TOXICITY, BIOACCUMULATION AND BIOSORPTION OF LEAD, CADMIUM AND ZINC IN CHAROPHYTES

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ABSTRACT

The ability for the use of common freshwater charophytes, *Chara aculeolata* and *Nitella opaca* in removal of cadmium (Cd), lead (Pb) and zinc (Zn) from wastewater was examined. Living *C. aculeolata* was more tolerant of Cd and Pb than *N. opaca*. The relative growth rate of *N. opaca* was drastically reduced at high concentrations of Cd and Pb although both were tolerant of Zn. Both macroalgae showed a reduction in chloroplast, chlorophyll and carotenoid content after Cd and Pb exposure, while Zn exposure had little effect. The bioaccumulation of both Cd and Pb was higher in *N. opaca* (1544.3 µg/g at 0.5 mg/L Cd, 21,657.0 µg/g at 10 mg/L Pb) whereas higher Zn accumulation was observed in *C. aculeolata* (6703.5 µg/g at 10 mg/L Zn). In addition, high BCF values (> 1000) for Cd and Pb were observed in both species. *C. aculeolata* showed a higher percentage of Cd and Pb removal (> 95%) than *N. opaca* and seemed to be a better choice for Cd and Pb removal from wastewater due to its tolerance to these metals.

The potential of dried *C. aculeolata* and *N. opaca* to biosorb Pb, Cd and Zn from a single-metal system, multiple-metal system and municipal wastewater were investigated. *C. aculeolata* and *N. opaca* performed well in the biosorption of all three metal ions, with preference towards Pb, followed by Cd and Zn. The process of Pb adsorption onto algal biomass followed the first-order rate kinetic (*N. opaca*) and intraparticle diffusion (*C. aculeolata*), in contrast to Cd and Zn whose biosorption kinetics fitted the second-order rate by both algae. According to the Langmuir isotherm model, *C. aculeolata* exhibited a slightly higher maximum uptake of Pb, Cd and Zn (106.4 mgPb/g, 36.1 mgCd/g, 15.2 mgZn/g) than those of *N. opaca* (102.0 mgPb/g, 27.6 mgCd/g, 13.4 mgZn/g). In the multiple-metal solution, an antagonistic effect by metal competition was observed. The ability of *C. aculeolata* to remove three metal ions was found to be high in real municipal water (81-100%). *C. aculeolata* in a continuous fixed bed column was capable of decreasing Pb and Cd concentrations from 10 to a value below the detection limit of 0.05 and 0.02 mg/L, respectively. Pb uptake capacity of *C. aculeolata* increased with increased bed depth and decreased flow rate. Cd uptake capacity increased with increased bed depth but remained constant at various flow rates. The Thomas model was found to fit with the experimental data for Pb and Cd (R^2>0.90). It can be concluded that *C. aculeolata* is a good biosorbent for treating wastewater with low concentrations of Pb and Cd contaminants.

KEY WORDS: CHAROPHYTES / PHYTOREMEDIATION / CADMIUM / LEAD / ZINC / BIOSORPTION

161 pages

การศึกษาความเป็นพิษ การสะสม และการดูดซับตะกั่ว แคดเมียมและสังกะสีในสาหร่ายไฟ
TOXICITY, BIOACCUMULATION AND BIOSORPION OF LEAD, CADMIUM AND ZINC IN CHAROPHYTES

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บทคัดย่อ

จากการศึกษาการกำจัดตะกั่ว แคดเมียม และสังกะสีที่ปนเปื้อนในน้ำโดยใช้สาหร่ายไฟสองชนิด พบว่า *Chara aculeolata* มีความทนทานต่อแคดเมียมและตะกั่วมากกว่า *Nitella opaca* อัตราการเจริญเติบโตของ *N. opaca* ลดลงอย่างมากในสารอาหารที่มีแคดเมียมหรือตะกั่วความเข้มข้นสูง สาหร่ายทั้งสองชนิดมีความทนทานต่อสังกะสี การศึกษาผลของโลหะหนักต่อปริมาณเม็ดสีพบว่าสาหร่ายทั้งสองชนิดมีปริมาณคลอโรพลาสต์ คลอโรฟิลล์ และแคโรทีนอยด์ลดลงเมื่อเลี้ยงในสารอาหารที่มีแคดเมียมและตะกั่ว สังกะสีมีผลกระทบน้อยต่อปริมาณเม็ดสี *N. opaca* มีการสะสมแคดเมียมสูงถึง 1544.3 ไมโครกรัมต่อกรัม และตะกั่วสูงถึง 21,657 ไมโครกรัมต่อกรัม ส่วน *C. aculeolata* มีการสะสมสังกะสีสูงถึง 6,703.5 ไมโครกรัมต่อกรัม สาหร่ายทั้งสองชนิดมีค่า biocon-centration factor ของแคดเมียมและตะกั่วสูงกว่า 1,000 *C. aculeolata* มีอัตราการกำจัดแคดเมียมและตะกั่วในน้ำมากกว่า *N. opaca* (มากกว่าร้อยละ 95) ดังนั้น *C. aculeolata* จึงเป็นสาหร่ายที่เหมาะสมต่อการกำจัดแคดเมียมและตะกั่วที่ปนเปื้อนในน้ำ และยังมีความทนทานต่อโลหะเหล่านี้ด้วย

จากการศึกษาการดูดซับตะกั่ว แคดเมียมและสังกะสี โดยสาหร่ายไฟแบบชีวมวลแห้งพบว่า *C. aculeolata* และ *N. opaca* สามารถดูดซับตะกั่วได้ดีที่สุด รองลงมาคือแคดเมียมและสังกะสีตามลำดับ *N. opaca* มีกลไกดูดซับตะกั่วแบบ first-order rate kinetic สาหร่าย *C. aculeolata* มีกลไกดูดซับตะกั่วแบบ intraparticle diffusion สาหร่ายทั้งสอง มีกลไกการดูดซับแคดเมียมและสังกะสีตาม second-order rate kinetic *C. aculeolata* มีค่าการดูดซับสูงสุดของตะกั่วที่ 106.4 มิลลิกรัมต่อกรัม แคดเมียม 36.1 มิลลิกรัมต่อกรัม และสังกะสี 15.2 มิลลิกรัมต่อกรัม ซึ่งมากกว่า *N. opaca* ที่มีการดูดซับสูงสุดของตะกั่วที่ 102 มิลลิกรัมต่อกรัม แคดเมียม 27.6 มิลลิกรัมต่อกรัม และสังกะสี 13.4 มิลลิกรัมต่อกรัม ในการศึกษาในสารละลายโลหะหนักผสมสามชนิดพบว่าสาหร่ายมีการดูดซับโลหะหนักน้อยลง *C. aculeolata* สามารถกำจัดโลหะหนักทั้งสามชนิดได้ร้อยละ 81-100 ในน้ำเสียชุมชน

จากการศึกษาการใช้ *C. aculeolata* แบบตรึงในคอลัมน์เพื่อบำบัดน้ำเสียพบว่า *C. aculeolata* สามารถลดปริมาณตะกั่วและแคดเมียมจาก 10 มิลลิกรัมต่อลิตร เหลือต่ำกว่า 0.05 และ 0.02 มิลลิกรัมต่อลิตร ตามลำดับ *C. aculeolata* สามารถดูดซับตะกั่วเพิ่มขึ้นเมื่อความสูงของชีวมวลแห้งในคอลัมน์เพิ่มขึ้นที่อัตราการไหลของน้ำต่ำ ส่วนการดูดซับแคดเมียมจะเพิ่มขึ้นเมื่อความสูงของชีวมวลแห้งในคอลัมน์เพิ่มขึ้นที่ช่วงอัตราการไหลของน้ำ 20-40 มิลลิลิตรต่อนาที จากการทดลองสามารถสรุปได้ว่า *C. aculeolata* เป็นสาหร่ายไฟที่มีชีวมวลเหมาะสมต่อการบำบัดน้ำเสียที่มีตะกั่วและแคดเมียมปนเปื้อนได้

161 หน้า

CONTENTS

CONTENTS (cont.)

CONTENTS (cont.)

CONTENTS (cont.)

CONTENTS (cont.)

CONTENTS (cont.)

CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF FIGURES (cont.)

LIST OF ABBREVIATIONS

ppm	part per million
g/ha	gram per hectare
ha	hectare
t/y	ton per year
ng/L	nanogram per liter
µg/L	microgram per liter
mg/L	milligram per liter
mg/kg	milligram per kiligram
g/L	gram per liter
mM	millimolar
M	molar
mL	milliliter
L	liter
g	gram
kg	kilogram
pm	picometer
nm	nanometer
cm	centimeter
m	meter
rpm	revolution per minute
min	minute
h	hour
wt	weight
SD	standard deviation
°C	degree celsius

CHAPTER I
INTRODUCTION

The presence of heavy metals in the environment is the problem of worldwide concern. The heavy metals, lead, copper, cadmium, zinc and nickel are the most common pollutants found in industrial effluent (Abdel-Halim *et al*., 2003; Amuda *et al*., 2007). High levels of these metals can cause harmful health effects. Various treatment technologies such as chemical precipitation, ion exchange and reverse osmosis are often inefficient and expensive (Volesky, 2001). The need for economical and effective methods for metal removal results in the search for alternative materials and methods. Many yeasts, algae, bacteria and various aquatic plants including clay are known to be able to concentrate metal species from dilute aqueous solutions (Chang *et al*., 1997; Volesky *et al*., 1993; Upatham *et al*., 2002; Singh *et al*., 2007).

It has been demonstrated that biosorption is a potential alternative to the traditional treatment process of metal ion removal. Biosorption uses the ability of the biological materials to accumulate heavy metals from waste streams by either metabolically mediated or purely physico-chemical pathways of uptake. The biosorption process has been demonstrated in a wide range of algae, such as *Spirogyra* sp., *Chlorella* sp., *Scenedesmus* sp., *Synechocystis*., *Ulotrix* sp. and *Padina* sp., for removal of different heavy metals from aqueous solutions (Gupta and Rastogi, 2008; Pavasant *et al*., 2006; Pena-Castro *et al*., 2004).

The potential of wetland macroalgae in aquatic phytoremediation is demonstrated in several studies. The process includes constructed wetland. Due to the relative novelty of the technology, much of the current research is still aimed at the selection of aquatic macroalgae species, based on the type of elements to be remediated, the local environmental and geographical conditions as well as the removal capabilities of the macroalgae (Bibi *et al*., 2010; Gao and Yan, 2012). Thailand has a large group of macroalgal species. In previous studies, several freshwater macroalgae that are highly efficient in absorbing and accumulating heavy metals were identified and evaluated for the phytoremediation of heavy metal-contaminated water.

Charophytes, the member of green algae of the Division Charophyta are abundant and ubiquitous in Thailand. They have a fast growth rate and high production of biomass. They have a higher calcifying potential than other aquatic plants. They contain $CaCO_3$ at levels as high as 60% of dry weight (Urbaniak, 2010). It is also reported that many heavily calcified charophytes get deposited in aquatic environments by taking up the nutrient from sediment. Presence of heavy metal may result in uptake ability of the charophytes. Exposure to heavy metal especially Cd may disturb calcification of these charophytes since these two metals have a similar ionic radius (Gomes and Asaeda, 2009). Therefore, heavy metal may have effect and interaction on other essential metals, which affects metal accumulation of charophytes

The use of macroalgae in heavy metal removal is inexpensive when compared with other systems. In addition, the climate and geographical condition in Thailand are suitable for the large production of the algal biomass. *Chara* and *Nitella* are among the most abundant charophytic macroalgae, growing in a wide range of fresh and brackish water bodies and blooming temporarily to permanently (Coops, 2002). In eutrophic areas, some *Chara* and *Nitella* species can reach nuisance levels, be detrimental to the diversity of other plants and animals, and require seasonal elimination by manual, biological or chemical methods (Królikowska, 1997; Petty, 2005). A survey of literature indicated that not much work has been done so far on the living and non-living biomass of charophytes for heavy metal removal. Therefore, the present study aimed to investigate the heavy metal removal ability or phytoremediation potential of two species of charophyte, *Chara aculeolata* and *Nitella opaca*.

CHAPTER II

OBJECTIVES

The objectives of the present study were as follows:

1) To determine the toxicity of Cd, Zn and Pb in two species of charophytes, *Chara aculeolata* and *Nitella opaca*.

2) To determine the metal accumulation in *C. aculeolata* and *N. opaca*.

3) To study the biosorption potential for Cd, Zn and Pb by *C. aculeolata* and *N. opaca* in batch system and packed bed column system.

4) To determine the metal removal from wastewater by *C. aculeolata* and *N. opaca*.

CHAPTER III
LITERATURE REVIEW

3.1 Heavy metals

Heavy metals are natural elements that are found in the bedrock throughout the world. Heavy metals such as Vi, Cr and Co are found with high levels in serpentine soils while calamine soils are abundant with Zn, Pb and Cd. Heavy metals are persistant and cannot be diluted from the environment. High background level by natural situation or global activities by human arises problem of heavy metal toxicity when their availability is high (Prasad, 1999). By human activities, enrichment of heavy metals in the environment such as air wastes, or deliberate dumping can concentrate heavy metals in the environment (Dean *et al.*,1972). Effluents from mining wastes increased several thousand folds of metal concentration in river water (Forsner and Wittman, 1979). Metals are transported in the environment by water and air, ultimately reaching the soil and sediment where they become bound. The time taken for them to become bound may be fairly long with high levels of metal bioavailability in soils at the beginning of the binding period and gradually decreases with longer period of time (Martin and Kaplan, 1998). Thus the greater problem of anthropogenically supplied metals, with their high bioavailability, possibly worse than slow weathering originally from bedrock, with their high background levels.

3.2 Cadmium (Cd)

3.2.1 Chemistry of Cd

Cd has atomic number of 48, relative atomic mass of 112.40, electronegativity of 1.69 and is crystal ionic radius of 109 pm (form of Cd^{2+} only) (Shannon, 1976). It is a metallic element in group IIb together with Zn in the periodic table. It is often impure state and present in various types of rocks, soils, water, including coal and petroleum. Other metal ores i.e. zinc, lead, and copper are the main natural sources of Cd.

Cd is usually in liquid phase due to its relatively high vapor pressure. However, when it is vaporized, it is oxidized quickly to produce Cd oxide in the air. For example, the reactive gases or vapor, in environment such as carbon dioxide, water vapor, sulfur dioxide, sulfur trioxide or hydrogen chloride, can react and produce Cd carbonate, hydroxide, sulfite, sulfate or chloride, respectively. These Cd salts may be formed in stacks with long period of time and emitted to the environment by weathering (EPA, 2012). One of its potential hazard is evaluated by its speciation and solubility in soil water and surface water. Practically insoluble forms of Cd salts are sulfide, carbonate or oxide. These forms can be converted to water-soluble salts in nature under the influence of oxygen and acids. The forms of Cd salts such as sulfate, nitrate, and halogenates are soluble in water (EPA, 2012).

3.2.2 Emission of Cd

3.2.2.1 Natural sources

Global Cd cycle originates naturally in earth's crust, which mobilizes Cd by volcanoes and weathering of rocks (Nriagu, 1989). Cd in atmosphere emitted by volcanoes and the weathering of rocks releases Cd to soils and aquatic systems. These processes rarely result in elevated Cd concentrations in any environmental compartment. Cd within the biosphere is translocated naturally by volcanoes, airborne soil particles, sea spray, biogenic material and forest fires. The estimation of total annual Cd emission to air from natural sources is about 150-2,600 tons while the total global anthropogenic air emission in 1995 is approximate 3,000 tons (Thornton, 1995). The air Cd emission from volcanic source could be estimated in spite of deep sea volcanism, which is also a source of environmental Cd release, remains to be quantified for the role in global Cd cycle (Nriagu, 1989).

Korte (1983) quantified the average Cd content in sea water (≤ 0.1 µg/L) while the river water (Mississippi, Yangtze, Amazon, and Orinoco sampled between 1976 and 1982) contained dissolved Cd < 1.1-13.5 ng/L (Shiller and Boyle, 1987). Concentrated Cd levels had been found up to 5 mg/kg in river and lake sediments but lower levels in marine sediments from 0.03 to 1 mg/kg (Korte,1983).

3.2.2.2 Anthropogenic sources

Drying of zinc concentrates and roasting, smelting, and refining of ores of metal production processes are the largest source of anthropogenic atmospheric Cd emissions. Waste incineration, production of Ni-Cd batteries, fossil fuel combustion, cement manufacturing and generation of dust by industrial processes are main source of anthropogenic sources of Cd emission in atmosphere (Bi *et al.*, 2006). In addition, mine water, concentrate processing water, leakages from mine tailings, process water from smelters, phosphate mining and related fertilizer production, and electroplating wastes are the largest contributors of Cd contamination in water (Bi *et al.*, 2006).

Landfills could be contaminated mainly by human activities, i.e. smelters, iron and steel plants, electroplating wastes, and battery production. Mine tailings of zinc mining also have the potential to transfer Cd to the surrounding environment (Bi *et al.*, 2006). The wordwide emission of Cd from human activities is shown in Table 3-1. Release of Cd to atmosphere, aquatic sources and land was quantified by Nriagu and Pacyna (1988).

3.2.3 Transport of Cd in environment

3.2.3.1 Atmospheric deposition

Dry deposition and precipitation are the processes which Cd is removed from the atmosphere. In rural areas of Scandinavia and Tennessee, USA, annual Cd deposition rates had been measured at about 0.4-0.9 g/ha (Laamanen, 1972; Andersson, 1977; Lindberg *et al.*, 1982). Agricultural soils in rural areas of the European Economic Community (EEC) had a value for the atmospheric Cd deposition of 3 g/ha per year (Hutton, 1982). Higher levels of relative Cd input for these areas was found at 5 g/ha per year from the application of phosphate fertilizers.

Mining industries often had elevated Cd deposition nearest smelter facilities and generally decreased rapidly with distance (Hirata, 1981). Many industrial sources emitted atmospheric Cd with wide dispersion and dilution along soil and water surface. For example, close to long established smelters, soil Cd concentrations commonly exceeded 100 mg/kg (Buchauer, 1972).

3.2.3.2 Transport from water to soil

Table 3-1 Total worldwide emissions of Cd from metal extraction, manufacturing processes, use and disposal of Cd-containing product.

	Emission to atmosphere (t/y)	**Emission to water (t/y)**	**Emission to land (t/y)**
Mining	0.6 - 3		
Pb production	39 - 195		
Cu-Ni production	1,700 - 3,400		
Zn-Cd production	920 - 4,600		
Secondary non-ferrous production	2.3 - 3.6		
Refuse incineration	59 - 1,436		
Phosphate fertilizer	68 - 274		
Cement production	8.9 - 534		
Total smelting and refining		0.01 - 3.6	
Iron and steel manufacturing	28 - 284		
Metal manufacturing processes		0.5- 1.8	
Chemical manufacturing processes		0.1 - 2.5	
Base metal mining and dressing		0 - 0.3	
Domestic wastewater		0.48 - 3.0	
Stream electric		0.01 - 0.24	
Municipal sewage sludge		0.08 - 1.3	0.02 - 0.34
Agricultural waste			0.2 - 6.4
Wastage of commercial product			0.78 - 1.6
Urban refuse			0.88 - 7.5

Table 3-1 Total worldwide emissions of Cd from metal extraction, manufacturing processes, use and disposal of Cd-containing product. (Cont.)

	Emission to atmosphere (t/y)	**Emission to water (t/y)**	**Emission to land (t/y)**
Coal fly ash and bottom ash			1.5 - 13
Animal wastes, manure			0.2 - 1.2
Fertilizer			0.03 - 0.25
TOTAL	3,100 - 12,040	2.1 - 17	5.6 - 38

(Source: Nriagu and Pacyna, 1988)

Land surrounding rivers can be contaminated with Cd anthropogenically by irrigation for agricultural purposes, by the dumping of dredged sediments, or naturally by flooding (Forstner, 1980; Sangster *et al.*, 1984). For example, excess of 70 mg/kg of soil Cd concentrations was found in land adjacent to the Neckar River, Germany, where the soil received dredged sediments to improve quality for agriculture (Forstner, 1980).

Industrial sources release Cd to fresh waters, where it is rapidly adsorbed by particulate matter in water. It may settle down or remain dissolved depending on locally environmental conditions. Settlement in sediment results in low concentrations of dissolved Cd in rivers that receive and transport large quantities of the metal (Yamagata and Shigematsu, 1970)

3.2.4 Toxic effects of Cd

Cd accumulates especially in the kidneys of human body. Kidney damage at renal tubule is probably the critical health effect for general population and occupational exposed workers. The accumulation of Cd in the renal cortex of kidney leads to impaired reabsorption of proteins, glucose, and amino acids by kidney (Nordic Council of Ministers, 2003). Cd exposure can lead to skeletal damage (osteoporosis) in both human and animal. High exposure of Cd in long term results in chronic obstructive airway disease in lung of exposed workers. The symptom may progress from early minor changes in ventilatory function to respiratory insufficiency. High exposure of Cd increases mortality rate from obstructive lung disease in workers (Nordic Council of Ministers, 2003). Other effects of Cd exposure are disturbances in calcium metabolism, hypercalciuria and formation of stones in the kidney (Nordic Council of Ministers, 2003).

The International Agency for Research on Cancer (IARC) classifies Cd as the carcinogenic agent to humans. Occupational exposure is linked to lung cancer and prostate cancer. The epidemiological data links Cd to cancer firstly at lung and then at prostate. Cancer in liver, kidney and stomach is considered equivocal when exposed to same dose of Cd (Nordic Council of Ministers, 2003).

3.3 Lead (Pb)

3.3.1 Chemistry of Pb

Pb has atomic number of 82, relative atomic mass of 207.20, electronegativity of 2.33 and crystal ionic radii are 133 pm for Pb^{2+} and 91.5 pm for Pb^{4+}(Shannon, 1976). Pb belongs to carbon group in the periodic table. It is a metallic element present naturally in ore which also contains Zn, Ag and commonly Cu. It can be extracted as a co-product of these metals. The main Pb minerals are galena (PbS) which contain 86.6% Pb, cerussite ($PbCO_3$) and anglesite ($PbSO_4$) (Australian Mines Atlas, 2012).

3.3.2 Emission of Pb

3.3.2.1 Natural sources

The estimation of total amount of Pb in earth's crust is 3.1 x 10^{14} tons. Pb usually occurs in all rocks, soils and dust with low concentration, ranging from 2 to 200 ppm. Relative high concentrations of Pb, are found in underlying parent rock with significant Pb content. Waters generally contain low Pb contents whereas significant amounts of Pb-rich dusts and vapors in the air are from windblown material and volcanoes. However, Pb release resulting from human activity has such a high level than those natural emissions. Global Pb emission by human activity is fairly high in comparison to global Cd emission to the environment (Thornton, 1995).

3.3.2.2 Anthropogenic sources

Metal mining and smelting are main sources of anthropogenic Pb emission. Emission of Pb to air is mainly generated from windblown dusts, loaded with ore particles from handling and storage of mining operation. The abundant Pb dust and particles are deposited in the near vicinity of the operation site. Refining and manufacturing of Pb, which involve heating Pb to above 500 ^{o}C, will potentially release Pb fume. In addition, processes of coal, oil and fossil fuel combustion, wood burning and cement manufacturing, have released Pb in to the atmosphere. Traces of Pb, which are released to the environment, can be estimated annually as high as 31,000-84,000 tons (Nriagu and Pacyna, 1988).

Transport of Pb to water is mainly from discharge of wastewater from ore recovery and treatment, release from tailing in mining process. The tailing is where sulphide oxide and acidic drainage water dissolve Pb in old mine working process. This is an important source of Pb contamination in the environment (Nriagu and Pacyna, 1988).

Historical smelting in uncontrolled conditions has resulted in considerable quantities of Pb as vapor prior to condense onto dust particles and deposit onto soil and water. This has caused local contamination of land around historical smelting operations (Maskall *et al*., 1995, 1996).

More than half of the Pb consumed today comes from recycling, rather than mining. Pb consumption in various manufactures are anthropogenic sources of Pb. These include battery, cable sheeting, rolled/extruded Pb (mainly sheet), ammunition, alloys, Pb compounds and petrol additives (Australian Mines Atlas, 2012). Table 3-2 shows total world-wide emissions of Pb from metal extraction and manufacturing processes and Table 3-3 presents emissions from use and disposal of Pb-containing products.

3.3.3 Transport of Pb in environment

3.3.3.1 Atmospheric deposition

Atmospheric input of Pb is from rainfall and dry deposition to sea and river. For example, the total annual wet and dry atmospheric flux was estimated at 175-950 tons around Ligurian sea and French Mediterranean coast. Pb has

Table 3-2 Total worldwide emissions of Pb from metal extraction and manufacturing processes.

	Emission to atmosphere in 1983 (t/y)	**Emission to water (t/y)**	**Emission to land (t/y)**
Mining	1,700 - 3,400	250 - 2,500	130,000 - 390,000
Pb production	11,700 - 31,200		
Cu-Ni production	11,050 - 22,100		
Zn-Cd production	5,520 - 11,500		
Secondary non-ferrous production	90 - 1,440		
Total non-ferrous smelting and refining	28,360 - 66,240	1,000 - 6,000	195,000 - 390,000
Iron and steel manufacturing	1,065 - 14,200	1,400 - 2,800	
Metals manufacturing processes		2,500 - 22,000	4,100 - 11,000
Chemical manufacturing processes		400 - 3,000	
Pulp and paper manufacturing		10 - 900	
Petroleum products manufacturing		0 - 120	
TOTAL	31,125 - 83,840	5,560 - 37,320	329,100 - 791,000

(Source: Nriagu and Pacyna, 1988; cited in Thornton, 1995)

high level of atmospheric deposition and a clearer transporation process with shorter time and space when compared to other heavy metals such as Cd, Cu and Zn (Migon *et al*., 1990)

3.3.3.2 Transport from water to soil

Table 3-3 Emission of Pb from use and disposal of Pb-containing products.

	Emission to atmosphere in 1983 (t/y)	**Emission to water (t/y)**	**Emission to land (t/y)**
Mobile sources	248,030		
Wastage of commercial products			195,000 - 390,000
Urban refuse	incineration of 1,400 - 2,800		18,000 - 62,000
Municipal sewage sludge	incineration of 240-300	2,900 - 16,000	2,800 - 9,700
Domestic waste water		1,500 - 12,000	
TOTAL	249,670 - 251,130	4,400 - 28,000	215,800 - 461,700

(Source: Nriagu and Pacyna, 1988; cited in Thornton, 1995)

In urban areas, Pb can contaminate from Pb particles in the air, Pb dust in the environment and can dissolve from Pb pipe and corrosion of Pb roof, etc. Pb remained in solution is eventually discharged into sewage system. Rainfall also leaches Pb into sewage system. Pb in treated water fraction is in the sewage sludge. The applying of sewage sludge to agricultural land can cause elevated Pb levels in soils. In addition, the historic use of Pb arsenate as a pesticide in orchards was a minor source of Pb contamination in soil (Alloway and Ayres, 1997).

3.3.3.3 Transport from land to underground water

When Pb recycling is not practical, disposal of Pb contaminated waste to landfill would seem to be the option. However, over long term of landfill, leachate, which potentially contains toxic metals and organic substances, can migrate through surrounding soil and contaminated soil and underground water resources (Alloway and Ayres, 1997). The greater leachate can occur in newer landfills with larger amounts of organic acids present from decomposing organic material (Williams, 1998).

3.3.4 Toxic effects of Pb

In human, Pb can result in a broad range of biological effects due to level and duration of exposure (Nordic Council of Ministry, 2003). The effects on neurology, metabolism and behavior in children are vulnerable than in adults. Pb effect on the central nervous system is the great concern with general population. Low level exposure of the fetus and developing child may lead to damage to the learning capacity and the neuro-psychological development (Goyer, 1986). Higher Pb contents in the blood of children correlates with their lower IQ. Low Pb blood levels cause slowing of nerve conduction velocity and impairment of psychological and neuro-behavioral functions has also been found after long-term Pb exposure of workers (Nordic Council of Ministry, 2003).

Pb is known to cause kidney damage and possible renal failure. Some damages are reversible but in chronic exposure to high Pb levels, decreasing of kidney function may progress. Renal effects have been seen among the general population when more sensitive indicators of function were measured (Nordic Council of Ministry, 2003).

The correlative evidence for carcinogenicity of Pb in human and its level of inorganic Pb compounds is inadequate. IARC classifies Pb as the agent with possible carcinogenicity to humans. (Nordic Council of Ministry, 2003).

3.4 Zinc (Zn)

3.3.1 Chemistry of Zn

Zn has atomic number of 30, relative atomic mass of 65.38, electronegativity of 1.65 and crystal ionic radius of 88 pm for Zn^{2+} (Shannon, 1976). Zn is a metallic element in the same group of Cd in the periodic table. Zn ore is usually found with Cu, Pb, Fe and mainly in forms of Zn-blende or sphalerite ((Zn,Fe)S), smithsonite ($ZnCO_3$) and hemimorphite or calamine ($Zn_4(Si_2O_7)(OH)_22(H_2O)$) (Porter, 1991).

3.4.2 Emission of Zn

3.4.2.1 Natural sources

The naturally global emission of Zn to atmosphere is estimated by Nriagu (1989) at 4.5 x 10^{10} tons. Due to globally consumption of Zn continuously, it is needed to be reserved for using in the future. Total global Zn reserve is estimated around 2.5 x 10^{8} tons.

3.4.2.2 Anthropogenic sources

Anthropogenic sources of Zn are from Zn mining and Zn-consumed industries, which produce, concentrate and emit Zn to the environment. Historic emission of Zn in atmosphere, water and land is shown in Table 3-4. In the past, global Zn emission from mine tailings was estimated to be annual production of 1.2 x 10^{7} tons (Tolcin, 2011). It was used to protect iron and steel from rust or to be anti-corrosive agent. Refined Zn metal nowadays is used in industries of galvanizing, Zn-based alloys, brass and bronze including alkaline, Zn carbon and Zn air batteries. The values of Zn mined from 2006 to 2010 by domestic production and use in USA remain constant with recycling and recovering metal from secondary materials such as electric arc furnace dust and galvanizing residues (Tolcin, 2011).

3.4.3 Transport of Zn in environment

3.4.3.1 Atmospheric deposition

Industry such as smelter is the major anthropogenic source of Zn. Atmospheric input of Zn in the vicinity of Zn-contributing industrial sources can deposit Zn in river. Enrichment of suspended solid phase of Zn was found to be two-time higher than the background values from industrial source (Nolting and Helder, 1991). The historic increased atmospheric deposition of Zn was a reason for its high concentration in the river. Zn can go off-shore direction with distribution in aqueous phase and dependence on the levels of riverine input (Nolting and Helder, 1991).

3.4.3.2 Transport from water to soil

The leached Zn in sediment can form solid phase at fairly high concentration in the river sediment (Nolting and Helder, 1991). Its concentration can be reached three fold higher than pre-industrial input that released it to the river.

3.4.4 Toxic effects of Zn

Table 3-4 Total worldwide emissions of Zn from metal extraction, manufacturing processes, use and disposal of Zn-containing product.

	Emission to atmosphere (t/y)	**Emission to water (t/y)**	**Emission to land (t/y)**
Mining	310-620		
Pb production	195-468		
Cu-Ni production	4,250-8,500		
Zn-Cd production	46,000-82,800		
Secondary non-ferrous production	270-1,440		
Refuse incinetation	2,800-8,400		
Phosphate fertilizer	1,370-6,850		
Cement production	1,200-6,000		
Total smelting and refining		7.6-44	
Iron and steel manufacturing	7,100-31,950		
Metal manufacturing processes		25-138	
Chemical manufacturing processes		0.2-5	
Base metal mining and dressing		0.02-6	
Domestic wastewater		15-81	
Stream electric		6-30	
Municipal sewage sludge		2.6-31	18-57
Agricultural waste			12-150
Wastage of commercial product			310-620
Urban refuse			22-97

Table 3-4 Total worldwide emissions of Zn from metal extraction, manufacturing processes, use and disposal of Zn-containing product. (Cont.)

	Emission to atmosphere (t/y)	**Emission to water (t/y)**	**Emission to land (t/y)**
Coal fly ash and bottom ash			112-484
Animal wastes, manure			150-320
Fertilizer			0.26-1.1
TOTAL	70,250-193,500	77-375	689-2,054

(Source: Nriagu and Pacyna, 1988)

Zn is essential metal for human with exceptional biological and public health importance. Zn deficiency causes many diseases in children such as growth retardation, delay of sexual maturation, infection susceptibility and diarrhea. These contribute to death in population of young children (Hambidge and Krebs, 2007). Zn is cofactor for many enzymes and proteins such as alcohol dehydrogenase and carbonic anhydrase in human (Maret, 2013). Thus, many nutrition reports suggest Zn sources in food for good biological and biochemical nutrition. Zn is essential for growth, immune-competence and neuro-behavioral development. However, high exposure or consumption of excess Zn can also cause some diseases such as ataxia or lacking of voluntary coordination of muscle movement and also Cu deficiency in the body.

3.5 Toxicity of heavy metals to macroalgae

The awareness of how different organisms are affected by heavy metal toxicity is increasing. For the assessment of toxicity, biological tests are being used. The organisms suitable for testing chemicals and discharges in the aquatic environment are needed to evaluate the metal toxicity and its persistence. Heavy metal effects on algae include cell lysis, growth inhibition, reduced photosynthesis, disrupted calcification, disturbance in sexual reproduction and change in bioluminescence and encystment (Haglund *et al.*, 1996). A growth and photosynthesis inhibition test methods in terms of EC_{50} and oxygen production using the macroalga *Gracilaria tenuistipitata* as the test organism was a choice to assess the toxicity of substances, including heavy metals (Cu, Cd, Ni, Pb, Cr and Hg), herbicides and complex wastewaters (from textile and chemical industries) (Haglund *et al*., 1996; Collén *et al*., 2003). The toxicity indicator such as EC_{50} values in this red macroalga for heavy metals was found between 0.05 and 17 mg/L.

Some heavy metals are well known in causing induction of oxidative stress, Cu and Cd cause decreased growth and induced oxidative stress in *Gracilaria* by increasing oxidation of lipids and oxidative damage to protein with increased content of protein carbonyl groups. The pollutant metals induce the production of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), singlet molecular oxygen ($^1O_2$) and hydroxyl radicals ($^{\cdot}OH$), which cause a disturbance in the cellular oxidative status by inducing oxidative damage to lipids, proteins and nucleic acid, eventually disturbing cell function. Formation of ROS is normal in process such as photosynthesis, but becomes a problem when its production is increased under some stress conditions. Photosynthetic organisms reduce ROS concentration through enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and through low molecular weight compounds such as ascorbate, glutathione, flavonoids, tocopherols and carotenoids. In stress response to exposure to heavy metals, which generate ROS, organisms can potentially detoxify metal toxicity by increasing their antioxidative defense either by enzymatic or by non-enzymatic system. In case of Cu, *G. tenuistipitata* induced CAT, APX and SOD while Cd cause induction of CAD activity. Both metals increased non-enzymatic β-carotene and lutein (Collén *et al.*, 2003).

The absence of some algal species such as brown kelp *Lessonia nigrescens* from a coastal environment resulted from chronic enrichment of Cu. Cu at similar levels to the impacted area interrupted the life cycle of *L. nigrescens* by preventing the normal development of the gametophytic microscopic phase (Contreras *et al*., 2007). Excess Cu could induce an oxidative condition in some tolerant seaweeds (Andrade *et al*., 2006a). In this case the persistance of seaweeds in the area affected to the mine wastes may be reduced to be moderated by an effective antioxidant system that buffers the lethal dose of Cu-induced oxidative stress. The biochemical response of *L.*

nigrescens in Cu stress condition showed induction of uncontrolled lipoperoxide accumulation, which may lead to cell damage and dysfunction (Contreras *et al*., 2007; 2009). The toxicity of heavy metals Pb and Cd in a green alga, *Cladophora fracta* showed damage and reduced number of chloroplasts, disintegrated cell wall and cell death (Lamai *et al*., 2005).

Another application of using seaweed as biomonitor of pollution by metals was also attractive though the mechanisms by which seaweeds detoxify and resist are not clear (Pawlik-Skowronska *et al*., 2007). The occurrence of non-enzymatic stress response can be used to indicate environmental pollution. Metal-complex-thiol peptide, phytochelatins (PC) in natural populations of brown, red and green seaweeds is a kind of biomarker of metal pollutions. It was synthesized from reduced glutathione (GSH) and involved in homeostasis and detoxification of metals in cells of higher plants, eukaryotic microalgae, some fungi and lichens (Zenk, 1996; Pawlik-Skowronska *et al*., 2002). The complex intracellular metals and metalloids are formed, with the binding to PCs and GSH. Exposure to high concentrations of Cd and Cu can induce PCs in several seaweed species. Pawlik-Skowronska *et al*. (2007) reported the first time PC found in native Phaeophyceae (*Fucus* sp.), Rhodophyceae (*Solieria chordalis*) and Chlorophyceae (*Rhizoclonium tortuosum*) but not in thalli of *Ulva* sp. and *Codium fragile* (Chlorophyceae). However, the involvement of these thiol peptides in metal homeostasis, detoxification and resistance to heavy metals varied between seaweed species that are growing under the same environmental conditions (Pawlik-Skowronska *et al*., 2007). The benthic macroalga *Vancheria* (Xanthophyceae) was investigated for the production of PCs in response to Cd uptake under laboratory conditions. In both *V. compacta* and *V. debaryana*, external Cd concentration strongly affected the level of PCs, which increased with time of Cd exposure. At low temperature in darkness, exposure of the algae to Cd resulted in significant decrease in PC content over the time interval tested (3 days) (Skowronski *et al*., 1998).

3.6 Bioaccumulation of heavy metals

It is essential to establish an appropriate terminology relative to the uptake and concentration of metallic species exhibited by different types of organisms and their products. The active mode of metal accumulation by living cells is usually designated as bioaccumulation, which is the process dependent on the metabolic activity of the cell and can be affected by the presence of the metallic ions. Metal tolerance may reflect the ability of an organism to survive in an environment with a high concentration of metals or to accumulate high concentration of metal without dying (Volesky, 1990).

Hyperaccumulation is the process that organisms accumulate high levels of metal ions in their tissues when they are grown in polluted water. Members of aquatic plants such as duckweed family could accumulate Cd ions with greater concentration than the external concentrations. These species possessed very high bioconcentration factors (BCF). The BCF provides an index of ability of the plant to accumulate the metal ions in relative to the metal ion concentration in surrounding environment. The maximal accumulation of heavy metal ions achieved by an organism species can possibly be classified as a hyperaccumulator, which will be a good bioremediator for those metal ions. To define hyperaccumulation, the following concentrations in the dry matter of any aboveground tissues have been suggested as threshold: 10,000 µg/g for Zn and Mn; 1,000 µg/g for Ni, Co, Cr, and Pb; and 100 µg/g for Cd and Se (Reeves *et al*., 1996). The BCF may sometimes be a better indicator than other index if the external metal ion level is too low (Boonyapookana *et al*., 2002).

The use of macroalgae as metal-removal material is an alternative application. There are many studies in metal accumulation of heavy metal in marine algal tissues such as *Ulva rigida* (Fe, Mn), *Padina gymnospora* (Zn), *Gracilaria tenuistipitata* (Cd), *Undaria pinnatifida* (Pb) and *Cladophora* sp. (Cd and Zn) when grown in polluted water. The advantages of using living algae to remove toxic metals from contaminated water are ubiquitous and abundant biomass. They have colonized almost all parts of the world with their fast growth rates, hence rapidly regenerate supply of metal-removal material (Lamai *et al*., 2005). However, there are only a few studies of bioaccumulation in freshwater macroalgae. The accumulation study of *Cladophora fracta* showed that Pb and Cd levels was increased in algal tissue when the exposure time and concentration were increased. At the same duration, the BCF of Pb was higher than that of Cd, indicating that higher Pb accumulation potential of *C. fracta* than that of Cd (Lamai *et al*., 2005).

The application of bioaccumulation and biosorption are mainly for removal of metal cations from the solutions. The first description of bioaccumulation as the bioremediation method of wastewater treatment was elaborated in 1970. The algae have ability of accumulation of metal cations and elimination of other competing orgamisms, hence these dense metal-like growth of algae was observed in the lagoon near the vicinity of Pb and Zn mining and milling works (Naja and Volesky, 2010). The distinction between bioaccumulation and biosorption is important in attempts to use biomass for metal sequestering/concentration purposes. The soluble chemicals present in aqueous environment, can be bound to the biomass firstly through biosorption, which is the process that sorbates interact with biomaterials at cellular surface. Later, bioaccumulation become accumulated the sorbates inside the cells (Chojnacka, 2010). The comparison between biosorption and bioaccumulation process is shown in Table 3-5. The first stage of metal interaction to biomaterials is biosorption and then subsequent stages of bioaccumulation with transport of pollutant into inside the cells and finally increases of metal concentration of cells. In cells, the binding sites for the pollutant metal are available. These lower residual concentration of external metal concentration. Bioaccumulating organisms should be resistant to high loads of pollutants and do not have mechanisms which protect them from excessive accumulation inside the cell (Deng and Wilson, 2001; Kocberber and Donmez, 2007). They should have mechanism of intracellular binding, such as metallothioneins and phytochelatins, which are synthesized in response to the presence of toxic metal ions. They complex those pollutant metal ions, thus exclude them from normal metabolic process.

3.7 Biosorption of heavy metals

Biosorption is a potential method for removal of metal from wastewater and for recovery of precious metals. Based on metal binding capacities of various biological materials such as algae, bacteria, fungi, yeast and aquatic plants, biosorption has proved to be a potential bioremediation (Veglio and Beolchini, 1997).

Wastewater from mining waste and industrial effluents are commonly applied with the traditional treatment, physical and chemical processes such as

Table 3-5 The comparison between biosorption and bioaccumulation process.

Biosorption	**Bioaccumulation**
Passive process	Active process
Biomass is not alive	Biomass is alive
Metals are bound with cellular surface	Metals are bound with cellular surface and interior
Adsorption	Absorption
Reversible process	Partially reversible process
Nutrients are not required	Nutrients are required
Single-stage process	Double-stage process
The rate is quick	The rate is slow
Not controlled by metabolism	Controlled by metabolism
No danger of toxic effect	Danger of toxic effect caused by contaminants
No cellular growth	Cellular growth occurs
Intermediate equilibrium concentration of metal ions	Very low equilibrium concentration of metal ions

(Source: Chojnacka, 2010)

chemical precipitation, electro-chemical and/or membrane processes. However, these applications sometimes have some technical or economic constraints, for example, in the case of metal contamination lower than 100 mg/L, these processes might be ineffective and/or expensive (Veglio and Beolchini, 1997). Alternative metal removal and/or recovery methods are being considered. Based on metal-sequestering properties of natural biomaterials, certain types of biomass can retain relatively high quantities of binding sites for metal ions by passive sorption and/or complexation. This is commonly known as biosorption.

Biosorption is the passive metal sequestering capability of the cell. It is caused by a number of different physico-chemical mechanisms, depending on a number of external environmental factors as well as on the type of a metal, its ionic form in the solution, and on the type of a particular active binding site responsible for sequestering the metal. An important feature of biosorption is that it can be responsible for binding and accumulating metallic species even when the cell is no longer metabolically active, or when it is dead. The remaining cell debris such as cell walls can still represent a potent biosorbent (Volesky, 1990).

Biosorption is a process that utilizes low cost biosorbents to sequester toxic heavy metals. The advantages of biosorption over the conventional methods (precipitation, adsorption with activated carbon, ion exchange, membrane processes, oxidation and reduction) are low operating cost, selectivity for specific metal, short operational time and no chemical sludge. Removing metal from wastewater required the use of efficient biosorbent materials. Many agricultural-based waste materials have been employed for the treatment of Pb wastes which include: coconut husk and shell (Tan *et al*., 1993; Amuda *et al*., 2007), seaweed (Kratochvil *et al*., 1998; Senthilkumar *et al*., 2007), sago waste (Quek *et al*., 1998), hazelnut shell (Cimino *et al*., 2000; Bulut and Tez, 2007), peanut hull (Brown *et al*., 2000), tree fern (Ho, 2005), black gram husk (Saeed *et al*., 2005), maize leaf (Babarinde *et al*., 2006), maize, sunflower waste (Zhang and Banks, 2006), rice husk (Zulkali *et al*., 2006), coffee beans (Kaikake *et al*., 2007), *Ficus religiosa* leaves (Qaiser *et al*., 2007), wheat bran (Ozer, 2007), almond shell (Bulut and Tez, 2007), tea waste (Amarasinghe and Williams, 2007), etc. The biosorption of Pb by *F. religiosa* leaves in powder showed the release of light metals like Cu, Mg, Na and K in the column effluents. These metals were released in exchange with the Pb binding to the biomass. This revealed that ion exchange was the major removal mechanism of biosorption in *F. religiosa* (Qaiser *et al*., 2009).

The use of dead, dried aquatic plants, for metal removal as a sample biosorbent material also has advantages in its high efficiency in detoxifying dilute effluents, minimization of the volume of chemical and/or biological sludge to be disposed of, no nutrient requirement and low cost. It is also possible to recover heavy metals, as once the metal biding reaches saturation, the biomass can be regenerated with desorbents (acid and/or hydroxide solutions) that discharge small volumes of concentrated heavy metal. The aquatic plants (*Spirodela intermedia*, *Lemma minor*, *Pistia stratiotes* and *Wolffia globosa*) were dried and studied in biosorptions of Cd, Ni, Cu, Zn, Pb and Cr (Miretzky *et al*., 2006; Upatham *et al*., 2002). In *S. intermedia*, *L. minor* and *P. stratiotes* the main mechanism involved in biosorption was also ion exchange between metals as counter ions present in the macrophyte biomass and heavy metal ions and protons taken up from water. Pb and Cd were efficiently removed by these three species, in spite of the presence of other metals in the water solution that competed for limited number of binding sites in the biomass surface. *S. intermedia* and *L. minor* biomass presented the higher average removal percentage than that of *P. stratiotes* (Miretzky *et al*., 2006). Aquatic plant, *Hydrilla verticillata* was shown to be an excellent biosobent for Cd. Metal ions were rapidly adsorbed and reached equilibrium within 20 minutes (Bunluesin *et al*., 2007). In continuous flow studies, *H. verticillata* in the fixed-bed column was capable of decreasing Cd concentration from 10 mg/L to below 0.02 mg/L. This knowledge can be applied for treating wastewater with a low concentration of Cd contaminants (Bunluesin *et al*., 2007).

Many biomaterials have been studied for the removal of metal contamination in water solution. Biosorptions of Cr. Cd, Cu, Pb, Zn and Ni by various biomaterials including: microalga *Scenedesmus incrasstulus* (Pena-Castro *et al*., 2004), cyanobacteria *Spirulina* (*Arthrospira*) *platensis* (Rangsayatorn *et al*., 2002), bacteria *Sphingomonas paucimobilis* (Tangaromsuk *et al*., 2002), *Bacillus brevis* (Kumar *et al*., 2009) and *Aeromonas hydrophila* (Hasan *et al*., 2009) and fungi *Penicillium simplicissimum* (Fan *et al*., 2008), were conducted in batch and/or immobilized biomaterial. Moreover, removal of heavy metals from industrial wastewater was also studied in various natural meterials such as animal bone powder, active carbon, Nile rose plant powder, commercial carbon and ceremics (Abdel-Halim *et al*., 2003).

Besides heavy metals, biosorptions of other toxic substance such as phenanthrene and dyes which also cause serious water pollution problems were studied in various biomaterials. Phenanthrene, a polycyclic aromatic hydrocarbon, which has mutagenic and carcinogenic properties can be adsorbed by dried brown seaweed *Sargassum hemiphyllum* (Chung *et al*., 2007). Biosorption of the basic dye (methylene blue), a pollution in form of reduced light penetration and photosynthesis was also studied in the giant duckweed *Spirodela polyrrhiza* (Waranusantigul *et al*., 2003).

3.7.1 Biosorption machanisms

The complexity of cell structure provides many ways for metal to be captured. Some mechanisms such as transport of the metal across cell membrane give intracellular accumulation and reaction associating with an active defense system of cells. Biosorption mechanism has been widely studied among microorganisms including microalgae and macroalgae. Although various biosorptions are still not very well understood, they may be classified by the following different criteria (Veglio and Beolchini, 1997).

According to the dependence on the cells' mechanism (Fig. 3-1) (Veglio and Beolchini, 1997)

1) Metabolism dependent
2) Non-metabolism dependent

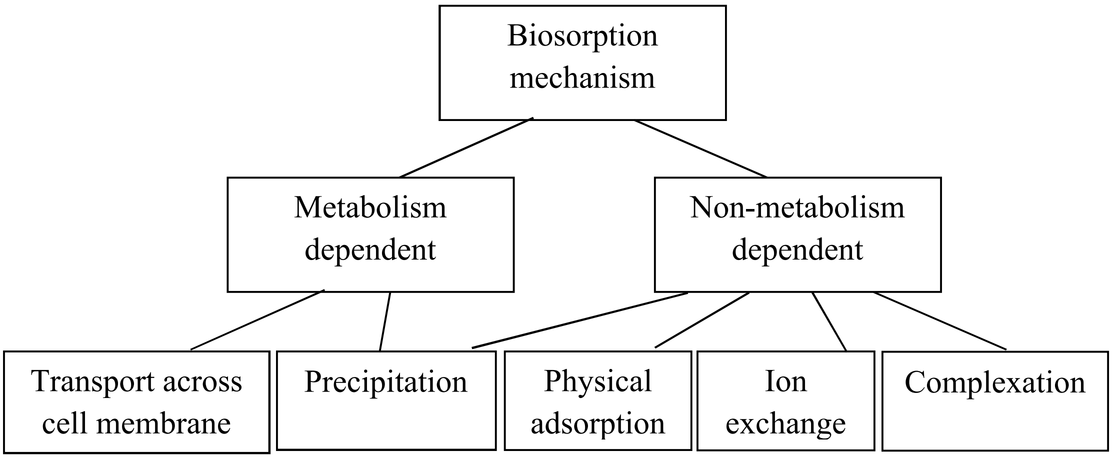


Figure 3-1 Biosorption mechanisms according to the dependence of cells' metalbolism.

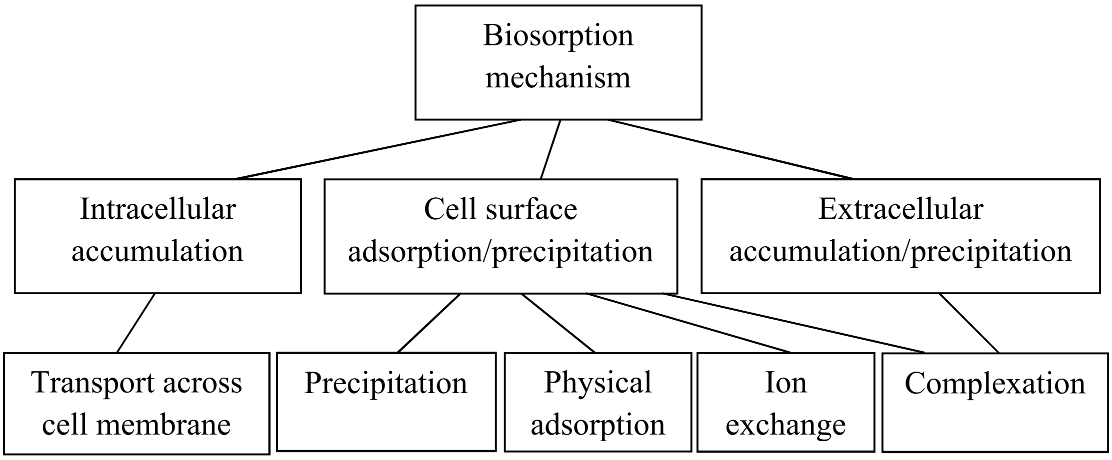


Figure 3-2 Biosorption mechanisms according to location where the metal is removed from the solution.

According to the location where the metal is removed from the solution (Fig 3-2) (Veglio and Beolchini, 1997)

1) Extracellular accumulation/ precipitation
2) Cell surface sorption/ precipitation
3) Intracellular accumulation

Biosorption from the cells' metabolism takes place only with viable cells having an active defense system to toxic metal. In the case of physico-chemical interaction between the metal and functional groups of the cell surface is not dependent on the metabolism. Cell wall of the dead biomass still composes mainly of polysaccharides, proteins and lipids and offers particular abundant metal-binding functional groups such as carboxylate, hydroxyl sulfate, phosphate and amino groups. Non-metabolism dependent biosorption is relatively rapid and can be reversible. In the most common mechanism, chemical characteristics of an ion exchange resin or of an activated carbon imply many advantages in the industrial application of biosorption (Veglio and Beolchini, 1997).

In the case of precipitation, metal takes place both in solution and on the cell surface. This may be dependent on cells' metabolism. In the presence of toxic metals, microorganism produces compounds which favor the precipitation process. On the other hand, precipitation may not depend on cells' metabolism. It may occur after a chemical interaction between the metal and the cell surface.

Each mechanism found in literature is reported below (Veglio and Beolchini, 1997).

3.7.1.1 Transport across the cell membrane

Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically essential ions such as K, Mg and Na. The metal transport system may become confused by the presence of heavy metal ions of the same charge and ionic radius. This kind of mechanism often takes place contemporary to biosorption phenomena not link to metabolic activity. Biosorption by living microorganisms comprises two basic steps. Firstly, an independent metabolism binding to cell walls, in which biosorption occurs and secondly, metabolism-dependent intracellular uptake, whereby metal ions are transported across the cell membrane into the cell and related to cellular accumulation (Veglio and Beolchini, 1997).

3.7.1.2 Physical adsorption

The physical adsorption is associated with the van der Waals' forces. Radionuclides present in the sea are accumulated by marine microorganisms through direct adsorption from the water (Tsezos and Volesky, 1981). Th and U biosorption by fungal biomass of *Rhizopus arrhizus* is also based on physical adsorption in cell-wall chitin structure (Tsezos and Volesky, 1982). In addition, U, Cd, Zn, Cu and Co biosorption by dead biomass of algae, fungi and yeasts take place through electrostatic interactions between ions in solution and cell walls (Kuyucak and Volesky, 1988).

3.7.1.3 Ion exchange

Cell walls of microorganisms including micro- and macroalgae contain polysaccharides as basic building blocks. With ion exchange properties of natural polysaccharides, bivalent metal ions can exchange with counter ions within the polysaccharides (Tsezos and Volesky, 1981). For example, alginates of marine algae usually occur as natural salts of K^+, Na^+, Ca^{2+} and/or Mg^{2+}. These metallic ions can exchange with the heavy metal ions such as Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, resulting in the biosorptive uptake of the metals (Kuyucak and Volesky, 1988).

3.7.1.4 Complexation

The metal removal from solution also takes place through complex formation on the cell surface after interaction between metal and active groups. Metal ions can bind to ligands or through chelation. *R. arrhizus* has a mechanism not only based on physical adsorption, but also on complex formations: metal ions coordinate with the nitrogen of chitin cell wall network (Tsezos and Volesky, 1981). Biosorption of Cu by *Chlorella vugaris* and *Zoogloea ramigera* took place through both adsorption and coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides (Aksu *et al*., 1992).

3.7.1.5 Precipitation

Precipitation may be either dependent on the cellular metabolism or independent of it. In the case of cellular metabolism, the metal removal from the solution is often associated with an active defense system of microorganisms.

In the presence of toxic metal, they produce compounds, which favor the precipitation process at cell surface (Veglio and Beolchini, 1997). For example, some *Arthrobacter* and *Pseudomonas* species can eliminate Cd from the solution by detoxicification systems that precipitate Cd on the cell surface (Scott and Palmer, 1990). In the case of non-metabolic mechanism, it may be consequence of the chemical interaction between the metal and the cell surface. For example, U biosorption by *R. arrhizus* has mechanism, which terminates step by firstly formation of complex uranium-chitin and followed by the complex hydrolysis and finally precipitation of the hydrolysis product (uranylhydroxide) in the cell wall (Tsezos and Volesky, 1982). Moreover, an additional mechanism was proposed such as entrapment of metals in the form of insoluble micro-deposits, can contribute to Cd biosorption by the biomass of marine algae (Holan *et al*., 1993).

From the literature, biosorption mechanisms are not various, but they can also take place simultaneously.

3.7.2 Biosorption system

3.7.2.1 Biosorption in batch system

Free or non-immobilized cells or dried cells of biomaterials are free in aqueous solution. Large contact of metal-bearing solutions with biomass is not practical due to solid/ liquid separation problems, however it is fundamental for the industrial application of biosorption since it gives information about equilibrium of the process (Veglio and Beolchini, 1997).

The adsorption or uptake is usually measured by parameter q (milligrams of metal accumulated per gram of biosorbant material). The result can be compared between this parameter and the highest observed value of the specific uptake/ adsorption, q_{max} (Veglio and Beolchini, 1997).

In fact, works of different authors cannot be compared directly, since the operating conditions are often different. Some factors affecting biosorption are influent to the equilibrium of the biosorption process (Veglio and Beolchini, 1997).

3.7.2.2 Biosorption by immobilized-live or -dead biomass

Although small particles of biomass contact large volume of metal containing aqueous solutions, immobilization of biomass can create a biomaterial with right size, mechanical strength, rigidity and porosity necessary for the operations. Various techniques are used for biomass immobilization. The techniques below are created for immobilzing microbial and algal biomass (Veglio and Beolchini, 1997).

1) Adsorption on inert supports

Support materials are used in towel fermentor to support the continuous culture for a period of time until a film of microorganism is apparent on the support surfaces for example reticulated foam biomass support particles are used for immobilization of *R. arrhizus* fungal biomass (Zhou and Kiff, 1991); production of *Enterobacter aerogenes* biofilm attached to activated carbon is used for immobilization of bacterium *Citrobacter* sp. (Macaskie *et al*., 1987); glass Raschig rings are used for immobilization of *Bacillus* sp. cells (Cotoras *et al*., 1993).

2) Entrapment in a polymeric matrix

Various polymers are used. Calcium alginate and polyacrylamide in the form of gel particles can be used to immobilize biomass. Polysulfone and polyethylenimine are proved to be the strongest immobilizing polymer (Veglio and Beolchini, 1997).

3) Covalent bonds to vector compounds

The most common vector compound forming stable cellular aggregates is silica gel in the form of gel particles. This technique is used for algal immobilization (Holan *et al*., 1993; Mahan and Holcombe, 1992).

4) Cell cross-linking

Various cross linkers are used to immobilize algae. The most common cross-linkers are formaldehyde, glutaric dialdehyde, divinylsulfone and formaldehyde-urea mixtures (Holan *et al*., 1993).

5) Alternative immobilization

Cross-flow microfiltration is used to retain *Saccharomyces cerevisiae* biomass by membrane during metal accumulation (Brady *et al*., 1994).

6) Packed bed column

A continuous flow column is used to pack the biomass particle. A packed bed column allows more efficient utilization of biosorbent capacity and results in a better quality of the wastewater effluent (Volesky and Prasetyo, 1994). The biomass was fixed and fluidized in bed reactors or columns, which have been used widely by chemical industry, pharmaceutical industry, food industry, wastewater treatment and for recovery of different substances (Schiewer and Volesky, 1996).

3.7.3 Factors affecting biosorption system

3.7.3.1 Temperature

Temperature in the range of 10-35°C seems not to influence biosorption (Vilar *et al*., 2005). However, there is evidence of Se biosorption by green alga (*Cladophora hutchinsiae*) indicated that the biosorption percentage decreased from 96% to 60% as temperature was increased from 20 to 50°C during the equilibrium time (Tuzan and Sarl, 2009).

3.7.3.2 pH

pH is the important factor in biosorptive process due to its effects on solution chemistry of the metals, the activity of functional groups in the biomass and the competition of metallic ions. At low pH the proton competes with metal ions for the active sites on biomass surface so, a remarkable inhibition is observed (Ho, 2005; Liu *et al*., 2009).

3.7.3.3 Sorbent dosage

Sorbent doses in solution affect amount of binding sites within the biomass for metal ions and the specific uptake/adsorption. The increasing sorbent dosage increases amounts of binding sites but the lower biomass concentrations increase the specific uptake/adsorption according to interference of active sites. Therefore, this factor was needed to be taken into account in any application of biomass as adsorbant (Veglio and Beolchini, 1997).

3.7.3.4 Ionic strength

Adding of an electrolyte seems not to influence adsorption due to some ions such as potassium does not compete directly with the covalent binding of heavy metals on the biosorbant surface (Vilar *et al.*, 2005). However, there is evidence showing that the influence of ionic strength on biosorption of Pb is important for *Gelidium* alga (Vilar *et al*., 2005). Changing the ionic strength by addition of an electrolyte influences adsorption in at least two ways (Vilar *et al*., 2005): (1) by affecting interfacial potential and therefore the activity of electrolyte ions and adsorption, and (2) by affecting the competition of the electrolyte ions and adsorbing anions for sorption sites.

3.7.3.5 Pretreatment of biomass

Before biosorption, pretreatment of algal biomass may be conducted to enhance the metal removal ability of the biomass. Various pretreating agents include acid pretreatment (HCl and HNO_3) (Gupta *et al.*, 2010), alkali pretreatment (NaOH) (Gupta and Rastogi, 2008), hot water, ammonium acetate, methanol, acetone, formaldehyde (Singh *et al.*, 2007) and $CaCl_2$ (Gupta and Rastogi, 2008; Gong *et al.*, 2005). Among these agents, NaOH pretreatment of algal biomass slightly enhances metal removal of Cu and Pb (Singh *et al.*, 2007) and $CaCl_2$ treated biomass provided the maximum metal uptake of Cr(III) which indicated good biosorbents than other treated and untreated biomass (Bishnoi *et al.*, 2007). Pretreatment by chemical modification of biomass with epichlorohydrin (EC_1 and EC_2), potassium permanganate (PC), glutaraldehyde (GA) and distilled water (DW) affected the biosorption of marine brown alga *Laminaria japonica*. The order of maximum metal uptakes for $Cd^{2+,}$ Cu^{2+} and Zn^{2+} was EC_1 >EC_2 >PC >DW >GA but the uptakes of Ni^{2+} were almost the same for these sorbents (Liu *et al.*, 2009).

3.7.3.6 Water hardness

The effects of hard and soft water on Cd accumulation by representative macroalgae, *Nitella flexilis*, plant *Elodea canadensis*, and animals, snails (*Ampullaria paludosa*), catfish (*Corydoras puntatus*) and guppies (*Lebistes reticulatus*) as a freshwater community were investigated. The initial rate of Cd uptake by these organisms was faster in hard water than in soft water; however, the total concentration of Cd was greater in those organisms cultured in soft water than those in hard water (Kinkade and Erdman, 1975).

3.7.4 Desorption and regeneration of biomass

After biosorption, the elution of metals could be interesting for the reutilization of exhausted biomass and recovery of the adsorbed metal. Desorption can be carried out by proton exchange using acids (for example HCl, HNO_3, H_2SO_4 and citric acid), by exchange with other ions ($CaCl_2$, NaCl, Na_2CO_3 and NaOH) or by chelating agent (EDTA) (Deng *et al.*, 2007a; Deng *et al.*, 2007b; Gupta *et al.*, 2006;

Mata *et al*., 2009; Onyancha *et al*., 2008; Gupta *et al*., 2010; Gong *et al*., 2005). The efficient eluent is one that desorbs the metal completely without deteriorating the biomass in case it will be reused.

3.7.5 Biosorption model

3.7.5.1 Biosorption kinetics

The kinetic study has the important practical task to determine the degree of utilization of the adsorption capacity as a function of the time of contact between the liquid and the solid. Therefore the different models are used to fit the obtained kinetic curves in order to define the rate parameters and explain the mechanism of mass transfer.

1) Pseudo-first order kinetic

The pseudo-first order rate equation of Lagergren has long been widely applied and developed a rate equation, which considered rate of ion-exchange adsorption from aqueous solutions (Lagergren, 1898; Boyd *et al*., 1947). In the case of diffusion through a boundary liquid film and adsorption kinetics as a chemical phenomenon, a mass action rate equation for adsorption kinetics as a chemical phenomenon and a diffusion equation for diffusion through a boundary liquid film are the same as the pseudo-first order rate equation of Lagergren (Boyd *et al*., 1947). Therefore, the rate-controlling step can be film diffusion or chemically ion-exchange. The ion exchange reaction can be described as follows:

$$M^{2+} + (\text{L-Biomass}) \Rightarrow (\text{M-Biomass}) + L^{2+} \quad (1)$$

Where, M^{2+} and L^{2+} represent the divalent metal cations sorbed and released from the biomass.

The pseudo-first order rate equation of Lagergren can be generally expressed as follows:

$$\frac{dqt}{dt} = Kad1\ (qe - qt) \quad (2)$$

where q_e and q_t are the sorption capacity at equilibrium and at time t, respectively (mg/g), K_{ad1} is the rate constant of pseudo-first order sorption (L/min).

After integration and applying boundary conditions t=0 to t=t and qt=0 to qt=qt, the integrated form of eq. 2 becomes linear as follows:

$$\log(q_e - q) = \log q_e - \frac{K_{ad1}}{2.303} t \quad (3)$$

The usefulness of eq.3 is ability to determine the parameters of first-order rate reaction where concentration of one substrate (divalent or biomass ion) plays an important role in rate-limiting step.

2) Pseudo-second order kinetic

Similar to the first-order rate law, the rate controlling step of pseudo-second order is ion exchange reaction and the model was based on chemisorption involving valence forces through sharing or exchange of electrons between sorbent and sorbate (Ho and Mckay, 1999). The ion exchange reaction can be described as eq.1. The difference of order kinetics is considered on the number of active sites occupied on the biomass.

The pseudo-second order rate equation of Ho can be generally written as follows:

$$\frac{dqt}{dt} = Kad2\ (qe - qt)^2 \quad (4)$$

where q_e and q_t are the sorption capacity at equilibrium and at time t, respectively (mg/g), K_{ad2} is the rate constant of pseudo-second order sorption (L/min).

After integtrating and rearranging eq.4, the linearized form of the pseudo-second order kinetic, which can be used to calculate the parameter of pseudo-second order kinetic, is expressed as follows:

$$\frac{t}{q} = \frac{1}{K_{ad2} q_e^2} + \frac{1}{q_e} t \quad (5)$$

The parameters of second-order rate determine whether both concentrations of divalent and biomass ions play important role in rate-limiting step.

3.7.5.2 Determination of biosorption rate-limiting step

Intraparticle diffusion

For porous sorbents, diffusion effect may be quite important and the physical meaning of the evaluated rate constants has to be consequently determined in order to get insight into the transfer mechanism. The intraparticle diffusion model has been applied and explained by two or three main mechanisms, the external surface adsorption or instantaneous adsorption occurs in the first step; the second step is the gradual adsorption step, where intraparticle diffusion is controlled; and the third step is the final equilibrium step, where the solute moves slowly from larger pores to micropores causing a slow adsorption rate (Wu *et al.*, 2009). The steps of adsorption in porous sorbent is shown in Fig. 3-3.

Intraparticle diffusion equation, which can be used to determine the parameter of rate-limiting step, is expressed as follows:

$$q = K_{id}t^{\frac{1}{2}} \qquad (6)$$

where q is the amount of metal adsorbed (mg/g) at time t (min) and K_{id} is constant for intraparticle diffusion.

3.7.5.3 Equilibrium models

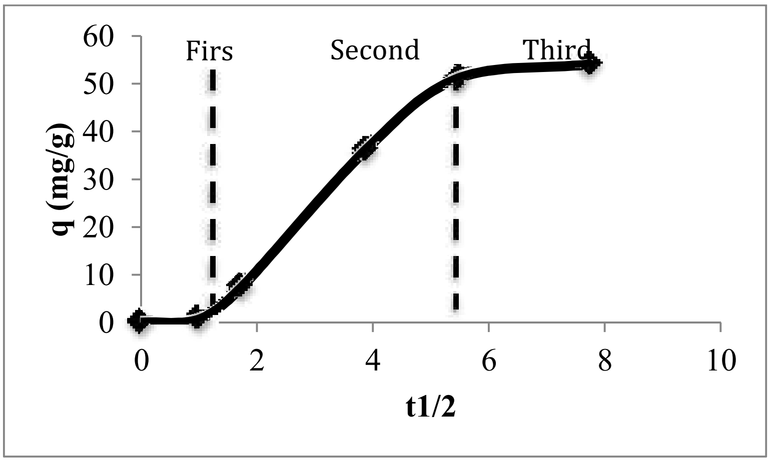


Figure 3-3 Steps of adsorption mechanisms in intraparticle diffusion model.

The equilibrium of the process is often described by fitting the experimental points with models usually used for the representation of the isotherm adsorption equilibrium. There are two widely accepted and easily linearized adsorption isotherm models used in the literature which were proposed, respectively, by Langmuir and Freundlich (Veglio and Beolchini, 1997; Volesky, 1990). These models can be applied only at a constant pH. In the case of variable pH, parameters should be considered to be functions of pH.

1) Langmuir model

The Langmuir model has many assumptions, namely:

- The surface consists of adsorption sites.
- All adsorbed species interact only with a site and not with each other.
- Adsorption is limited to a monolayer.

- Adsoption energy of all sites is identical and independent of the presence of adsorbed species on neighboring sites

A general form of the Langmuir model equation is

$$qe = \frac{qm\,KL\,Ce}{1+KLCe} \tag{7}$$

Where C_e is the metal residual concentration in solution; q_m is the maximum specific uptake corresponding to sites saturation; K_L is the ratio of adsorption/desorption rates or a constant related to the affinity between the sorbent and sorbate.

A Langmuir model can be linearized as

$$\frac{Ce}{qe} = \frac{1}{qm\,KL} + \frac{1}{qm}\,Ce \tag{8}$$

The values of q_m and K_L can be determined from a plot of C_e/q_e vs C_e, having a slope of $1/q_m$ and an intercept of $1/q_mK_L$. Another linear form can be obtained by dividing eq.8 by C_e:

$$\frac{1}{qe} = \frac{1}{qm\,KL\,Ce} + \frac{1}{qm} \tag{9}$$

Plotting $1/q_e$ against $1/C_e$, a straight line having a slope of $1/q_mK_L$ and an intercept of $1/q_m$ is obtained.

2) Freundlich model

The Freundlich model has some assumptions (Volesky, 1990):

- The surface consists of heterogeneous adsorption sites.
- Adsorption is a multi-layer.
- The linearized model is infinitude, thus and limited on estimation of maximum adsorption capacity.

The Freundlich adsorption model does not become linear at low concentrations but remains concave to the concentration axis; also, it does not show a saturation or limiting value. The general form of this model is

$$qe = KF\,Ce^{1/n} \tag{10}$$

Where K_F is a constant related to the capacity of adsorbent for adsorbate (l/g), and n is a constant that is a function of the strength of adsorbent.

This can be linearized by taking the logarithm of both sides of eq.10 to give

$$\log qe = \log KF + \frac{1}{n}\log Ce \tag{11}$$

Plotting log q_e vs log C_e, a straight line is obtained with a slope of $1/n$ and intercept of log K_F. The linear form of this isotherm can be obtained by plotting the data on log-log paper. For irreversibly adsorbed chemicals, $1/n$ (the slope of the line) is zero. For favorably adsorbed chemicals, $1/n$ is between 0 and 1. For unfavorably adsorbed chemicals, $1/n$ is greater than 1. For chemicals that are not adsorbed, $1/n$ approaches infinity (or is very large)

3) Thomas model

For biosorption in fixed bed column, the performance of the columns is analyzed using the effluent concentration versus time of volume curves. The plot is usually referred to as the breakthrough curve, which is a function of the column flow characteristics, sorption equilibrium and mass transfer factors. Typically, breakthrough curve is shown in Fig. 3-4. Breakthrough occurs when the effluent concentration reaches 5% of the influent value. Exhaustion of biosorption bed is assumed to have occurred when the effluent concentration is equal to 95% of the influent value. At complete exhaustion, the effluent concentration is equal to the influent concentration (Naddafi *et al.*, 2007).

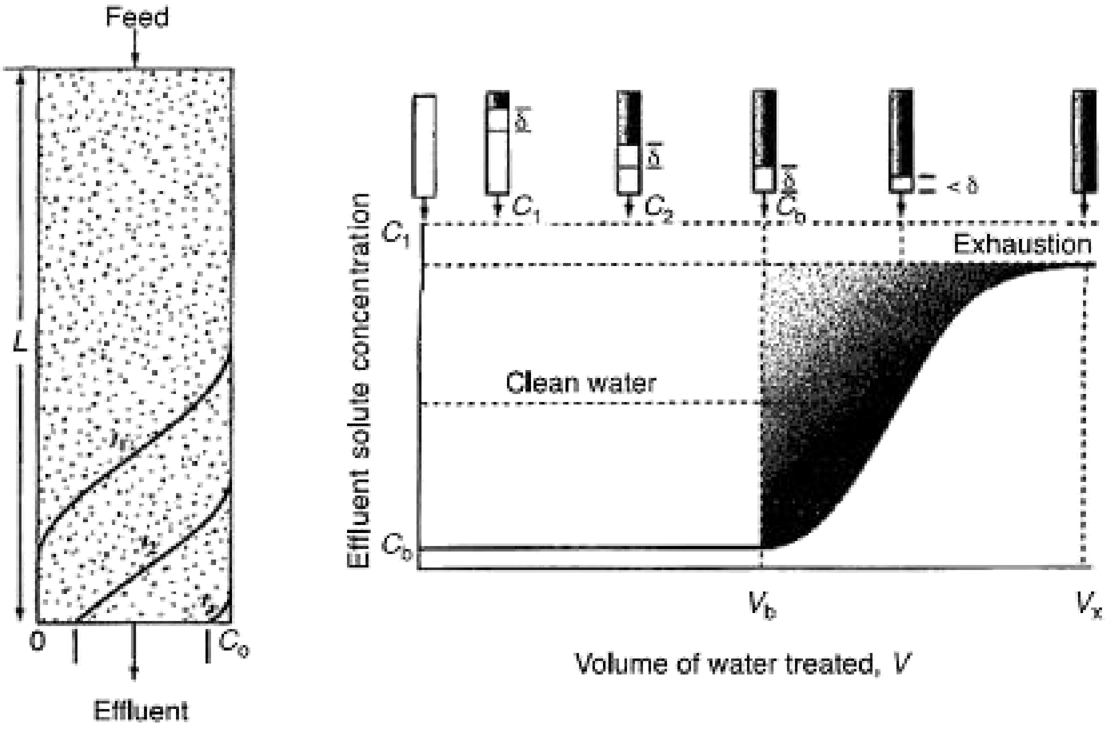


Figure 3-4 Downflow of packed bed column (left) and breakthrough curve of continuous packed bed column (right).

Successful design of column requires prediction of breakthrough curve and maximum sorption capacity. Various mathematical models can be used to describe fixed bed adsorption. Among these, the Thomas model is simple and widely used by several investigators (Yan and Viraranghavan, 2001; Aksu and Gönen, 2003). The Thomas or reaction model assumes Langmuir kinetics of adsorption-desorption, no axial dispersion and the rate driving force obeys second-order reversible reaction kinetics. Thomas solution also assumes a constant separation factor but it is applicable to either favorable or unfavorable isotherm (Yan and Viraranghavan, 2001; Aksu and Gönen, 2003). The Thomas model has the following form:

$$\frac{Cb}{C1} = \frac{1}{1+\exp(Kth/F(QmM-C1V))} \qquad (12)$$

where C_1 and C_b are metal ion concentrations (mg/L) in the influent and effluent, respectively, K_{th} is Thomas model constant (L/mg.h), Q_m is maximum biosorption capacity, M is sorbent mass (g), F is volumetric flow rate (L/h) and V is the volume of metal solution passed through the column.

Linear form of Thomas model is expressed as

$$\ln\left(\frac{C1}{Cb} - 1\right) = \frac{KthQmM}{F} - KthC1t \qquad (13)$$

When we plot ln [(C_1/C_b)-1] against time t (min), the maximum sorption capacity (Q_m) can be calculated from intercept $K_{th}Q_mM/F$ and K_{th} can be calculated from slope $K_{th}C_1$.

3.8 Biosorption of heavy metals by macroalgae

3.8.1 Marine macroalgae

Many workers have reported the potential of marine macroalgae for biosorption of toxic metals. Brown, red and green seaweeds were evaluated for the uptake or biosorption capacities for Pb, Cd, Cu, Zn and Ni ranged between 0.61-1.97 mmol/g. The biosorption performance of brown alga *Cysteseira baccata* has been studied for the removal of Cd and Pb. The maximum metal uptake or biosorption capacity was around 0.9 mmol/g and was affected by pH of the solution. Ionic strength of solution (calcium and sodium nitrate salts) was found to affect the metal biosorption (Lodeiro *et al*., 2006). Another brown seaweed *Laminaria japonica* which chemically-modified by cross-linking with epichlorohydrin was found to have the highest maximum uptake capacity of Pb around 1.67 mmol/g compared to other pretreatments, i.e., oxidizing by potassium permanganate and washing by distilled water (Luo *et al*., 2006). Most common biosorption isotherms usually follow Langmuir and Freundlish models. However, there are other isotherms such as Dubinin-Radushkevich model which was applied to describe biosorption isotherm of Cd by red alga*, Ceramium virgatum* (Sarl and Tuzen, 2008). The marine green macroalga, *Chaetomorpha linum*, was also investigated for the biosorption capacities of Cu and Zn. This dried algal material showed very high maximum capacities of Cu and Zn around 1.46 and 1.97 mmol/g, respectively. The kinetic data was also studied to indicate the fast biosorption rate in similarity to other algal material, *C. baccata* and *C. virgatum* (Ajjabi and Chouba, 2009; Lodeiro *et al*., 2006; Sarl and Tuzen, 2008). The kinetic examination of the equilibrium data showed the biosorption of metal ions onto *C. linum* biomass followed the pseudo-second-order kinetic model. Macroalgal materials mostly used for sorption of heavy metals were dried and studied in monometal system. Green macroalga *Caulerpa lentilifera* was investigated for the sorption isotherm and showed the affinity sequence of $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$ (Pavasant *et al*., 2006) similar to that of *Padina* sp.(Pb > Cu > Cd > Zn > Ni). However, another brown seaweed *Sargassum* sp. had a different affinity of Pb > Zn > Cd > Cu > Ni (Sheng *et al*., 2004).

3.8.2 Freshwater macroalgae

Biosorption utilizes the ability of some materials to accumulate heavy metals from aqueous solution by either metabolically mediated or physico-chemical uptake pathway. Biomass material can be used to adsorb heavy metals present at very low concentration. There are many reports on the biosorption of heavy metals by freshwater algae with varying removal efficiencies, maximum adsorption capacities (q_m) and binding constants. *Cladophora* sp., *Spirogyra* sp. and *Oedogonium* sp. are green algae readily available as source of biomass for heavy metal removal from wastewater. The maximum biosorption capacities of freshwater macroalgae are mentioned in Table 3-6.

Table 3-6 Maximum sorption capacity of freshwater macroalgae.

Freshwater macroalgae	**Metal**	**Maximum biosorption capacity (mg/g)**	**References**
Oedogonium hatei	Ni	42.2	Gupta *et al.*, 2010
Oedogonium hatei	Cr (VI)	35.2	Gupta and Rastogi, 2009
Oedogonium sp.	Cd	88.9	Gupta and Rastogi, 2008
Cladophora fascicularis	Pb	198.5	Deng *et al.*, 2007a
Cladophora hutchinsiae	Se	74.9	Tuzen and Sarl, 2009
Cladophora sp.	Cu	819	Doshi *et al.*, 2008
Spirogyra neglecta	Ni	504	Singh *et al.*, 2007
	Cr (III)	347	
	Cr (VI)	168	
	Cu	115.3	
Spirogyra sp.	Pb	116.1	Gupta and Rastogi, 2008
	Pb	140	
Spirogyra sp.	Cr (III)	30.21	Bishnoi *et al.*, 2007
Spirogyra sp.	Cu	133.3	Gupta *et al.*, 2006
S. condensata	Cr (III)	14	Onyancha *et al.*, 2008
Rhizodonium nieroglyphicum	Cr (III)	11.81	Onyancha *et al.*, 2008

3.9 Application of vegetation in constructed wetland

National and constructed or artificial wetlands are simple and inexpensive biofiltration and purification system for treatment of contaminated wastewater, especially in rural area. The wetland functions in removal of pollutants physically, chemically and biologically by various mechanisms. Suspended solids are sedimentated and filtered while soluble organics are degraded by either aerobic and anaerobic microbes. Matrix, microorganism and plant in wetland can sorb, uptake, fix and change form of phosphorus and nitrogen. The process of nitrification and denitrification occurs in constructed wetland. Metals are removed from wastewater by adsorption and cation exchange, complexation, precipitation, plant uptake and microbial oxidation/ reduction. Vegetation is most important in these functions since it provides surfaces and optimal environment for microbial growth and results in filtration system (Cooper *et al*., 1996).

3.9.1 Types of constructed wetland

Flow patterns in wetland systems are different among types of wetland, i.e. subsurface-flow wetland, surface-flow wetland and tidal-flow wetland. Subsurface-flow system is soil-based technology that removes contaminants in wastewater by filtration through root zone mixing between macrophyte root, microorganism and substrate soil (Fig. 3-5). The inlet pipes are buried and distributed wastewater throughout the treatment zone and the outlet collects treated water at the base of the soil media (Shutes, 2003).

The most common type of constructed wetland is surface-flow system, which is mainly used to treat large volume of wastewater such as municipal wastewater (Fig. 3-6). The system supports the variety of vegetation among plants and macroalgae. The water flows above the surface of the substrate materials growing macrophytes. The system is designed for nutrient polishing from wastewater (Shutes, 2003). Unlike from continuous flow wetland, tidal-flow wetland is operated for flood and drain cycling system. The system is designed to adsorb ammonium ion during flood period and fix it in thin biofilms during drain period when exposed to atmospheric oxygen inducing nitrification (Fig. 3-7). The next flood cycle proceeds with desorption of nitrate ion into the flooded water where it is then denitrified by bacteria. This developed technique is to create the condition for nitrification and denitrification cycle (Ye *et al*., 2011).

3.9.2 Design of constructed wetland

Treatment levels of wastewater are usually to set-up 3 levels (Tibaijuka, 2008). First, preliminary treatment mainly removes coarse materials such as large

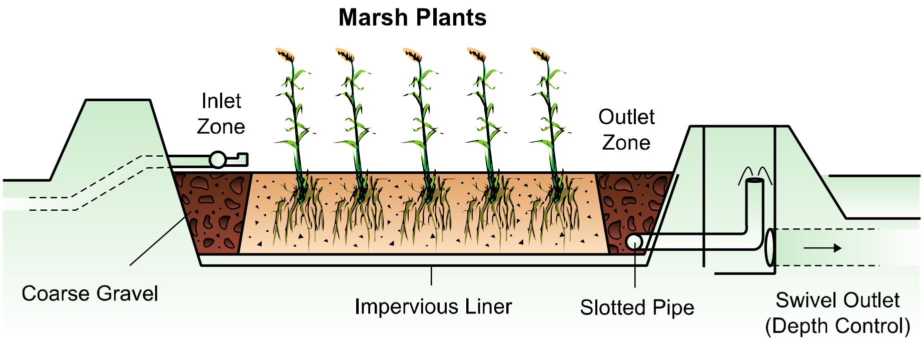


Figure 3-5 Subsurface-flow wetland (Kadlec and Knight, 1996).

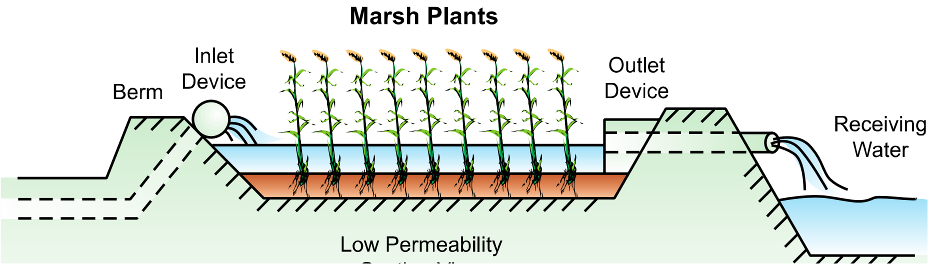
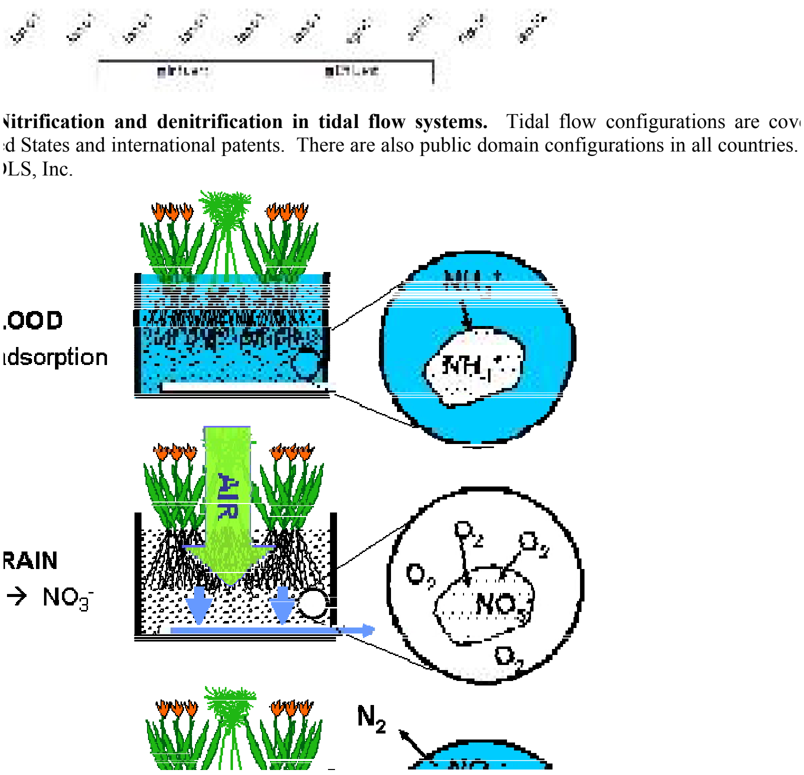


Figure 3-6 Surface-flow wetland (Kadlec and Knight, 1996).



itrification and denitrification in tidal flow systems. Tidal flow configurations are cov
d States and international patents. There are also public domain configurations in all countries.
LS, Inc.

Figure 3-7 Tidal-flow wetland (Ye *et al.*, 2011).

solids, rags and grit from the liquid phase. The treatment is to prevent preclude operation during further treatment in wetland. Screen and grit chamber in preliminary treatment remove sand, gravel and other heavy materials. Second, the suspended matter left in raw wastewater is settled by gravity in primary treatment process. The septic tank is designed to remove the suspended particles in sludge and separate scum containing organic pollutants from the effluent. Anaerobic baffle reactor is an improved septic tank to increase removal of solids and organic pollutants by increasing the area for contact between wastewater and microbial biomass that degrades some organic matter anaerobically. The last treatment is wetland, the secondary or tertiary process. The constructed wetland initiates firstly in lining the basin with impermeable layer that prevents the leakage of waste material, secondly filling with substances that provide the space for water flow and finally planting vegetation. The construction of inlet and outlet structures is dependent on the design and type of the constructed wetland. The inlet should distribute wastewater throughout the entire area of the wetland while the outlet should collect the effluent in a correct flow direction (Tibaijuka, 2008).

3.9.3 Vegetation

The selection of macrophytes for vegetation in wetland is important and different due to type of the constructed wetland. In response to water presence and absence, wetland macrophytes and macroalgae are divided into 3 groups. Terrestrial plant does not tolerate flooding while amphibious plant tolerates flooding and drying. This kind of plant can be fluctuation tolerators, which does not change in their living form when water level has changed or they can be fluctuation responders, which change their growth form such as leaf shape, petiole or internode length. In surface flow wetland, the submerged plant and macroalgae are suitable and are selected for vegetation (Leck and Brock, 2000).

3.9.4 Application of macroalgae in constructed wetland

Macroalgae grown in freshwater and marine habitats are in the forms of floating or attaching to substrate such as rocks, sediment and other algae (Raven *et al*., 1999). They are typically autotrophic organisms that can be primary producer in the wetland system. *Ulva lactuca* and *Laminaria* species were studied for the application in purification of aquaculture effluent, which contained high level of nutrients (nitrogen and phosphorus). *U. lactuca* has faster growth rate with high photosynthetic capacity and relative rapid nutrient uptake in comparison to other macroalgae (Lartigue *et al.*, 2003; Ale *et al.*, 2010). *U. lactuca* has been reported to take up nitrogen in forms of both NH^{4+} and NO^{3-} (Lartigue *et al.*, 2003). With high growth rate and productivity, *U. lactuca* biomass can uptake phosphate as high as 0.1 g PO_4^{3-} $/m^2h^1$ (Tsagkamilis *et al.*, 2009).

3.10 Application of biomaterial in wastewater treatment plant

Municipal wastewater contains wastes including garbage, rubbish, ashes, street cleaning, dead animals and medical waste while industrial waste contributes wastewater from processes of industry, manufacturing, mining or agricultural operations. The water quality of municipal and industrial wastewater has to be improved by wastewater treatment plant. The typical plant functions in removal of suspended solid by sedimentation, biodegradation of organics and nutrients including nitrogen and phosphate by microbial degradation and killing pathogenic bacteria by chlorination (Khopkar, 2004).

3.10.1 Design of municipal wastewater treatment plant

Preliminary treatment of wastewater is operated to separate large suspended solids prior to primary treatment. The primary treatment removes total suspended solids by physical settling or filtration with primary clarifier and reduces the biological oxygen demand (BOD) with a biological treatment process in primary sludge (Fig. 3-8). The primary effluent flows further to the aeration tank where the secondary treatment takes place by microbial degradation of organic matter. The secondary treatment contains attached growth process or suspended growth process by microorganisms. The quality of effluent is finally increased by disinfection with chemicals such as chlorine. This step removes the pathogen and harmful organics causing the diseases from other bacteria, virus and parasite in the water (Khopkar, 2004).

The advanced treatment is additionally conducted in some wastewater treatment plant. The most common technology is granular activated carbon (GAC)

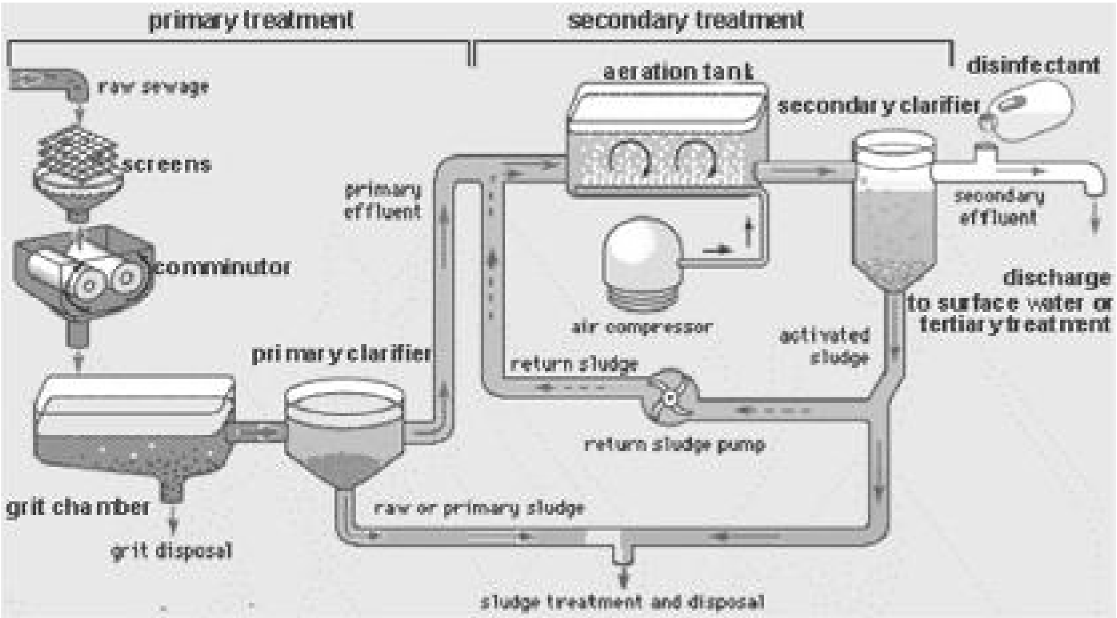


Figure 3-8 Primary and secondary treatment system of municipal wastewater treatment plant

adsorption for tertiary treatment of municipal and industrial wastewater. GAC is used to adsorb the small quantities of soluble organics, which are aromatic solvents, polynuclear and chlorinated aromatics, phenolics, aromatic and aliphatic amines, surfactants, soluble organic dyes, fuels, chlorinated solvents, aliphatic and aromatic acids and pesticides/ herbicides. GAC also adsorbs inorganic compounds such as nitrogen, sulfides and heavy metals remaining in the wastewater after secondary treatment (EPA, 2000). The advanced treatment of municipal wastewater is shown in Fig. 3-9 as column filters after primary and secondary treatments. The plant is operated for production of water supply to the urban area.

3.10.2 Configuration of column filter

Adsorption system in advanced or tertiary treatment process consists of a steel column or a steel or concrete rectangular tank filled with activated carbon. A typical downflow contactor receives wastewater from the top and discharges into the bottom direction. Wastewater is flown with pressure and gravity, hence reduce and conserve energy in operation (EPA, 2000).

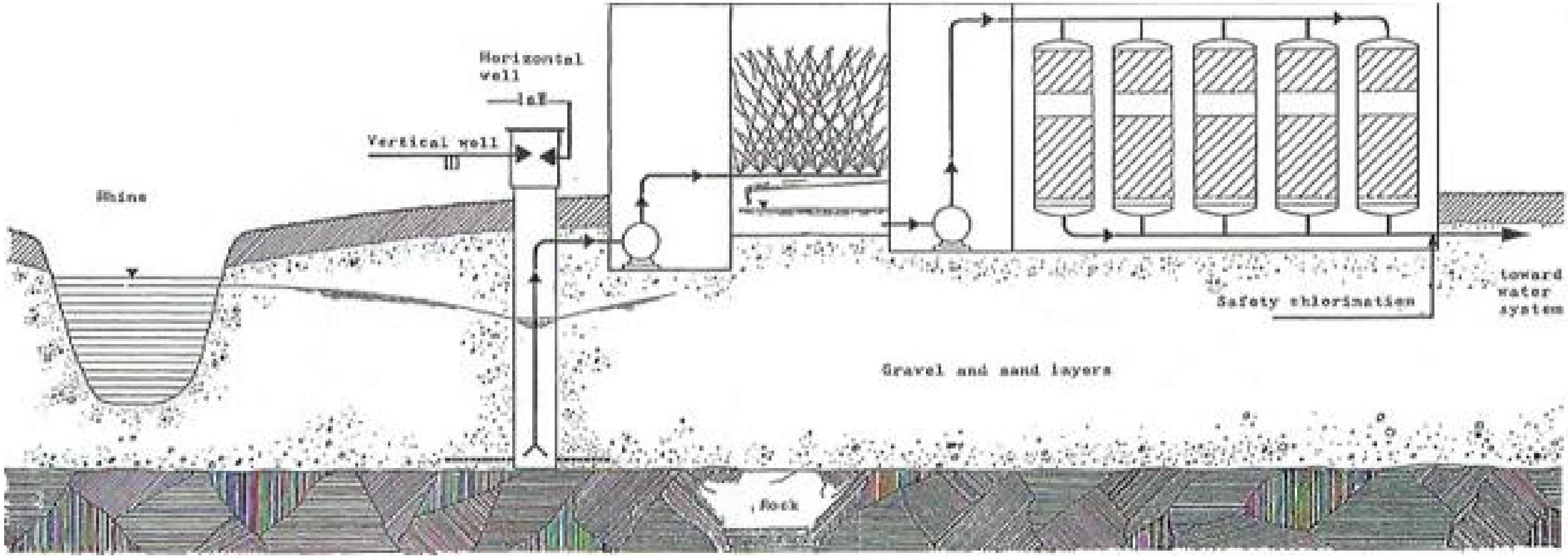


Figure 3-9 Water work with column filter in advanced (tertiary) water treatment plant.

In water purification by filtration system, the operation device can be set-up in both downflow and upflow modes. Downflow mode may face with the problems of clogging solids in filter bed and reducing flow rate to an unacceptable level. Hence, pre-treated water is needed to minimize the remaining particles in wastewater. Upflow filter operation then overcomes this problem. A sump area feeds wastewater below the column filter. This allows space for particulates to settle down at the bottom of the sump area before flowing upward through column filter and existing at the top (Pratap *et al*., 2007).

3.10.3 Application of biomaterial as filter media

Typical filter medium for municipal water treatment is activated carbon, which has internal walls of pores in the particle forming by thermal activation (EPA, 2000). The commercial medium filters that are used in household purification can be several layers with different density and site distribution of the media. A typical configuration from top to bottom is in the following order: anthracite, granite and sand. In treatment of heavy metal contaminated wastewater, many biosorbents have been proved to remove toxic substances efficiently. Flute reed has been reported for its ability to remove dye from textile wastewater (Inthorn *et al*., 2010). Aquatic plant (Bunluesin *et al*., 2007), cyanobacteria (Chen *et al*., 2005) and various macroalgae such as *Ulva reticulate*, *Sargassum glaucescens* and *Spirogyra aequinoctialis* have been used as biosorbents for removal of heavy metals from wastewater by column filter system (Vijayaraghavan *et al*., 2005; Naddafi *et al*., 2007; Sulaymon *et al*., 2013).

3.11 Waste disposal and management

The sludge from municipal waste is usually digested in aerobic and/or anaerobic condition to reduce the waste volume. The leftover waste material from sludge and column carbon can be managed before disposal to landfills.

3.11.1 Waste incineration

Waste incinerator functions in reduction of large volume of municipal solid waste and reduction of reactivity of toxic metals and organic compounds. Heavy metals are minimized or concentrated while organic compounds are completely destructed. Heavy metals can change form when they are burnt to the different speciation and also can distribute to various phase leaving an incinerator. The operation conditions in incinerator such as combustion temperature, gas atmosphere and chlorine content in the flue gas has affected to metal speciation and partitioning to another phase. Metal compounds that have high vapor pressure and low boiling point such as Hg are easily volatilized and enriched in fly ash and flue gas. Among Cd, Pb and Zn, Zn has relatively low vaporization but Pb easily vaporizes when the condition has high chlorine content. Large portion of heavy metals in incinerator is remained in bottom ash and boiler ash (Abanades *et al.*, 2002; Lu-Shi *et al.*, 2004).

3.11.2 Regeneration of spent activated carbon

Advanced treatment by column filter in wastewater treatment plant has advantage of adsorption and regeneration of activated carbon. The regeneration process reduces solid waste volume caused by spent carbon and saves the carbon cost. In this treatment system, the carbon contactor is equipped in connection with separated compartments that remove the spent carbon to be regenerated and load the virgin or regenerated carbon to the contactor. The carbon is typically transported hydraulically by pumping as slurry with water or compressed air. The repeat regenerated carbon is destroyed and degraded during regeneration process. Adsorption equilibrium of reused carbon must be determined to predict long-term system performance (EPA, 2000).

3.11.3 Landfill

Landfill is the final step of waste disposal. Under state regulation, the toxic solid waste must be treated to reduce the level of toxicity characteristic before land disposal. The toxicity characteristic is needed to be lower than the regulatory levels, for example, the regulatory levels of maximum concentration of Cd and Pb are 1 and 5 mg/L, respectively in leachate from the solid waste (EPA, 2004). Several test methods such as Toxicity Characteristic Leaching Procedure (TCLP) test and Total Constituent Analysis (TCA) test are acceptable for evaluation of the concentration of toxic substances for hazardous and non-hazardous waste. The toxic wastes need to be analyzed for the characteristics of ignitability, corrosivity and reactivity before disposal as hazardous waste. In case of heavy metal, TCLP test is used to simulate and evaluate the landfill leaching in a sanitary landfill. Acid rain can leach toxic metal into water extract. The method uses buffer acid solution to extract and then determines the metal concentration in the TCLP extract. TCA test is used for classifying hazardous and non-hazardous waste. If amount of total constituent (TC) exceeds the criteria that possesses the toxic characteristic, the waste is hazardous and if the concentration of TC extracts exceed the permissible level, the waste is needed to be treated further prior to land disposal (EPA, 2004).

Modern landfills are constructed to be able to monitor the risk of contaminants under the regulations. Landfill should be located in restrictive area away from faults, wetland and flood plains. It should prevent groundwater contamination with composite liner blocking underlying soil from leachate and with leachate collection and removal system. Groundwater monitoring is required to determine waste material leakage from the landfill. The harmful landfill gas produced from decomposition of waste material especially in new landfill should be monitored. Frequently waste covering with layer of soil can reduce odor, control litter, insects and rodents and protect public health. Landfill closure and post-closure care are also required to cover landfill and provide long-term care of landfill site. Municipal waste landfills receive household waste, non-hazardous sludge, industrial solid waste and construction and demolition debris. Industrial hazardous waste and household hazardous waste such as paints, cleaners/ chemicals, motor oil, batteries and pesticide are not allowed to be disposed as municipal waste. It is needed to be separated landfill or management (EPA, 1997). The reuse of solid waste material from municipal treatment sludge as fertilizer and from column filter as regenerated media help reduction of large volume of municipal solid waste in landfill.

3.12 Charophytes: freshwater macroalgae

Chara and *Nitella* belong to the Family Characeae, Order Charales. This order is macroscopic and consists of algae with creeping rhizoidal branches of limited growth. Each whorl of secondary branches (branchlets) has limited growth. It has complex and unique type of advanced oogamous reproduction.

3.12.1 Genus *Chara* sp.

Chara is macroscopic, usually lime-encrusted with similar organization to other members of the order. Branches of unlimited growth are composed of elongated, single celled internodes and multicellular nodes with branchlets (rarely branches of unlimited growth) arising from the short cells of nodes. The cortex is single-layered over intermodal cells, rarely partially or ecoticate, showing varying degrees of development of primary, secondary and tertiary cell rows. The primary row is always distinguished by the presence of spine cells. Branchlets are undivided, subtended by a single or double ring of unicellular outgrowths (stipulodes); simplified cortication is over intermodal cells; rings of unicellular bract-cells develop at nodes; the terminal segment is single-celled or in chains not separated by nodal cells. Reproduction is by an advanced form of oogamy, the oogonium always above the antheridium when together on a branchlet (monoecious species). Oosporangium each consists of an

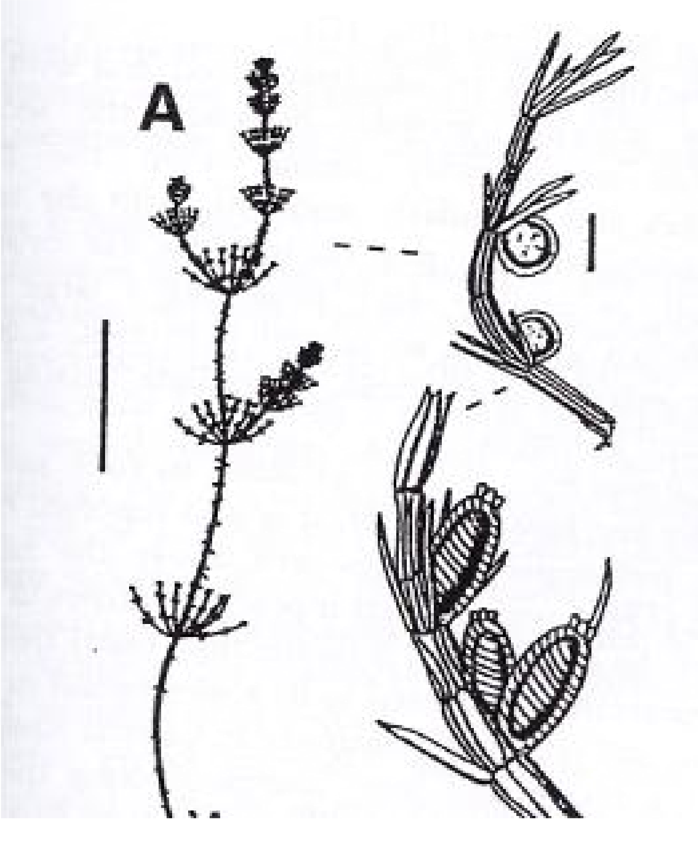


Figure 3-10 Macro- and microscopic feature of *Chara* sp.

oogonium surrounded by eight spirally twisted sterile cells, bearing a crown of five cells. Antheridia are usually spherical consisting of eight shield-shaped cells borne on the end of a shortstalk (Wehr and Sheath, 2003).

Thalli are often large (up to 1 m) and coarse, especially when heavily lime-encrusted. It is most abundant in hard water or alkaline ponds and lakes. It is found occasionally in the shallows of slow-flowing rivers and in spring seepage areas and known to grow to depths as great as 12 m. Often *Chara* species have a strong odor, hence its common name in North America is skunkweed or muskweed (Wehr and Sheath, 2003).

3.12.2 Genus *Nitella* sp.

Nitella is macroscopic, only lightly lime-encrusted or not at all; it is similar in organization to other members of the order but not as erect as *Chara*. Branches of limited growth ecoticate, hence spines are absent. Branchlets are not subtended by stipulodes and bract-cells are absent, forked one or more times into similar single-celled rays and/or one- to three-celled ultimate rays (sometimes devided two, three, or

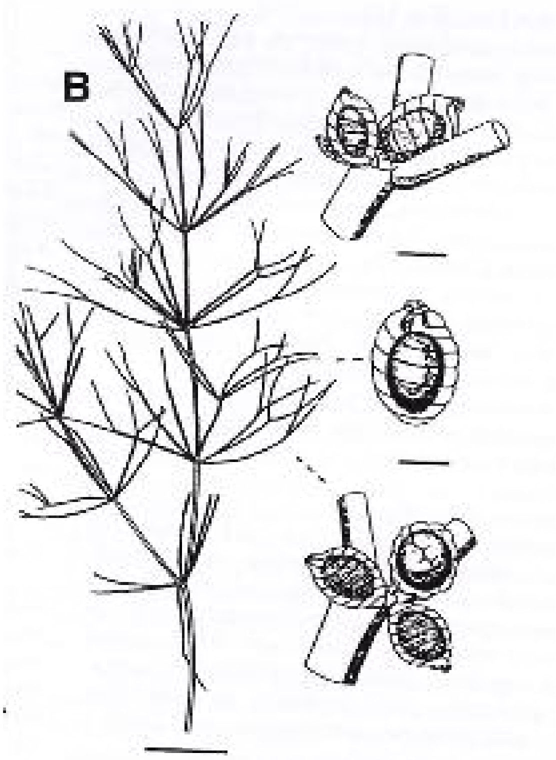


Figure 3-11 Macro- and microscopic feature of *Nitella* sp.

four times). Oogonia are produced at the forking of branchlets and occasionally at the base of a whorl of branchlets. Oogonia arise laterally with two to three together but antheridia are solitary and terminal. Oogonia are with a crown of 10 cells in two tiers and laterally compressed (Wehr and Sheath, 2003).

Nitella is widely distributed and usually more common than *Chara* in softer water areas and acid lakes, including bog lakes where the water is stained brown. It is also common in streams throughout temperate regions of North America (Wehr and Sheath, 2003).

Under hard water conditions, most aquatic plants are capable of precipitating calcite ($CaCO_3$). However, in most cases, the $CaCO_3$ is dispersed and not associated with the plant themselves. Furthermore, charophytes have a higher calcifying potential than other aquatic plants. They contain $CaCO_3$ at levels as high as 60% per dry weight. It is also reported that many heavily calcified charophytes get deposited in aquatic environments (Gomes and Asaeda, 2009).

Both macroalgae are a group of non-vascular hydrophytes with worldwide distribution. In the last few decades, anthropogenic inputs of metals have exceeded natural inputs. However, few laboratory studies have demonstrated the potential of charophytes in the bioaccumulation and biosorption of heavy metals (Bibi *et al*., 2010).

CHAPTER IV
MATERIALS AND METHODS

4.1 Algal materials

Samples of *Chara aculeolata* Kutzing and *Nitella opaca* C. Agardh (ex Bruzelius) were collected prior to the experiment. *C. aculeolata* was found in Bueng Boraphet Reservoir, Nakhon Sawan Province, Thailand, while *N. opaca* was commonly found in natural ponds in Bangkok. The whole thalli were washed with tap water and distilled water to remove all the debris and epiphytes. Water samples at the collecting sites were analyzed for water quality standards (AOAC, 2006).

4.2 Culture conditions

Both species of macroalgae were grown in 20 L glass aquaria (23 cm × 45 cm × 28 cm) containing 10% Hoagland's nutrient solution (EPA, 1975) under controlled conditions (25±2^{o}C, 2200 lux, 12 h/12 h light and dark cycle).

Modified Hoagland's nutrient solution

Stock solution

Macronutrients

Indicated amounts of each following chemical was added and then adjusted the volume by adding distilled water to 1000 mL

$Ca(NO_3)_2$	164.10	g/L
KNO_3	101.10	g/L
$Mg (SO_4)$	120.39	g/L
KH_2PO_4	136.09	g/L

Micronutrients

Indicated amounts of the following chemical was added and then adjusted the volume by adding distilled water to 1000 mL

H_3BO_3	2.86	g/L

Figure 4-1 *C. acueolata* in subculture.

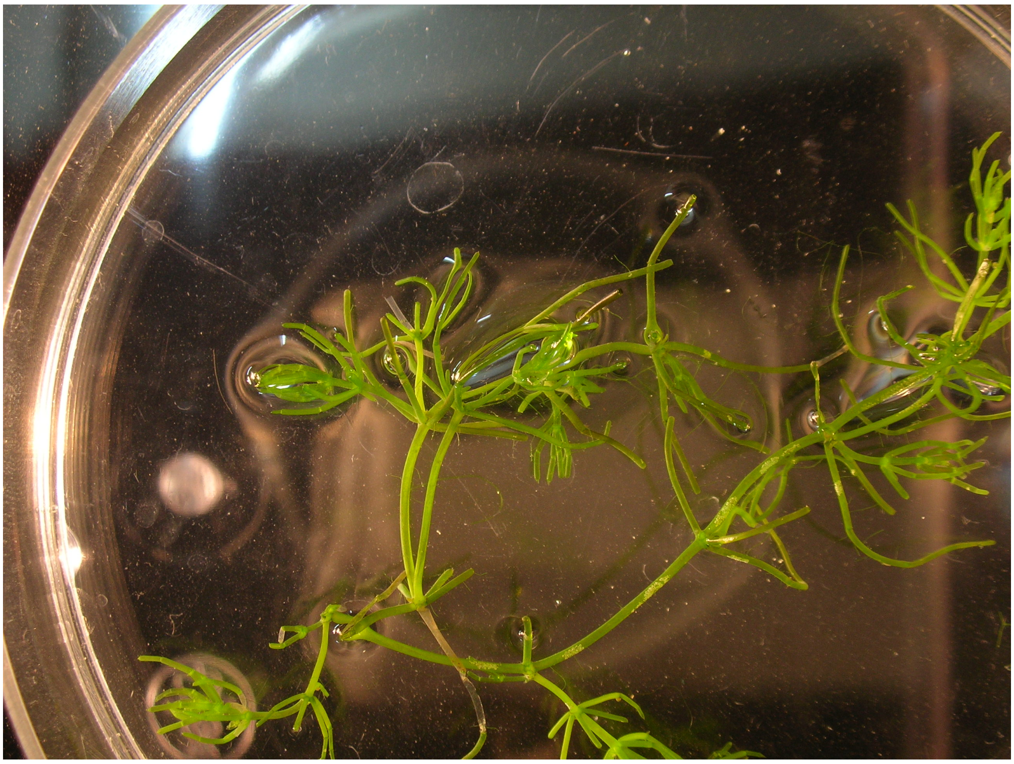


Figure 4-2 *N. opaca* in subculture.

$MnCl_2.4H_2O$	1.81	g/L
$ZnSO_4.4H_2O$	0.22	g/L
$CuSO_4.5H_2O$	0.08	g/L
H_2MoO_4	0.02	g/L

Preparation of $Fe_3.6H_2O$

$Fe_3.6H_2O$ 13.5 g was dissolved in 500 mL distilled water

Preparation of 10% Hoagland's nutrient solution

The following stock solutions were mixed and then adjusted the volume by adding distilled water to 1000 mL

$Ca(NO_3)_2$	0.5	mL
KNO_3	0.5	mL
Mg (SO_4)	0.2	mL
KH_2PO_4	0.1	mL
$FeCl_3.6H_2O$	0.1	mL
Micronutrient solution	0.1	mL

Preparation of low phosphate 10% Hoagland's nutrient solution

The following stock solutions were mixed and then adjusted the volume by adding distilled water to 1000 mL

$Ca(NO_3)_2$	0.500	mL
KNO_3	0.500	mL
Mg (SO_4)	0.200	mL
KH_2PO_4	0.005	mL
$FeCl_3.6H_2O$	0.100	mL
Micronutrient solution	0.100	mL

The pH of the solution was 5.5. After one-week acclimatization, algae were exposed to various concentrations of heavy metals (Cd, Pb, Zn).

4.3 Chemicals

For living biomass, The 10% Hoagland's nutrient solution was supplemented with two nominal concentrations of Cd (0.25 and 0.5 mg/L of Cd prepared from $Cd(NO_3)_2$ standard solution), Pb (5 and 10 mg/L of Pb prepared from

$Pb(NO_3)_2$ standard solution), and Zn (5 and 10 mg/L of Zn prepared from $Zn(NO_3)_2$ standard solution). The initial pH of all solutions was 5.5. The tested concentrations of Cd, Pb and Zn were different according to different levels of their toxicity and water quality standard.

For dried biomass, standard solutions of $Pb(NO_3)_2$, $Cd(NO_3)_2$ and $Zn(NO_3)_2$ used were of analytical grades. The stock solutions of 1000 mg/L metal ions were diluted with Milli Q water to make metal ion solutions of desired concentrations for the adsorption. Each test metal solution was adjusted to desirable pH values with 0.1 M HCl and 0.1 M NaOH before testing the algal biosorption.

4.4 Instruments

1. Flame atomic absorption spectrophotometer (FAAS)

The FAAS instrument (Variance SpectrAA 55B, Varian Australia Pty Ltd, Australia) used was a Varian SpectrAA 55B double beam atomic absorption spectrophotometer with a deuterium background correction. The Cd, Pb and Zn deuterium lamp current were 4 mA. The monochromators were set at wavelength of 228.8, 283.3 and 213.9 nm, respectively.

2. pH meter

A Hanna instruments model HI 221, a pH meter with a glass combination electrode, was used for all pH experiments. Commercial standard buffers (Merck) of pH 4.00±0.02, 7.00±0.02 and 10.00±0.02 were employed for the instrumental calibration.

3. Analytical balance

A Kern balance model ALS 220-4N was used for weighing chemicals and algal materials

4. Spectrophotometer

A CECIL CE7200, Aquarius spectrophotometer was used to estimate the absorption spectra of extracted pigment solutions for chlorophyll determination

5. Centrifuge

A Heraeus centrifuge, model Biofuge pico was used to separate the residue from the supernatant after chlorophyll extraction.

6. Light microscope

A digital compound transmission light microscope (Olympus CH40, Olympus optical Co Ltd, Japan) was used to study toxicity symptoms of algae.

7. Rotary incubator shaker

8. Peristaltic pump

A pump (model 77200-62; Cole Palmer Instrument Company, Barrington, IL, USA) was used to flow metal solution to algal packed bed column.

4.5 Experiments

4.5.1 Experiment I: Heavy metal toxicity

Apical tips or thalli of the stock *C. aculeolata* and *N. opaca* (4-5 internodes, 8-10 cm length, 5 g fresh weight) with uniform morphology were harvested and each transferred into 1 L Erlenmeyer's flasks containing various concentrations of Cd, Pb or Zn. Algae cultured in the medium without heavy metals served as controls. There were 3-6 replicates for each treatment. The experiment was carried out for 6 days. The cultured metal solutions were changed on day 3.

4.5.1.1 Effect on relative growth rate

Algae were harvested after 6 days of exposure to determine the growth rates. Control and treated algae were blotted gently to drain excess water and the fresh weights were recorded. The relative growth rate (RGR) of macroalgae was calculated following the formula:

$$RGR = \left(\frac{w1-w0}{w0}\right)/\left(\frac{W1-W0}{W0}\right) \tag{1}$$

where, w_1 (g), W_1 (g) represent the fresh weight at time T_1; w_0 (g), W_0 (g) represent the fresh weight at time T_0; w, W represent different metal treatment and the control, respectively (Gao and Yan, 2012).

4.5.1.2 Effect on pigment contents

Total chlorophyll, chlorophyll *a*, *b*, and carotenoid contents in algae from each treatment were determined after 6 days of exposure by the absorption spectra of algal extract in a spectrophotometer according to the methods described by Arnon (1949), MacKinney (1941) and Jeffrey *et al*. (1997).

The thalli were weighed and chlorophylls were extracted by homogenizing thalli twice in a tissue grinder with 80% acetone. The homogenate was quantitatively transferred to a 1.5 mL microcentrifuge tube and centrifuged at 3000 g for 3 minutes. The filtered extract was placed in the dark and immediately diluted to a 1 mL in a microcentrifuge tube with acetone. The absorbance of the extract was measured at 663, 645 and 480 nm where maximum absorption by chlorophyll a, b and carotenoid occurred. The instrument was set at zero absorbance with a tube containing acetone before reading was made with the solution of pigments.

The concentration of chlorophyll a, b and carotenoid in mg/L was calculated by the formula

$$\text{Chlorophyll a in mg/L} = 12.7 \times A663 - 2.69 \times A645 \quad (2)$$

$$\text{Chlorophyll b in mg/L} = 22.9 \times A645 - 4.68 \times A663 \quad (3)$$

$$\text{Total chlorophyll in mg/L} = 8.02 \times A663 + 20.2 \times A645 \quad (4)$$

$$\text{Carotenoid (astraxantin) in mg/L} = 4 \times A480 \quad (5)$$

4.5.1.3 Toxicity symptoms

Toxicity symptoms caused by heavy metals were searched for in both algal species after 3 and 6 days of exposure under a digital compound transmission light microscope. The criteria such as reduction of chloroplast, softening of thallus, and detachment of corticating cells were used to evaluate the severity of toxicity symptoms (mild, moderate, severe).

4.5.2 Experiment II: Heavy metal accumulation

4.5.2.1 Metal accumulation

After 6 days, algae from each flask were harvested separately and analyzed for their accumulation of Cd, Pb and Zn. The procedure of digestion of algal materials was performed according to Anderson (1991) and Katz and Jennis (1983). Algae were washed with 5 mmol/L EDTA for 10 min to remove heavy metals bound to the external cell surface (Vasconcelos and Leal, 2001), dried at $60^{o}C$ until a constant weight was obtained, digested in conc HNO_3 at $200^{o}C$ prior to conc HCl at $150^{o}C$, and impurities removed by filtration (APHA, 1998). After digestion, metal concentrations in algae and solutions were determined by a flame atomic absorption spectrophotometer.

4.5.2.2 Percentage of metal removal

The percentage metal removal was calculated from uptake (*U*, %):

$$U = \left(\frac{C0-C1}{C0}\right) \times 100 \quad (6)$$

where, C_0 (mg/L) and C_1 (mg/L) are initial and remaining concentrations of metal in the medium (Abdel-Halim *et al.*, 2003).

4.5.2.3 Bioconcentration factor (BCF)

The bioconcentration factor is used to determine the ability of algae in concentrating heavy metals or in acting as hyperaccumulator. The value is defined as the ratio of metal concentration in dry biomass to the initial metal concentration in the external medium (Raskin *et al.*, 1994). The BCF values for Cd, Pb and Zn were determined.

4.5.3 Experiment III: Biosorption of Pb, Cd and Zn in *C. aculeolata* and *N. opaca* in batch culture

4.5.3.1 Preparation of dried algal biomass

The charophytes (*Chara aculeolata* and *Nitella opaca*) were used as biosorbent for the biosorption of Pb(II), Cd(II) and Zn(II) ions. *C. aculeolata* was collected from Bueng Boraphet reservoir, Nakhon Sawan province, Thailand and *N. opaca* were gathered from natural ponds in Bangkok. Samples were cleaned with tap and distilled water to remove debris and epiphytes, and then dried at 60^{o}C in a hot air oven for 24 h. The dried algal biomass were later ground, sieved with a 2-mm mesh sieve and stored in a desiccator before use.

4.5.3.2 Biosorption experiments for single-metal systems

All experiments were carried out in triplicate. The negative control consisted of solutions without algal biomass.

1) Effect of algal dosage

The influence of sorbent dosage on biosorption of Pb, Cd and Zn was studied by varying the amounts of algal biomass, 0.1, 0.2, 0.5, 1, 2, 3 and 4 g/L, added to the 10 mg/L metal solutions. The reaction mixture was shaken on a rotary shaker at 150 rpm for 24h at 25±2 °C and filtered through a 0.45 μm Whatman membrane filter. Each filtrate was acidified below pH 2 and determined for remaining metal using a FAAS. The efficiency of biosorbent was determined by metal removal efficiency (Lawal *et al.*, 2010) calculated as follows:

$$\text{Removal efficiency } (\%) = \frac{(C_i - C_e)}{C_i} \times 100 \qquad (7)$$

where C_i is the initial metal ion concentration (mg/L); C_e is the final or equilibrium concentration of metal ion in solution (mg/L).

2) Effect of pH

The initial pH of each metal solution was adjusted to the desired pH (2-6) using 0.1 M HCl and 0.1 M NaOH. 2 g/L dried biomass of *C. aculeolata* or 3 g/L of *N. opaca* each were mixed with 10 mg/L metal solutions. The efficiency of biosorbent was determined by metal removal efficiency (eq.7).

3) Effect of contact time

To determine the contact time required for biosorption equilibrium, dried *C. aculeolata* (2 g/L) or *N. opaca* (3 g/L) each were added to 10 mg/L metal solutions at pH 4 for various durations (3, 15, 30, 60, 90, 120, 180 and 360 min). Aliquots of 10 ml solution were withdrawn at different time intervals, filtered and analyzed for the metal content using an FAAS, until no more metal was removed and the equilibrium was achieved.

The amount of metal ions sorbed by the biomass (q) was determined by using the equation as follows (Fan *et al.*, 2008):

$$q = \frac{(C_i - C_e)V}{M} \qquad (8)$$

where q is the amount of metal sorbed or uptake (mg/L); C_i and C_e are the initial and equilibrium metal concentrations in the solutions (mg/L), respectively; V is the solution volume (L); and M is the mass of algae (g).

In kinetic studies, the aliquots of metal solutions were taken out at different time intervals, filtered and analyzed for the remaining metal ions. The Langergren's pseudo-first-order and Ho's pseudo-second-order models were applied to test the fitness of experimental data and to examine the biosorption mechanism of Pb, Cd and Zn ions onto dried algal biomass. The linear forms of the pseudo-first-order rate equation and the pseudo-second-order equation (Fan *et al.*, 2008) can be expressed as follows:

Langergren's pseudo-first-order rate :

$$\log(q_e - q) = \log q_e - \frac{K_{ad1}}{2.303} t \quad (9)$$

Ho's pseudo-second-order rate :

$$\frac{t}{q} = \frac{1}{K_{ad2} q_e^2} + \frac{1}{q_e} t \quad (10)$$

where q_e and q (mg/g) are the amounts of metal ions sorbed at equilibrium time and time t (min), respectively; K_{ad1} is the specific rate constant of the first-order model and can be calculated by the slope of a plot of log (q_e-q) versus t; K_{ad2} is the specific rate constant of the second-order model and can be determined by the intercept of a plot of t/q versus t.

Intraparticle diffusion equation was determined using the equation given by Weber and Morris (Waranusantigul *et al.*, 2003).

$$q = K_{id} t^{\frac{1}{2}} \quad (11)$$

where q is the amount of metal adsorbed (mg/g) at time t (min); K_{id} can be determined from the slope of the linear portion of a plot of q versus $t^{1/2}$.

4) Sorption isotherm

To establish a relationship between the amounts of metal ions sorbed and the equilibrium concentrations of metal ions in solution, adsorption capacity of algal biomass in each metal solution of Pb(II), Cd(II) and Zn(II) was determined at various initial metal concentrations (1, 5, 10, 50, 100, 200, 300 and 400 mg/L).

The metal concentrations in the solution were quantified by FAAS and the data obtained were then used to test the fitness to Langmuir and Freundlich isotherms for the adsorption process at equilibrium. The amount of metal ions sorbed by the biomass (q) was determined by using the eq.8. The equilibrium sorption capacity were determined by using the linear forms of Langmuir and Freundlich (Davis *et al.*, 2003) presented as follows:

Langmuir model :

$$\frac{C_e}{q_e} = \frac{1}{Q_m K_L} + \frac{C_e}{Q_m} \quad (12)$$

Freundlich model :

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (13)$$

where q_e and Q_m are the amount of metal adsorbed at equilibrium and the amount of maximum metal uptake (mg/g), respectively; K_L is Langmuir equilibrium constant (L/mg) relating to the free energy of biosorption or the affinity between the sorbent and the sorbate; K_F is Freundlich equilibrium constant $(L/g)^{1/n}$ relating to the biosorption capacity; and n is empirical parameter relating to the biosorption intensity, which varies with the heterogeneity of the material.

4.5.3.3 Biosorption experiments for multiple-metal systems

The metal biosorption in multi-metal conditions were conducted to imitate the realistic contamination. Adsorption capacity (q) of algal biomass in mixed metal solution consisting of Pb(II), Cd(II) and Zn(II) at equal volumes was studied at 1, 5, 10, 50, 100 and 200 mg/L of initial metal concentrations and determined by using eq.11. Langmuir and Freundlich isotherms (eq. 12-13) were applied to study adsorption at equilibrium in comparison to single-metal solutions.

4.5.3.4 Desorption study

To determine the most effective desorbing agent for elution of heavy metals from algal biomass**,** 2 g/L of dried *C. aculeolata* and *N. opaca* were added to 50 ml of 10 mg/L Pb or Cd solution. The mixtures were shaken at 150 rpm, 25±2 °C for 3 h and then filtered through 0.45 μm Whatman filter paper. The filtrates were analyzed for metal concentration by using FAAS to determine metal removal percentage as eq. 7. Pb and Cd laden biomasses were added to various solutions of desorbing agents; 0.1 M HCl, 0.1 M HNO_3, 0.1 M $CaCl_2$, 1 M NaOH and 0.05 M nitrilotriacetic acid (NTA). 0.1 M $CaCl_2$ solution was adjusted to pH 3 with HCl (Vijayaraghavan *et al.*, 2005). NaOH was used as control because it was the solvent for NTA. The mixtures were shaken at 150 rpm, 25±2 °C for 3 h and filtered through 0.45 μm Whatman filter paper. The filtrates were analyzed for metal concentration by using FAAS to determine metal desortion percentage as eq.15.

$$\text{Metal removal (\%)} = \frac{Madsorption}{Mtotal} \times 100 \qquad (14)$$

$$\text{Desorption efficiency (\%)} = \frac{Mdesorption}{Madsorption} \times 100 \qquad (15)$$

4.5.4 Experiment IV: Metal removal from wastewater by *C. aculeolata* and *N. opaca*

Actual municipal wastewater was used to evaluate the practicality of the biomass. Samples of municipal wastewater influent were collected from two different wastewater treatment facilities in Bangkok, Thailand (Dindaeng plant and Siphaya plant), at the same period of time and stored in plastic bottles. The grab samplings from the DinDaeng plant and the Siphaya plant were designated as municipal water-1 and municipal water-2, respectively. The wastewater samples were analyzed for water quality (temperature, pH, DO, BOD, electrical conductivity and TSD) prior to filtration to remove solid sediment. The filtrate was then analyzed for Pb, Cd and Zn contents using an FAAS. The water quality and concentrations of metals and anions of the two municipal water samples are shown in Table 1. Standard solutions of $Pb(NO_3)_2$, $Cd(NO_3)_2$ and $Zn(NO_3)_2$ were added to the wastewater to the final concentrations of 2 mgPb/L, 0.3 mgCd/L and 6 mgZn/L in accordance to 10 fold above wastewater quality standard. 2 g/L of *C. aculeolata* and 3 g/L of *N. opaca* were added to wastewater. The reaction mixture was shaken on a rotary shaker at 150 rpm for 24h at 25±2 ^{o}C and filtered through a 0.45 μm Whatman membrane filter. Each filtrate was determined for remaining metal using a FAAS.

4.5.5 Experiment V: Biosorption of Pb, Cd and Zn in *C. aculeolata* and *N. opaca* in packed column

Continuous-flow sorption experiments were conducted in a glass column with 1 cm ID and 50 cm length. Dry biomasses of *C. aculeolata* and *N. opaca* were ground and sieved for particle sizes of 0.2-0.6 mm. *C. aculeolata* biomass of different weight (0.5, 1.0 and 1.5 g) was packed in the column. The packed bed volumes were 1.93, 3.46 and 5.15 ml, respectively and bed depths were 3.29, 6.49 and 10.1 cm, respectively. For *N. opaca*, 0.2 g of dry biomass with bed volume of 1.95 ml was used (bed depth of 3.3 cm). 10 mg/L of Pb or Cd solutions (pH 4) were pumped through the column using a peristaltic pump with a flow rate of 40 ml/min (*C. aculeolata*) and 20 ml/min (*N. opaca*). The samples were periodically collected and analyzed for metal concentrations by using FAAS. The biosorption saturation capacity of the packed column was reached when no metal sorption occurred or the effluent reached equilibrium.

Table 4-1 Water quality parameters and composition of two municipal wastewater samples.

Parameter	Municipal water-1	Municipal water-2
Temperature (^oC)	32.6 ± 0.2	30.5 ± 0.0
pH	7.2 ± 0.0	7.3 ± 0.0
DO (mg/L)	0.5 ± 0.4	0.3 ± 0.1
BOD (mg/L)	0.5 ± 0.4	0.3 ± 0.1
Electrical conductivity (µS/cm)	568.5 ± 0.7	504.5 ± 2.1
TDS (g/L)	0.3 ± 0.0	0.3 ± 0.0
Metal (mg/L)		
Pb	ND	ND
Cd	ND	ND
Zn	0.058	0.076
Mn	ND	0.05
Na	45.1	28.1
K	8.7	7.28
Mg	10.4	7.39
Ca	35.8	35.4
Anion (mg/L)		
Cl	70.2	30.6
NO3	5.6	1.9
NO2	0.89	0.75
SO4	55.2	43.0
PO3	0.6	0.7

ND = Not detectable, Method detection limit: Pb = 0.05 mg/L; Cd = 0.002 mg/L; Mn = 0.025 mg/L

4.5.5.1 Effect of bed depth and the usage rate on Pb and Cd biosorption

The known quantity of *C. aculeolata* biomass was placed in the column yield the desired bed depth of sorbent. 0.5 g of the biomass yields bed depth of 3.29 cm and 1 g yields 6.49 cm. Pb or Cd ion solutions having the initial concentration

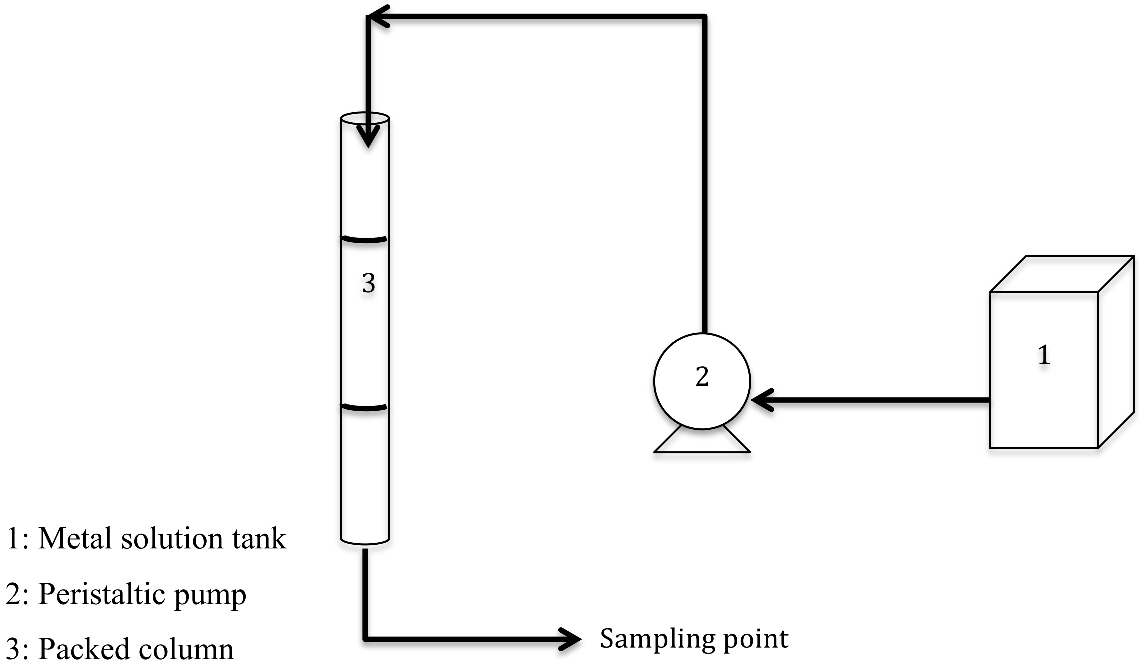


Figure 4-3 An experimental set-up of the fixed bed column system.

of 10 mg/L, pH 4 was pumped downward through the column at a flow rate of 40 ml/min. The effluent was collected and analyzed for metal concentration.

To determine the quantity of the biomass for the next experiment, the graphs of usage rate against EBCT were plotted. The point at the lowest usage rate was identified (Inthorn *et al.*, 2010). Usage rate and EBCT were in accordance to the equation as follows:

$$EBCT = BV/F \tag{16}$$

$$Usage\ rate = M/Vb \tag{17}$$

where BV is bed volume (ml), F is flow rate (ml/min), M is sorbent mass (g) and V_b is volume at breakpoint of biosorption bed ($V_b = 0.05\ V_o$) (ml).

4.5.5.2 Effect of flow rate and bed depth service time (BDST) on Pb and Cd biosorption

To study the effect of flow rate on metal bisorption, metal solutions were passed through the column with various flow rates of 20, 30, 40 and 60 ml/min. Bed depth service time model was applied to determine sorption capacity (Q) of metal ion by biomass. The linear relationship between bed depth (Z) and bed depth service time (Tb) was developed. When Tb was plotted versus Z, the sorption capacity of system can be determined by slope and the rate constant (K) can be determined by intercept. The equation (Inthorn *et al*., 2010) is expressed as follows:

$$Tb = \frac{Q}{C1V}Z - \frac{1}{KC1}\ln\left(\frac{C1}{Cb} - 1\right) \qquad (18)$$

where Tb is bed depth service time (h), Q is sorption capacity (mg/g), Z is bed depth (cm), C1 is initial metal concentration (mg/L), V is linear velocity (cm/h), K is rate constant and Cb is breakthrough metal ion concentration (mg/L).

4.5.5.3 Effect of Pb and Cd concentration on metal biosorption

The sorption equilibrium was studied by varied metal ion concentrations (5, 10 and 20 mg/L of Pb or Cd solution). The solution was passed to the 0.5 g packed column with a flow rate of 40 ml/min for Pb solution and of the 1 g packed column at 20 ml/min for Cd solution. The effluents were analyzed for metal ion concentrations. Successful design of a column sorption process required prediction of the maximum equilibrium sorption capacity or breakthrough curve for the effluent (Yan and Viraraghavan, 2001). Various mathematical models can be used to describe fixed bed adsorption. Among these, the Thomas model is simply and widely used by several investigators (Vijayaraghavan *et al*., 2005; Yan and Viraraghavan, 2001). The linearized form of Thomas model can be expressed as follows:

$$\ln\left(\frac{C1}{Cb} - 1\right) = \frac{KthQmM}{F} - KthC1t \qquad (19)$$

where C_1 is initial metal concentration (mg/L), C_b is metal concentration at breakpoint of biosorption (mg/L), Kth is Thomas model constant (L/mg.h), Qm is maximum biosorption capacity (mg/g), F is volumetric flow rate (L/h) and t is time (h).

4.5.4.4 Effect of binary metal solution on Pb and Cd biosorption

For the determination of the synergistic/anagonistic effect of cations present in the effluent metal solution, Pb concentrations at 10 mg/L was passed through 0.5 g of *C. aculeolata* biomass and the same Cd concentration was passed through 1 g biomass on the packed column. Effluents were collected periodically and analyzed for Pb and Cd concentrations.

4.5.5.5 Reusability

Packed bed experiment was conducted at room temperature (25±2 °C) in a 1-cm ID glass column, packed with 0.5 and 1 g of *C. aculeolata* dry biomass and obtained bed volume of 1.93 and 3.46 ml, respectively. 1 L of Pb or Cd 10 mg/L (pH 4) were pumped through the column at a flow rate of 40 ml/min or 20 ml/min, respectively using a peristaltic pump. The effluent was collected and analyzed for Pb or Cd concentration by using FAAS. For desorption process, 1 L of 0.1 HCl was used to rinse the Pb or Cd laden biomass. The desorbed and regenerated *C. aculeolata* column was used for two more cycles.

4.6 Statistical analysis

The mean relative growth rate, pigment contents and metal concentrations in dried biomass and residual metal concentrations were calculated and subjected to analysis of variance (ANOVA) with differences determined using Tukey HSD' s multiple comparisons test on the SPSS for Windows program. The 0.05 level of probability was used as the criterion of significance.

CHAPTER V

RESULTS

5.1 Toxicity of Pb, Cd and Zn in *C. aculeolata* and *N. opaca*

5.1.1 Relative growth rate

C. aculeolata and N. opaca showed similar growth responses to Cd and Pb. A concentration-dependent decrease in RGR of both algal species was observed (Table 5-1). Significant reduction in RGR was observed when the Cd and Pb concentrations were increased ($P \leq 0.05$). N. opaca showed greater sensitivity to these metals and the RGRs of both species were severely inhibited, especially at high Cd concentration (0.5 mg/L). However, C. aculeolata showed more sensitivity to Zn than N. opaca since there was a significant decrease in RGR of C. aculeolata ($P \leq 0.05$) when exposed to high concentration of Zn.

5.1.2 Toxicity symptoms

In general, high concentrations of metals caused several toxicity symptoms including reduction of chloroplast resulting in chlorosis, softening of the algal thallus, and detachment of corticating cells in the cortex around central cells of C. aculeolata (Fig. 5-1). Effects of heavy metals were characterized as mild, moderate, and severe (Table 5-2). C. aculeolata exhibited severe reduction of chloroplast when exposed to Cd while N. opaca was more sensitive to Pb at high concentration. Both Cd and Pb caused softening of thallus in both algal species. Zn appeared to have a very mild effect on reduction of chloroplast but caused a detachment of corticating cells in C. aculeolata at high concentration (10 mg/L).

5.1.3 Pigment contents

Both C. aculeolata and N. opaca showed significant reductions in total chlorophyll, chlorophyll a and b, and carotenoid contents with the increased metal

Table 5-1 Relative growth rates (RGR) of the *C. aculeolata* and *N. opaca* exposed to different levels of Cd, Pb or Zn after 6 days.

Algal species	**RGR (% of control)**						
	Control	**Cd**		**Pb**		**Zn**	
	0	**0.25 mg/L**	**0.5 mg/L**	**5 mg/L**	**10 mg/L**	**5 mg/L**	**10 mg/L**
C. aculeolata	100.0	19.2^{a}	17.4^{a}	35.1	-15.5^{a}	73.0	28.1^{a}
N. opaca	100.0	-44.9^{ab}	-86.1^{ab}	-23.1^{a}	-59.9^{a}	90.2	76.9

Results are means ± SD of 4--6 independent thalli. Significant differences are indicated: ^{a} $P \leq 0.05$ versus control without heavy metal; ^{b} $P \leq 0.05$ versus treated *C. aculeolata*.

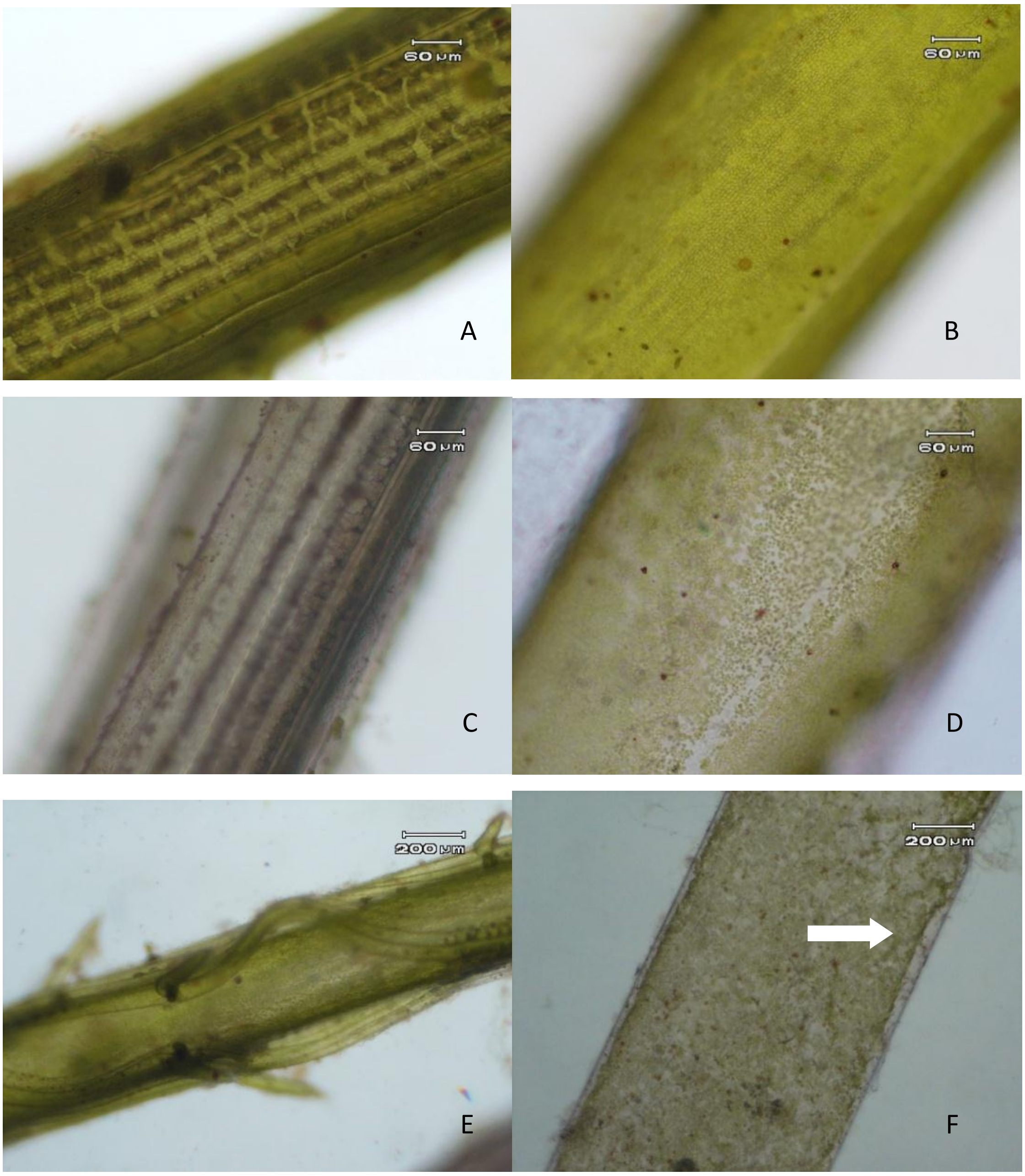


Figure 5-1 Light micrographs of untreated *C. aculeolata* (A), untreated *N. opaca* (B). Treated macroalgae showing reduction of chloroplast in *C. aculeolata* (C), reduction of chloroplast in *N. opaca* (D), detachment of corticating cells (E) and softening of thallus (F)

Table 5-2 Toxicity symptoms observed in *C. aculeolata* and *N. opaca* exposed to Cd, Pb and Zn for 6 days. *

Algal species	Metal	Concentration (mg/L)	Toxicity symptoms		
			Reduction of chloroplast	Softening of thallus	Detachment of corticating cells
C. aculeolata	Cd	0.25	+++	++	-
		0.5	+++	++	-
	Pb	5	+	++	-
		10	++	++	-
	Zn	5	+	-	-
		10	+	-	+
N. opaca	Cd	0.25	+	+++	-
		0.5	++	+++	-
	Pb	5	+	++	-
		10	+++	+++	-
	Zn	5	+	-	-
		10	+	-	-

* Effects of heavy metals were compared on the stem of the thalli.

+: mild effect with >80% of chloroplast and <20% of thallus shrinkage; ++: moderate effect with 50% of chloroplast and 50% thallus shrinkage; +++: severe effect with <20% of chloroplast and >80% of thallus shrinkage; -: no effect.

Table 5-3 The effects of Cd, Pb and Zn on contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoid of *C. aculeolata* at different concentrations after 6-day exposure.

Metal concentration (mg/L)		Pigment content (mg/g fresh wt)			
		Total chlorophyll	Chlorophyll a	Chlorophyll b	Carotenoid
Control		69.17±6.67	45.64±4.27	23.55±2.94	11.95±1.31
Cd	0.25	37.09±5.51^{a}	22.51±3.73^{a}	14.49±1.84^{a}	6.68±0.74^{a}
	0.5	19.89±1.46^{ab}	12.26±1.11^{ab}	7.58±0.37^{ab}	3.83±0.29^{ab}
Pb	5	54.88±5.41^{a}	36.82±3.41^{a}	18.21±1.97	10.55±1.10
	10	34.55±1.68^{ab}	21.00±1.68^{ab}	13.14±0.75^{a}	8.37±0.75^{a}
Zn	5	57.06±3.15	37.23±1.74	19.97±1.73	11.01±0.96
	10	60.54±1.57	39.70±2.23	20.93±1.00	12.64±0.33

Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus control without Cd, Pb or Zn; b, $P \leq 0.05$ versus lower concentration of same metal.

Table 5-4 The effects of Cd, Pb and Zn on contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoid of *N. opaca* at different concentrations after 6-day exposure.

Metal concentration (mg/L)		Pigment content (mg/g fresh wt)			
		Total chlorophyll	Chlorophyll a	Chlorophyll b	Carotenoid
Control		52.68±2.34	36.50±2.03	16.19±0.97	11.26±0.71
Cd	0.25	31.77±1.47[a]	20.36±1.24[a]	11.05±0.56[a]	7.03±0.34[a]
	0.5	31.40±2.51[a]	20.08±1.48[a]	10.95±0.97[a]	6.30±0.82[a]
Pb	5	23.36±4.36[a]	14.30±3.07[a]	8.87±1.29[a]	4.02±0.49[a]
	10	13.86±0.24[a]	8.90±0.26[a]	4.90±0.14[ab]	2.47±0.10[a]
Zn	5	49.59±8.21	33.41±5.52	16.26±2.71	8.91±1.71
	10	45.48±5.74	31.06±4.43	14.47±1.30	8.20±1.28[a]

Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus control without Cd, Pb or Zn; b, $P \leq 0.05$ versus lower concentration of same metal.

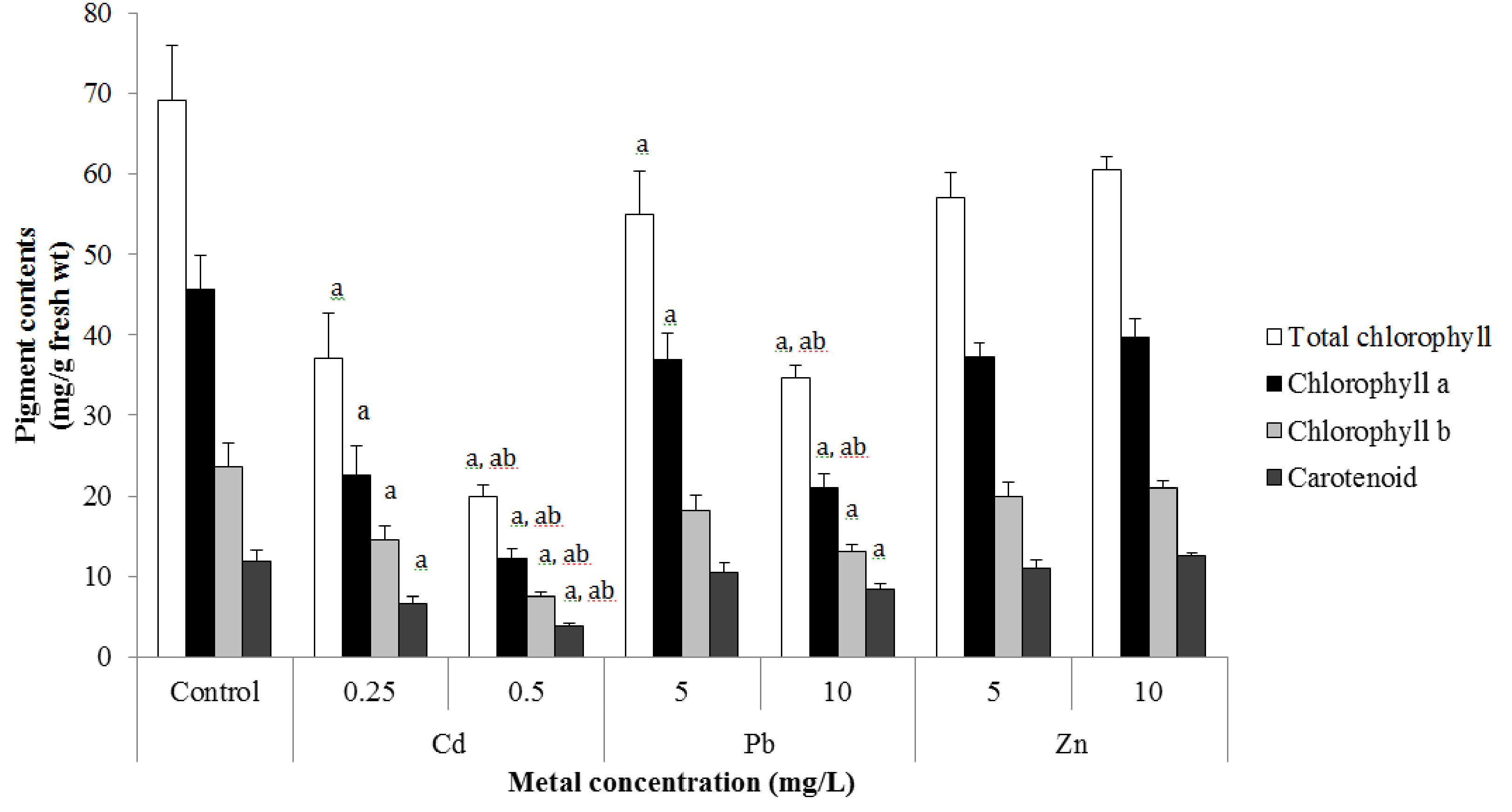


Figure 5-2 The effects of Cd, Pb and Zn on contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoid of *C. aculeolata* at different concentrations after 6-day exposure. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus control without Cd, Pb or Zn; b, $P \leq 0.05$ versus lower concentration of same metal.

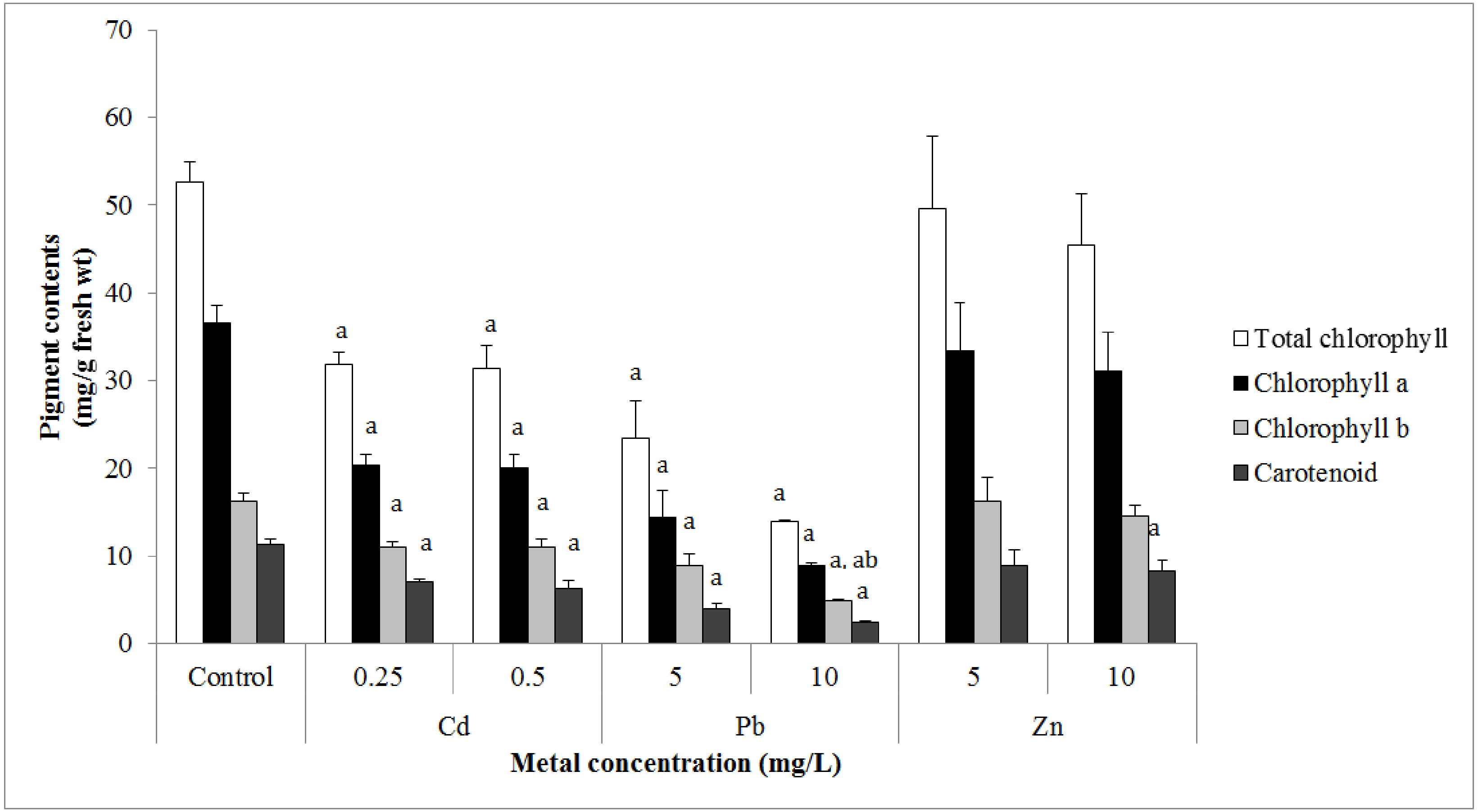


Figure 5-3 The effects of Cd, Pb and Zn on contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoid of *N. opaca* at different concentrations after 6-day exposure. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus control without Cd, Pb or Zn; b, $P \leq 0.05$ versus lower concentration of same metal.

concentrations ($P \leq 0.05$; Tables 5-3, 5-4, Figs. 5-2, 5-3). In general, *C. aculeolata* was more sensitive to Cd at high concentration (0.5 mg/L) while *N. opaca* was more sensitive to Pb. Zn had no effect on pigment contents of *C. aculeolata* ($P > 0.05$) but caused a significant decrease in carotenoid content of *N. opaca* ($P \leq 0.05$). *C. aculeolata* showed the lowest pigment contents (19.9 and 3.8 mg/g for total chlorophyll and carotenoid, respectively) when exposed to high Cd concentration. *N. opaca* showed the lowest pigment contents (13.9 and 2.5 mg/g for total chlorophyll and carotenoid, respectively) when exposed to 10 mg/L Pb.

5.2 Bioaccumulation of Pb, Cd and Zn in *C. aculeolata* and *N. opaca*

5.2.1 Percentage of metal removal

High percentages of Cd and Pb removal were observed in *C. aculeolata* and *N. opaca* (Table 5-5). A total removal of Cd (100%) was found in *C. aculeolata* at 0.25 mg/L Cd on day 3 but the proportion of Cd removed decreased with increasing concentration ($P \leq 0.05$). As shown with the results on growth, *N. opaca* was more sensitive to both Cd and Pb than was *C. aculeolata*. All *N. opaca* thalli died at 0.5 mg/L Cd and 10 mg/L Pb. In contrast, a very low percentage of Zn removal was observed in both species of charophytes.

5.2.2 Bioaccumulation

The present study showed a concentration-dependent accumulation of Cd, Pb and Zn in *C. aculeolata* and *N. opaca* tissues (Table 5-6; Figs. 5-4, 5-5, 5-6). The metal accumulation in algae increased linearly with increasing concentration of metals in the medium. In *C. aculeolata*, the coefficient of correlation, R^2 values between metal concentration in solution and that accumulated by algae for Cd, Pb and Zn were 0.982, 0.946 and 0.952, respectively. In *N. opaca*, the R^2 values were 0.820, 0.968 and 0.717 for Cd, Pb and Zn, respectively. At low concentrations in the medium, *N. opaca* accumulated significantly more Cd and Pb than *C. aculeolata* ($P \leq 0.05$). However, neither algal species showed any significant difference in Cd and Pb accumulation at high concentration of metals ($P > 0.05$). In contrast, *C. aculeolata* accumulated

Table 5-5 Percentage of metal removal from the culture media by *C. aculeolata* and *N. opaca* exposed to different metal concentrations after 3 and 6 days.

Metal	Concentration (mg/L)	Percentage of metal removal			
		Day 3		Day 6	
		C. aculeolata	*N. opaca*	*C. aculeolata*	*N. opaca*
Cd	0.25	100.0±0	60.7±6.5 [a]	98.5±2.5	5.4±2.1 [a,b]
	0.5	91.4±4.8 [c]	23.4±2.4 [a,c]	77.7±7.8 [b,c]	0 [a,b]
Pb	5	96.8±1.3	93.2±1.0	90.6±0.9	76.5±3.2 [a,b]
	10	94.1±0.6	88.5±3.0	95.0±1.6	43.2±7.7 [a,b,c]
Zn	5	17.7±4.2	0 [a]	3.7±1.5	0
	10	0 [c]	0	0	0

Results are means ± SD of 4-6 independent thalli. [a] $P \leq 0.05$ versus *C. aculeolata*; [b] $P \leq 0.05$ versus day 3; [c] $P \leq 0.05$ versus lower concentration of same metal.

Table 5-6 The accumulation of Cd, Pb and Zn by *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days.

Metal concentration (mg/L)		Metal accumulation (µg/g)	
		C. aculeolata	*N. opaca*
Control		0	0
Cd	0.25	493.26±87.47	1,399.51±54.75[a]
	0.5	1,288.15±55.39[b]	1,544.28±450.07
Pb	5	5,601.88±802.69	14,271.32±1,463.87[a]
	10	19,022.92±2,812.74[b]	21,656.98±2,124.81[b]
Zn	5	4,487.86±424.87	1,892.04±226.56[a]
	10	6,467.19±586.81[b]	1,812.85±379.24[a]

Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus *C. aculeolata*; b, $P \leq 0.05$ versus lower concentration.

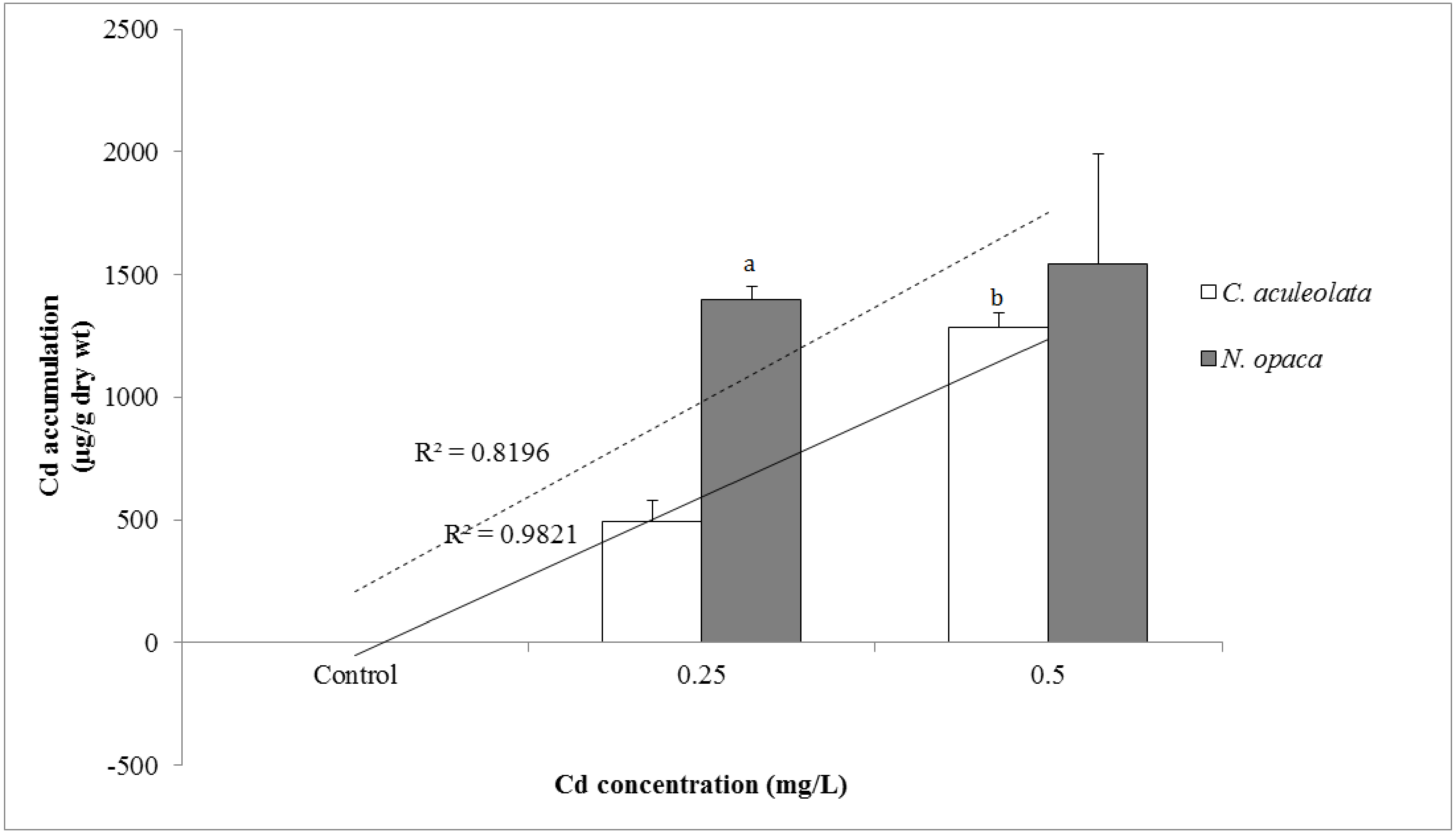


Figure 5-4 The accumulation of Cd by *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus *C. aculeolata*; b, $P \leq 0.05$ versus lower concentration. Linear regression line: solid line, *C. aculeolata*; dash line, *N. opaca*.

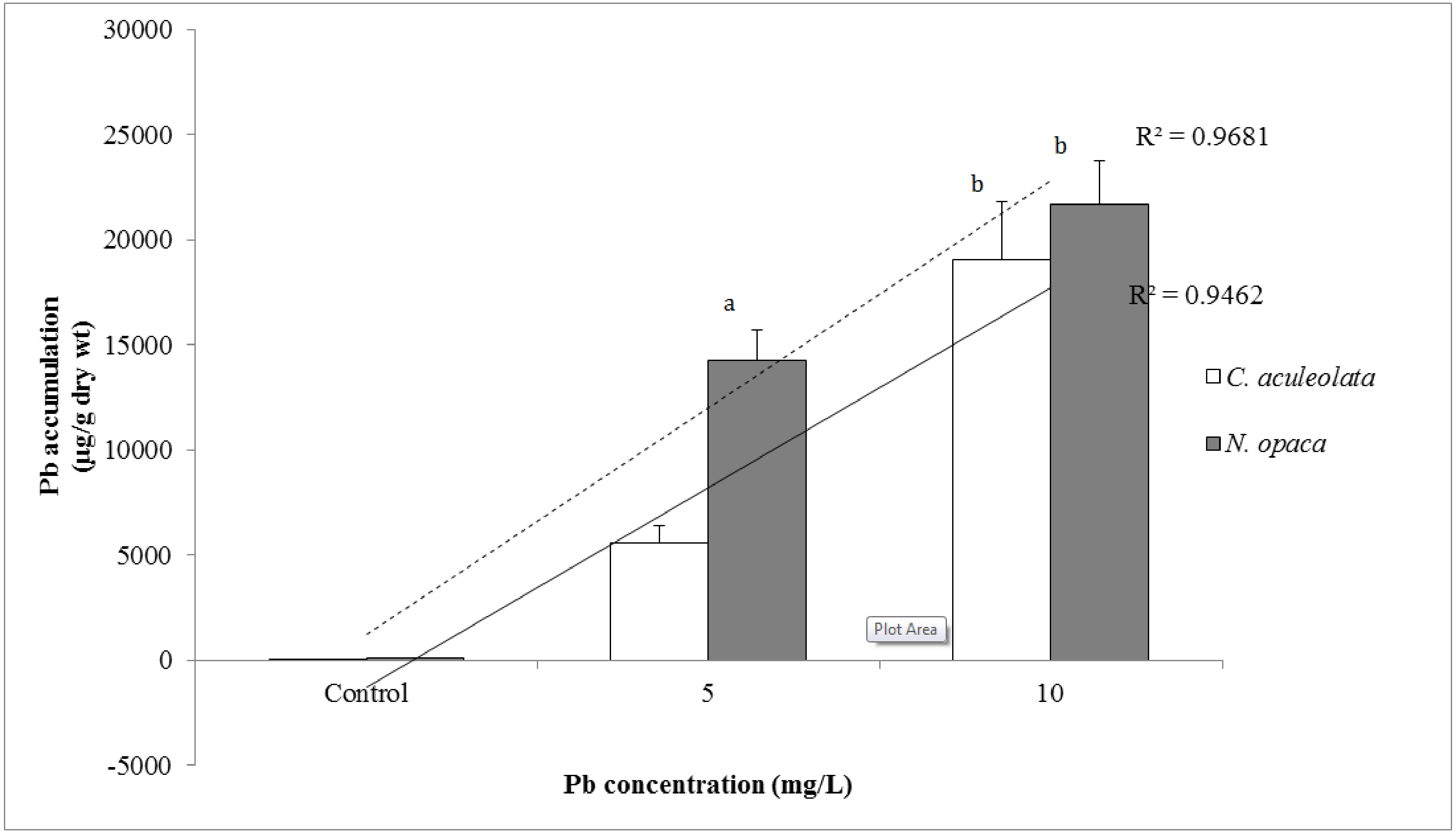


Figure 5-5 The accumulation of Pb by *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus *C. aculeolata*; b, $P \leq 0.05$ versus lower concentration. Linear regression line: solid line, *C. aculeolata*; dash line, *N. opaca*.

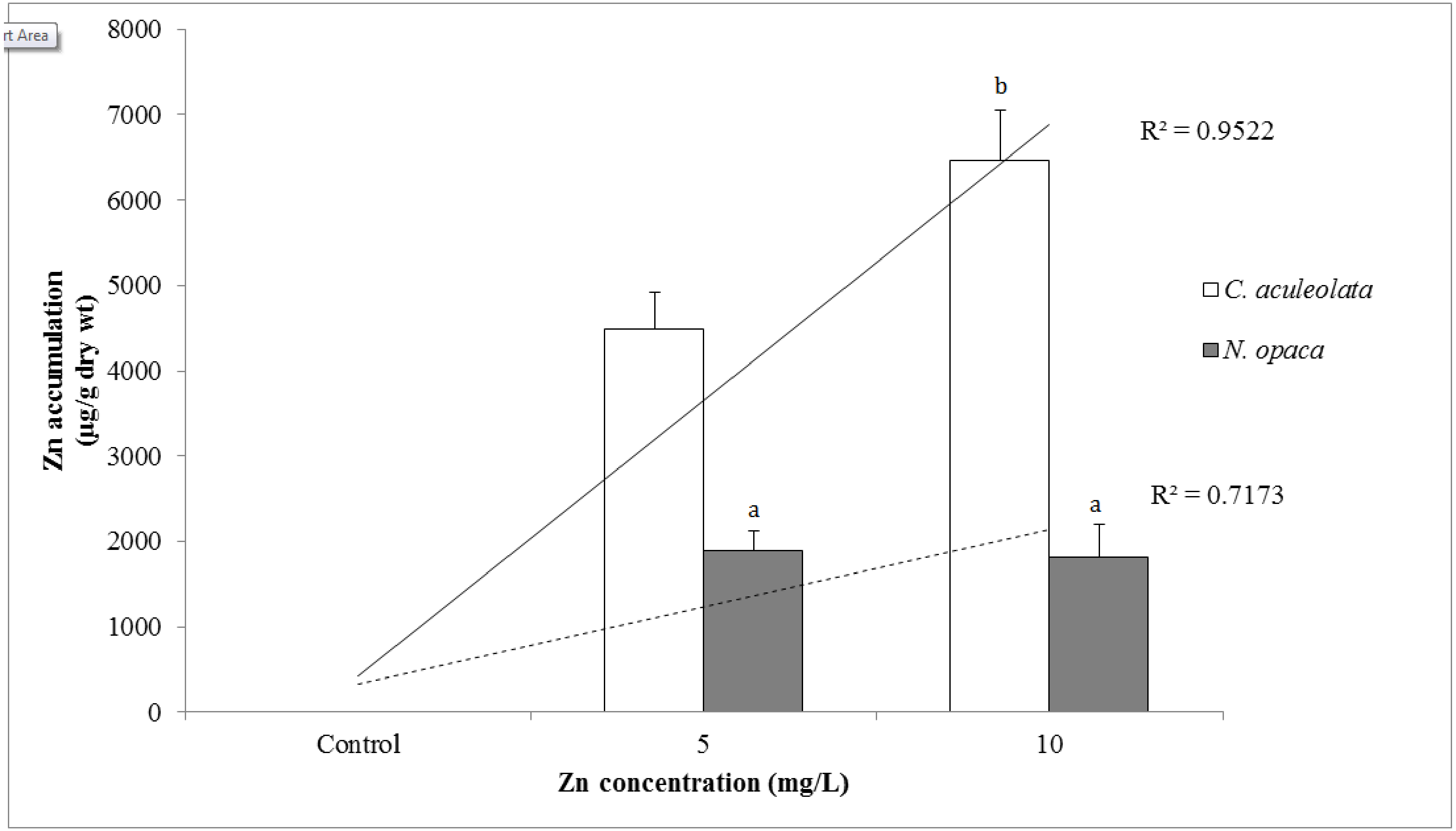


Figure 5-6 The accumulation of Zn by *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus *C. aculeolata*; b, $P \leq 0.05$ versus lower concentration. Linear regression line: solid line, *C. aculeolata*; dash line, *N. opaca*.

Table 5-7 The bioconcentration factors (BCF) for Cd, Pb and Zn in *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days.

Metal concentration (mg/L)		Bioconcentration factor	
		C. aculeolata	*N. opaca*
Cd	0.25	3,023.06±536.07	8,577.19±335.52[a]
	0.5	3,394.33±145.96	4,069.26±1,185.96[b]
Pb	5	1,313.97±188.28	3,347.45±343.36[a]
	10	2,116.01±312.87	2,409.01±236.35
Zn	5	1,027.83±97.31	433.32±51.89
	10	873.51±79.26	244.86±51.22

Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus *C. aculeolata*; b, $P \leq 0.05$ versus lower concentration of same metal.

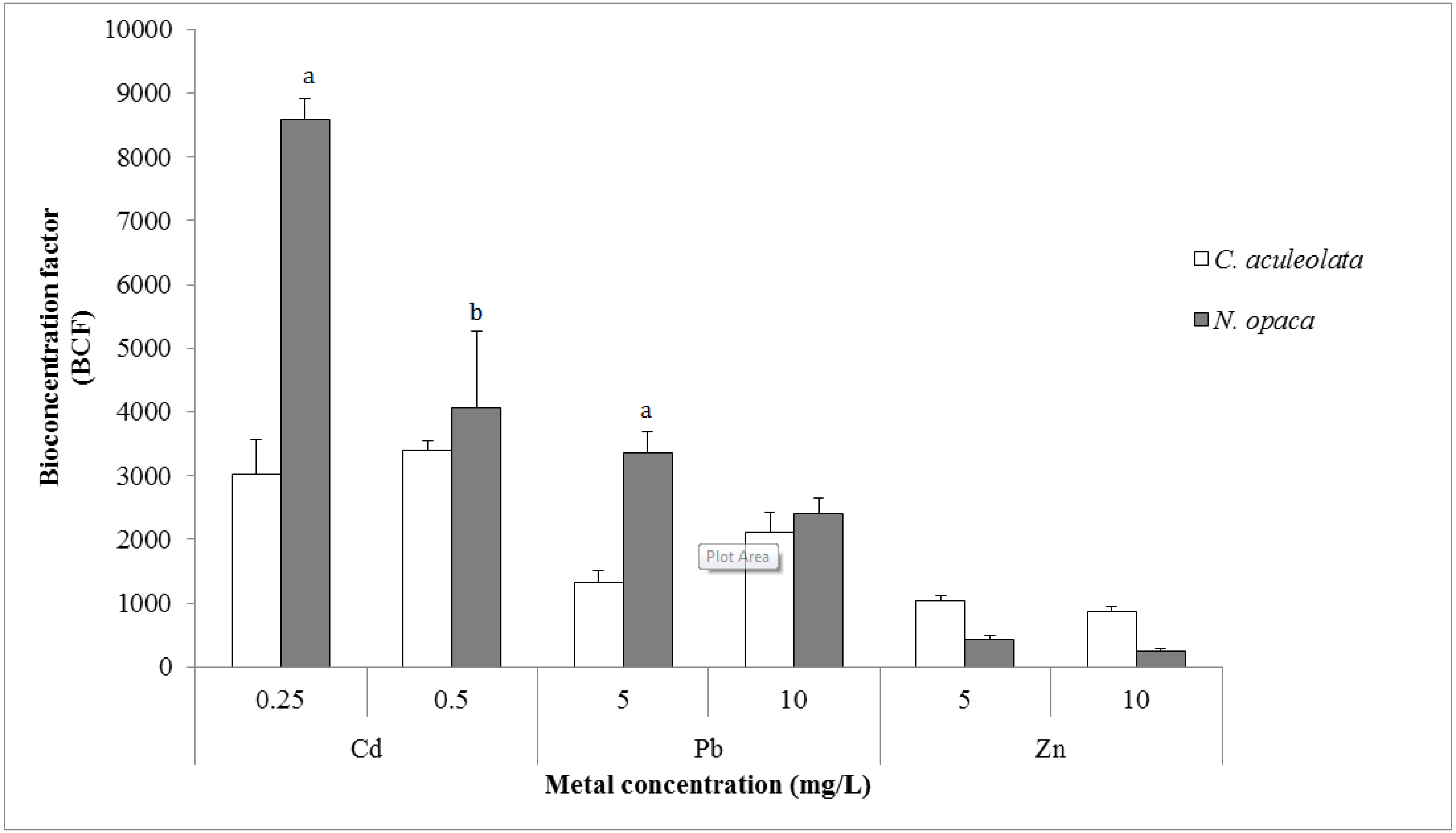


Figure 5-7 The bioconcentration factors (BCF) for Cd, Pb and Zn in *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leq 0.05$ versus *C. aculeolata*; b, $P \leq 0.05$ versus lower concentration of same metal.

significantly more Zn than *N. opaca* both at high and low Zn concentration (Fig. 5-6). *N. opaca* displayed the highest accumulation of Cd at 0.5 mg/L (1544.3 µg/g) and Pb at 10 mg/L (21,657.0 µg/g), while *C. aculeolata* displayed the highest accumulation of Zn at 10 mg/L (6467.2 µg/g) after 6 days of exposure (Table 5-6).

5.2.3 Bioconcentration factor

C. aculeolata and *N. opaca* showed a similar trend of bioconcentration of Cd, Pb and Zn to that observed in the growth and toxicity studies. *N. opaca* was more sensitive to Cd and Pb than *C. aculeolata* but both algal species showed similar response to Zn (BCF values < 1000; Table 5-7; Fig. 5-7). Even though *N. opaca* displayed significantly high BCFs for Cd (8577.2) and Pb (3347.5) at low concentrations ($P \leq 0.05$), these were not significantly different from those of *C. aculeolata* at high Cd concentration. There was no significant decrease in BCFs with increasing concentrations of Cd, Pb and Zn in *C. aculeolata*, indicating that the alga maintained its ability to accumulate these metals even when metal concentrations were increased.

5.3 Biosorption of Pb, Cd and Zn in *C. aculeolata* and *N. opaca* in batch culture

5.3.1 Biosorption of metal ions from synthetic solutions

5.3.1.1 Effect of sorbent dosage

The results showed that the sorbent dosage was not critical in Pb removal, as the maximum efficiency was attained by as little as 0.1 g/L *C. aculeolata* biomass and 0.2 g/L *N. opaca* biomass (Table 5-8; Figs. 5-8, 5-9). In contrast, the removal efficiency of Cd and Zn increased with the sorbent dosage and reached their maximum when 2 g/L *C. aculeolata* and 3 g/L *N. opaca* biomasses were used. Therefore, these optimal sorbent dosages were selected for further studies.

5.3.1.2 Effect of initial pH

Both *C. aculeolata* and *N. opaca* exhibited maximum sorption percentage of all three metal ions when the solution was initially adjusted to pH 4

Table 5-8 Removal efficiency affected by algal dosage on biosorption of Pb, Cd and Zn by *C. aculeolata* and *N. opaca*.

Algal dosage (g/L)	Removal efficiency (%)					
	C. aculeolata			*N. opaca*		
	Pb	Cd	Zn	Pb	Cd	Zn
0.1	98.5±1.0	44.3±9.4^{a}	28.9±7.9^{a}	89.8±8.5	13.2±0.4^{a}	13.0±8.6^{a}
0.2	99.3±0.5	58.4±0.9^{b}	39.3±3.3^{b}	99.7±0	37.1±1.7^{ab}	24.3±0.5^{a}
0.5	97.4±1.8	74.7±0.4^{c}	60.6±2.8^{c}	99.7±0	67.1±3.0^{b}	54.8±0.3^{b}
1	95.8±3.7	83.3±0.9^{cd}	75.4±0.7^{d}	99.7±0	81.3±0.3^{c}	70.0±2.9^{c}
2	95.5±4.0	90.5±0.7^{de}	84.0±0.4^{e}	99.7±0	84.7±0.3^{c}	78.6±4.0^{cd}
3	96.4±2.5	91.9±0.4^{de}	86.0±0.7^{e}	99.7±0	85.6±0.3^{c}	83.7±0.6^{d}
4	97.9±1.4	92.5±0.3^{d}	83.9±1.1^{e}	99.7±0	86.1±0.1^{c}	84.0±0.3^{d}

(initial metal concentration, 10 mg/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Significant differences are indicated as a, b, c, d and e, the homogeneity subsets compared among values from each metal by one way ANOVA, Tukey HSD test ($P \leq 0.05$).

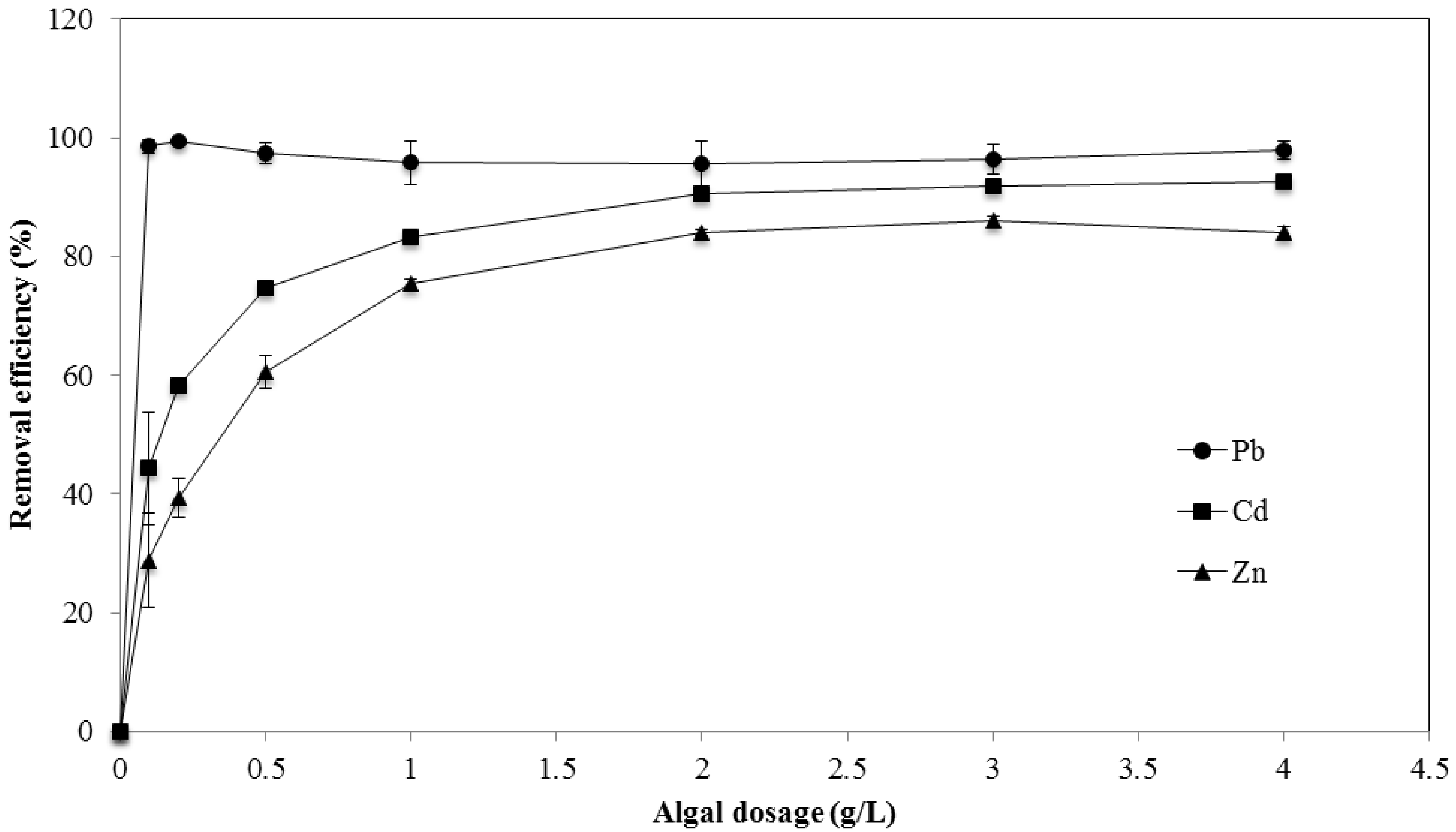


Figure 5-8 Effect of algal dosage on biosorption of Pb, Cd and Zn by *C. aculeolata* (initial metal concentration, 10 mg/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Error bars represent standard deviation of the mean (n = 3).

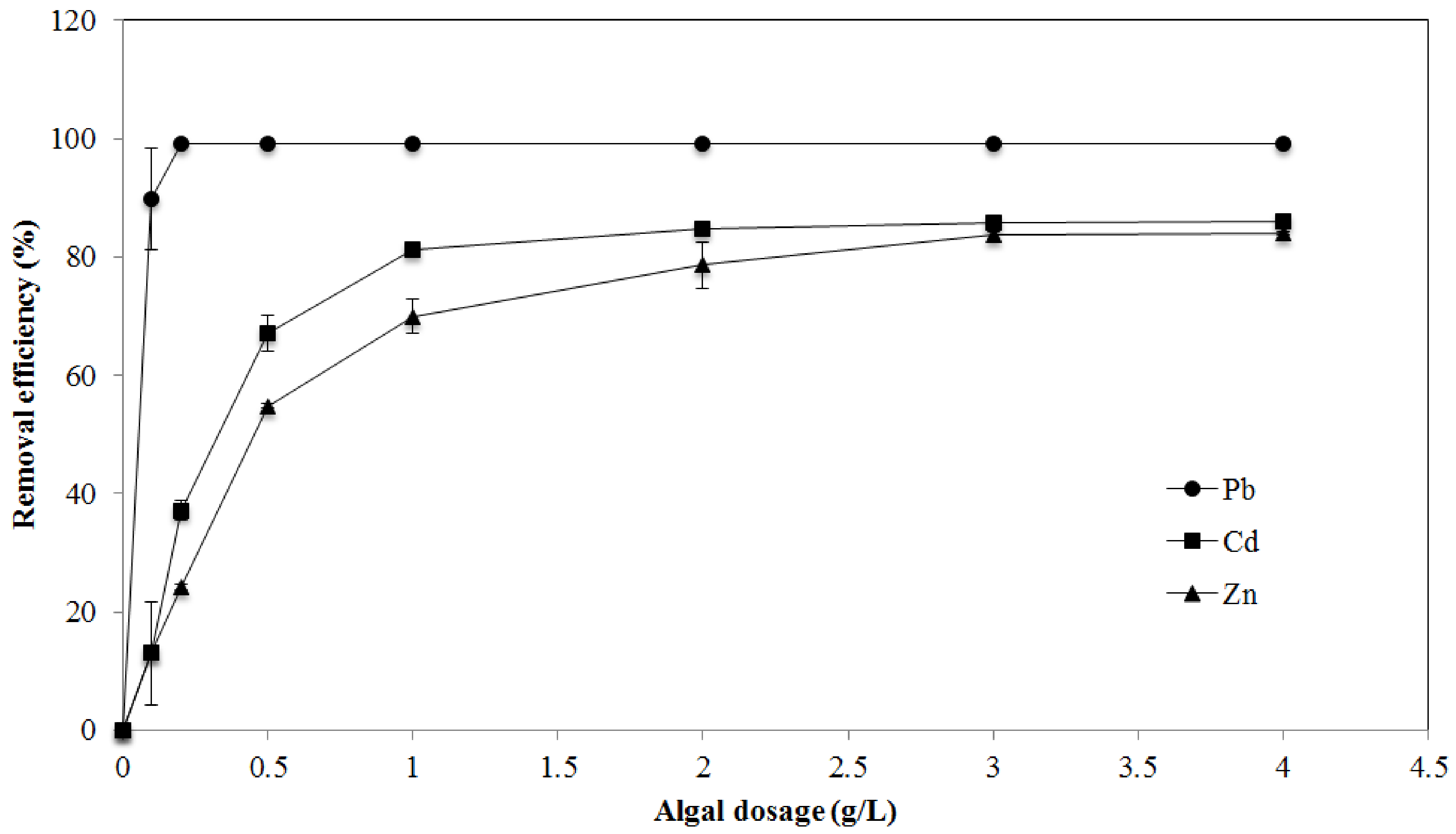


Figure 5-9 Effect of algal dosage on biosorption of Pb, Cd and Zn by *N. opaca* (initial metal concentration, 10 mg/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Error bars represent standard deviation of the mean (n = 3).

Table 5-9 Removal efficiency affected by initial pH on biosorption of Pb, Cd and Zn by *C. aculeolata* and *N. opaca*.

pH	Removal efficiency (%)					
	C. aculeolata			*N. opaca*		
	Pb	Cd	Zn	Pb	Cd	Zn
2	99.3 ± 0.2^{a}	77.1 ± 1.5^{a}	78.2 ± 2.3^{ab}	90.6 ± 0.5^{a}	4.9 ± 4.1^{a}	0 ± 0^{a}
3	99.2 ± 0.3^{a}	93.4 ± 0.1^{bc}	87.4 ± 0.3^{b}	99.5 ± 0^{a}	75.5 ± 2.0^{b}	49.4 ± 0.5^{b}
4	97.5 ± 0.9^{a}	94.6 ± 0.4^{b}	87.9 ± 0.5^{b}	99.2 ± 0^{a}	90.6 ± 0.2^{c}	65.0 ± 0.8^{c}
5	62.2 ± 10.2^{b}	94.2 ± 0.4^{bc}	86.3 ± 0.7^{b}	74.2 ± 25.1^{a}	90.8 ± 1.7^{c}	72.9 ± 0.4^{c}
6	74.2 ± 14.3^{b}	92.5 ± 0.6^{c}	84.2 ± 1.0^{b}	0 ± 0^{b}	88.9 ± 1.0^{c}	64.9 ± 0.4^{c}
7	-	92.6 ± 0.3^{c}	85.0 ± 0.3^{b}	-	87.3 ± 1.3^{c}	17.9 ± 4.9^{d}
8	-	92.3 ± 0.6^{c}	67.9 ± 10.5^{a}	-	88.3 ± 1.4^{c}	3.7 ± 5.2^{a}

(initial metal concentration, 10 mg/L; *Chara* dose, 2 g/L; *Nitella* dose, 3 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Significant differences are indicated as a, b, c and d, the homogeneity subsets compared among values from each metal by one way ANOVA, Tukey HSD test ($P \leq 0.05$).

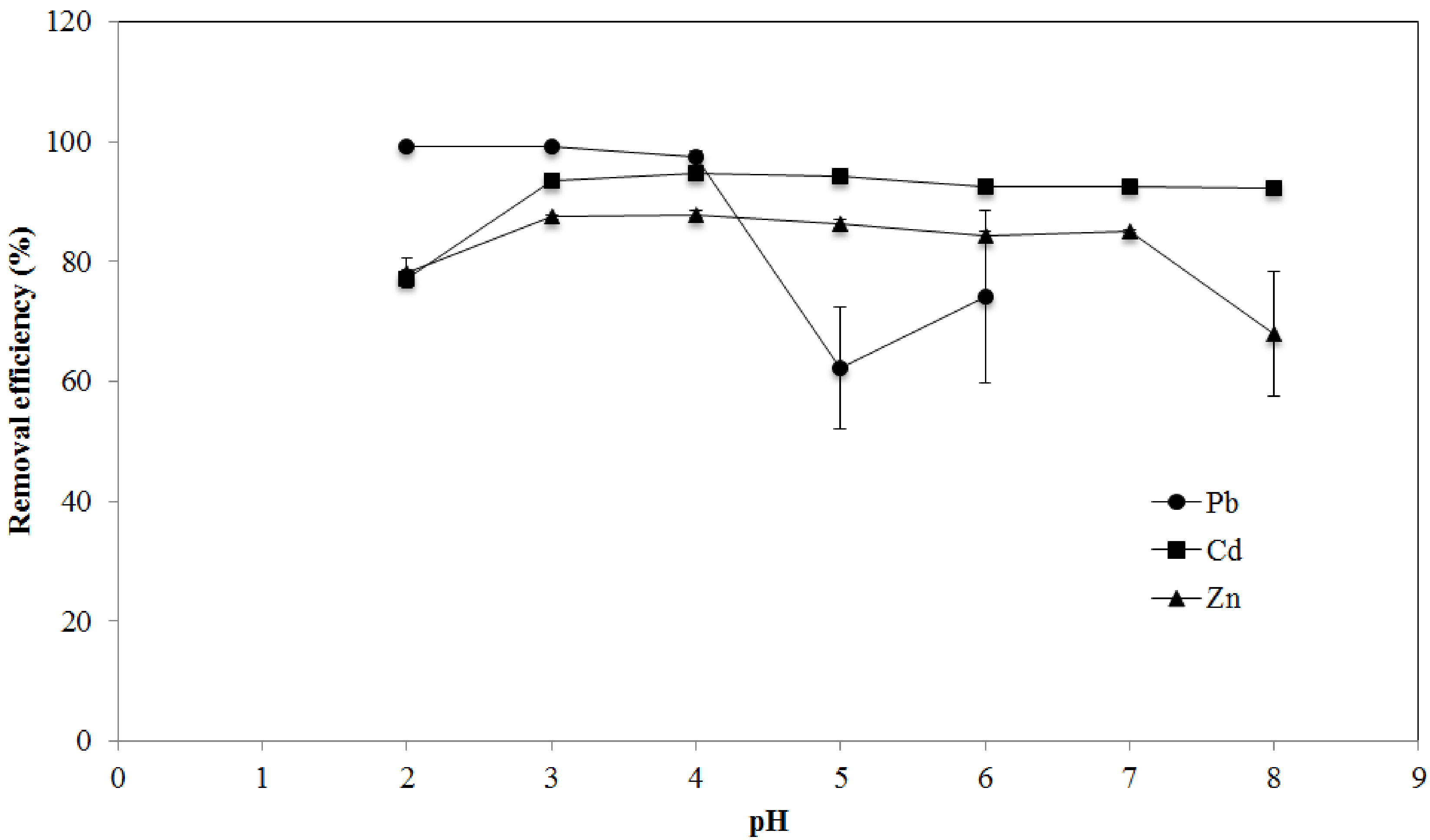


Figure 5-10 Effect of initial pH on biosorption of Pb , Cd and Zn by *C. aculeolata* (initial metal concentration, 10 mg/L; *Chara* dose, 2 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Error bars represent standard deviation of the mean (n = 3).

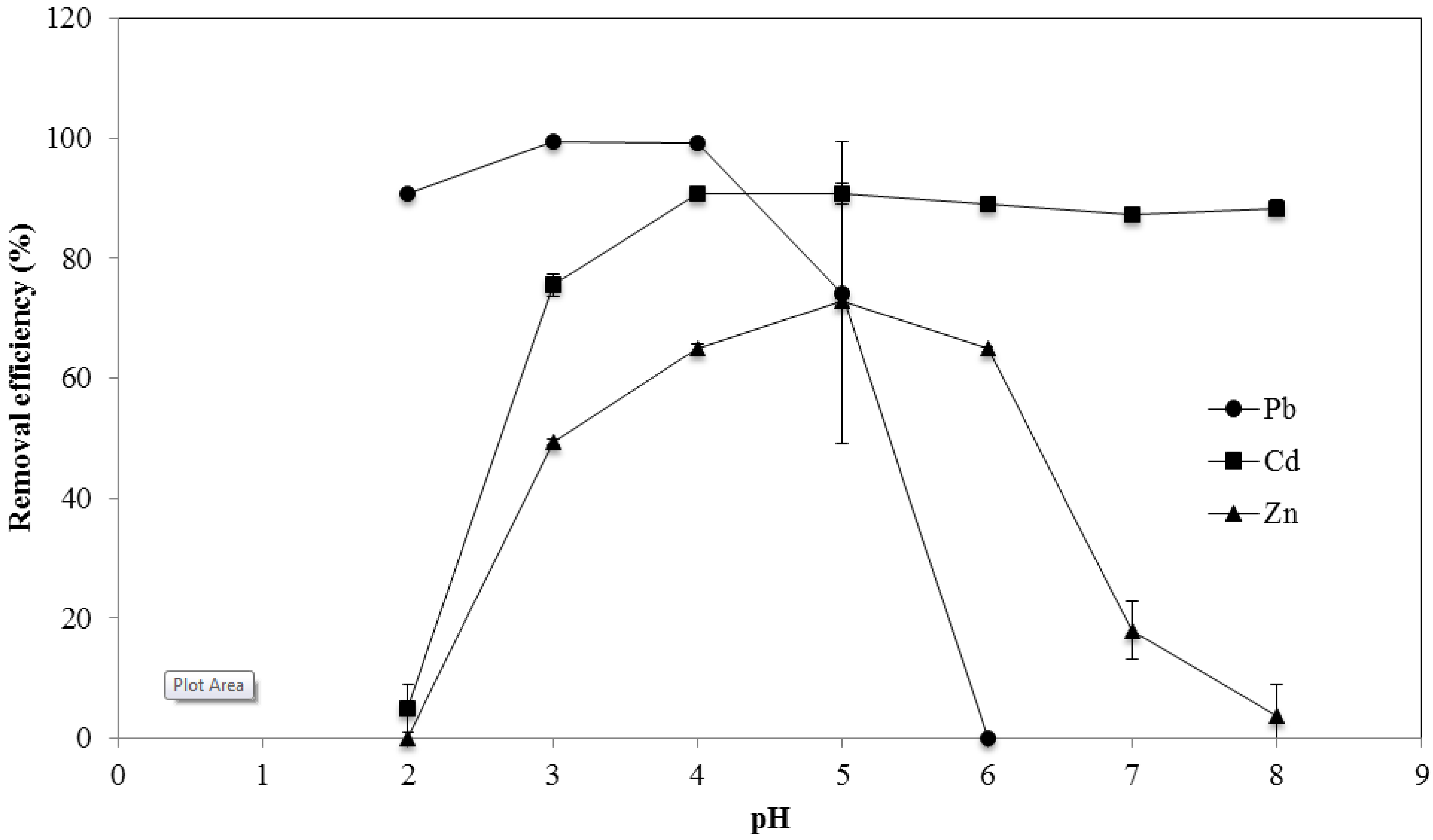


Figure 5-11 Effect of initial pH on biosorption of Pb, Cd and Zn *N. opaca* (initial metal concentration, 10 mg/L; *Nitella* dose, 3 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Error bars represent standard deviation of the mean (n = 3).

Table 5-10 Removal efficiency affected by contact time on biosorption of Pb, Cd and Zn by *C. aculeolata* and *N. opaca*.

Contact time (min)	Removal efficiency (%)					
	C. aculeolata			*N. opaca*		
	Pb	Cd	Zn	Pb	Cd	Zn
3	74.4±3.2^a	43.7±0.3^a	48.1±3.8^a	91.3±0.4^a	64.0±2.1^a	67.5±2.9^a
15	99.2±0^b	82.4±0.7^b	72.5±2.6^b	99.2±0^b	84.6±0.6^b	82.6±0.1^b
30	99.2±0^b	86.9±0.3^c	82.8±0.1^c	99.2±0^b	85.8±0.2^b	83.0±0.0^b
60	99.2±0^b	87.7±0.4^c	83.1±0.2^c	99.2±0^b	85.7±0.3^b	83.1±0.0^b
90	99.2±0^b	89.7±0.0^d	83.7±0.0^c	99.2±0^b	86.4±0.0^b	83.0±0.2^b
120	99.2±0^b	89.9±0.1^d	83.6±0.7^c	99.2±0^b	86.3±0.5^b	82.9±0.1^b
180	99.2±0^b	90.5±0.1^d	83.9±1.1^c	99.2±0^b	86.1±0.2^b	83.7±1.2^b
360	99.2±0^b	90.2±0.3^d	84.9±0.3^c	99.2±0^b	86.2±0.3^b	82.7±0.0^b

(initial metal concentration, 10 mg/L; *Chara* dose, 2 g/L; *Nitella* dose, 3 g/L; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Significant differences are indicated as a, b, c and d, the homogeneity subsets compared among values from each metal by one way ANOVA, Tukey HSD test ($P \leq 0.05$).

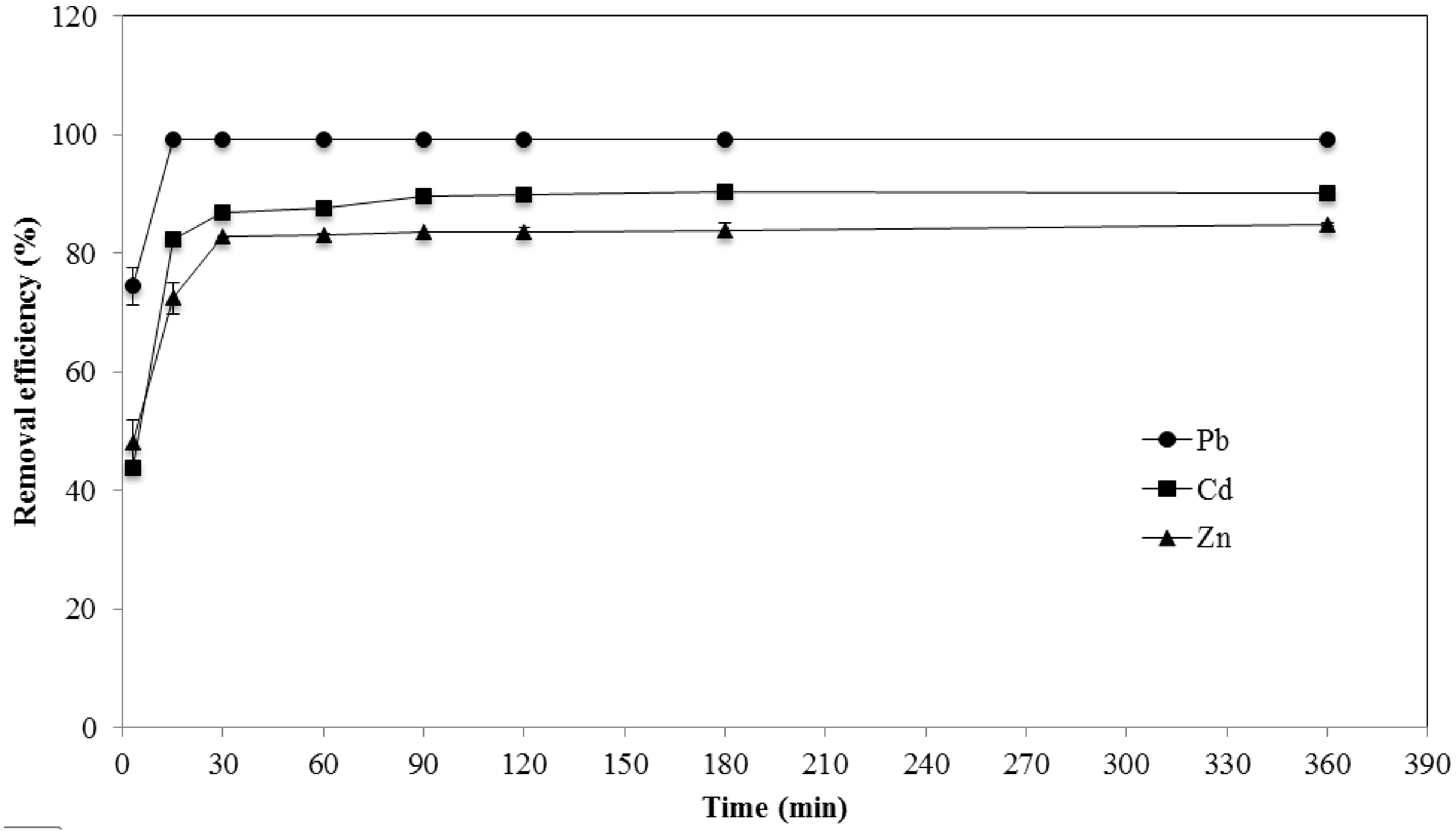


Figure 5-12 Effect of contact time on biosorption of Pb, Cd and Zn by *C. aculeolata* (initial metal concentration, 10 mg/L; *Chara* dose, 2 g/L; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Error bars represent standard deviation of the mean (n = 3).

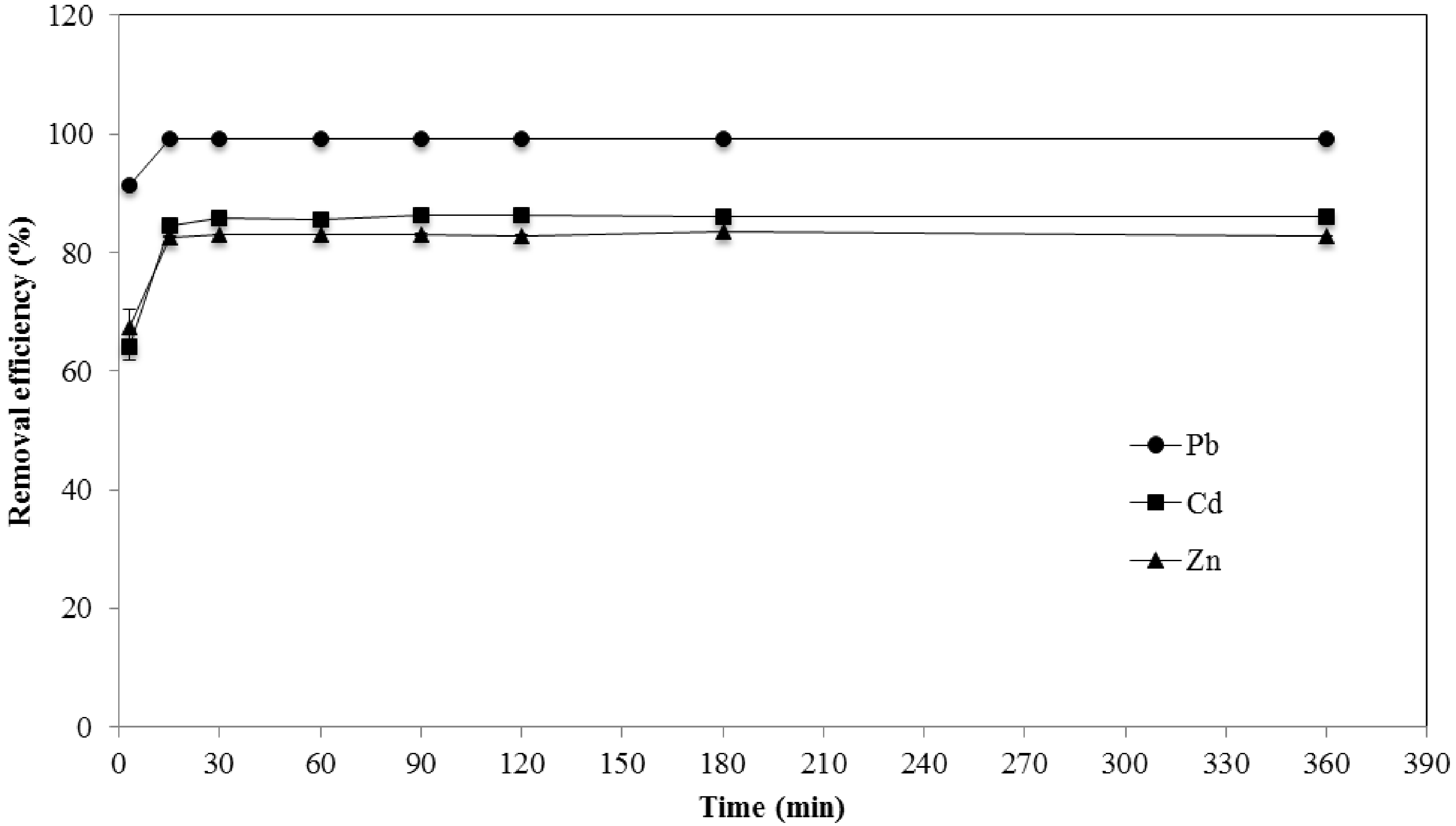


Figure 5-13 Effect of contact time on biosorption of Pb, Cd and Zn by *N. opaca* (initial metal concentration, 10 mg/L; *Nitella* dose, 3 g/L; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Error bars represent standard deviation of the mean (n = 3).

(Table 5-9; Figs. 5-10, 5-11). At the highest biosorption efficiency, 98, 95 and 88% of Pb, Cd and Zn were removed by *C. aculeolata*, respectively and 100, 91 and 65% removed by *N. opaca*, respectively.

For *C. aculeolata*, biosorption of Cd was highest and not strongly affected by initial pH in the solutions above pH 3. A similar trend was observed for Zn, but its removal efficiency was reduced in the solution above pH 7. For *N. opaca*, the effects of pH on biosorption of Cd and Zn were more pronounced. Cd was efficiently removed in the solutions above pH 4, whereas the removal efficiency of Zn was optimal between pH 4 and 6. High removal efficiency of Pb by biomass of *C. aculeolata* and *N. opaca* was highest between pH 2 and pH 4. Beyond pH 4, the Pb removal efficiency dropped significantly possibly due to Pb precipitation as $Pb(OH)_2$ in the solution that contains algal biomass with increased pH. Because pH 4 yielded the optimal biosorption for all three metals by both algal biomasses, this pH was chosen for further studies.

5.3.1.3 Influence of contact time

The biosorption of *C. aculeolata* reached equilibrium within 15 min for Pb, 90 min for Cd and 30 min for Zn (Table 5-10; Figs. 5-12, 5-13). The equilibrium for *N. opaca* was reached relatively sooner, within 15 min for all three metals.

5.3.1.4 Sorption kinetics

The sorption kinetics of Cd and Zn onto both algal biomass fitted well with the pseudo-second-order model, exhibiting higher correlation coefficients (R^2) than those of the pseudo-first-order model (Table 5-11; Figs. 5-14, 5-15). The sorption kinetics of Pb fitted slightly better to the pseudo-first-order model for *N. opaca* (Fig.5-16). When the performances of the two algal biomass were compared, the results showed that the kinetic constants (K_{ad1} and K_{ad2}) for all three metals sorbed by *N. opaca* were greater than those by *C. aculeolata*, indicating faster rates of metal biosorption by *N. opaca* biomass. The theoretical metal uptake (q_e) values obtained from the second-order model were similar to the experimental metal uptake (q_{exp}) data for sorption of Cd and Zn onto the algal biomass.

The best fit of the experimental data to intraparticle diffusion model was obtained in Pb sorption by *C. aculeolata* (Table 5-11). The plots of q versus $t^{1/2}$

Table 5-11 Parameters in pseudo-first-order, pseudo-second-order kinetics and intraparticle diffusion for adsorption of Pb, Cd and Zn ions by *C. aculeolata* and *N. opaca*.

Heavy metal	***C. aculeolata***								
	q_{exp}	**First-order kinetic**			**Second-order kinetic**			**Intraparticle diffusion**	
Pb	54.05	**K_{ad1}**	**q_e**	**R^2**	**K_{ad2}**	**q_e**	**R^2**	**K_{id}**	**R^2**
Pb	54.05	0.10	62.52	0.9865	3.73	75.19	0.9341	11.79	0.9910
Cd	3.69	0.10	2.07	0.9436	1.57	3.78	0.9989	0.49	0.8858
Zn	4.48	0.17	4.36	0.9572	1.96	4.68	0.9993	0.51	0.9782
Heavy metal	***N. opaca***								
	q_{exp}	**First-order kinetic**			**Second-order kinetic**			**Intraparticle diffusion**	
Pb	27.83	**K_{ad1}**	**q_e**	**R^2**	**K_{ad2}**	**q_e**	**R^2**	**K_{id}**	**R^2**
Pb	27.83	0.18	32.6	0.9903	5.01	31.35	0.9895	4.76	0.9010
Cd	2.37	0.13	1.54	0.9199	3.42	2.38	0.9998	0.17	0.8541
Zn	2.98	0.19	1.67	0.9159	6.52	3.01	0.9999	0.15	0.8318

q_{exp}, metal uptake obtained from the experiments (mg/g); q_e, the theoretical metal uptake (mg/g); K_{ad1}, the first-order rate constant; K_{ad2}, the second-order rate constant; R^2, correlation coefficient.

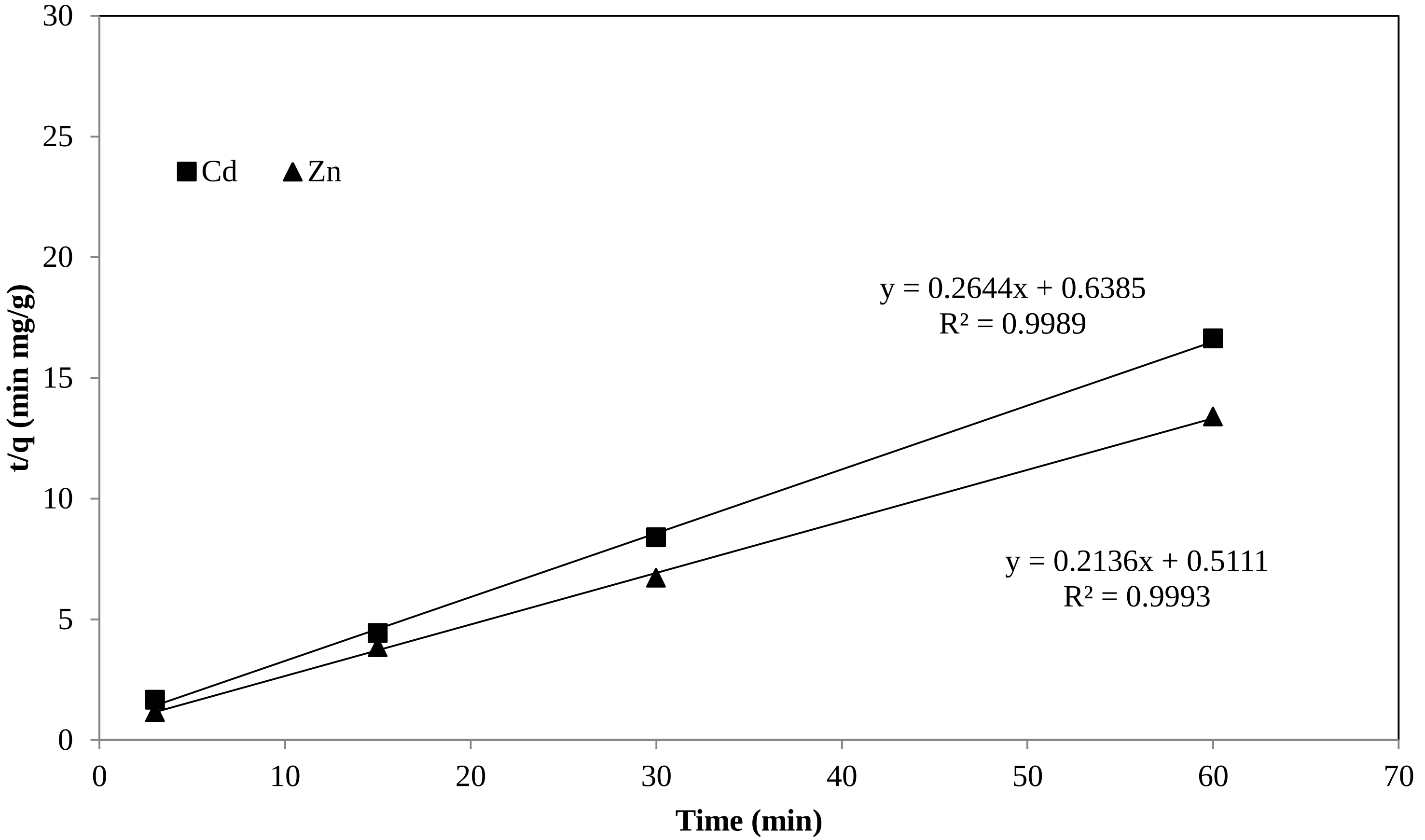


Figure 5-14 Linear portions of the fitted data to the pseudo-second order kinetic. *C. aculeolata* was exposed to Pb, Cd and Zn at pH 4; *Chara* dose, 2 g/L; temperature, 25±2 ^{o}C; agitation rate, 150 rpm. Data points derive from triplicate experiments.

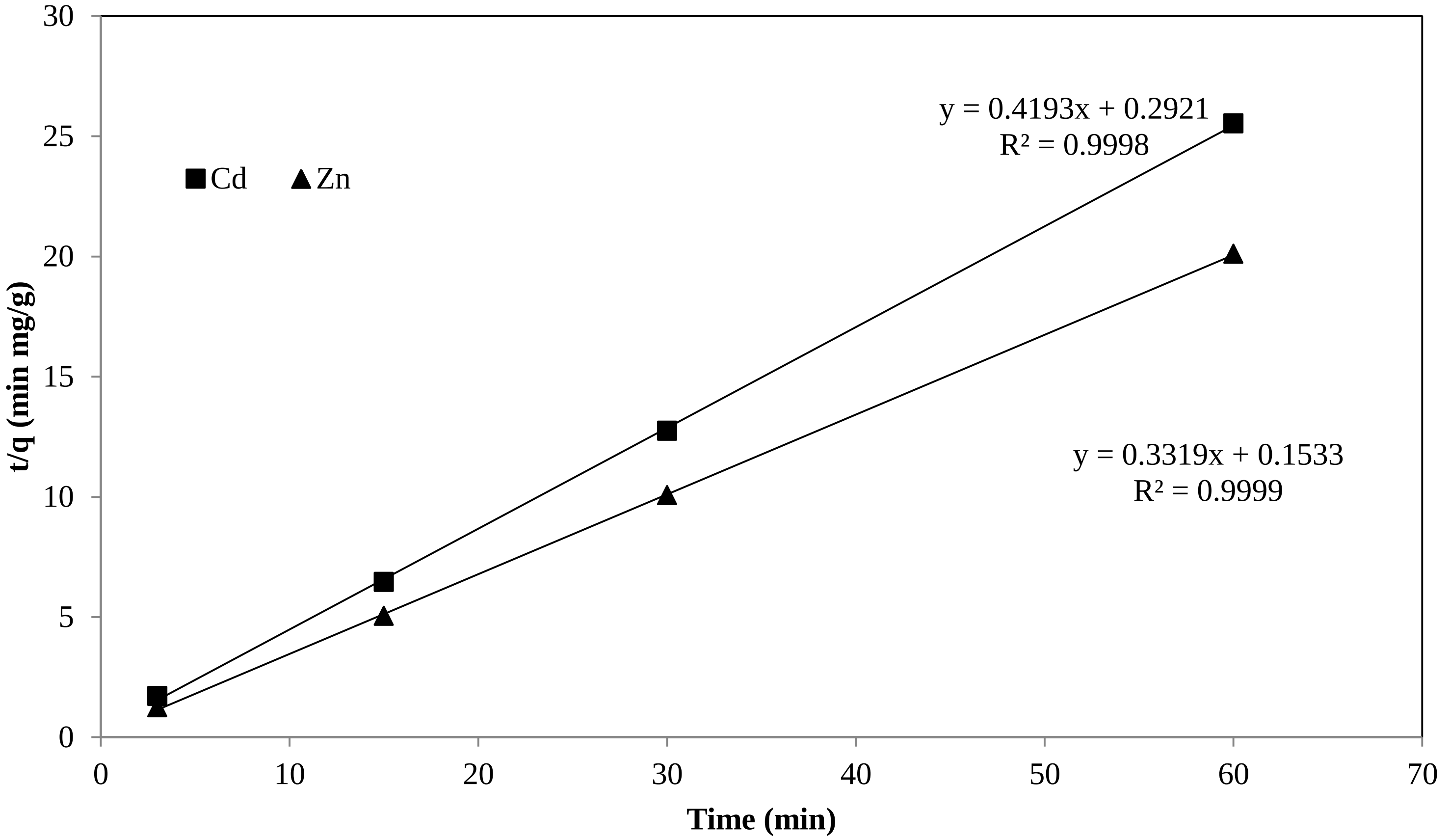


Figure 5-15 Linear portions of the fitted data to the pseudo-second order kinetics. *N. opaca* was exposed to Pb, Cd and Zn at pH 4; *Nitella* dose, 3 g/L; temperature, 25±2 ^{o}C; agitation rate, 150 rpm. Data points derive from triplicate experiments.

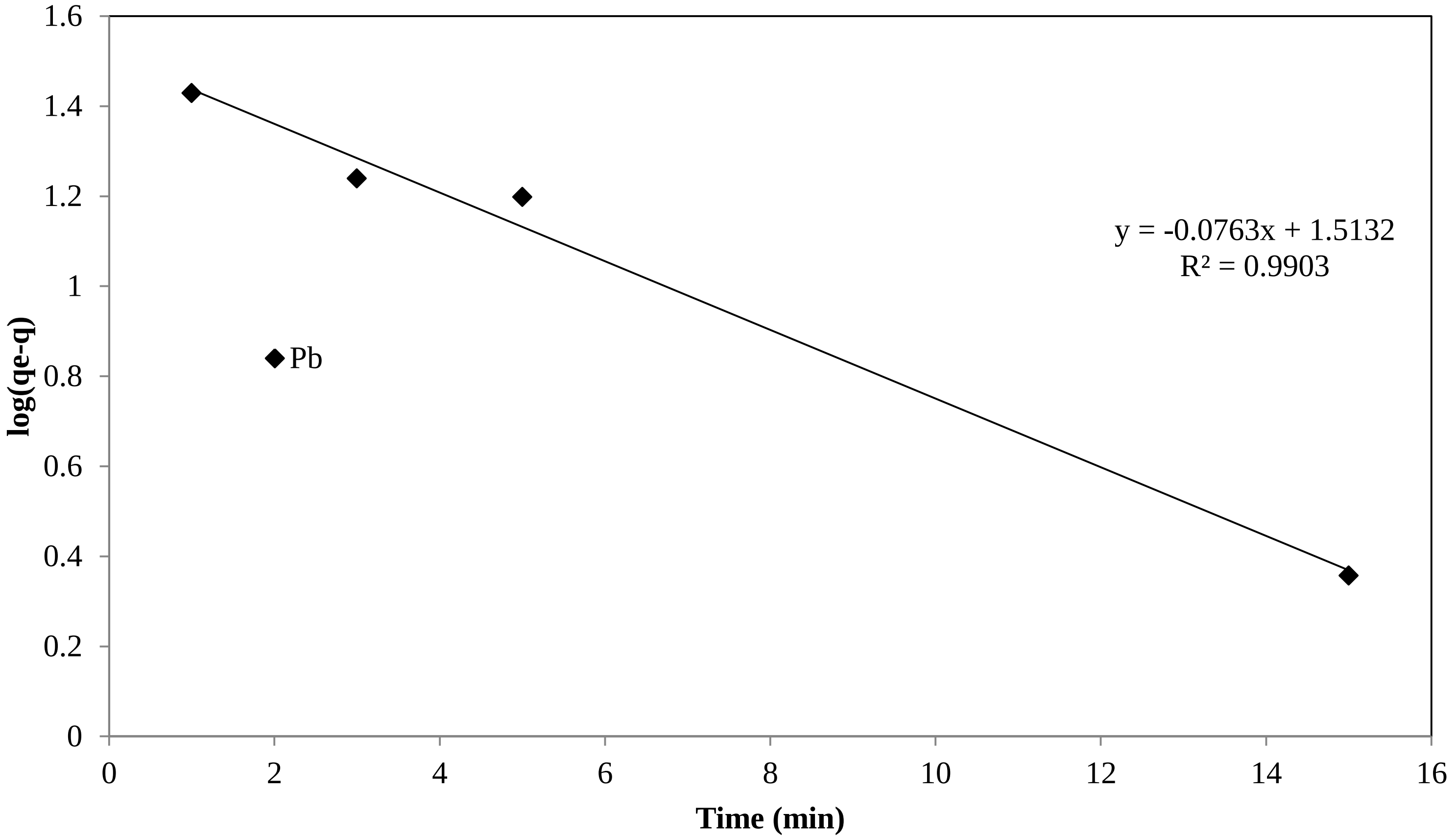


Figure 5-16 Linear portions of the fitted data to the pseudo-first order kinetic. *N. opaca* was exposed to Pb, Cd and Zn at pH 4; *Nitella* dose, 3 g/L; temperature, 25±2 °C; agitation rate, 150 rpm. Data points derive from triplicate experiments.

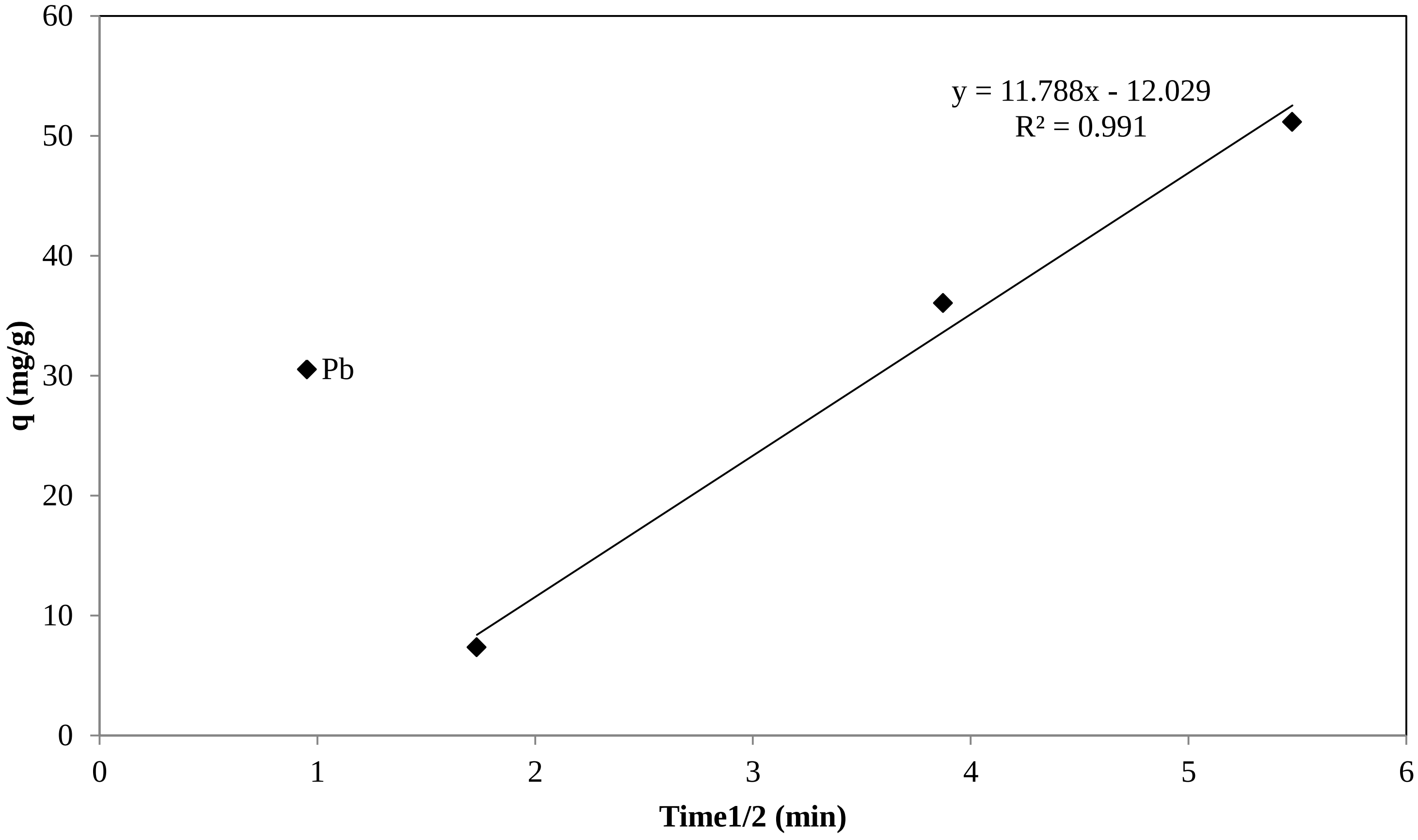


Figure 5-17 Linear portions of the fitted data to the intraparticle diffusion. *C. aculeolata* was exposed to Pb, Cd and Zn at pH 4; *Chara* dose, 2 g/L; temperature, 25±2 ^{o}C; agitation rate, 150 rpm. Data points derive from triplicate experiments.

for Pb tested exhibited an initial curve portion corresponding to the boundary layer sorption, followed by a linear portion (Fig.5-17) indicating the effect of intraparticle diffusion, before the sorption process reached an equilibrium. The results indicated that intraparticle diffusion plays a significant role in Pb sorption through pores of *C. aculeolata* biomass sorbents after the initial adsorption of ions onto the sorbent's surface. The rate constants for intraparticle diffusion were examined to assess the mobility of metal ions through micropores of the biomass. The intraparticle diffusion constants (K_{id}) calculated from slope of the linear portion are shown in Table 5-11. The K_{id} values were higher for Pb sorption by both algal biomass than for Cd and Zn, suggesting that Pb was more easily transported within the biomass matrix. The K_{id} values of dried *C. aculeolata* biomass were higher than those of *N. opaca* for all three metal ions.

5.3.1.5 Biosorption isotherms

The Langmuir isotherm was generated by plotting the biosorptive capacity (q_e) of algal biomass against the residual metal concentration (C_e) at equilibrium (Table 5-12; Figs. 5-18, 5-19). The results showed that the uptake of all three metal ions by both algal biomass initially correlated with the increase in metal concentration in solution until the sorbent capacity eventually became saturated. The maximum adsorption capacity (Q_m) and Langmuir constant (K_L) for each metal and algal biomass are shown in Table 5-12. In addition to the Langmuir model which assumes single-layer coating of metal ions on the sorbent's surface, Table 5-12 also showed parameters that were derived according to the Freundlich model which describes adsorption behavior on heterogeneous surfaces. The results showed that our data for both algal biomass fitted well with the Langmuir model than did the Freundlich model (higher R^2 values, Table 5-12), indicating that the surface of algal biomass exhibited rather homogenous binding property. The maximum adsorption (Q_m) and the biosorption capacity (K_F) of two charophytes are in the order of Pb>Cd>Zn. The experimental q_e values (100.3, 33.1 and 15.6 mg/g Pb, Cd and Zn, respectively in *C. aculeolata* and 80.6, 23.2 and 11.8 mg/g Pb, Cd and Zn, respectively in *N. opaca* obtained in Figs. 5-18 and 5-19 were similar to Q_m (Table 5-12) indicating a good prediction of Q_m values from Langmuir model. Almost all graphs reached saturation at higher equilibrium concentrations, except for Pb uptake by *N. opaca*

Table 5-12 Biosorption isotherm constants for the adsorption of single-metal solutions and multiple-metal solutions by *C. aculeolata* and *N. opaca*.

Langmuir model

Heavy metal	**Single-metal solution**						**Multi-metal solution**					
	C. aculeolata			***N. opaca***			***C. aculeolata***			***N. opaca***		
	Q_m	**K_L**	**R^2**	**Q_m**	**K_L**	**R^2**	**Q_m**	**K_L**	**R^2**	**Q_m**	**K_L**	**R^2**
Pb	106.38	0.0513	0.9814	102.04	0.0309	0.9487	48.31	2.6203	0.9949	53.19	1.7091	0.9814
Cd	36.10	0.0409	0.9787	27.62	0.0529	0.9969	7.79	0.1013	0.9998	9.14	0.3743	1.0000
Zn	15.15	0.1745	0.9645	13.42	0.0804	0.9734	4.05	0.5558	0.9909	1.48	0.2721	0.9837

Freundlich model

Heavy metal	**Single-metal solution**						**Multi-metal solution**					
	C. aculeolata			***N. opaca***			***C. aculeolata***			***N. opaca***		
	n	**K_F**	**R^2**	**n**	**K_F**	**R^2**	**n**	**K_F**	**R^2**	**n**	**K_F**	**R^2**
Pb	3.59	22.05	0.8164	1.78	5.95	0.8303	5.07	24.36	0.9777	3.34	23.39	0.9891
Cd	2.14	3.04	0.9618	1.73	1.89	0.9619	2.40	2.43	0.8880	1.61	1.55	0.8698
Zn	2.00	2.00	0.8745	2.19	1.73	0.9367	1.96	1.51	0.9348	1.32	1.06	0.8596

Q_m, Maximum adsorption capacity (mg/g); K_L, Langmuir constant; K_F, Freundlich constant; n, biosorption intensity.

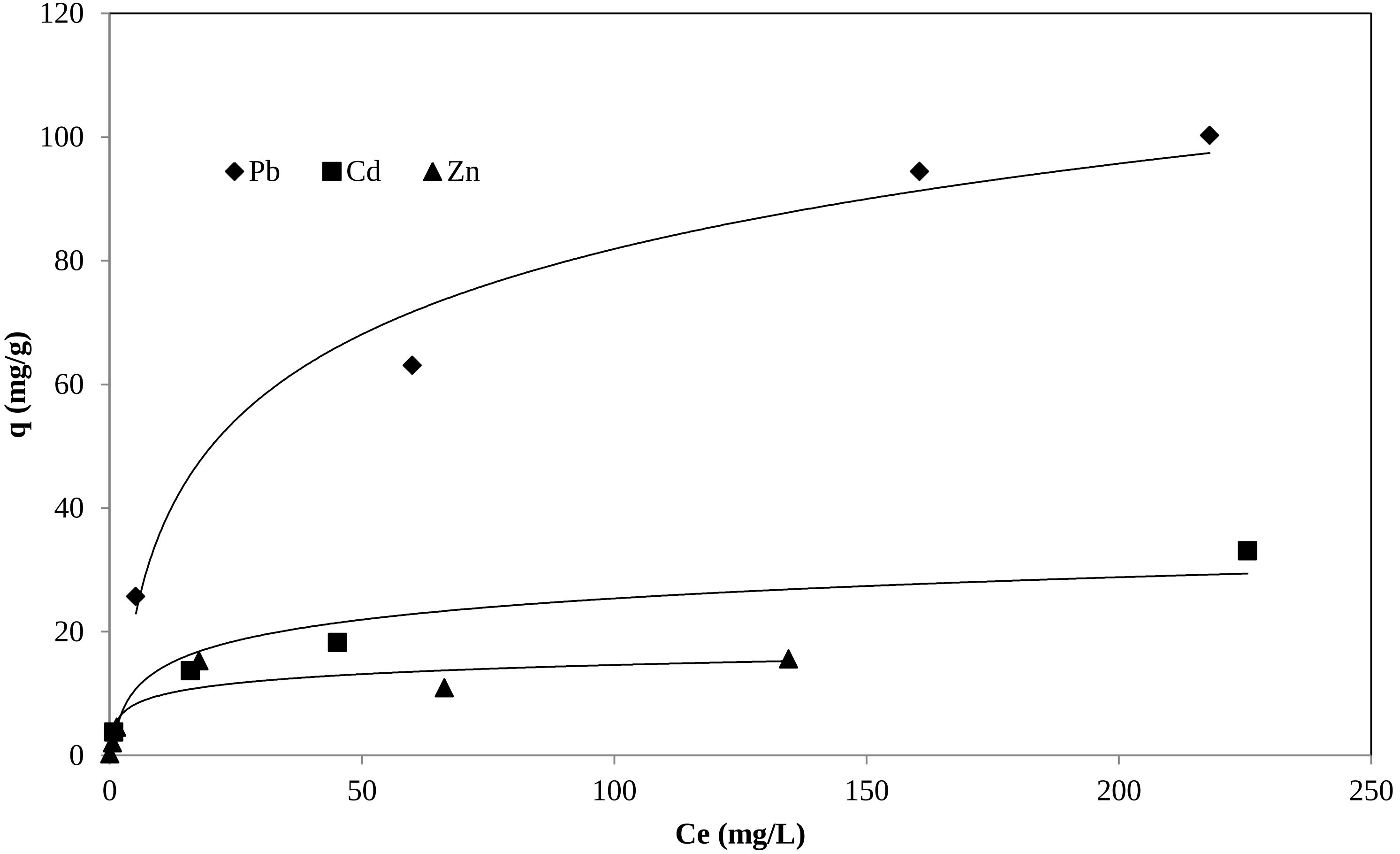


Figure 5-18 Biosorption isotherms for single-metal solutions by using *C. aculeolata* (pH 4; *Chara* dose, 2 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Data points derive from triplicate experiments.

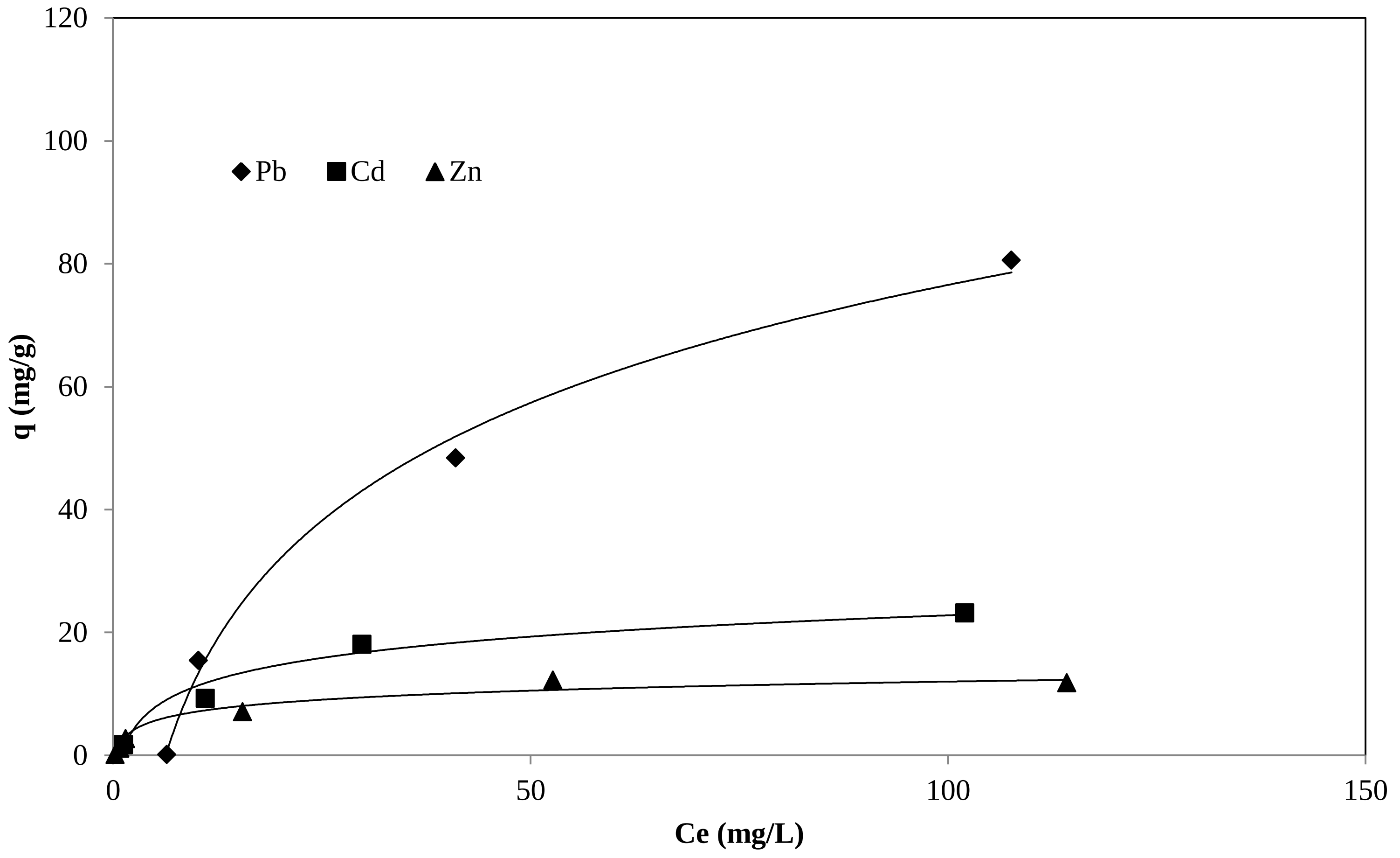


Figure 5-19 Biosorption isotherms for single-metal solutions by using *N. opaca* (pH 4; *Nitella* dose, 3 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Data points derive from triplicate experiments.

biomass. Its q_e value was lower than the Q_m indicating that the biomass was not fully covered by the Pb ions.

5.3.2 Biosorption of metal ions from multi-metal solutions

Figures 5-20 and 5-21 represented the plots of q_e against C_e in multiple-metal biosorption. The plots exhibited concave curves, which did not attain any plateau, indicating the further biosortion potential of both charophytes. The experimental q_e values (47.5 mgPb/g, 7.6 mgCd/g and 6.8 mgZn/g in *C. aculeolata* and 52.9 mgPb/g, 8.3 mgCd/g and 7.0 mgZn/g in *N. opaca*) were decreased in the sorption of the metal mixture, when compared to the single metal solution (Figs. 5-18, 5-19), suggesting the antagonistic effect in the multiple sorption onto algal biomass. Cd uptake was decreased to a maximum of 4.6 times in *C. aculeolata* followed by Zn (3.7 times) and Pb (2.2 times) whereas *N. opaca* reduced Zn adsorbed to a maximum of 9.1 times, followed by Cd (3.0 times) and Pb (1.9 times).

In the metal mixture, the metal adsorption at equilibrium onto *C. aculeolata* and *N. opaca* fitted well to the Langmuir isotherm model (Table 5-12). The results confirmed that the metal sorption process was monolayer coated by Pb, Cd and Zn ions onto algal biomass. In this study, the Langmuir and Freundlich constants (Q_m and K_F values) in the metal mixture indicated the order of metal preference (Pb>Cd>Zn) similar to that in the single-metal solutions.

5.3.3 Application of charophytes to municipal wastewater

The results of heavy metal removal from municipal wastewater by *C. aculeolata* and *N. opaca* are shown in Table 5-13. Pb and Zn ions in synthetic and municipal wastewater were removed effectively by both algae and were detected under the wastewater standard in Thailand (Table 5-13). *C. aculeolata* could remove Cd ions better than *N. opaca* under realistically municipal wastewater with low BOD values. The percentages for removal of Pb, Cd and Zn ions were 81-100% by *C. aculeolata* and 72-100% by *N. opaca*.

5.3.4 Desorption study

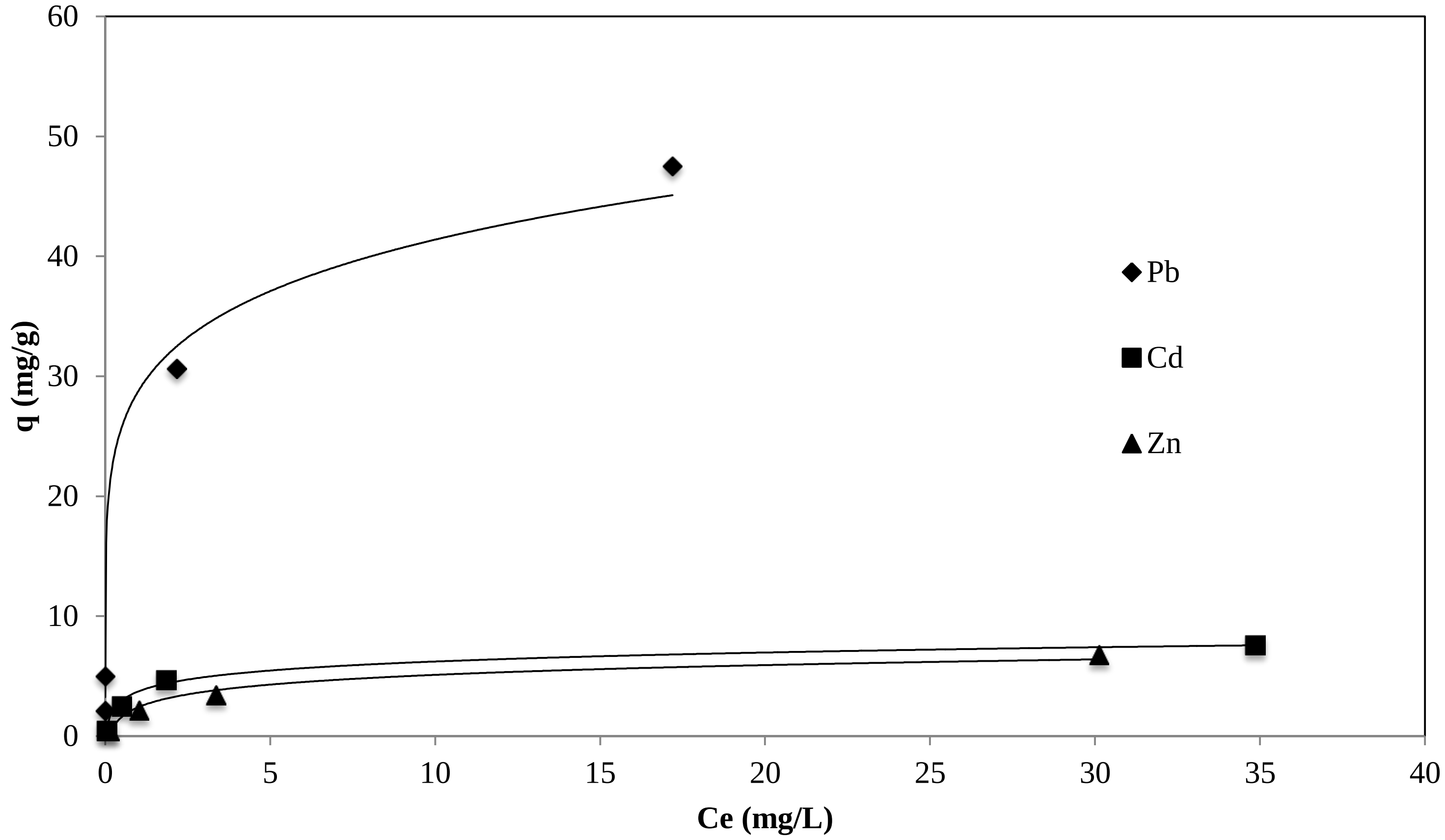


Figure 5-20 Biosorption isotherms for multiple-metal solutions by using *C. aculeolata* (pH 4; *Chara* dose, 2 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Data points derive from triplicate experiments.

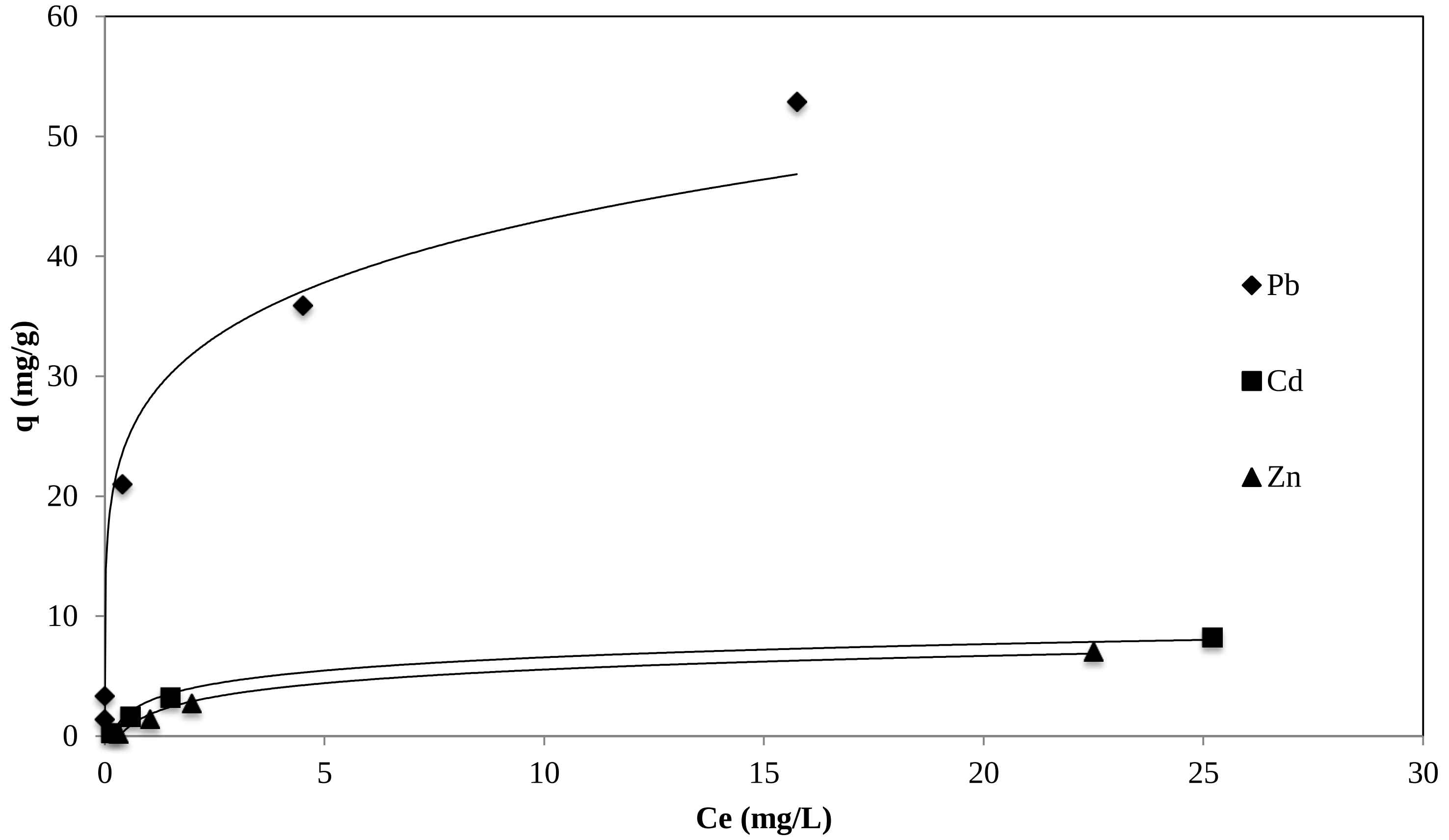


Figure 5-21 Biosorption isotherms for multiple-metal solutions by using *N. opaca* (pH 4; *Nitella* dose, 3 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm). Data points derive from triplicate experiments.

Table 5-13 Removal of heavy metal ions from synthetic mixture and municipal wastewater by *C. aculeolata* and *N. opaca*.

Pollutant	**Synthetic solution**			**Municipal water-1**			**Municipal water-2**			**Permissible limit in Thailand (mg/L)**
	Initial concen-tration (mg/L)	**Final concentration (mg/L)**		**Initial concen-tration (mg/L)**	**Final concentration (mg/L)**		**Initial concen-tration (mg/L)**	**Final concentration (mg/L)**		
		C.aculeolata	***N.opaca***		***C.aculeolata***	***N.opaca***		***C.aculeolata***	***N.opaca***	
Pb	1.143	ND (100)	ND (100)	1.615	ND (100)	ND (100)	1.513	ND (100)	ND (100)	0.20
Cd	0.387	0.038 (90.2)	0.050 (87.1)	0.401	0.072 (82.1)	0.104 (74.0)	0.396	0.072 (82.0)	0.110 (72.1)	0.03
Zn	5.804	0.734 (87.3)	1.086 (81.3)	6.315	1.221 (80.7)	1.167 (81.5)	6.371	1.134 (82.2)	1.147 (82.0)	5.00

ND = Not detectable, Method *detection limit: Pb* = 0.05 mg/L

The numbers in parenthesis are the percentage of metal removal.

Table 5-14 Pb and Cd desorption from *C. aculeolata* and *N. opaca* biomass.

Desorbing agent	Pb desorption		Cd desorption	
	C. aculeolata	*N. opaca*	*C. aculeolata*	*N. opaca*
HCl	61.3 ± 1.8^{ab}	61.5 ± 4.3^{ab}	98.2 ± 2.5^{a}	83.5 ± 4.5^{ab}
HNO_3	60.8 ± 3.8^{ab}	55.5 ± 1.9^{c}	100.0 ± 0^{a}	93.3 ± 2.7^{ab}
$CaCl_2$	10.8 ± 1.0^{e}	20.4 ± 0.2^{e}	67.2 ± 23.3^{bc}	87.7 ± 1.4^{ab}
NaOH	41.5 ± 0.5^{d}	40.8 ± 3.9^{d}	25.6 ± 1.1^{cd}	15.2 ± 0.6^{e}
NTA	65.7 ± 3.2^{a}	45.7 ± 0.1^{d}	44.3 ± 1.6^{cde}	50.3 ± 4.4^{cd}

(initial metal concentration, 10 mg/L; *Chara* dose, 2 g/L; *Nitella* dose, 3 g/L; contact time, 6 h; temperature, 25±2 ^{o}C; agitation rate, 150 rpm; pH 4). Significant differences are indicated as a, b, c, d and e, the homogeneity subsets compared among values from each metal by one way ANOVA, Tukey HSD test ($P\leq0.05$).

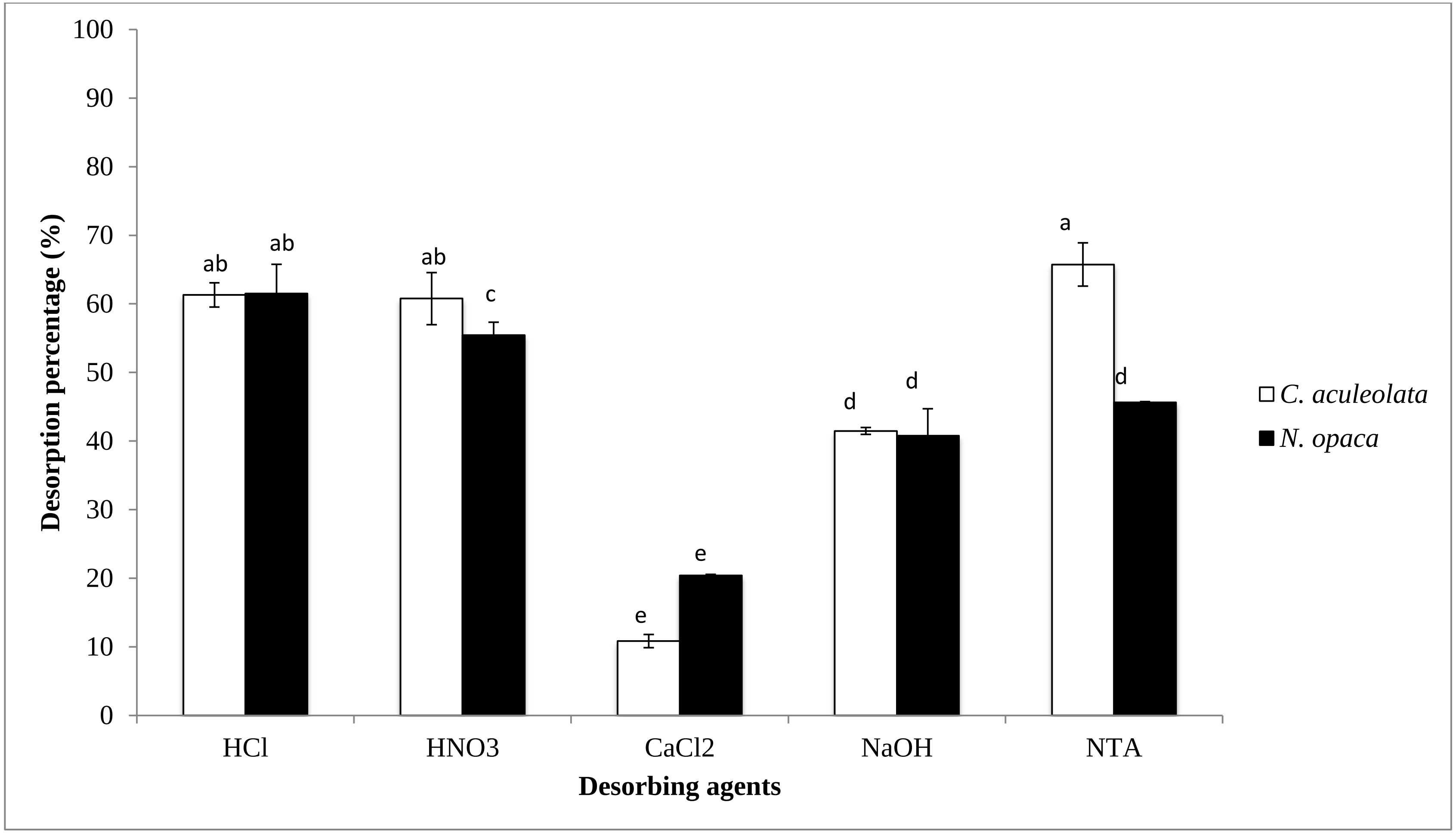


Figure 5-22 Pb desorption from *C. aculeolata* and *N. opaca* biomass. Significant differences are indicated as a, b, c, d and e, the homogeneity subsets by one way ANOVA, Tukey HSD test ($P \leq 0.05$).

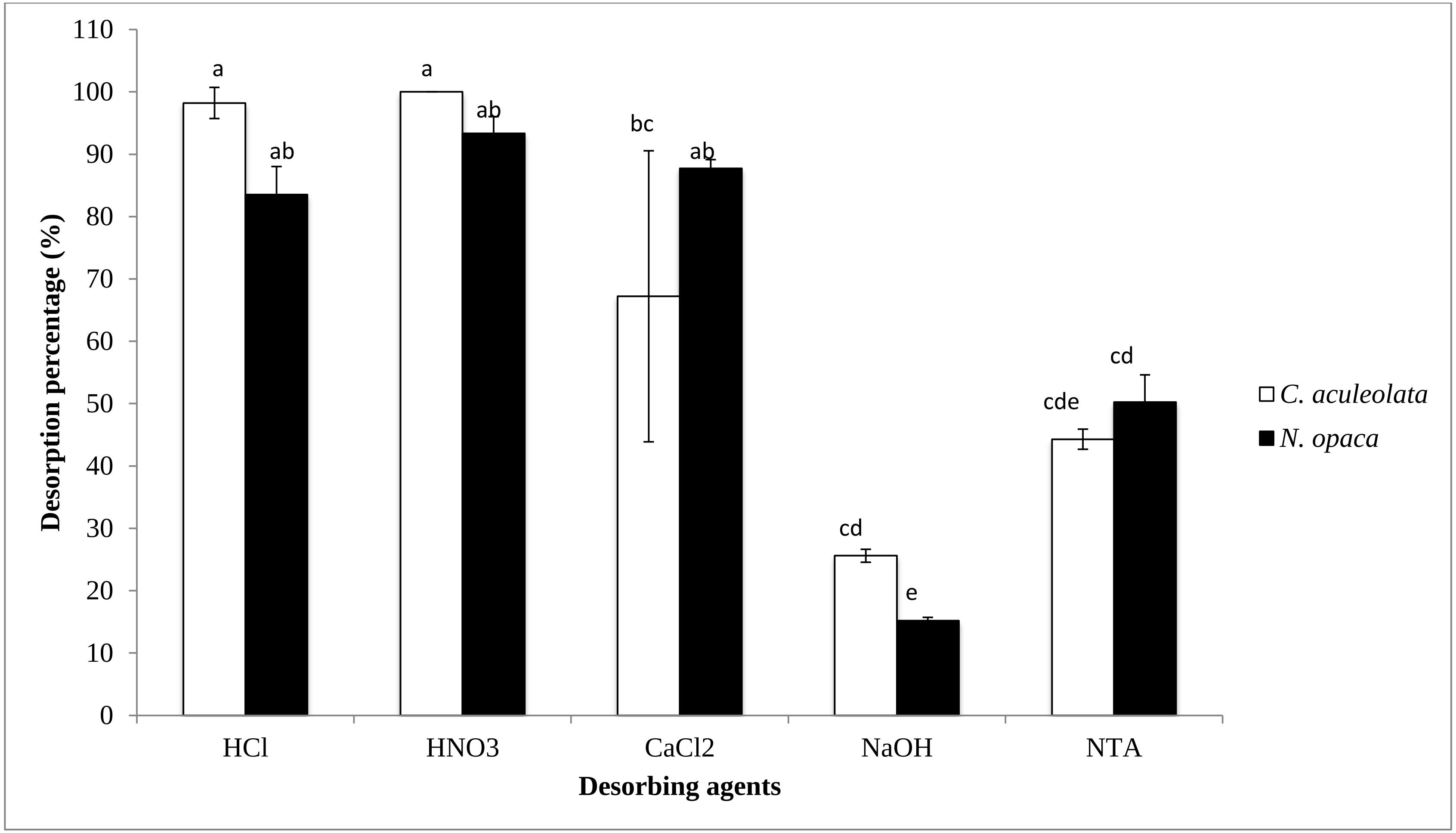


Figure 5-23 Cd desorption from *C. aculeolata* and *N. opaca* biomass. Significant differences are indicated as a, b, c, d and e, the homogeneity subsets by one way ANOVA, Tukey HSD test ($P \leq 0.05$).

Table 5-14; Figs. 5-22 and 5-23 show Pb and Cd desorption from metal-laden *C. aculeolata* and *N. opaca* biomasses. HCl and HNO_3 showed high desorption percentage in both Pb (61.31 and 60.78 %, respectively for *C. aculeolata*, and 61.51 and 55.45 %, respectively for *N. opaca*) and Cd (98.22 and 100 %, respectively for *C. aculeolata*, and 83.54 and 93.32 %, respectively for *N. opaca*) indicating that they are effective agents for elution of Pb and Cd from algal biomass. NTA showed high desorption percentage for elution of Pb from *C. aculeolata* and $CaCl_2$ could elute Cd from both *C. aculeolata* and *N. opaca* biomass with relatively high percentage in comparison to acid. The results of this study indicated that acid (0.1 M HCl and HNO_3) was more effective than Ca salt and base (NaOH and NTA) for elution of Pb and Cd ions from the algal biomass.

5.2 Biosorption of Pb, Cd and Zn in *C. aculeolata* and *N. opaca* in packed column

5.4.1 Pb and Cd breakthrough curves by charophytes

The breakthrough curve of Pb biosorption by *C. aculeolata* and *N. opaca* is shown in Table 5-15; Fig. 5-24. The results showed the ratio of the effluent concentration to initial concentration (C/Co; Table 5-15 and Fig.5-24). According to the breakthrough curve, the breakthrough point is 5 % effluent concentration of influent concentration. The exhaustion point is 70% effluent concentration of influent Pb concentration by *C. aculeolata* and 90% effluent concentration of influent Pb concentration by *N. opaca* (Fig. 5-24) The breakthrough and exhaustion points of Pb sorption by *C. aculeolata* occurred in throughput volumes of 1,750 ml and 3,250 ml, respectively, whereas those by *N. opaca* were obtained at 500 and 2,750 ml, respectively. The effluent concentration ratio of *N. opaca* (C/Co = 0.90) was more than that of *C. aculeolata* (C/Co = 0.70). The phenomenon was dependent on sorption mechanism by different algal biomass. Pb sorption by *N. opaca* was by ion exchange sorption while *C. aculeolata* sorped Pb with the possible chemical sorption (Figs. 5-16, 5-17). At the same bed depth of algal biomass, the breakthrough volume for Pb by

Table 5-15 Pb sorption by *C. aculeolata* and *N. opaca* packed column.

Volume (mL)	Pb sorption	
	C. aculeolata	*N. opaca*
0	0	0
250	0	0
500	0	0.02
750	0	0.09
1000	0	0.23
1250	0	0.36
1500	0.01	0.51
1750	0.04	0.63
2000	0.12	0.70
2250	0.23	0.71
2500	0.33	0.84
2750	0.51	0.93
3000	0.51	0.87
3250	0.63	0.92
3500	0.65	0.91
3750	0.68	0.91
4000	0.65	0.87
4250	0.69	0.90
4500	0.68	0.94
4750	0.63	0.88

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/C_o) (*Chara* bed depth, 3.29 cm at flow rate 40 ml/min ; *Nitella* bed depth, 3.30 cm at flow rate 20 ml/min; Pb concentration, 10 mg/L).

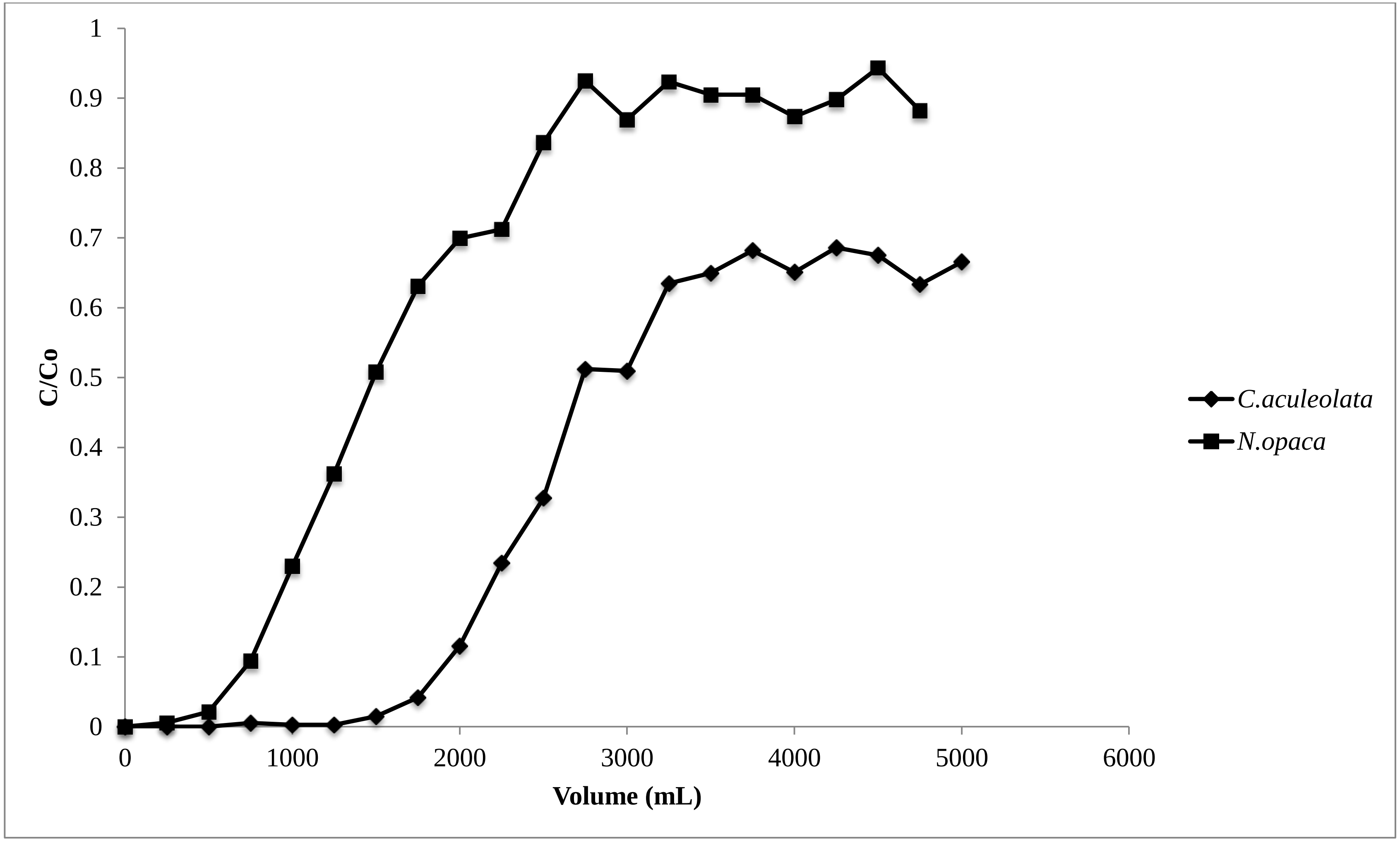


Figure 5-24 Pb sorption by *C. aculeolata* and *N. opaca* packed column.

Table 5-16 Effect of bed depth of packed column on Pb and Cd sorption by *C. aculeolata*.

Volume (mL)	Bed depth			
	Pb (3.29 cm)	Pb (6.49 cm)	Cd (3.29 cm)	Cd (6.49 cm)
0	0	0	0	0
500	0	0.01	0.14	0
1000	0	0.01	0.45	0.01
1500	0.01	0.04	0.69	0.17
2000	0.12	0.13	0.85	0.44
2500	0.33	0.22	0.78	0.66
3000	0.51	0.30	0.74	0.76
3500	0.65	0.30	0.80	0.78
4000	0.65	0.28	0.76	0.83
4500	0.68	0.21	0.81	0.83
5000	0.67	0.20	0.76	0.86

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/Co) (flow rate, 40 ml/min; metal concentration, 10 mg/L).

Table 5-17 Relationship between usage rate and EBCT on Pb and Cd sorption by *C. aculeolata*.

Metal ion	EBCT (min)	Usage rate (g/L)
Pb	0.048	0.29
	0.087	0.33
Cd	0.048	2
	0.087	1

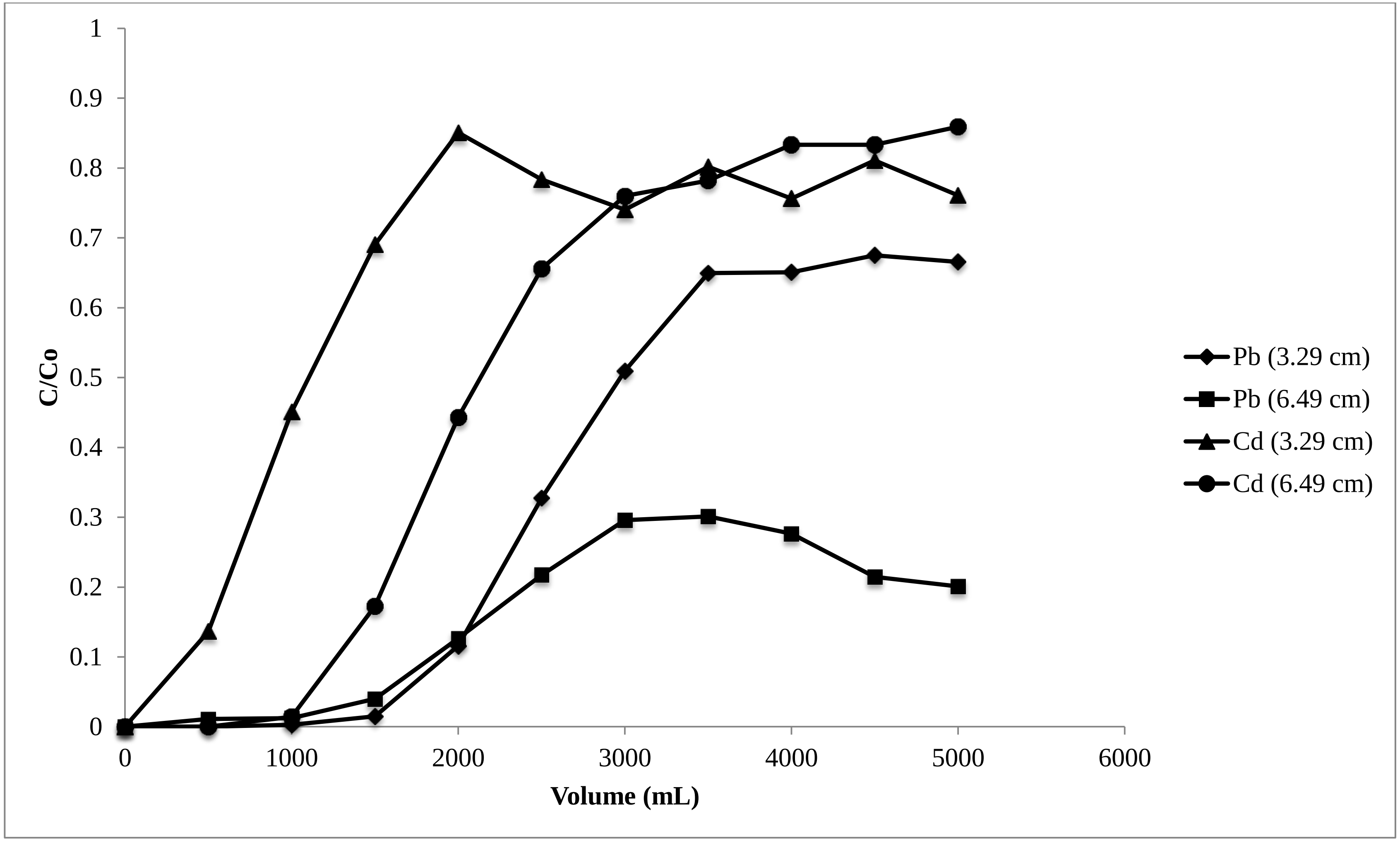


Figure 5-25 Effect of bed depth of packed column on Pb and Cd sorption by *C. aculeolata*.

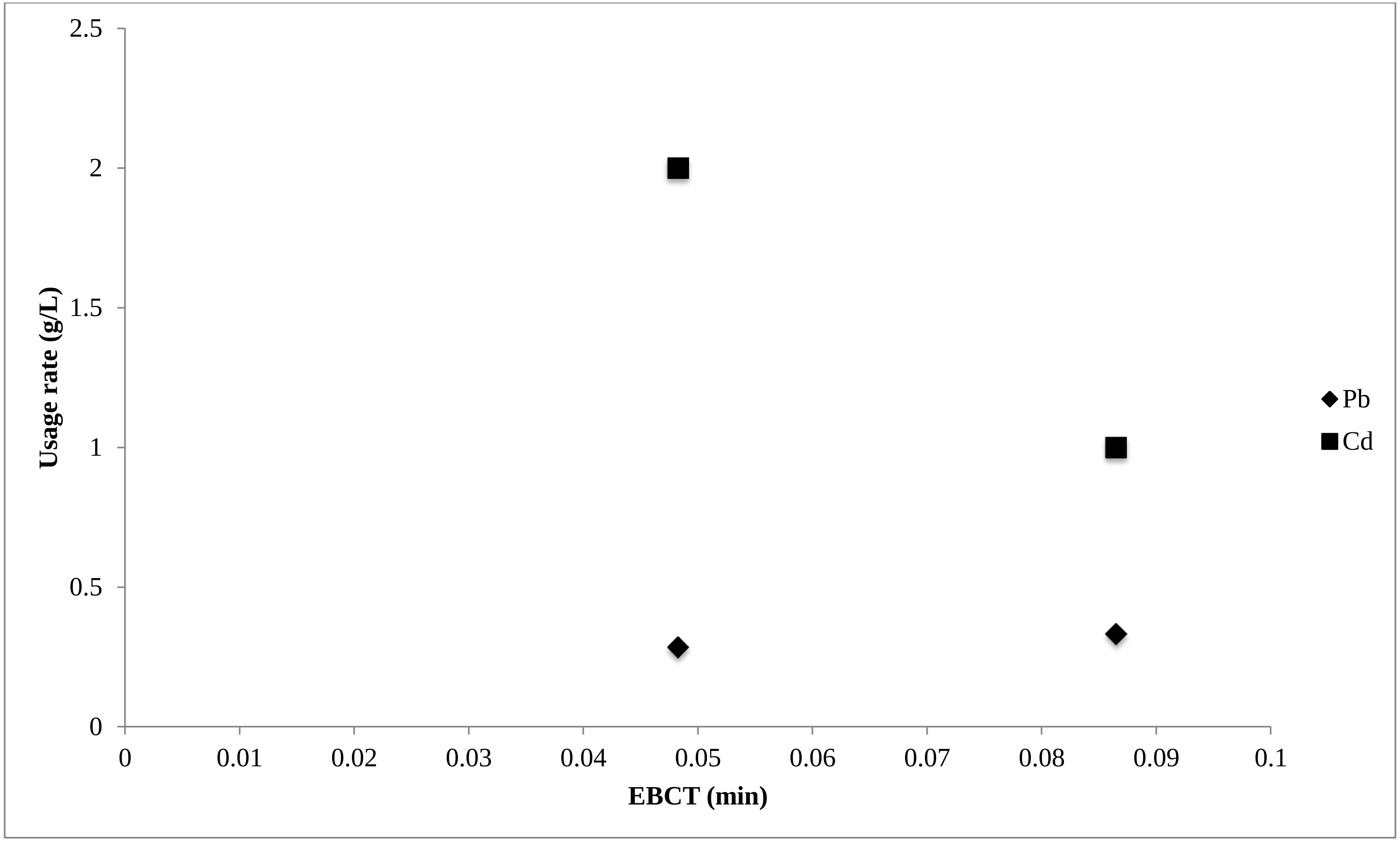


Figure 5-26 Relationship between usage rate and EBCT on Pb and Cd sorption by *C. aculeolata*.

C. aculeolata was 3.5 times more than that by *N. opaca* indicating a good property of *C. aculeolata* as a biosorbent for Pb removal in packed column. In addition, very low bulk density of *N. opaca* (0.13 g/ml) compared with *C. aculeolata* (0.26 g/ml) and cramped property of *N. opaca* when passing metal solutions were observed.

5.4.2 Effects of bed depth and usage rate on Pb and Cd biosorption

The breakthrough curves for biosorption of Pb and Cd by *C. aculeolata* biomass at different bed depths are shown in Table 5-16 and Fig. 5-25. The results showed that the uptake of Cd was increased with an increase in bed depth from 3.29 to 6.49 cm. The breakthrough and exhaustion volume of Cd sorption increased with the increase in bed depth due to an increased amount of binding sites for metal ions. The breakthrough of Pb sorption by the biomass remained relatively constant. However, the bed depth strongly affected the ratio of effluent concentration. 30% of the exhaust effluent concentration of Pb was observed in 6.49-cm packed bed column in comparison with higher exhaust effluent concentration of Pb (70%) by 3.29-cm packed bed column. In the present study, *C. aculeolata* biomass had much higher relative affinity for Pb than for Cd ions.

Table 5-17 and Fig. 5-26 show the data and plot of usage rate against EBCT. As the EBCT increased, the usage rate of Pb by *C. aculeolata* biomass was increased. In practical application of a fixed bed column, a short EBCT has facilitated smaller reaction volume ensuring efficiency and economy (Metcalf and Eddy Inc., 2003). The results indicated that short EBCT for Pb was observed with less usage rate of *C. aculeolata* biomass. Therefore, the usage rate with bed depth of 3.29 cm was used for Pb biosorption in the next experiment. Conversely, the usage rate of Cd by the biomass was decreased when the EBCT increased. Slightly higher EBCT for Cd was found with progressively decreased usage rate. Thus, the usage rate with bed depth of 6.49 cm was chosen for the next Cd biosorption experiment.

5.4.3 Effects of flow rate and BDST on Pb and Cd biosorption

The breakthrough curves for biosorption of Pb and Cd at different flow rates are shown in Table 5-18, 5-19; Figs. 5-27, 5-28. In general, for both metal ions, the breakthrough curves were constant with increasing flow rates. For Pb sorption, the

Table 5-18 Effect of flow rate through packed column on Pb sorption by *C. aculeolata*.

Volume (mL)	Flow rate (ml/min)		
	30	40	60
0	0	0	0
250	0	0	0
500	0	0	0
750	0	0	0
1000	0.01	0	0
1250	0.02	0	0
1500	0.04	0.01	0.02
1750	0.07	0.04	0.05
2000	0.12	0.12	0.11
2250	0.18	0.23	0.20
2500	0.25	0.33	0.34
2750	0.31	0.51	0.41
3000	0.39	0.51	0.53
3250	0.38	0.63	0.61
3500	0.51	0.65	0.64
3750	0.51	0.68	0.71
4000	0.54	0.66	0.73
4250	0.46	0.69	0.70
4500	0.48	0.68	0.74
4750	0.45	0.63	0.69
5000	0.43	0.67	0.73

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/Co) (bed depth, 3.29 cm; Pb concentration, 10 mg/L).

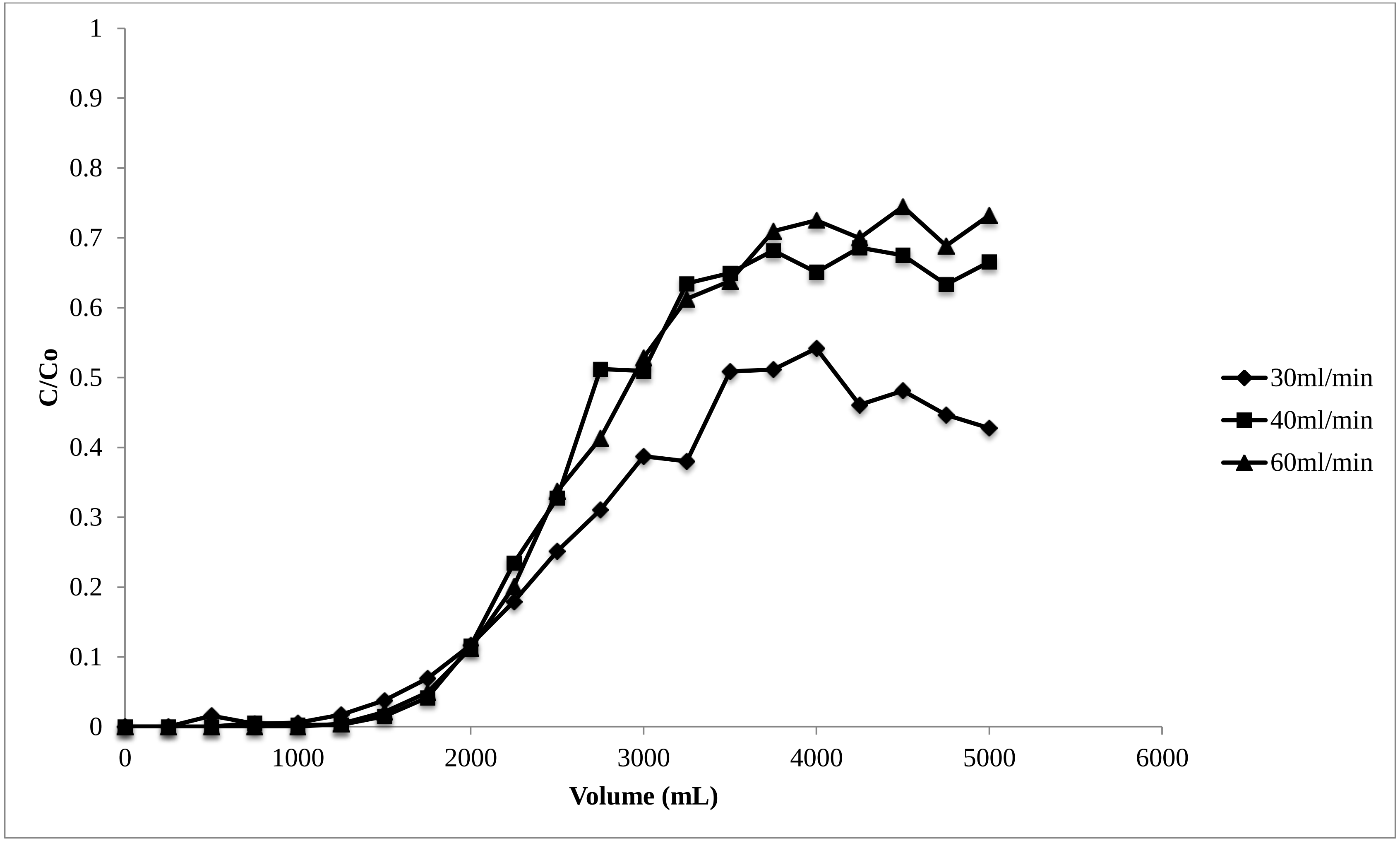


Figure 5-27 Effect of flow rate through packed column on Pb sorption by *C. aculeolata*.

Table 5-19 Effect of flow rate through packed column on Cd sorption by *C. aculeolata*.

Volume (mL)	Flow rate (ml/min)		
	20	30	40
0	0	0	0
500	0	0	0
1000	0.02	0.03	0.01
1500	0.14	0.14	0.17
2000	0.33	0.33	0.44
2500	0.56	0.57	0.66
3000	0.72	0.68	0.76
3500	0.78	0.74	0.78
4000	0.83	0.76	0.83
4500	0.84	0.76	0.83
5000	0.78	0.75	0.86
5500	0.81	0.79	0.85
6000	0.81	0.77	0.83
6500	0.89	0.81	0.84

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/Co) (bed depth, 6.49 cm; Cd concentration, 10 mg/L).

Table 5-20 Sorption capacity of Pb by *C. aculeolata* packed column at different flow rates.

Flow rate (ml/min)	Q (mg/g)	K (L/mg.h)
60	81.46	4.69
40	52.35	2.44
30	109.80	0.54

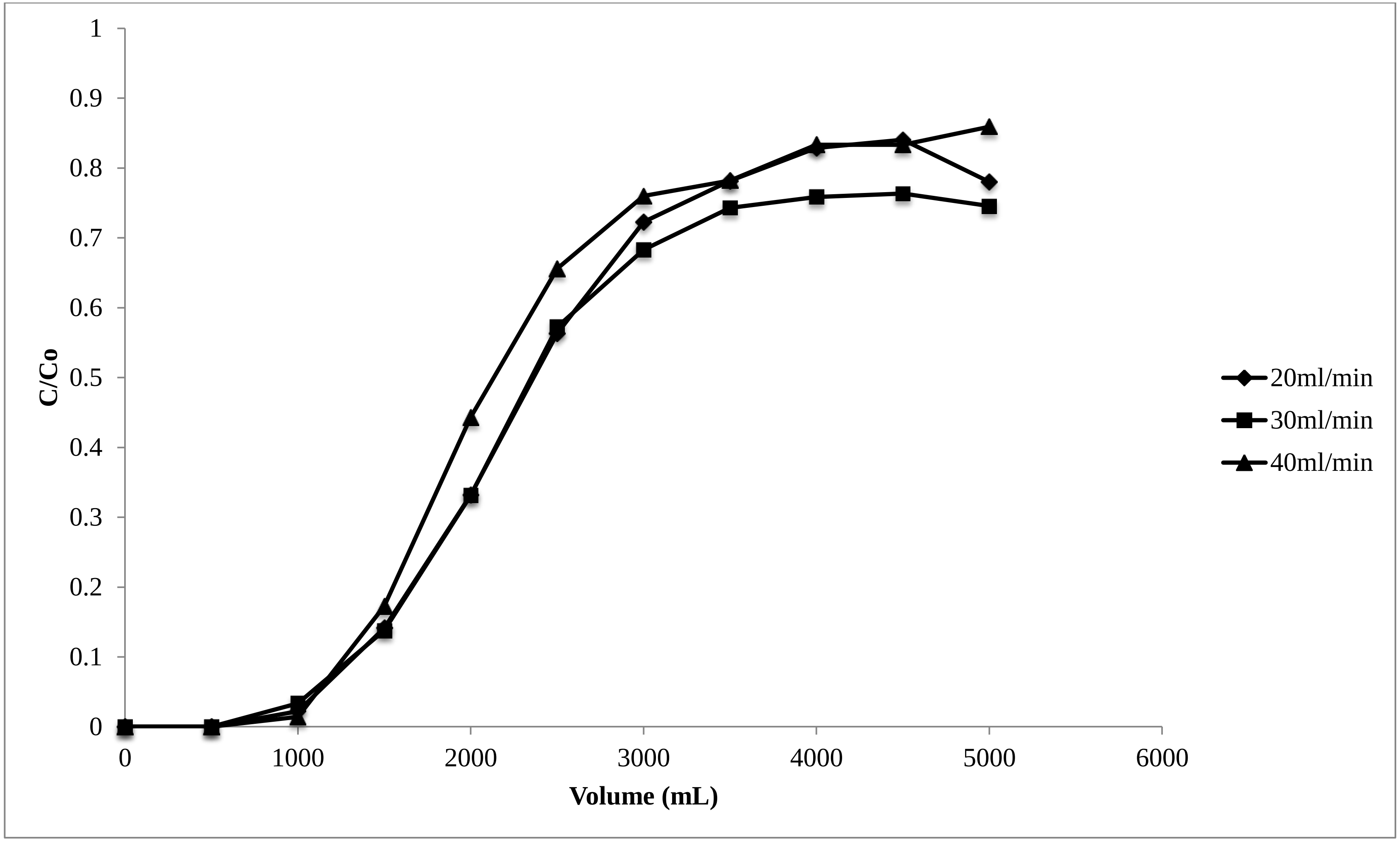


Figure 5-28 Effect of flow rate through packed column on Cd sorption by *C. aculeolata*.

slow flow rate at 30 ml/min decreased the exhaust effluent concentration indicating higher Pb uptake by the biomass (Fig.5-27). The Pb sorption capacity of the bed at different flow rates, which was calculated from the linear relationship between bed depth and BDST, are shown in Table 5-20. The Pb uptake capacity by *C. aculeolata* biomass was slightly different between the flow rates of 40 and 60 ml/min. When the flow rate was decreased to 30 ml/min, the sorption capacity of the biomass was increased as 109.8 mg Pb/g DW. However, the rate constants (K), which characterize the rate of Pb ion transfer from the fluid phase to the solid phase, were increased with the increased flow rate. For Cd biosorption, the flow rate had no effect on the breakthrough curves indicating a rapid reaction between Cd ions and biosorbent with a wide range of flow rates (20-40 ml/min). The sorption capacity and rate constant for Cd ions (38.37 mg/g and 1.66 L/mg.h, respectively) were less than those for Pb ions (51.61 mg/g and 2.42 L/mg.h, respectively) at flow rate of 40 ml/min. The results suggested the rate of Pb transfer to biosorbent was faster than that of Cd.

5.4.4 Effect of Pb and Cd concentration on metal biosorption

The result and breakthrough curves for the effect of Pb or Cd concentration on metal biosorption of *C. aculeolata* biomass are presented in Table 5-21, 5-22; Figs. 5-29 and 5-30. For both Pb and Cd ions at low concentration, the breakthrough volume was increased. The breakthrough volumes of Pb sorption at initial Pb concentration of 5, 10 and 20 mg/L with flow rate of 40 ml/min were 3,250, 1,750 and 750 ml, respectively (Table 5-21; Fig. 5-29) and those for initial Cd concentration of 5, 10 and 20 mg/L with flow rate of 20 ml/min were 2,000, 1,000 and 750 ml, respectively (Table 5-22; Fig. 5-30). All breakthrough volumes of Pb were more than those of Cd. The maximum sorption capacity of Pb and Cd was shown in Table 5-23. The results indicated that when the Pb and Cd concentrations were increased, the maximum sorption capacities for both metal ions increased. At the same initial concentration, the maximum Pb sorption capacity was much more than that of Cd indicating higher capacity of *C. aculeolata* biomass in the packed column for Pb ions.

5.4.5 Effect of binary metal solution on Pb and Cd biosorption

The effect of Cd ion on Pb biosorption was presented in Table 5-24, Fig. 5-31. The breakthrough curves for biosorption of single Pb ion and for the biosorption of mixture (Pb and Cd) were different. Pb breakthrough point of single Pb was 1,750 ml whereas the point for Pb in mixed solution was 1,250 ml. The results indicated that Cd ion had interference to Pb biosorption. Similarly, the presence of Pb had an antagonistic effect on Cd biosorption (Fig.5-31). The Cd breakthrough point of single Cd was 250 ml while the point for Cd in mixed solution was less than 250 ml. The effluent concentration of Cd in the binary solution was higher than its effluent concentration in the single Cd solution. The phenomenon was a result of the Pb ion replacement of some Cd ions in the binding sites and release of Cd ions into the solution.

5.4.6 Reusability

Table 5-25 shows the removal efficiency obtained in three sorption cycles with the original and regenerated biosorbent. After the first cycle, Pb sorption by *C. aculeolata* biomass was reduced with the lowest efficiency of 98.03%, whereas Cd sorption by the biomass remained constant as 100% when the biomass was used in cycle 2 and 3. The Pb desorption time in the third cycle was increased (1.5 h) in comparison to the second cycle (1.3 h). Similarly, the recycling time was also increased from 1.1 to 1.9 h for Pb biosorption. For Cd, the recycling time was reduced from 1.6 h to 1 h in cycle 2 and 3, respectively, while desorption time was increased from 1.2 (cycle 2) to 1.8 h (cycle 3). The results indicated the ability of *C. aculeolata* biomass to be reused as the biosorbent for removal of Pb and Cd ions.

Table 5-21 Effect of Pb concentration on Pb biosorption by *C. aculeolata* packed column.

Volume (mL)	Pb concentration (mg/L)		
	5	10	20
0	0	0	0
250	0	0	0
500	0	0	0.01
750	0	0	0.04
1000	0	0	0.17
1250	0	0	0.42
1500	0	0.01	0.68
1750	0	0.04	0.76
2000	0	0.12	0.81
2250	0	0.23	0.84
2500	0	0.33	0.86
2750	0.01	0.51	0.86
3000	0.02	0.51	0.85
3250	0.03	0.63	0.88
3500	0.05	0.65	0.88
3750	0.06	0.68	0.87
4000	0.08	0.65	0.88
4250	0.13	0.69	0.88
4500	0.13	0.68	0.86
4750	0.18	0.63	0.89
5000	0.19	0.67	0.89

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/Co) (bed depth, 3.29 cm; flow rate; 40 mL/min).

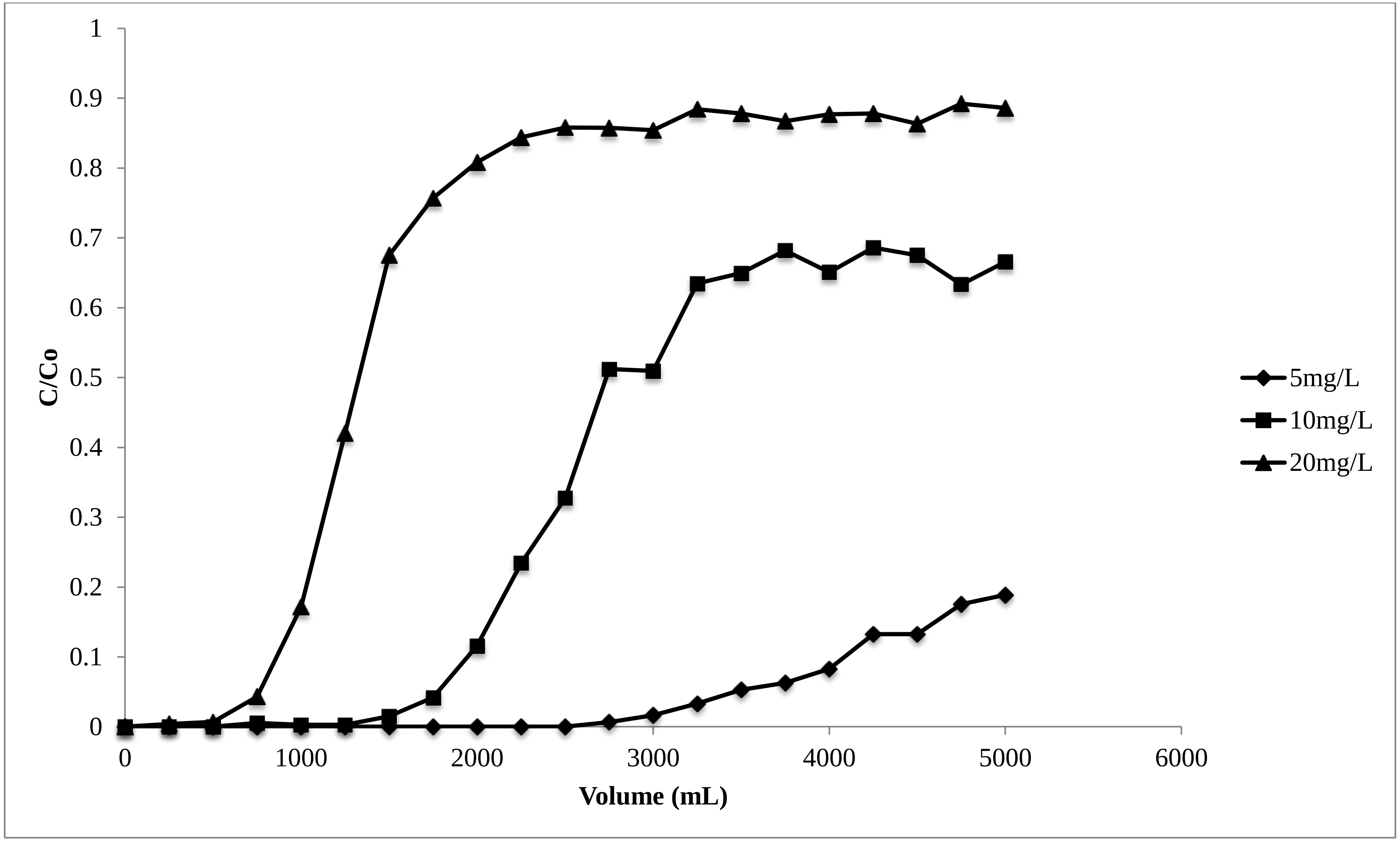


Figure 5-29 Effect of Pb concentration on Pb biosorption by *C. aculeolata* packed column.

Table 5-22 Effect of Cd concentration on Cd biosorption by *C. aculeolata* packed column.

Volume (mL)	Cd concentration (mg/L)		
	5	10	20
0	0	0	0
500	0	0	0
1000	0	0.02	0.23
1500	0	0.14	0.81
2000	0.03	0.33	0.95
2500	0.13	0.56	0.99
3000	0.24	0.72	1.01
3500	0.40	0.78	0.97
4000	0.57	0.83	0.96
4500	0.65	0.84	1.01
5000	0.85	0.78	0.98

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/Co) (bed depth, 6.49 cm; flow rate, 20 mL/min).

Table 5-23 Maximum sorption capacity of Pb and Cd by *C. aculeolata* packed column.

Metal concentration (mg/L)		Qm (mg/g)	R^2
Pb	5	42.99	0.923
	10	105.63	
	20	250.47	
Cd	5	16.33	0.9666
	10	37.85	
	20	79.74	

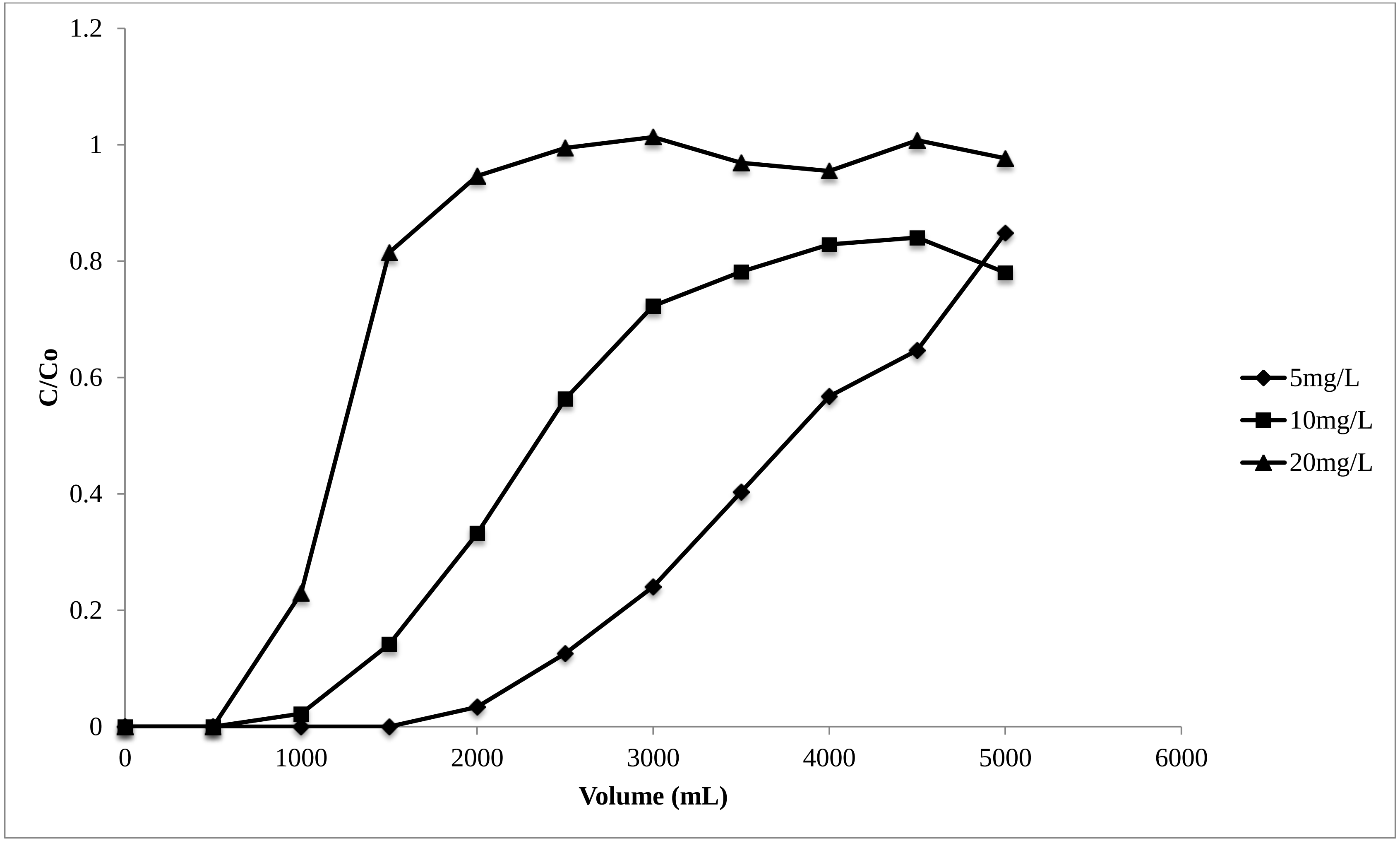


Figure 5-30 Effect of Cd concentration on Cd biosorption by *C. aculeolata* packed column.

Table 5-24 Pb and Cd sorption in binary metal solution by *C. aculeolata* packed column.

Volume (mL)	Heavy metal			
	Pb	Pb (mix)	Cd	Cd (mix)
0	0	0	0	0
250	0	0	0.04	0.07
500	0	0.01	0.14	0.29
750	0	0.01	0.27	0.69
1000	0	0.03	0.45	0.86
1250	0	0.04	0.61	1.06
1500	0.01	0.08	0.69	1.13
1750	0.04	0.14	0.79	1.10
2000	0.12	0.20	0.85	1.06
2250	0.23	0.30	0.79	1.05
2500	0.33	0.43	0.78	1.10
2750	0.51	0.53	0.79	1.04
3000	0.51	0.60	0.74	1.02
3250	0.63	0.65	0.77	1.02
3500	0.65	0.70	0.80	0.98
3750	0.68	0.74	0.75	0.99
4000	0.65	0.78	0.76	0.98
4250	0.69	0.76	0.82	0.92
4500	0.68	0.78	0.81	0.97
4750	0.63	0.78	0.80	0.96
5000	0.67	0.76	0.76	0.95

Result was shown on the ratio of effluent metal concentration and influent metal concentration (C/Co) (bed depth, 3.29 cm; flow rate, 40 mL/min).

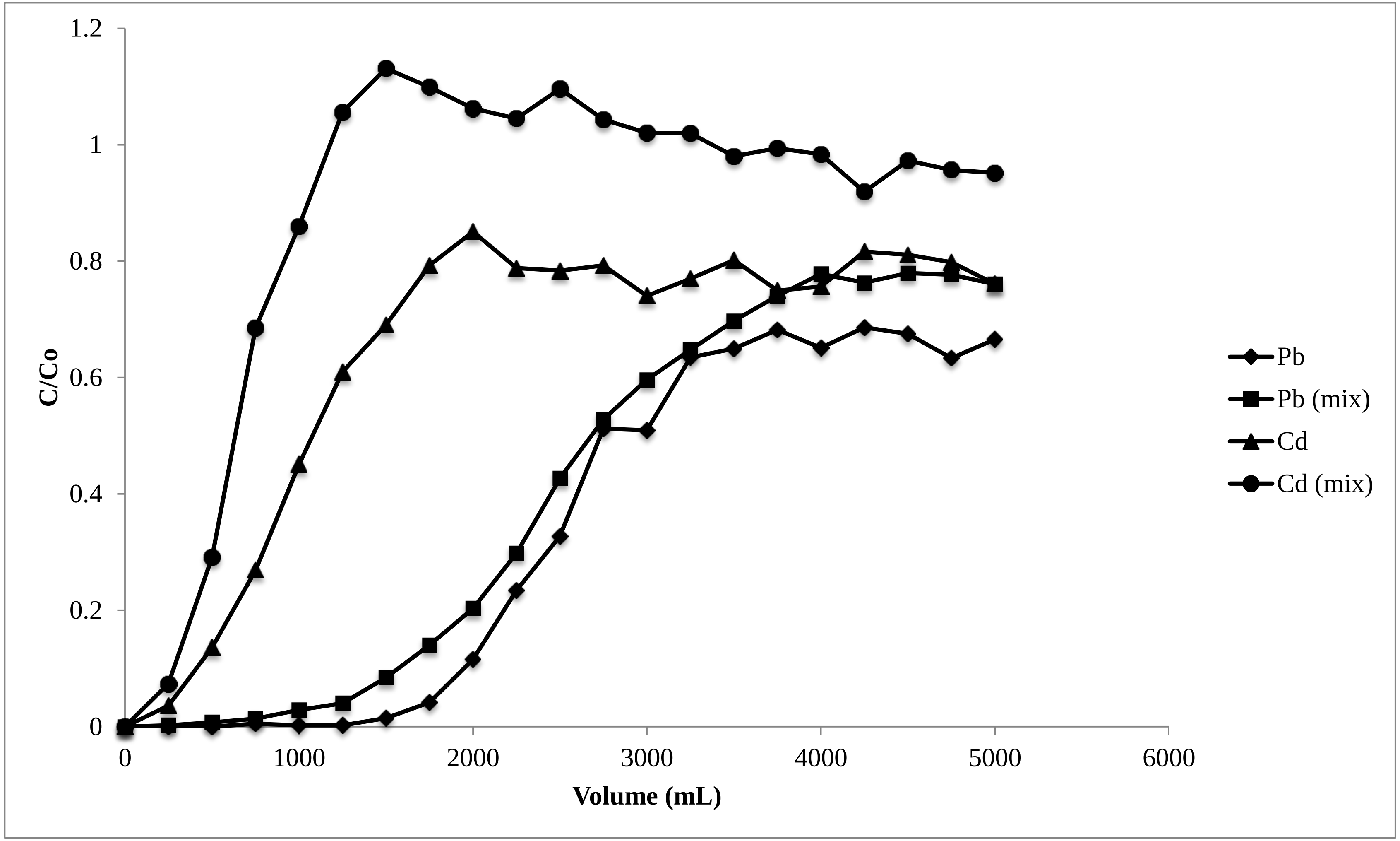


Figure 5-31 Pb and Cd sorption in binary metal solution by *C. aculeolata* packed column.

Table 5-25 Reusability of *C. aculeolata* packed bed column.

Metal	Cycle	Removal efficiency (%)
Pb	1	100
	2	98.51
	3	98.03
Cd	1	100
	2	100
	3	100

CHAPTER VI
DISCUSSION

6.1 Heavy metal toxicity

Both species of charophytes, *C. aculeolata* and *N. opaca* showed different responses to heavy metals in terms of their growth performance, pigment contents and toxicity symptoms. *C. aculeolata* was more tolerant of Cd and Pb than *N. opaca*. However, similar RGRs were observed when the algae were exposed to Zn. The toxicity of metals in term of growth reduction was in the order of Zn<Pb<Cd. Similar trends have been observed by Rai *et al*. (1981) in other species of macroalgae, and by Lamai *et al*. (2005) in *Cladophora fracta*, a freshwater chlorophyte. Both Cd and Pb at high levels cause decreased growth and induce oxidative stress in marine macroalgae (Collén *et al*., 2003) and in the freshwater species *Chara globularis* and *Hydrodictyon reticulatum* (Gao and Yan, 2012). Oxidative stress can induce cytotoxicity and cell death, as reported for tobacco cells by Garnier *et al*. (2006). Although Zn at high concentration (5 mg/L) inhibits growth of several marine macroalgae (Filho *et al*., 1997), *C. aculeolata* and *N. opaca* were not affected, and could be considered as Zn-tolerant. Cellular mechanisms for detoxification and tolerance include binding to cell wall and extracellular exudates, reduction uptake or efflux pumping of metals at the plasma membrane, chelation of metals in cytosol by peptides and compartmentalization of metals in vacuole (Hall, 2002). Charophytes in this study had low Zn accumulation in their tissue, therefore, the detoxification may be due to binding at cell wall or efflux pumping out the cell. Pawlik-Skowronska (2003) has reported on elevated Zn resistance in green alga *Stigeoclonium tenue* isolated from ditch containing mining water in comparison to same species isolated from unpolluted lake water. The ecotypes of algal species might influence their difference in tolerance to metals and therefore other aspects of their metal metabolism, such as accumulation. Both algal species in this study grew in clean freshwater with low BOD values (Table 6-1). *C. aculeolata* found in Bueng Boraphet reservoir required less nutrients than *N.*

Table 6-1 Environmental status of the stations from where *C. aculeolata* and *N. opaca* were collected.

Species	*C. aculeolata*	*N. opaca*
Temperature (^{o}C)	29.5±0.5	28.1±0.1
pH	6.9±0.4	6.9±0.2
DO (mg/L)	5.7±1.4	2.1±1.6
BOD (mg/L)	2.9±0.4	1.9±0.7
Electrical conductivity (µS/cm)	289.8±17.6	423.7±44.0
Alkalinity (mg/L as $CaCO_3$)	103.8±2.0	142.6±14. 7
TDS (g/L)	0.2±0.0	0.2±0.0
NO_3^--N (mg/L)	0.1±0.0	2.9±2.1
NO_2^--N (mg/L)	0.006±0.001	0.002±0.001
NH_4^+-N (mg/L)	0.034±0.014	1.083±1.684
Ortho-phosphate (mg/L)	0.025±0.003	0.571±0.184

Results are means ± SD of 3--4 stations.

opaca (Table 6-1). Therefore, in nutrient-limiting medium contaminated with heavy metals, *C. aculeolata* survived better than *N. opaca*.

Metal tolerance may be assessed by the degree of toxicity symptoms such as reduction in photosynthetic pigments (chlorophyll and carotenoids) leading to chlorosis, and by softening and detachment of algal cells. *C. aculeolata* and *N. opaca* exposed to Cd, Pb, Zn for 6 days showed significant decreases in chlorophyll *a*, *b*, total chlorophyll and carotenoid contents with increases in metal accumulations. The decrease in chlorophyll concentration was possibly due to increased chlorophyllase activity, disorganization of chloroplast membranes and thylakoids, and inactivation of electron transport of PSII (Boonyapookana *et al*., 2002). As in the present study, both Cd and Pb have been reported to lower chlorophyll contents of *C. fracta* and chlorophyll fluorescence in several marine macroalgae (Lamai *et al*., 2005; Baumann *et al*., 2009). Cd and Pb do also cause the disintegration and disorganization of thylakoid membranes and chloroplast membranes in photosynthetic organisms such as plants, cyanobacteria, micro- and macroalgae (Visviki and Rachlin, 1994;

Rangsayatorn *et al*., 2002). No decline in chlorophyll contents was observed in charophytes treated with Zn, except for a carotenoid reduction in *N. opaca* exposed to high Zn concentration in this study. Baumann *et al*. (2009) have reported that high Zn concentration does affect the PSII of several marine macroalgae.

6.2 Heavy metal accumulation

Macroalgae with good phytoremediation potential should display an ability to accumulate heavy metals at high concentration, a fast metal removal rate, and also high BCF (bioconcentration factor) values. More studies have been focused on marine macroalgae due to their identification as easily available, abundant and effective biosorbent biomass (Kuyucak and Volesky, 1990). Recent studies on the metal bioaccumulation in freshwater macroalgae such as *Chara*, *Nitella*, *Cladophora*, *Enteromorpha* and *Microspora* have revealed the higher accumulation of metals in *Cladophora* and *Chara* (Axtell *et al*., 2003; Lamai *et al*., 2005; Gomes and Asaeda, 2009; Bibi *et al*., 2010; Al-Homaidan *et al*., 2011; Gao and Yan, 2012). Different species of macroalgae have been found to have varying metal accumulation capacity and removal rates. Our study revealed that *C. aculeolata* and *N. opaca* possessed the potential to accumulate heavy metals in their thalli with bioaccumulation increasing with increase in external concentration in the order of Pb>Zn>Cd. *N. opaca* and *C. aculeolata* accumulated higher concentration of these metals at all concentrations tested, i.e., 21,657 µg/g for Pb (at 10 mg/L), 6467 µg/g for Zn (at 10 mg/L), and 1544 µg/g for Cd (at 0.5 mg/L). These were much higher than those reported for freshwater charophytes in other studies: 2540 µg/g Zn at 1 mg/L after 35 days for *Nitella graciliformis* (Bibi *et al*., 2010), 3650 µg/g Pb at 160 mg/L after 15 days for *Chara globularis*, and 1660 µg/g Pb at 1.61 mg/L after 15 days for *Chara corallina* (Gao and Yan, 2012). Harvesting habitat, experimental conditions, and major nutrients used may have affected patterns of metal accumulation in these members of the same family (Charophyceae) in addition to differences among species (Kinkade and Erdman, 1975; Lee and Wang, 2001). The high concentrations of metals accumulated by charophytes in this study were by far the highest concentrations encountered.

A BCF value of > 1000 can be used to indicate hyperaccumulating capacity (Boonyapookana *et al*., 2002). Based on the BCF values, *C. aculeolata* and *N. opaca* could be considered as Cd hyperaccumulator (BCF 3000-8000), and moderate Pb accumulators (1300-3300). In comparison, *C. fracta* shows BCF values of 1190-1230 and 1200 for Pb and Cd, respectively (Lamai *et al*., 2005). As in other studies (Wang and Dei, 1999; Lamai *et al*., 2005), BCF decreased with increasing metal concentrations, probably due to growth reduction induced by higher metal concentration. The accumulation potential of *C. aculeolata* and *N. opaca* based on the BCF values was in the order of Cd>Pb>Zn.

Different aquatic plants and macroalgae display varying metal removal rates. The present results revealed that the metal removal was up to 100% for Cd, 96.8% for Pb, and 17.7% for Zn in *C. aculeolata* at low metal concentration and 3 days of exposure. In *N. opaca*, they were 60.7%, 93.2%, and 0%, respectively. *C. aculeolata* was a Cd and Pb remover while *N. opaca* was only a Pb remover and neither species was a Zn remover. In comparison, another macroalga, *Microspora* shows a total removal rate of over 95% for Pb after 10 days of exposure (Axtell *et al*., 2003). *Salvinia minima* (aquatic fern) and *Spirodela punctata* (aquatic plant) removed 70%-90% of Pb and Zn in 2 days of exposure to concentrations of 1-8 mg/L (Srivastav *et al*., 1993). High percentage of Cd and Pb removal in this study was probably the result of high metal adsorption at outer cells and high uptake into intracellular cytoplasm.

In aquatic ecosystem, many metal-bearing waste streams contain substances such as organic matter, alkaline earth metals, and dissolved organic carbon (DOC) that may decrease the removal capability of the metal ions by algae (Brauckmann, 1990; Eilbeck and Mattock, 1987). DOC (such as humic acid, fulvic acid) stays dissolved in aquatic system or in soil solution under natural conditions (Harter and Naidu, 1995). DOC reduces metal adsorption onto soil surfaces or algal cell surface by competing more effectively for the free metal ion and forming soluble organo-metallic complexes (Guisquiani *et al*., 1998). Macroalgae such as charophytes are usually used in the secondary wastewater treatment for removal of residual free metal ions (Stottmeister *et al*., 2006). Hence, the practical removal of metals by these algae requires additional analysis of degree of DOC that affects metal removal since the concentration of DOC varies with season, type of water, and chemical and biological processes such as microbial decomposition (Karlik and Szpakowska, 2001).

6.3Biosorption in batch system

In this study, two species of charophytes, *C. aculeolata* and *N. opaca* were compared for their biosorptive capacity. The effects of operation conditions in batch study such as pH, algal dosage and contact time were determined. pH affects the solubility and the availability of heavy metal ions in the aqueous phase (Vilar *et al*., 2005; Ho, 2005). Biomass dosage generally increases overall surface area of the biosorbent, which in turn increases the number of metal binding sites (Waranusantigul *et al*., 2003). The ideal biosorbents are able to rapidly adsorb high concentrations of heavy metals from wastewater. The results of the present study showed that heavy metal removal process was influenced significantly by variation of pH. Both *C. aculeolata* and *N. opaca* exhibited the maximum sorption percentage of all three metal ions (Pb, Cd, Zn) at pH 4. As pH increased to more than 4, Pb biosorption capacity decreased dramatically while Cd and Zn biosorption had wider range of optimal pH (4-6). The removal efficiency for Pb was dramatically reduced due to precipitation of the metal in the solution (Morris *et al*., 1997). The functional groups on the cell wall of the biomass and its ionic state at these pHs determine the extent of biosorption (Crist *et al*., 1981; Davis *et al*., 2003; Pérez *et al*., 2008). Microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulfate, phosphate and amino groups (Ahalya *et al*., 2003). In the present study, the increase in biosorption efficiency was due to the increase in availability of metal binding sites resulting from an increase in algal dosage, which is in agreement with Waranusantigul *et al*. (2003) and Namasivayam and Ranganathan (1995). The maximal biosorptions of the metal ions by *C. aculeolata* and *N. opaca* were attained at algal dosage of 2 g/L and 3 g/L, respectively. Low algal dosage (2-3 g/L) of these charophytes is a promise for the application of a large volume of wastewater treatment in comparison with *Ceramium virgatum* (10 g/L) and seed husk waste (20 g/L) (Lawal *et al*., 2010; Sari and Tuzen, 2008). The equilibrium times of *N. opaca* for the three metals were much faster than those of *C. aculeolata* and other species of green, brown and red seaweeds (Gupta and Rastogi, 2008; Sari and Tuzen, 2008; Sheng *et al*., 2004; Vijayaraghavan *et al*., 2009). The very fast sorption observed with *N. opaca* represents an advantageous aspect when water treatment systems are designed.

The predominant scientific basis for sorbent selection is the equilibrium isotherm and the adsorption rate is generally secondary in importance. In the present study, the actual fit to the experimental data by pseudo-first-order and pseudo-second-order models indicated that the sorption of Cd and Zn by *C. aculeolata* and *N. opaca* was the ion-exchange adsorption (Lee *et al*., 2007). *C. aculeolata* might perform chemical adsorption with Pb ions, which was taken place by a sequence of steps. The rate-determining step of chemical adsorption is needed to be monitored to see how the system is affected by other factors (e.g. metal concentration, sorbent size, sorption system). In this study, the adsorption capacity of metals (Pb, Cd, Zn) of *C. aculeolata* was higher than that of *N. opaca*. The biosorption capacity of these algae was fairly high in comparison with other green freshwater macroalgae, e.g. *Spirogyra hyalina* (31.3 mgPb/g and 18.2 mgCd/g), *Cladophora rivularis* (48.1 mgPb/g) and *C. fracta* (0.3 mgCd/g and 2.5 mgZn/g) (Jafari and Senobari, 2012; Ji *et al*., 2012; Kumar and Oommen, 2012). In comparison to single-metal solution, the maximum sorption capacity (Q_m) in this study was compared to other sorbents (Table 6-2). A clear reduction of biosorption capacity of charophytes in multi-metal system was revealed in agreement with other organisms, e.g. some fungi, plants and other macroalgae. Under various sorption conditions, agrowastes such as maize cob and black gram husk seem to need more biomass for maximizing adsorption. *Sargassum* sp. has been reported as a well-established biosorbent for a variety of metal ions in the single-metal solutions (Vijayaraghavan *et al*., 2009). In this study, *C. aculeolata* and *N. opaca* are promising in metal biosorption from the mixture of metal solutions and showed a great potential as adsorbents especially for Pb and Cd ions.

6.4Biosorption of charophytes in wastewater

Municipal wastewater samples had very low DO but high electrical conductivity and TDS. They contained a mixture with high level of Na, K, Ca, Cl and

Table 6-2 The maximum sorption capacity (mg/g), Q_m, of various sorbents.

Sorbent	**Biomass (g/L)**	**Single-metal solution**			**Multi-metal solution**			**Other components**	**Reference**
		Pb	**Cd**	**Zn**	**Pb**	**Cd**	**Zn**		
Maize cob and husk	20	465.7	493.7	495.5	-	-	-	-	(Igwe *et al*., 2005)
Sargassum sp.	3	214.0	-	24.2	44.2	-	7.3	Cu, Mn	(Vijayaraghavan *et al*., 2009)
C. aculeolata	2	106.38	36.10	15.15	48.31	7.79	4.05	-	This study
N. opaca	3	102.04	27.62	13.42	53.19	9.14	1.48	-	This study
Black gram husk	10	49.97	39.99	33.81	-	-	-	-	(Saeed *et al*., 2005)
P. simplicissimum	1	30.37	21.50	25.54	13.38	6.94	18.72	-	(Fan *et al*., 2008)
Humid *Tremella fuciformis* (jelly fungi)	48	24.40	22.76	20.13	24.68	10.04	13.58	-	(Pan *et al*., 2010)
Mangifera indica L (mango)	5	24.40	-	18.93	19.29	-	15.09	-	(Ashraf *et al*., 2010)
Auricularia polytricha (jelly fungi)	4	21.19	21.83	14.16	10.27	2.37	3.18	-	(Pan *et al*., 2010)
Sphagnum moss	1.67	18.6	-	9.46	13.7	-	1.28	Cu, Ni	(Zhang and Banks, 2005)
Activated carbon derived from bagasse	6	-	38.03	31.11	-	29.77	19.02	Cu	(Mohan and Singh, 2002)

SO_4 (Table 4-1). Municipal water-1 contained higher levels of Na and Cl than those of municipal water-2. The metal removal efficiency of *C. aculeolata* and *N. opaca* was higher in municipal water-2 (82-100%) than that in municipal water-1 (80.7-100%). Biosorption activity was interfered by light metals, e.g. K, Na, Ca and Mg. They reduce mobility of heavy metals, compete for binding sites with the metal ions under study, suppress the metal ion uptake by electrostatic competition and significantly reduce metal uptake (Murithi *et al.*, 2012; Vilar *et al.*, 2005). In this study, the Pb and Zn contents were removed below the allowable limit. However, Cd content in the effluent was affected by variety of ions in wastewater. The presence of anions, such as Cl, NO_3 and SO_4, can lead to two incidents: an enhancement of sorption or a reduction of sorption, depending on the formation of complexes with higher or lower affinity for the sorbent than the free metal ions (Volesky and Schiewer, 1999). In many studies, Cl ion has been reported for the reduction effect on metal biosorption (Deng *et al.*, 2009; Diniz and Volesky, 2005). In the present study, *C. aculeolata* and *N. opaca* effectively adsorbed Pb and Zn ions in municipal wastewater. *C. aculeolata* could remove Cd ions in a comparable level with *C. fascicularis*, which reduced the levels of Cd under the discharge standard of Cd in wastewater in China (Deng *et al.*, 2007). The permissible levels of heavy metals in wastewater are varied with their toxic levels, sources of discharged wastewater and the setting values among various countries (EPA, 2003; Homchean, 1972).

6.5Biosorption of charophytes in packed column

Many parameters such as particle porosity, surface area, bulk density and swelling degree are very important in the modeling of breakthrough curves (Sulaymon *et al.*, 2013). In the present study, *C. aculeolata* had a higher bulk density than *N. opaca* and had a good property for metal sorption in the fixed bed column. Types and quantity of functional groups detected on the surface of different biomass affect sorption mechanisms by varying the structural properties of the biosorbent. The functional groups are the potential sites for sorption and the uptake of metal depends on various factors such as abundance of sites, their accessibility, chemical state, and affinity between the sorption site and metal ion. In algae, hydroxyl and carboxyl groups are very effective for capturing the heavy metal ions due to their negative charges (Sheng *et al*., 2004). The functional groups presented in protein, polysaccharide, fatty acid including P-O-C links of organic phosphate groups play important roles in metal sorption by the algal biomass (Jafari and Senobari, 2012; Sulaymon *et al*., 2013).

Column parameters, bed depth and flow rate, affected the breakthrough curve of biosorption in this study. Biosorption kinetics of biosorbent indicate the biosorption mechanism and the characteristic properties such as biosorption reaction and porosity in biomass. The reaction of metal biosorption in *C. aculeolata* may be complexation and/or precipitation of Pb and Cd with active sites in the biomass. Pb may precipitate in the biomass. In the presence of phosphate and chloride, Pb solubility is limited with low concentration of Pb ions and the partition coefficient for Pb may reflect precipitation reaction rather than adsorption reaction (EPA, 2005). Anionic substances such as phosphate, chloride, and carbonate are known to influence Pb reactions either by precipitation of minerals of limited solubility or by reducing adsorption through complex formation (EPA, 2005). In this study, increase of bed depth with slow flow rate increased Pb uptake by *C. aculeolata* biomass. The increasing equilibrium solution concentration relates to decreasing Pb adsorption and tends to form metal ion complexation. Zachara *et al*. (1992) reported that Cd forms monocarbonate complexes in low concentrations of CO_3. Based on $CdCO_3$ solubility, the formation of $Cd(CO_3)_3^{4-}$ and the increase of $CdCO_3$ solubility with the increase in CO_3 concentration were found. Since *C. aculeolata* biomass contains abundant CO_3, Cd sorption by the biomass might have aqueous Cd^{2+}-CO_3^{2-} with strong interactions that could be eluted reversibly by desorbing agents.

In the present study, Pb and Cd sorption by *C. aculeolata* biomass could be eluted from metal-load biomass by desorbing agents. The results suggested that pH of desorbing agents might affect the elution of both Pb and Cd from the algal biomass. The application of the biodegraded-chelating agent, NTA, is limited by its solubility in NaOH solution with the high pH. Lower pH, which increases metal solubility, increased desorption percentage of both metal ions. In high pH solution, the decrease in metal desorption efficiency is due to the formation of insoluble hydroxylated complexes of the metal ions. Pb(II) is present as $Pb(OH)_2$ at pH above 6 (Morris *et al*., 1997). Similarly, beyond pH 9, Cd(II) precipitates as $Cd(OH)_2$ (Reed and Matsumoto, 1993). The efficient removal of sorbed metals from *C. aculeolata* biomass is important to determine its long-term use for repeated extraction-elution cycles. In this study, NTA was attractive for practical application due to the biodegradable property while HCl was used as desorbing agent due to its ability of high desorption percentage. The limitation of acid is corrosion and could destroy the structure of binding sites for heavy metals (Puranik and Paknikar, 1999). For regeneration and reuse of biosorbent in column, which offers an economical method for removal of heavy metals from wastewater stream, require the effective and environmental friendly desorbing agent.

CHAPTER VII

CONCLUSIONS

Both species of charophytes, *C. aculeolata* and *N. opaca* showed a preference for bioaccumulation in the order of Cd>Pb>Zn. While *C. aculeolata* removed both Cd and Pb at relatively similar rates, *N. opaca* showed a preference for Pb removal. The primary limitation on this bioaccumulation and removal potential was the lethal dosages apparent at 0.5 mg/L Cd and 10 mg/L Pb in both species. *N. opaca* was more sensitive to low dose of Cd and Pb.

Phytoremediation, the use of tolerant macroalgae that accumulate metals at high rates may offer an effective, inexpensive and environmental friendly option of heavy metal remediation. *C. aculeolata* should be a better choice than *N. opaca* due to its tolerance to many metals, and ideally should be used to treat large volume of wastewater with low concentration of metals such as the treatment of wastewater in constructed wetland.

The dried biomass of *C. aculeolata* and *N. opaca* can be used as biosorbent for removal of heavy metals from wastewater. Both algal biomass showed a preference for the biosorption in the order of Pb>Cd>Zn. The maximum removal efficiency by *C. aculeolata* required a lower dosage of 2 g/L in comparison to that of *N. opaca* (3 g/L). The limitation of this removal potential was the narrow range of pH condition for Pb biosorption but with a broader range for Cd and Zn. The kinetics data from both algae showed a quite rapid sorption of Pb, Cd and Zn ions with different manners; ion exchange adsorption with Cd and Zn, and chemical adsorption with Pb by *C. aculeolata* but ion exchange adsorption with all metal ions by *N. opaca*. The rate constant for intraparticle diffusion found in metal adsorption onto *C. aculeolata* was relatively high when compared to *N. opaca* suggesting the structural porosity of *C. aculeolata* biomass. This porosity increases the sorbent area for metal sorption sites like a conventional activated carbon. The maximum sorption values (Q_m) obtained in both *C. aculeolata* and *N. opaca* were slightly different and are promising in multi-metal condition. *C. aculeolata* should be a better choice than *N. opaca* due to its greater porosity, adsorption capacity in a comparable biomass and metal removal efficiency in real wastewater (81-100%). *C. aculeolata* showed an excellent performance of removal of Pb and Zn from municipal wastewater.

Column study confirmed that *C. aculeolata* had good property for biosorption in packed bed column in comparison to *N. opaca* biomass. Column parameters such as bed depth, flow rate and metal concentration had the effect on breakthrough curve of biosorption by *C. aculeolata*, which related to biosorption kinetic and sorbent characteristic such as porosity, bulk density and functional groups or active sites of the biomass. The selective uptake of Pb and Cd in a binary solution showed much higher relative affinity for Pb than for Cd. For study of reusability, the efficiency of biosorbent regeneration by 0.1 M HCl was achieved very high about 98% for Pb and 100% for Cd in the third reused cycle. Metal desorption in this study is useful for application of regenerating spent biomass and for metal recovery and recycling after algal sorption from wastewater.

Suggested further studies:

1) Physical properties of *C. aculeolata* biomass such as particle size and porosity, and its chemical property such as the functional groups (binding sites) need to be studied to clarify the mechanism of metal biosorption in *C. aculeolata* biomass.

2) The application of *C. aculeolata* packed column for metal removal from real wastewater is also needed to evaluate the practical use.

3) Toxicity Characteristic Leaching Procedure (TCLP) and Total Constituent Analysis (TCA) tests are needed to simulate the metal leaching from the biomass after land disposal and to evaluate the appropriate method for disposal of the repeat regenerated algal biomass.

REFERENCES

Abanades, S., Flamant, G., Gagnepain, B. and Gauthier, D. (2002). Facts of heavy metals during municipal solid waste incineration. *Waste Management and Research*, 20, 55-68.

Abdel-Halim, S. H., Shehata, A. M. A. and El-Shahat, M. F. (2003). Removal of lead ions from industrial wastewater by different types of natural materials. *Water Research*, 37, 1678-1683.

Ahalya, N., Ramachandra, T.V. and Kanamadi, R.D. (2003). Biosorption of heavy metals, *Research Journal of Chemistry and Environment*, 7, 1-8.

Ajjabi, L. C. and Chouba, L. (2009). Biosorption of Cu^{2+} and Zn^{2+} from aqueous solutions by dried marine green macroalga *Chaetomorpha linum*. *Journal of Environmental Management*, 90(11), 3485-3489.

Aksu, Z., Sag, Y. and Kutsal, T. (1992). The biosorption of copper (II) by *Chlorella vulgaris* and *Zoogloea ramigera*. *Environmental Technology*, 13, 579-586.

Aksu, Z., Gönen, F. (2003). Biosorption of phenol by immobilized activated sludge in a continuous packed bed: prediction of breakthrough curves. *Process Biochemistry*, 39, 599-613.

Ale, M. T., Minkkelson, J. D. and Meyer, A. S. (2010). Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. *Journal of Applied Phycology*, 23, 345-351.

Al-Homaidan, A. A., Al-Ghanayem, A. A. and Alkhalifa, A. H. (2011). Green algae as bioindicators of heavy metal pollution in Wadi Hanifah stream, Riyadh, Saudi Arabia. *International Journal of Water Resources and Arid Environments*, 1, 10-15.

Alloway, B.J. and Ayres, D.C. (1997). *Chemical Principles for Environmental Pollution*. Blackie Academic and Professional, Glasgow, 190-220.

Amarasinghe, B. M. W. P. K. and Williums, R. A. (2007). Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater. *Chemical Engineering Journal*, 132, 299-309.

Amuda, O. S., Giwa, A. A. and Bello, I. A. (2007). Removal of heavy metal from industrial wastewater using modified activated coconut shell carbon. *Biochemical Engineering Journal*, 36, 174-181.

Andersson, A. (1977). Heavy metals in Swedish soils: on their retention, distribution and amounts. *Sweden Journal of Agricultural Research,* 7, 7-20.

Anderson, R. (1991). *Sample Pretreatment and Separation*. John Wiley and Sons, Singapore.

Andrade, S., Contreras, L., Moffett, J. W. and Correa, J. A. (2006a). Kinetics of copper accumulation in *Lessonia nigrescens* (Phaeophyceae) under conditions of environmental oxidative stress. *Aquatic Toxicology*, 78, 398-401.

AOAC. (2006). *Official Method of Analysis*, 18th ed. AOAC International, Texas.

APHA, AWWA, WEF. (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th ed. Port City Press, Washington D.C..

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts polyphenoloxidases in *Beta vulgaris*. *Plant Physiology*, 24, 1-14.

Ashraf, M.A., Maah, M.J. and Yusoff, I. (2010). Study of mango biomass (*Mangifera indica* L) as a cationic biosorbent. *International Journal of Environmental Science and Tecchnology*, 7, 581-590.

Australian Mines Atlas. (2012). Lead factsheet. Mineral resources, Mines and Processing Centre, Australian Government Geoscience Australia. http://www.australian minesatlas.gov.au/education/fact_sheets/lead.html.

Axtell, N. R., Sternberg, S. P. K. and Claussen, K. (2003). Lead and nickel removal using *Microspora* and *Lemna minor*. *Bioresource Technology*, 89, 41-48.

Babarinde, N. A. A., Babalola, J. O. and Sanni, R. A. (2006). Biosorption of lead ions from aqueous solution by maize leaf. *International Journal of Physical Sciences*, 1, 23-26.

Baker, A. J. M., McGrath, S. P., Reeves, R. D. and Smith, J. A. C. (2000). Metal hyperaccumulator plants: A review of the ecology and physilogy of a biological resource for phytoremediation of metal-polluted soil. In Terry, N. and Banuelos, G. eds. *Phytoremediation of Contaminated Soil and Water*. Lewis Publishers, Boca Raton, Florida.

Baumann, H. A., Morrison, L. and Stengel, D. B. (2009). Metal accumulation and toxicity measured by PAM-chlorophyll fluorescence in seven species of marine macroalgae. *Ecotoxicology and Environmental Safety*, 72, 1063-1075.

Bi, X., Feng, X., Yang, Y., Qiu, G. and Li, G. (2006). Quantitative assessment of Cd emission from zinc smelting and its influences on the surface soils and mosses in Hezhang County, Southwestern China. *Atmospheric Environment*, 40(22), 4228-4233.

Bibi, M. H., Asaeda, T. and Azim, E. (2010). Effects of Cd, Cr, and Zn on growth and metal accumulation in an aquatic macrophyte, *Nitella graciliformis*. *Chemistry and Ecology*, 26, 49-56.

Bishnoi, N. R., Kumar, R., Kumar, S. and Rani, S. (2007). Biosorption of Cr(III) from aqueous solution using algal biomass *Spirogyra spp*. *Journal of Hazardous Materials*, 145(1-2), 142-147.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D. and Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environmental Science and Technology*, 31, 860-865.

Boonyapookana, B., Upatham, E. S., Kruatrachue, M., Pokethitiyook, P. and Singhakaew, S. (2002). Phytoaccumulation and phytotoxicity of cadmium and chromium in duckweed *Wolffia globosa*. *International Journal of Phytoremediation*, 4, 87-100.

Boyd, G.E., Adamson, A.W. and Myers, L.S. (1947). The exchange adsorption of ions from aqueous solutions by organic zeolites II kinetics. *Journal of the American Chemical Society*, 69, 2836-2848.

Brady, D., Rose, P. D. and Duncan, J. R. (1994). Communication to the editor: The use of hollow fiber cross-flow microfiltration in bioaccumulation and continuous removal of heavy metals from solution by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, 44, 1362-1366.

Brauckmann, B. M. (1990). Industrial solutions amenable to biosorption. In: Volesky B ed. *Biosorption of Heavy Metals*. CRC Press, Florida.

Brown, P., Jefcoat, A., Parrish, D., Gill, S. and Graham, E. (2000). Evaluation of the adsoptive capacity of peanut pellets for heavy metals in solution. *Advances in Environmental Research*, 4, 19-29.

Buchauer, M.J. (1972). The effects of zinc and cadmium pollution on forest vegetation. *Bulletin of New Jersey Academic Science*, 17(2), 42.

Bulut, Y. and Tez, Z. (2007). Adsorption studies on ground shells of hazelnut and almond. *Journal of Hazardous Materials*, 149, 35-41.

Bunluesin, S., Kruatrachue, M., Pokethitiyook, P., Upatham, S. and Lanza, G. R. (2007). Batch and continuous packed column studies of Cd biosorption by *Hydrilla verticillata* biomass. *Journal of Bioscience and Bioengineering*, 103(6), 509-513.

Chang, J. S., Law, R. and Chang, C. C. (1997). Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. *Water Research*, 31, 1651-1658.

Chen, J. Z., Tao, X. C., Xu, J., Zhang, T., Liu, Z. L. (2005). Biosorption of lead, cadmium and mercury by immobilized *Microcystis aeruginosa* in a column. *Process Biochemistry*, 40, 3675-3679.

Chojnacka, K. (2010). Biosorption and bioaccumulation – the prospects for practical applications. *Environment International*, 36, 299-307.

Chung, M. K., Tsui, M. T. K., Cheung, K. C., Tam, N. F. Y. and Wong, M. H. (2007). Removal of aqueous phenanthrene by brown seaweed *Sargassum hemiphyllum*: Sorption-kinetic and equilibrium studies. *Separation and Purification Technology*, 54(3), 355-362.

Cimino, G., Passerini, A. and Toscano, G. (2000). Removal of toxic cations and Cr (VI) from aqueous solution by hazelnut shell. *Water Research*, 34, 2955-2962.

Collén, J., Pinto, E., Pedersén, M. and Colepicolo, P. (2003). Induction of oxidative stress in the red macroalga *Gracilaria tenuistipitata* by pollutant metals. *Archives of Environmental Contamination and Toxicology*, 45, 337-342.

Contreras, L., Medina, M. H., Andrade, S., Oppliger, V. and Correa, J. A. (2007). Effects of copper on early developmental stage of *Lessonia nigrescens* Bory (Phaeophyceae). *Environmental Pollution*, 145, 75-83.

Contreras, L., Mella, D., Moenne, A. and Correa, J. A. (2009). Differential responses to copper-induced oxidative stress in the marine macroalgae *Lessonia nigrescens* and *Scytosiphon lomentaria* (Phaeophyceae). *Aquatic Toxicology*, 94(2), 94-102.

Cooper, P. E., Job G. F., Green, M. B., Shutes R. B. E. (1996). *Reed Beds and Constructed Wetlands for Wastewater Treatment*. Water Research Centre Publication, Swindon, 154-184.

Coops, H. (2002). Ecology of charophytes: an introduction. *Aquatic Botany*, 72, 205-208.

Cotoras, D., Viedma, P. and Pimentel, J. (1993). Biosorption of metal ions by attached bacterial cells in a packed-bed bioreactor. In Torma, A. E., Apel, M. L. and Brierley, C. L. eds. *Biohydrometallurgical technologies, vol II: The minerals, metals and materials Society*. Metadata, Hannover.

Crist, R.H., Oberholser, K., Shank, N. and Nguyen, M. (1981). Nature of bonding between metallic ions and algal cell walls. *Environmental Science and Technology*, 15, 1212-1217.

Davis, T.A., Volesky, B. and Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, 37, 4311-4330.

Dean, J.G., Bosqui, F.L. and Lanouette, V.H. (1972). Removing of heavy metal from wastewater. *Environmental Science and Technology, 6*, 518-522.

Deng, L., Su, Y., Su, H., Wang, W. and Zhu, X. (2007a). Sorption and desorption of lead (II) from wastewater by green algae *Cladophora fascicularis*, *Journal of Hazardous Materials*, 143, 220-225.

Deng, L., Zhu, X., Wang, X., Su, Y. and Su, H. (2007b). Biosorption of copper(II) from aqueous solutions by green alga *Cladophora fascicularis*. *Biodegradation*, 18(4), 393-402.

Deng, L., Zhang, Y., Qin, J., Wang, X. and Zhu, X. (2009). Biosorption of Cr (VI) from aqueous solutions by nonliving green alga *Cladophora albida*. *Minerals Engineering*, 22, 372-377.

Deng, X., Wilson, D. B. (2001). Bioaccumulation of mercury from wastewater by genetically engineered *Escherichia coli*. *Applied Microbiology and Biotechnology*, 56, 276-279.

Diniz, V. and Volesky, B. (2005). Effect of counter-ions of lanthanum biosorption by *Sargassum polycystum*. *Water Research*, 39, 2229-2236.

Doshi, H., Seth, C., Ray, A. and Kothari, I. (2008). Bioaccumulation of heavy metals by green algae. *Current Microbiology*, 56(3), 246-255.

Eilbeck, W. J. and Mattock, G. (1987). *Process in Wastewater Treatment*. Ellis Harwood, Chichester.

Environmental Protection Agency. (1975). Test methods for assessing the effects of chemical on plants. In: Rubinstein, R., Smith, J. eds. EPA-560-17-008, Final Report, 175-182.

Environmental Protection Agency. (1997). Landfill reclamation. http://www.epa.gov/wastes/nonhaz/municipal/landfill/land_rcl.pdf. EPA-530-F-97-001.

Environmental Protection Agency. (2000). *Wastewater Technology Fact Sheet*. National Science Center for Environmental Publications, Washington D.C. EPA-832-F-00-017.

Environmental Protection Agency. (2003). Water quality criteria. http://www.epa.gov/waterscience/criteria/goldbook.pdf.

Environmental Protection Agency. (2004). Methods for evaluating solid waste, physical/ chemical methods. http://www.epa.gov/wastes/hazard/test methods/sw846/pdf/chap7.pdf.

Environmental Protection Agency. (2005). Partition coefficients for lead. http://www.epa. gov/radiation/docs/kdreport/vol2/402-r-99-004b_appf.pdf.

Environmental Protection Agency. (2012). Cadmium fact sheet. http://www.epa.gov/osw/hazard/wastemin/minimize/factshts/cadmium.pdf.

Fan, T., Liu, Y., Feng, B., Zeng, G., Yang, C., Zhou, M., Zhou, H., Tan, Z. and Wang, X. (2008). Biosorption of cadmium(II), zinc(II) and lead(II) by *Penicillium simplicissimum*: Isotherms, kinetics and thermodynamics, *Journal of Hazardous Materials*, 160, 655-661.

Filho, G. M. A., Karez, C. S., Andrade, L. R., Yoneshigue-Valentin, Y. and Pfeiffer, W. C. (1997). Effects on growth and accumulation of zinc in six seaweed species. *Ecotoxicology and Environmental Safety*, 37, 223-228.

Forstner, S. and Wittman, G. T. W. (1979). *Metal Pollution in the Aquatic Environment*. Springer-verlag, Berlin.

Forstner, U. (1980). Cd in the environment, part I. In: Nriagu, J.O. ed. *Cd in Polluted Sediments*. John Wiley and Sons, New York, 305-363.

Gao, Y. and Yan, X. (2012). Response of *Chara globularis* and *Hydrodictyon reticulatum* to lead pollution: their survival, bioaccumulation, and defense. *Journal of Applied Phycology*, 24, 245-251.

Garnier, L., Simon-Plas, F., Thuleau, P., Agnel, J., Blein, J., Ranjeva, R. and Montillet J.L. (2006). Cadmium affects tobacco cells by a series of three waves of reactive oxygen species that contribute to cytotoxicity. *Plant, Cell and Environment*, 29, 1956-1969.

Gomes, P. I. A. and Asaeda, T. (2009). Phycoremediation potential of chromium (VI) by *Nitella* and impact of calcium encrustation. *Journal of Hazardous Materials*, 166, 1332-1338.

Gong, R., Ding, Y., Liu, H., Chen, Q. and Liu, Z. (2005). Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. *Chemosphere*, 58(1), 125-130.

Goyer, R.A. (1986). Toxic effects of metals. In: Klaassen, C. D., Amdur, M. O, and Doull, J. eds. *The Basic Science of Poisons*. 3rd ed. McGraw-Hill, New York.

Guisquiani, P. L., Concezzi, L., Businelli, M. and Macchioni, A. (1998). Fate of pig sludge liquid fraction in calcareous soil: agricultural and environmental implications. *Journal of Environmental Quality*, 27, 364-371.

Gupta, V. K., Rastogi, A., Saini, V. K. and Jain, N. (2006). Biosorption of copper(II) from aqueous solutions by *Spirogyra* species. *Journal of Colloid and Interface Science*, 296(1), 59-63.

Gupta, V. K. and Rastogi, A. (2008). Biosorption of lead from aqueous solutions by green algae *Spirogyra* species: kinetics and equilibrium studies, *Journal of Hazardous Materials*, 152, 407-414.

Gupta, V. K. and Rastogi, A. (2009). Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions. *Journal of Hazardous Materials*, 163(1), 396-402.

Gupta, V. K., Rastogi, A. and Nayak, A. (2010). Biosorption of nickel onto treated alga (*Oedogonium hatei*): Application of isotherm and kinetic models. *Journal of Colloid and Interface Science*, 342(2), 533-539.

Haglund, K., Björklund, M., Gunnare, S., Sandberg, A., Olander, U. and Pedersén, M. (1996). New method for toxicity assessment in marine and brackish environments using the macroalga *Gracilaria tenuistipitata* (Gracilariales, Rhodophyta). *Hydrobiologia*, 326-327(1), 317-325.

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Environmental Botany*, 53, 1-11.

Hambidge, K. M. and Krebs, N. F. (2007). Zinc deficiency: a special challenge. *Journal of Nutrition*, 137(4), 1101-1105.

Harter, R. D. and Naidu, R., (1995). Role of organic-metal complexation in metal sorption by soils. *Advances in Agronomy*, 55, 219-263.

Hasan, S. H., Srivastava, P. and Talat, M. (2009). Biosorption of Pb(II) from water using biomass of *Aeromonas hydrophila*: Central composite design for optimization of process variables. *Journal of Hazardous Materials*, 168(2-3), 1155-1162.

Hirata, M. (1981). Annaka: Land polluted mainly by fumes and dust from a zinc smelter. In: Kitagishi, K. and Gamare, I. eds. *Heavy Metal Pollution in the Soils of Japan*, Japan Scientific Societies Press, Tokyo.

Ho, Y. S. and Mckay, G. (1999). Pseudo-second order model for sorption processes, *Process Biochemistry*, 34, 451-465.

Ho, Y. S. (2005). Effect of pH on lead removal from water using tree fern as the sorbent, *Bioresource Technology*, 96, 1292-1296.

Holan, Z. R., Volesky, B. and Prasetyo, I. (1993). Biosorption of cadmium by biomass of marine algae. *Biotechnology and Bioengineering*, 41, 819-825.

Homchean, K. (1972). Industrial estate authority of Thailand strategy for environmental compliance. http://www.inece.org/5thvol1/homchean.pdf.

Hutton, M. (1982). Cd in the European Community: A prospective assessment of sources, human exposure and environmental impact. Monitoring and Assessment Research Centre, London, Report No. 26.

Igwe, J.C., Ogunewe, D.N. and Abia A.A. (2005). Competitive adsorption of Zn (II), Cd (II) and Pb (II) ions from aqueous and non-aqueous solution by maize cob and husk, *African Journal of Biotechnology*, 4, 1113-1116.

Inthorn, D., Tipprasertsin, K., Thiravetyan, P. and Khan, E. (2010). Color removal from textile wastewater by using treated flute reed in a fixed bed column. *Journal of Environmental Science and Health part A*, 45, 637-644.

Jafari, N. and Senobari, Z. (2012). Removal of Pb(II) ions from aqueous solutions by *Cladophora rivularis* (Linnaeus) Hoek. *Scientific World Journal*, doi:10. 1100/2012/793606.

Jeffrey, S. W. (1997). Chlorophyll and carotenoid extinction coefficients. In: Jeffrey, S. W., Mantoura, R. F. C. and Wright, S. W. eds. *Phytoplankton Pigments in Oceanography*. UNESCO Publishing, Paris.

Ji, L., Xie, S., Feng, J., Li, Y., Chen, L. (2012). Heavy metal uptake capacities by the common freshwater green alga *Cladophora fracta*. *Journal of Applied Phycology*, 24, 979-983.

Kadlec, R.H. and Knight, R.L. (1996). *Treatment Wetlands*. Boca Raton and CRC Press, Florida.

Kaikake, K., Hoaki, K., Sunada, H., Dhakal, R. P. and Baba, Y. (2007). Removal characteristics of metal ions using degreased coffee beans: adsorption equilibrium of cadmium (II). *Bioresource Technology*, 98, 2787-2791.

Karlik, B. and Szpakowska, B. (2001). Labile organic matter and heavy metals in waters of agricultural landscape. *Polish Journal of Environmental Studies*, 10, 85-88.

Khopkar, S. M. (2004). *Environment Pollution Monitoring and Control*. New Age International, New Delhi.

Kratochvil, D., Pimentel, P. and Volesky, B. (1998). Removal of trivalent and hexavalent chromium by seaweed biosorbent. *Environmental Science and Technology*, 32, 2693-2698.

Katz, S. A. and Jennis, S. W. (1983). *Regulatory Compliance Monitoring by Atomic Absorption Spectroscopy*. Verlac Chemie International Incorporation, Florida.

Kinkade, M. L. and Erdman, H. E. (1975). The influence of hardness components (Ca^{2+} and Mg^{2+}) in water on the uptake and concentration of cadmium in a simulated freshwater ecosystem. *Environmental Research*, 10, 308-313.

Kocberber, N., Donmez, G. (2007). Chromium (IV) bioaccumulation capacities of adapted mixed cultures isolated from industrial saline wastewater. *Bioresource Technology*, 98, 2178-2183.

Korte, F. (1983). Ecotoxicology of Cd: general overview. *Ecotoxicology and Environmental Safety*, 7, 3-8.

Królikowska, J. (1997). Eutrophication processes in a shallow, macrophyte-dominated lake – species differentiation, biomass and the distribution of submerged macrophytes in Lake Luknajno (Poland). *Hydrobiologia*., 342/343, 411-416.

Kumar, R., Singh, R., Kumar, N., Bishnoi, K. and Bishnoi, N. R. (2009). Response surface methodology approach for optimization of biosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp. *Bacillus brevis*. *Chemical Engineering Journal*, 146(3), 401-407.

Kumar, J. I. N. and Oommen C. (2012). Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyaline*. *Journal of Environmental Biology*, 33, 27-31.

Kuyucak, N. and Volesky, B. (1988). Biosorbents for recovery of metals from industrial solutions. *Biotechnology Letters*, 10(2), 137-142.

Kuyucak, N. and Volesky, B. (1990). Biosorption by algal biomass. In: Volesky, B. ed. *Biosorption of Heavy Metals*. CRC Press, Florida.

Laamanen, A. (1972). Functions, progress and prospects for an environmental subarctic base level station. *Environmental Health*, 9: 17-25.

Lagergren, S. (1898) Zur theorie der sogenannten adsorption gelöster stoffe, Kungliga Svenska Vetenskapsakademiens, *Handlinger*, 24(4), 1-39.

Lamai, C., Kruatrachue, M., Pokethitiyook, P., Upatham, E. S. and Soonthornsarathool, V. (2005). Toxicity and accumulation of lead and cadmium in filamentous green alga *Cladophora fracta* (O.F. Müller ex Vahl): A laboratory study. *ScienceAsia*, 31, 121-127.

Lartigue, J., Neill, A., Hayden, B. L., Pulfer, J. and Cebrian, J. (2003). The impact of salinity fluctuations on net oxygen production and inorganic nitrogen uptake by *Ulva lactuca* (Chlorophyceae). *Aquatic Botany*, 75, 339-350.

Lawal, O. S., Sanni, A. R., Ajayi, I. A. and Rabiu, O. O. (2010). Equilibrium, thermodynamic and kinetic studies for the biosorption of aqueous lead(II) ions onto the seed husk of *Calophyllum inophyllum*. *Journal of Hazardous Materials*, 177, 829-835.

Leck, M. A. and Brock, M. A. (2000). Ecological and evolutionary trends in wetlands: evidences from seeds and seed banks in New South Wales, Australia and New Jersey, USA. *Plant Species Biology*, 15, 97-112.

Lee, I. H., Kuan, Y. C. and Chern, J. M. (2007). Equilibrium and kinetics of heavy metal ion exchange. *Journal of the Chinese Institute of Chemical Engineers*, 38, 71–84.

Lee, W. and Wang, W. (2001). Metal accumulation in the green macroalga *Ulva fasciata*: effects of nitrate, ammonium and phosphate. *The Science of the Total Environment*, 278, 11-22.

Lindberg, S. E., Turner, R. R. and Lovett, G. M. (1982). *Process of Atmospheric Deposition of Metals and Acids to Forests.* Paper presented at Annual Meeting of the Air Pollution Control Association, New Orleans, Conference Proceedings 75.

Liu, Y., Cao, Q., Luo, F. and Chen, J. (2009). Biosorption of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions from aqueous solutions by pretreated biomass of brown algae. *Journal of Hazardous Materials*, 163(2-3), 931-938.

Lodeiro, P., Barriada, J. L., Herrero, R. and Sastre de Vicente, M. E. (2006). The marine macroalga *Cystoseira baccata* as biosorbent for Cd(II) and lead(II) removal: Kinetic and equilibrium studies. *Environmental Pollution*, 142(2), 264-273.

Luo, F., Liu, Y., Li, X., Xuan, Z. and Ma, J. (2006). Biosorption of lead ion by chemically-modified biomass of marine brown algae *Laminaria japonica*. *Chemosphere*, 64(7), 1122-1127.

Lu-shi, S., Abanades, S., Lu, J. D., Flamant, G., Gauthier, D. (2004). Volatilization of heavy metals during incineration of municipal solid waste. *Journal of Environmental Science*, 16(4), 635-639.

Macaskie, L. E., Wates, J. M. and Dean, A. C. R. (1987). Cadmium accumulation by a *Citrobacter* sp. immobilized on gel and solid supports; applicability to the treatment of liquid wastes containing heavy metal cations. *Biotechnology and Bioengineering*, 30, 66-73.

MacKinney G, 1941. Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, 144, 315-323.

Mahan, C. A. and Holcombe, J. A. (1992). Immobilization of algae cells on silica gel and their charracterization for trace metal preconcentration. *Analytical Chemistry*, 64, 1933-1939.

Maret, W. (2013). Zinc and the zinc proteome. *Metallomics and the Cell Metal Ion in Life Sciences*, 12, 479-501.

Martin, H.W. and Kaplan, D.L. (1998). Temporal changes in Cd, tantalum and vanadium mobility in soil and phytoavailability under field conditions. *Water Air and Soil Pollution*, 101, 339-410.

Maskall, J. E., Whitehead, K. and Thornton, I. (1995). Heavy metal migration in soils and rocks at historical smelting sites. *Environmental Geochemistry and Health*, 17, 127-138.

Maskall, J. E., Whitehead, K., Gee, C. and Thornton, I. (1996). Long-term migration of metals at hidtorical smelting sites. *Applied Geochemistry*, 11, 43-51.

Mata, Y. N., Blazquez, M. L., Ballester, A., Gonzalez, F. and Munoz, J. A. (2009). Sugar-beet pulp pectin gels as biosorbent for heavy metals: Preparation and determination of biosorption and desorption characteristics. *Chemical Engineering Journal*, 150(2-3), 289-301.

Metcalf and Eddy Inc. (2003). *Wastewater Engineering: Treatment and Reuse*, 4th ed., McGraw-Hill, New York.

Migon, C., Morelli, J., Nicolas, E. and Copin-Montegut, G. (1991). Evaluation of total atmospheric deposition of Pb, Cd, Cu and Zn to the Ligurian sea. *The Science of the Total Environment*, 105, 135-148.

Miretzky, P., Saralegui, A. and Fernandez Cirelli, A. (2006). Simultaneous heavy metal removal mechanism by dead macrophytes. *Chemosphere*, 62(2), 247-254.

Mohan, D. and Singh, K.P. (2002). Single- and multi-component adsorption of cadmium and zinc using activated carbon derived from bagasse-an agricultural waste, *Water Research*, 36, 2304-2318.

Morris, E. G., Vincent, B. and Snowden, J. M. (1997). Adsorption of lead ions onto *N*-isopropylacrylamide and acrylic acid copolymer microgels. *Journal of Colloid and Interface Science*, 190, 198-205.

Murithi, G., Onindo, C. O. and Muthakia, G. K. (2012). Kinetic and equilibrium study for the sorption of Pb (II) ions from aqueous phase by water hyacinth (*Eichhornia crassipes*). *Bulletin of the Chemical Society of Ethiopia*, 26, 181-193.

Naddafi, K., Nabizadeh, R., Saeedi, R., Mahvi, A. H., Vaezi, F., Yaghmaeian, K., Ghasri, A. and Nazmara, S. (2007). Biosorption of lead(II) and cadmium(II) by protonated *Sargassum glaucescens* biomass in a continuous packed bed column. *Journal of Hazardous Materials*, 147, 785-791.

Naja, G. M. and Volesky, B. (2010). Treatment of metal-bearing effluents: removal and recovery. In: Wang, L. K., Chen, J. P., Hung, Y. T. and Shammas, N. K., eds. *Handbook in Heavy Metals in the Environment*. Boca Raton, Taylor and Francis and CRC Press, Florida.

Namasivayam, C. and Ranganathan, K. (1995). Removal of Cd (II) from wastewater by adsorption on “waste” Fe (II) Cr (II) hydroxide, *Water Research*, 29, 1737-1744.

Nriagu, J. O. and Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, 333, 134-139.

Nriagu, J. O. (1989). A global assessment of natural sources of atmospheric trace metals. *Nature*, *338,* 47-48.

Nolting, R. F. and Helder, W. (1991). Lead and zinc as indicators for atmospheric and riverine particle transport to sediments in the Gulf of Lions. *Oceanologica Acta*, 14(4), 357-367.

Nordic Council of Ministers. (2003). Cd review, http://www.who.int/ifcs/documents/forums/forum5/nmr_Cd.pdf, 8-15

Nordic Council of Ministry. (2003). Lead review. http://www.who.int/ifcs/documents/forums/forum5/nmr_lead.pdf, 15-16.

Onyancha, D., Mavura, W., Ngila, J. C., Ongoma, P. and Chacha, J. (2008). Studies of chromium removal from tannery wastewaters by algae biosorbents, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*. *Journal of Hazardous Materials*, 158(2-3), 605-614.

Ozer, A. (2007). Removal of Pb (II) ions from aqueous solutions by sulphuric acid-treated wheat bran. *Journal of Hazardous Materials*, 141, 753-761.

Pan, R., Cao, L., Huang, H., Zhang, R. and Mo, Y. (2010). Biosorption of Cd, Cu, Pb, and Zn from aqueous solutions by the fruiting bodies of jelly fungi (*Tremella fuciformis* and *Auricularia polytricha*). *Applied Microbiology and Biotechnology*, 88, 997-1005.

Pavasant, P., Apiratikul, R., Sungkhum, V., Suthiparinyanont, P., Wattanachira, S. and Marhaba, T. F. (2006). Biosorption of Cu^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+} using dried marine green macroalga *Caulerpa lentillifera*. *Bioresource Technology*, 97(18), 2321-2329.

Pawlik-Skowronska, B., Sanita di Toppi, L., Favali, M. A., Fossati, F., Pieszel, J. and Skowronski, T. (2002). Lichens respond to heavy metals by phytochelatin synthesis. *New Phytologist*, 156, 95-102.

Pawlik-Skowronska, B. (2003). Resistance, accumulation and allocation of zinc in two ecotypes of the green algae *Stigeoclonium tenue* Kutz. Coming from habitats of different heavy metal concentrations. *Aquatic Botany*, 75, 189-198.

Pawlik-Skowronska, B., Pirszel, J. and Brown, M. T. (2007). Concentrations of phytochelatins and glutathione found in natural assemblages of seaweeds depend on species and metal concentrations of the habitat. *Aquatic Toxicology*, 83(3), 190-199.

Petty, D.G. (2005). *Aquatic Plant Management: Best Management Practices in Support of Fish and Wildlife Habitat.* The Aquatic Ecosystem Restoration Foundation (AERF), Georgia, 9-25.

Pena-Castro, J. M., Martinez-Jeronimo, F., Esparza-Garcia, F. and Canizares-Villanueva, R. O. (2004). Heavy metals removal by the microalga *Scenedesmus incrassatulus* in continuous cultures. *Bioresource Technology*, 94(2), 219-222.

Pérez, J.A.M., García-Ribera, R., Quesada, T., Aguilera, M., Ramos-Cormenzana, A. and Monteoliva-Sánchez, M. (2008). Biosorption of heavy metals by the exopolysaccharide produced by *Paenibacillus jamilae*. *World Journal of Microbiology and Biotechnology*, 24, 2699-2704.

Porter, F. C. (1991). *Zinc Handbook: Properties, Processing, and Use in Design*. CRC Press, Dekker.

Prasad, M. N. V. (1999). *Heavy Metal Stress in Plants*. Spinger-Verlag, Berlin.

Pratap, M., Khambhammettu, U., Clark, S. and Pitt, R. (2007). *Stormwater Polishing: Upflow vs. Downflow Filters*. Paper presented at World Environmental and Water Resources Congress, 1-12. doi:10.1061/40927(243)582.

Puranik, P. R. and Paknikar, K. M. (1999). Biosorption of lead, cadmium and zinc by *Citrobactor* strain MCM B-18: characterization studies. *Biotechnology Progress*, 15, 228-237.

Qaiser, S., Saleemi, A. R. and Ahmad, M. M. (2007). Heavy metal uptake by agro based waste materials. *Electronic journal of Biotechnology*, 10, 409-416.

Qaiser, S., Saleemi, A. R. and Umar, M. (2009). Biosorption of lead from aqueous solution by *Ficus religiosa* leaves: Batch and column study. *Journal of Hazardous Materials*, 166(2-3), 998-1005.

Quek, S. Y., Wase, D. A. and Froster, C. F. (1998). The use of sago waste for sorption of lead and copper. *Water Science and Technology*, 24, 251-256.

Rai, L., Gaur, J. P. and Kumar, H. D. (1981). Physiology and heavy metal pollution. *Biological Reviews*, 56, 99-151.

Rangsayatorn, N., Upatham, E. S., Kruatrachue, M., Pokethitiyook, P., Lanza, G. R. (2002). Phytoremediation potential of *Spirulina* (*Arthrospira*) *platensis*: biosorption and toxicity studies of cadmium. *Environmental Pollution*, 119, 45-53.

Raskin, I., Kumar, P. B. A. N., Dushenkov, S. and Salt, D. (1994). Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 28, 115-126.

Raven, P. H., Evert, R. F. and Eichhorn, S. E. (1999). *Biology of Plants*. W.H. Freeman and Company, New York.

Reed, B. E. and Matsumoto, M. R. (1993). Modeling cadmium adsorption by activated carbon using the Langmuir and Freundlich isotherm expressions. *Separation Science and Technology*, 28, 2179-2195.

Reeves, R. D., Baker, A. J. M. and Brooks, R. R. (1996). Abnormal accumulation of trace metals by plants. *Mining Environmental Management*, 3(3), 4-8.

Saeed, A., Iqbal, M. and Akhtar, M.W. (2005). Removal and recovery of lead(II) from single and multimetal (Cd, Cu, Ni, Zn) solutions by crop milling waste (black gram husk). *Journal of Hazardous Materials*, 117, 65-73.

Salt, D. E., Smith, R. D., Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology*, 49, 643-668.

Sangster, B., de Groot, G., Loeber, J. G., Derks, H. J. G. M., Krajnc, E. I. and Savelkoul, T. J. F. (1984). Urinary excretion of Cd, protein, beta-2-microglobulin and glucose in individuals living in a Cd-polluted area. *Human Toxicology*, 3, 7-21.

Sari, A. and Tuzen, M. (2008). Biosorption of cadmium(II) from aqueous solution by red algae (*Ceramium virgatum*): Equilibrium, kinetics and thermodynamic studies, *Journal of Hazardous Materials*, 157, 448-454.

Schiewer, S. and Volesky, B. (1996). Modeling multi-metal ion exchange in biosorption. *Environmental Science and Technology*, 30, 2921-2927.

Scott, J. A. and Palmer, S. J. (1990). Sites of cadmium uptake in bacteria used for biosorption. *Applied Microbiology and Biotechnology*, 33, 221-225.

Senthilkumar, R., Vijayaraghavan, K., Thilakavathi, M., Iyer, P. V. R. and Velan, M. (2007). Application of seaweeds for the removal of lead from aqueous solution. *Biochemical Engineering Journal*, 33, 211-216.

Setia, R. C., Kaur, N., Setia, N. and Nayyar, H. (2008). Heavy metal toxicity in plants and phytoremediation. In: Setia, R. C., Setia, N. and Nayyar, H. eds. *Crop Improvement: Strategies and Applications*. International Publishing House Pvt. Ltd., New Deli, 206-218

Shannon, R. D. (1976). Revised effective ionic radii and systematic studies of interatomic distances in halides and chaleogenides. *Acta Crystallographica*, 32, 751-767.

Sheng, P. X., Ting, Y. P., Chen, J. P. and Hong, L. (2004). Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: characterization of biosorptive capacity and investigation of mechanisms, *Journal of Colloid and Interface Science*, 275, 131-141.

Shiller, A. M., Boyle, E. A. (1987). Variability of dissolved trace metals in the Mississippi River. *Geochimica et Cosmochimica Acta*, 51, 3273-3277.

Shutes, B. (2003). *The Use of Constructed Wetlands for Wastewater Treatment*. Wetland International, Salangor.

Singh, A., Kumar, D. and Gaur, J. P. (2007). Copper(II) and lead(II) sorption from aqueous solution by non-living *Spirogyra neglecta*. *Bioresource Technology*, 98(18), 3622-3629.

Skowronski, T., De Knecht, J. A., Simon, J. and Verkleij, J. A. C. (1998). Phytochelatin synthesis in response to Cd uptake in *Vaucheria* (Xanthophyceae). *European Journal of Phycology*, 33, 87-91.

Srivastav, R. K., Gupta, S. K., Nigam, K. D. P. and Vasudevan, P. (1993). Use of aquatic plants for the removal of heavy metals from wastewater. *International Journal of Environmental Studies*, 45, 43-50.

Stottmeister, U., Buddhawong, S., Kuschk, P., Wiessner, A. and Mattusch, J. (2006). Constructed wetlands and their performance for treatment of water contaminated with arsenic and heavy metals. In: Twardowska, I. ed. *Soil and Water Pollution, Monitoring, Protection and Remediation*. Springer, Dordrecht.

Sulayman, A. H., Mohammed, A. A., Al-Musawi, T. J. (2013). Column biosorption of lead, cadmium, copper and arsenic ions onto algae. *Journal of Bioprocessing and Biotechniques*, 3, doi: 10.4172/2155-9821.1000128.

Tan, W. T., Ool, S. T. and Lee, C. K. (1993). Removal of Cr (VI) from solution by coconut husk and palm pressed fibres. *Environmental Tecnology*, 14, 277-282.

Tangaromsuk, J., Pokethitiyook, P., Kruatrachue, M. and Upatham, E. S. (2002). Cd biosorption by *Sphingomonas paucimobilis* biomass. *Bioresource Technology*, 85(1), 103-105.

Thornton, I. (1995). *Metals in the Global Environment: Facts and Misconceptions*. International Council on Metals and the Environment, Ottawa.

Tibaijuka, A. K. (2008). *Constructed Wetlands Manual*. United Nations Human Settlements Programme, Nairobi.

Tolcin, A. C. (2011). Mineral commodity summaries 2009: zinc. US Geological Survey.

Tsagkamilis, P., Danielidis, D., Dring, M. J. and Katsaros, C. (2010). Removal of phosphate by the green seaweed *Ulva lactuca* in a small-scale sewage treatment plant (Ios Island, Aegean Sea, Greece). *Journal of Applied Phycology*, 22, 331-339.

Tsezos, M. and Volesky, B. (1981). Biosorption of uranium and thorium. *Biotechnology and Bioengineering*, 23, 583-604.

Tsezos, M. and Volesky, B. (1982). The mechanism of uranium biosorption by *Rhizopus arrhizus*. *Biotechnology and Bioengineering*, 24, 385-969.

Tuzen, M. and Sarl, A. (2009). Biosorption of selenium from aqueous solution by green algae (*Cladophora hutchinsiae*) biomass: Equilibrium, thermodynamic and kinetic studies. *Chemical Engineering Journal*, 158(2), 200-206.

Upatham, E. S., Boonyapookana, B., Kruatrachue, M., Pokethitiyook, P. and Parkpoomkamol, K. (2002). Biosorption of cadmium and chromium in duckweed *Wolffia globosa*. *International Journal of Phytoremediation*, 4(2), 73-86.

Urbaniak, J. (2010). Estimation of carbonate and element content in charophytes – methods of determination. *Polish Journal of Environmental Studies*, 19, 413-417.

Vasconcelos, M. T. S. D. and Leal, M. F. C. (2001). Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Marine Chemistry*, 74, 65-85.

Veglio, F. and Beolchini, F. (1997). Removal of metals by biosorption: a review. *Hydrometallurgy*, 44(3), 301-316.

Vijayaraghavan, K., Jegan, J., Palanivelu, K. and Velan, M. (2005). Biosorption of copper, cobalt and nickel by marine green alga *Ulva reticulata* in a packed column. *Chemosphere*, 60, 419-426.

Vijayaraghavan, K., Teo, T. T., Balasubramanian, R. and Joshi, U.M. (2009). Application of *Sargassum* biomass to remove heavy metal ions from synthetic multi-metal solutions and urban storm water runoff. *Journal of Hazardous Materials*, 164, 1019-1023.

Vilar, V. J. P., Botelho, C. M. S. and Boaventura, R. A. R. (2005). Influence of pH, ionic strength and temperature on lead biosorption by *Gelidium* and agar extraction algal waste. *Process Biochemistry*, 40, 3267-3275.

Visviki, J. and Rachlin, J. W. (1994). Acute and chronic exposure of *Dunaliella salina* and *Chlamydomonas bullosa* to copper and cadmium: effects on ultrastructure. *Archives of Environmental Contamination and Toxicology*, 26, 154-162.

Volesky, B. (1990). Biosorption and biosorbents. In: Volesky, B. ed. *Biosorption of Heavy Metals*. CRC Press, Florida, 3-5.

Volesky, B., May, H. and Holan, Z. (1993). Cadmium biosorption by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, 41, 826-829.

Volesky, B. and Pradetyo, I. (1994). Cadmium removal in a biosorption column. *Biotechnology and Bioengineering*, 43, 1010-1015.

Volesky, B. and Schiewer, S. (1999). Biosorption of metals. In Flickinger, M. and Drew, S. W. ed. *Encyclopedia of Bioprocess Technology*. Wiley, New York, 433-453.

Volesky, B. (2001). Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*, 59, 203-216.

Wang, W. X. and Dei, R. C. H. (1999). Kinetic measurements of metal accumulation in two marine macroalgae. *Marine Biology*, 135, 11-23.

Waranusantigul, P., Pokethitiyook, P., Kruatrachue, M. and Upatham, E.S. (2003). Kinetics of basic dye (methylene blue) biosorption by giant duckweed (*Spirodela polyrrhiza*), *Environmental Pollution*, 125, 385-392.

Wehr, J. D. and Sheath, R. G. (2003). Filamentous and plantlike green algae. In: Wehr, J. D. and Sheath, R. G. eds. *Freshwater Algae of North America: Ecology and Classification*. Academic press, New York, 338-339.

Williams, P. (1998). *Waste Treatment and Disposal*. John Wiley and Sons, Chichester.

Wu, F. C., Tseng, R. L. and Juang, R. S. (2009). Initial behavior of intraparticle diffusion model used in the desorption of adsorption kinetics. *Chemical Engineering Journal*, 153, 1-8.

Yamagata, N. and Shigematsu, I. (1970). Cd pollution in perspective. *Bulletin of Institute of Public Health (Tokyo)*, 24, 18-24.

Yan, G. and Viraraghavan, T. (2001). Heavy metal removal in a biosorption column by immobilized *Mucor rouxii* biomass. *Bioresource Technology*, 78, 243-249.

Ye, J., Song, Y. H., Peng, J. F., Gao, H. J., Qiu, G. L., Duan, L., Zhang, P. Y. and Zeng, G. M. (2011). *Performance of the Tidal-flow Wetland for Wastewater Treatment in Low Temperature Seasons*. Paper presented at International Conference on Computer Distributed Control and Intelligent Environment Monitoring.

Zachara, J. M., Rai, D., Felmy, A. R., Cowan, C. E., Smith, S. C., Moore, D. A. and Resch, C. T. (1992). Aqueous complexation, precipitation and adsorption reactions of cadmium in the geologic environment. Electric Power Research Institute, California.

Zenk, M. H. (1996). Heavy metal detoxification in heigher plants: a review. *Gene*, 179, 21-30.

Zhang, Y. and Banks, C. (2005). The interaction between Cu, Pb, Zn and Ni in their biosorption onto polyurethane-immobilised *Sphagnum* moss. *Journal of Chemical Technology and Biotechnology*, 80, 1297-1305.

Zhang, Y. and Banks, C. (2006). A comparison of the properties of polyurethane immobilized *Sphagnum* moss, seaweed, sunflower waste and maize for the biosorption of Cu, Pb, Zn and Ni in cotinuous flow packed columns. *Water Research*, 40, 788-798.

Zhou, J. L. and Kiff, R. J. (1991). The uptake of copper from aqueous solution by immobilized fungal biomass. *Journal of Chemical Technology and Biotechnology*, 52, 317-330.

Zulkali, M. M. D., Ahmad, A. L. and Norulakmal, N. H. (2006). *Oryza sativa* L. husk as heavy metal adsorbent: optimization with lead as model solution. *Bioresource Technology*, 97, 21-25.