vulgaris* (IFRPD1118), *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* and 2 strains of cyanobacteria, *Tolythrix tenuis* and *Calothrix parietina* were selected for study in aqueous solution. This was because *Chlorella vulgaris* (IFRPD1118), *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus* had high tolerance on plate, high removal capacity and high concentration factor. *Tolythrix tenuis* and *Calothrix parietina*

were filamentous strains which had high removal capacity and easy to separate by filtration.

5. Cyanobacteria, *Tolypothrix tenuis* and *Calothrix parietina* could remove Hg, Cd and Pb faster than green algae *Chlorella vulgaris* (IFRPD1118), *Chlorella vulgaris* (CCAP 211/11B) and *Scenedesmus acutus*.

6. *Tolypothrix tenuis* had highest maximum adsorption capacity 26.86 mg Hg/g dry wt. at minimum concentration of 1.04 ppm, *Scenedesmus acutus* had highest maximum adsorption capacity 109.57 mg Cd/g dry wt. at minimum concentration of 109 ppm and *Chlorella vulgaris* (IFRPD1118), had highest maximum adsorption capacity 126.66 mg Pb/g dry wt. at minimum concentration of 129.5 ppm which more than other strains.

## 6.2 Suggestion

To apply this system in real wastewater treatment system filamentous cyanobacteria were more suitable for use in outdoor application than green algae. This was due to the fast growth speed of cyanobacteria and could be separated more easily by using simple filtration while green algae need high-speed centrifugation system, which costly to separate the biomass from wastewater.

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**APPENDIX**

Culture medium (M 18) consisting of : (per liter)

1. $\text{NaNO}_3$	1.50 g
2. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	380 mg
3. $\text{K}_2\text{HPO}_4$	120 mg
4. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	110 mg
5. $\text{NaCl}$	70 mg
6. $\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O} (n \sim 11-14)$	10 mg
7. $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	9 mg
8. $\text{H}_3\text{BO}_3$	3 mg
9. $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	2 mg
10. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.30 mg
11. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08 mg
12. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.04 mg

The pH was adjusted to 7.5.

## BIOGRAPHY

<b>NAME</b>	Miss Nalin Sidtitoon
<b>DATE OF BIRTH</b>	24 February 1975
<b>PLACE OF BIRTH</b>	Bangkok, Thailand
<b>INSTITUTION OF ATTENDED</b>	Mahidol University, 1993-1996: Bachelor of Science (Public Health; Sanitary Science) Mahidol University, 1997-2000: Master of Science (Environmental Sanitation)
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