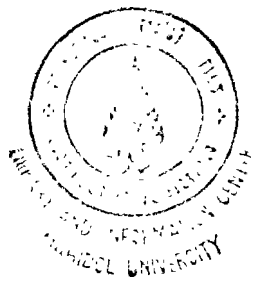

TOXICITY AND BIOSORPTION OF CHROMIUM AND CADMIUM BY USING DUCKWEED *WOLFFIA GLOBOSA* HARTOG & PLAS

BENJAPORN BOONYAPOOKANA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(ENVIRONMENTAL BIOLOGY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2000

ISBN 974-663-776-2

Thesis
entitled

TOXICITY AND BIOSORPTION OF CHROMIUM AND CADMIUM BY USING DUCKWEED *WOLFFIA GLOBOSA* HARTOG & PLAS

Benjaporn Boonyapookana
.....
Miss Benjaporn Boonyapookana
Candidate

Suchart Upatham
.....
Prof.Suchart Upatham, Ph.D.
Major-advisor

M. Kruatrachue
.....
Prof.Maleeya Kruatrachue, Ph.D.
Co-advisor

.....
Asst.Prof.Prayad Pokethitiyook, Ph.D.
Co-advisor

.....
Prof.Liangchai Limlomwongse, Ph.D.
Dean
Faculty of Graduate Studies

Praneet Damrongphol
.....
Assoc.Prof.Praneet Damrongphol, Ph.D.
Chairman
Master of Science Programme
in Environmental Biology
Faculty of Science

Thesis
entitled

TOXICITY AND BIOSORPTION OF CHROMIUM AND CADMIUM BY USING DUCKWEED *WOLFFIA GLOBOSA* HARTOG & PLAS

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Environmental Biology)

on

March 27, 2000

Benjaporn Boonyapookana
Miss Benjaporn Boonyapookana
Candidate

Suchart Upatham
Prof.Suchart Upatham, Ph.D.
Chairman

M. Kruatrachue
Prof.Maleeya Kruatrachue, Ph.D.
Member

Puangpaka Soon
Asst.Prof.Puangpaka Soontornchainaksaeng, Ph.D.
Member

Asst.Prof.Prayad Pokethitiyook, Ph.D.
Member

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

Amaret Bhumiratana, Ph.D.
Dean
Faculty of Science
Mahidol University

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and deep appreciation to my advisor, Professor Dr. Suchart Upatham, for his guidance, invaluable advice, supervision and encouragement throughout. I would like to extend my gratefulness to my co-advisors Professor Dr. Maleeya Kruatrachue, and Assistant Professor Dr. Prayad Pokethitiyok, Department of Biology, Faculty of Science, Mahidol University and Assistant Professor Dr. Puangpaka Soontornchainaksaeng, Department of Plant Science, Faculty of Science, Mahidol University for their guidance. I would like to thank Acharn Karavich Nathalang, lecturer in the Department of Biology for providing specimens of *Wolffia globosa* and for his advice in this study.

I wish to thank the Central Instrument Facilities, Faculty of Science, Mahidol University for the use of the flameless atomic absorption spectrophotometer and Department of Biology for partial financial support during this study.

Finally, my appreciation is also expressed to all of my friends, of whom I will always remember for their help, friendship, encouragement and enjoyment. I am also very grateful to my family for their love and understanding throughout the period of hard work.

Benjaporn Boonyapookana

4037169 SCEB/M : MAJOR : ENVIRONMENTAL BIOLOGY ;
M.Sc. (ENVIRONMENTAL BIOLOGY)
KEY WORD : TOXICITY/BIOSORPTION/CADMIUM/CHROMIUM/
DUCKWEED/*WOLFFIA GLOBOSA*

BENJAPORN BOONYAPOOKANA: TOXICITY AND BIOSORPTION OF CHROMIUM AND CADMIUM BY USING DUCKWEED *WOLFFIA GLOBOSA* HARTOG & PLAS. THESIS ADVISORS: SUCHART UPATHAM, Ph.D., MALEEYA KRUATRACHUE, Ph.D., PRAYAD POKETHITIYOOK, Ph.D., 193 p. ISBN 974-663-776-2

The aquatic plant *Wolffia globosa* Hartog & Plas, abundant in nature, is a rootless duckweed, was used for toxicity test and biosorption of cadmium (II) and chromium (VI) in synthetic solutions. *Wolffia globosa* were cultured in 3% Hoagland's nutrient medium which was supplemented with 1, 2, 4 and 8 mg/L of cadmium (II) and chromium (VI) and were separately harvested after 3, 6, 9 and 12 days. The effects of cadmium (II) and chromium (VI) on the biomass productivity and the chlorophyll content in *W. globosa* indicated that there were significant decreases ($P<0.05$) in the biomass productivity and the chlorophyll content when the exposure times to and concentrations of cadmium (II) and chromium (VI) were increased. The accumulation of the above heavy metals in the plant showed that there were significant increases ($P<0.05$) in toxicity level in the plant tissue when the exposure times and concentrations were increased. *Wolffia globosa* exhibited a higher accumulation (higher in BCF) of cadmium (II) than that of chromium (VI), suggesting that this plant species has more a greater propensity to absorb cadmium (II).

The biosorptions of cadmium (II) and chromium (VI) by using dried *Wolffia globosa* biomass were investigated using batch technique. The effects of concentration and pH solution on the adsorption isotherm were measured by determining the adsorption isotherm at initial cadmium (II) and chromium (VI) concentrations from 10 to 400 mg/L and pH values between 1.5 and 6 for chromium (VI) and between 4 and 7 for cadmium (II). The adsorption equilibria were found to follow Langmuir models. The maximum adsorption capacity (X_m) at pH 7 in *W. globosa*-Cd (II) system was estimated to be 80.65 mg/g, while the removal achieved at pH4, pH 5, and pH 6 were lower (35.09, 48.78, and 65.39 mg/g). In *W. globosa*-Cr (VI) system, the maximum adsorption capacity (X_m) at pH 1.5 was estimated to be 73.53 mg/g, while the removal achieved at pH 3, pH 5, and pH 6 were lower (47.39, 33.11, and 12.85 mg/g). The effects of contact times to cadmium (II) and chromium (VI) sorption indicated that cadmium (II) and chromium (VI) were absorbed rapidly and more efficiently at lower concentrations.

4037169 SCEB/M : สาขาวิชา : ชีววิทยาสภาวะแวดล้อม ; วท.ม. (ชีววิทยาสภาวะแวดล้อม)

เบญจภรณ์ บุณยพุกกณะ: การทดสอบความเป็นพิษและการดูดซับของโลหะโครเมียมและแคดเมียมโดยไข่น้ำ (TOXICITY AND BIOSORPTION OF CHROMIUM AND CADMIUM BY USING DUCKWEED *WOLFFIA GLOBOSA* HARTOG & PLAS). คณะกรรมการควบคุมวิทยานิพนธ์: สุชาติ อุปถัมภ์, Ph.D., มาลียา เครือตราชู, Ph.D., ประหยัด โภคฐิติยุกต์, Ph.D., 193 หน้า. ISBN 974-663-776-2

การทดสอบความเป็นพิษและการดูดซับโลหะหนักโครเมียมและแคดเมียมในสารละลายสังเคราะห์โดยใช้ไข่น้ำ (*Wolffia globosa* Hartog & Plas) ซึ่งเป็นพืชน้ำจำพวกแหนไม่มีรากนั้น ได้ทำการทดลองโดยใช้สารละลายอาหาร Hoagland ที่เติมโลหะหนักแคดเมียมและโครเมียมที่ความเข้มข้นต่างๆ ดังนี้คือ 1, 2, 4 และ 8 มิลลิกรัมต่อลิตร โดยทำการเก็บตัวอย่างต้นพืชมาวิเคราะห์ในวันที่ 3, 6, 9 และ 12 ผลการวิจัยแสดงให้เห็นว่าเมื่อเวลาที่ใช้ในการดูดซับและความเข้มข้นของโลหะหนักแคดเมียมและโครเมียมเพิ่มขึ้น ผลผลิตมวลชีวภาพและปริมาณคลอโรฟิลล์ในไข่น้ำจะลดลง และการสะสมของโลหะหนักในเนื้อเยื่อพืชจะเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ไข่น้ำสามารถสะสมโลหะหนักแคดเมียมได้มากกว่าโลหะหนักโครเมียม แสดงให้เห็นว่าไข่น้ำมีความสามารถในการเลือกจับโลหะหนักแคดเมียมได้ดีกว่า

การทดลองการดูดซับโลหะหนักแคดเมียมและโครเมียมโดยใช้ไข่น้ำที่อบแห้งนั้น ได้ทำการทดลองโดยใช้วิธี batch ความเข้มข้นและค่าพีเอชของสารละลายที่มีผลต่อการดูดซับจะวัดจากความเข้มข้นเริ่มต้นของสารละลายโลหะหนักที่ 10 ถึง 400 มิลลิกรัมต่อลิตร และค่าพีเอชระหว่าง 1.5 ถึง 6 สำหรับโลหะหนักโครเมียม และค่าพีเอชระหว่าง 4 ถึง 7 สำหรับโลหะหนักแคดเมียม สมดุลย์ของการดูดซับจะเป็นไปตามแบบจำลองของ Langmuir ไข่น้ำสามารถดูดซับโลหะหนักแคดเมียมได้มากที่สุดที่ค่าพีเอชเท่ากับ 7 โดยมีค่าการดูดซับสูงสุดคือ 80.65 มิลลิกรัมต่อกรัม ในขณะที่ ค่าพีเอชเท่ากับ 4, 5 และ 6 จะมีค่าการดูดซับสูงสุดต่ำกว่า ได้แก่ 35.09, 48.78 และ 65.39 มิลลิกรัมต่อกรัม ตามลำดับ ในขณะเดียวกันไข่น้ำสามารถดูดซับโลหะหนักโครเมียมได้มากที่สุดที่ค่าพีเอชเท่ากับ 1.5 โดยมีค่าการดูดซับสูงสุดคือ 73.53 มิลลิกรัมต่อกรัม ในขณะที่ค่าพีเอชเท่ากับ 3, 5 และ 6 จะมีค่าการดูดซับสูงสุดต่ำกว่า ได้แก่ 47.39, 33.11 และ 12.85 มิลลิกรัมต่อกรัม ตามลำดับ สำหรับผลของเวลาที่มีต่อการดูดซับโลหะหนักแคดเมียมและโครเมียมในสารละลายนั้น พบว่ามีการดูดซับอย่างรวดเร็วและจะมีประสิทธิภาพในการดูดซับสูงเมื่อทดลองกับสารละลายโลหะหนักที่มีความเข้มข้นต่ำ

CONTENTS

LIST OF TABLES

Page

Page

LIST OF FIGURES

Page

Page

Page

CHAPTER I

INTRODUCTION

During the past two centuries, as the world's population has grown to ten times its former size, heavy metals are the cause of a great deal of water pollution problem and pose a major threat to the health and well-being of millions of people and global ecosystems. "Heavy metals" are a general collective term applying to the group of metals and metalloids with an atomic density greater than 6 g/cm^3. Unlike most organic pollutants, heavy metals occur naturally in rock-forming and ore minerals, and so there is a range of normal background concentrations of these elements in soils, sediments, waters, and living organisms (1). The primary production of heavy metals in 1930 and 1985 has increased over the 55-year period. Nickel showed the greatest increase (35 times), followed by chromium (17 times) and cadmium (14 times), whereas that for mercury production had not even doubled (2). The uses of heavy metals are in electroplating other metals or alloys for protection against corrosion, and in the manufacture of storage batteries, glass ceramic, pigment in paint, leather tanning agents, and steel processing (3).

The release of various heavy metals into aquatic environments is a worldwide problem of increasing magnitude. Primary sources of these metals are stack emissions and wastewater from zinc smelting, incineration of plated metals, and coal fire power plants. Additional sources of these metals are sewage sludge and municipal wastewater that are contaminated through industrial sources. Heavy metals produced undesirable effects, even if they are present in extremely minute quantities, on humans, animals,

and plants life. The toxic effects have been known for a very long time. For the affected species, heavy metals can affect their survival, reproduction, physiological change, and also behavior. For example, in plants, the most general symtoms of cadmium are stunting and chlorosis (4). In humans, acute exposure to cadmium leads to nausea, vomiting, salivation, diarrhea, and muscular cramps (5). Chromium reduced growth and chlorophyll content in some aquatic plants (6). Zinc, lead and mercury affected the nervous system, kidneys, liver and caused respiratory, gastro-intestinal disorders in humans and animals (5,7). Because of the widespread use of these metals in several industries in Thailand, cadmium (II) and chromium (VI) were selected as toxicants for the present study.

There are several conventional methods used at present to polish the contaminated water that contains heavy metals, including such different techniques as chemical precipitation, electrodeposition, solvent extraction, ultrafiltration, ion exchange resins, and other methods which are expensive and frequently inefficient to reach the minimum desirable metal concentrations (8). The use of aquatic plants to remove heavy metals is simple, economic, and environmentaliy friendly technologies. Some plants have a natural ability to absorb and hyperaccumulate trace element in their tissue (9,10). This ability is being harnessed to remove toxic heavy metals from contaminated waters in a process referred to as phytoremediation. Several aquatic plants that are highly effective in absorbing and accumulating various toxic heavy metals have been identified in the last two decades and are being evaluated for the phytoremediation of waters polluted with heavy metals.

Lemnaceae (duckweeds) are small floating plants which propagate rapidly, highly sensitive to many surrounding factors and their have potential use as an indicator of water pollution or their utilization in the purification of contaminated water (11). Duckweed plants are divided into four genera, *Spirodela*, *Lemna*, *Wolffiella*, and *Wolffia* (12). *Wolffia* is a widely occurring rootless duckweed and the smallest of all the duckweed species. This plant is easy to harvest, abundantly available, and has a potential for the treatment of water contaminated with heavy metals (12). In the present study, duckweed, *Wolffia globosa* Hartog & Plas (water eggs or khai-nam) was used to determine the toxicity and biosorption of chromium and cadmium.

The objectives of this study were as follows:

1. To determine the chromium (VI) and cadmium (II) toxicity by using *Wolffia globosa*.
2. To determine the chromium (VI) and cadmium (II) uptake potential by using *W. globosa*.
3. To determine the removal of chromium (VI) and cadmium (II) from aqueous solution by dried *W. globosa* biomass.

CHAPTER II

LITERATURE REVIEW

A. Cadmium (Cd)

Cadmium is one of the most toxic heavy metals and is considered non-essential for living organisms. This element is found at low concentrations in natural environments, occurring in the earth's crust at an average concentration of 0.2 mg/kg (5), but human activities have led to increased levels in all the continents, e.g., high concentrations of cadmium have recently been found in kidneys of the penguin from the Antarctic (13). Because of its high toxicity, the presence of cadmium as contaminant in the environment is the severe problem.

1. Sources and chemistry of cadmium

1.1. Sources

Estimates of the worldwide anthopogenic input of cadmium to freshwater range from 2.1 to 1.7×10^3 metric tons per year (Table 2-1). The major specific sources on a worldwide basis are atmospheric deposition, smelting and refining of nonferrous metals, manufacturing processes related to chemicals and metals, and domestic wastewater. Only about 15 % of the atmospheric deposition comes from natural sources, such as volcanoes, windborne soil particles, and biogenic particles (15).

Table 2-1. Worldwide anthopogenic input of cadmium to freshwater (14).

Source	**Input (thousand metric tons/yr)**
Atmospheric deposition	0.9-3.6
Smelting and refining nonferrous metals	0.01-3.6
Manufacturing processes	
• chemicals	0.1-2.5
• metals	0.5-1.8
Domestic wastewater	
• central	0.2-1.8
• noncentral	0.3-1.2
Discharge of sewage sludge	0.1-1.3
Steam electricity production	0.01-0.24
Base metal mining and dressing	0-0.3
Total input	2.1-17

The dominant worldwide source in oceans is atmospheric precipitation, giving 4.0×10^{14} metric tons/yr, followed by river water discharge (3.2×10^{13} metric tons/yr), river-suspended particulate discharge (1.8×10^{10} metric tons/yr), and atmospheric particulate deposition (5×10^{8} metric tons/yr) (16). Hutton and Symon (17) noted that approximately 43 metric tons of cadmium per year entered the UK coastal waters. Of this, 33.3 metric tons came from sewage and sewage sludge,

5.7 metric tons from the production and use of phosphate fertilizers, 2.6 metric tons from nonferrous metal production, and 1.7 metric tons from iron and steel production.

1.2. Chemistry

Cadmium is relatively mobile in aquatic systems, existing as Cd^{2+}, $Cd(OH)_2$ (aq), $Cd(OH)_3^-$, $Cd(OH)_4^{2-}$, and $CdCO_3$, and in various other organic and inorganic complexes. In many freshwater, the affinity of ligands to complex with cadmium follows the order: humic acids, CO_3^{2-}, OH^-, Cl^-, and SO_4^{2-}. The solubility of calcium hydroxide complexes decreases as pH values increase, owing to the formation of solid $Cd(OH)_2$ according to the reaction:

$$Cd^{2+} + 2OH^- \rightleftharpoons Cd(OH)_2$$

Since the 2+ valency state predominates in freshwater, redox potential has little effect on speciation (5).

Sorption to suspended solids such as clay is an important, often dominant fate process in freshwater. Coprecipitation with hydrous iron, aluminum-manganese oxides, and carbonate material also occurs, and periodically dominates fate processes. For example, Samanidou and Fytianos (18) found that the main species in the sediments to the Axios River (Greece) were in association with Fe-Mn hydrous oxides, accounting for 22-43 % of total Cd, followed by associations with carbonate at 14-35 %.

Other processes, such as photolysis and volatilization, are not widely regarded as important in the environmental fate of cadmium.

2. Uses of cadmium

Following its discovery by Stromeyer in 1817, the use of cadmium was developed very slowly in the nineteenth century. Until the end of the First World War, the development of electroplating to protect corrosion in iron and steel led to a greatly increased demand. Due to its cost and waste disposal problems, as well as its toxicity, the use of cadmium in electroplating has decreased to less than half, whereas zinc is used instead. However, cadmium is still used in electroplating.

Nickel-cadmium batteries are the second-largest application. They have an advantage of being able to be operated in any position at very low temperature, and have long life.

Cadmium also is used as pigment in coloring glass, plastics, textiles, paper, rubber, in printing inks, ceramic glaze and fireworks because it is very stable to heat and light. In addition, sizable mounts are used in low-melting-point alloys, similar to Wood's metal, and in automatic fire sprinklers, and relatively smaller uses are in brazing alloys, solders and bearing. Some of cadmium compounds are used in plastics as stabilizer and to improve high-temperature properties.

Because of its ability to converse solar energy to electrical power, cadmium is utilized in semi-conductors and photoelectric cells. Cadmium has a great neutron-absorbing capacity, especially the isotope 113. For this reason, it is used in control rods and shielding for nuclear reactors.

In dentistry, the powder of cadmium is used as an amalgam by the mixtures of one part of cadmium per four parts of mercury. Many cadmium salts, especially the oxide and iodide, are used as anthelmintic in swine and poultry. During

the early 1900s, cadmium salts were used sporadically in treating human syphilis and tuberculosis.

3. Fate and transport of cadmium in environmental media

3.1. Cadmium in the atmosphere

Cadmium is emitted in a minor amount into the atmosphere from industrialization in the forms of dust and aerosol. The deposition of a significant percentage of these particles occurs in the vicinity of point sources, exponentially decreases with a distance but depends upon the meteorological parameters. The total annual input of cadmium into the atmosphere has been estimated to be approximately 7,000 metric tons. With this number, only about 10 % come from natural sources (19). The major sources of atmospheric emission are non-ferrous metal production and processing, fossil fuel-combustion, waste incineration, and iron and steel production.

3.2. Cadmium in the aquatic environment

The input of cadmium into freshwater is due directly to the contribution from wastewater of lead-zinc mining, zinc-cadmium plants, plastic and steel production, nickel-cadmium battery plant, and other miscellaneous sources, such as photovoltaic cells and control rods for nuclear reactor processing. The indirect input of cadmium is from precipitation, atmosphere, washing out from weathering of minerals, soil, sewage sludge deposits, and waste dumps. Cadmium concentrations in industrial effluents may vary from 0 to 1,000 mg/L, whereas that in municipal wastewater is commonly less than 10 g/L (20).

There is an evidence that cadmium contents are associated with fine sediments. The sediments of non-polluted rivers show cadmium contents ranging

from 0.04 to 0.8 mg/kg, whereas in the polluted waters, the level ranges from 30 to 400 mg/kg. The result of cadmium pollution in water is the accumulation in aquatic food chain with an accumulation factor up to 10^4 (19).

3.3. Cadmium in soils and sediments

Cadmium in soil derived from igneous rock, metamorphic rock, and sedimentary rock is less than 1 mg/kg, except in the contamination from the sources contaminated with significantly high cadmium contents, such as black shales. Sources of soil contamination by cadmium are mining and smelting of cadmium and zinc, atmospheric pollution from metallurgical industries, disposal of wastes containing cadmium, such as incineration of plastic container and batteries, sewage sludge application to land, and burning of fossil fuels.

Within soil profiles, cadmium tends to be present at higher concentration in the surface horizon which is partly of the atmospheric deposition, phosphatic fertilizers, and cycling through plants. The relatively high humus content also contributes to the high adsorptive capacity of cadmium in the surface horizon due to the formation of chelates with humic acids. Additionally, cadmium is also bound to clay minerals. In addition, plants also uptake cadmium directly from wet precipitation. The highest cadmium concentration is found in lichens, litter, humus, and moss. Cadmium uptake in plants depend not only on the total cadmium content in soil, but also on the soil profile and the ratio of the cation exchange capacity of the organic and inorganic contents to the total cadmium content of soil (19).

4. Toxic effect of cadmium

4.1. Toxic effect on plants

Cadmium and, in particular, the free cadmium ion are highly toxic to most plant and animal species. The EC_{50} (effective concentration) for duckweed *Lemna minor* is only 0.2 mg/L, compared to 0.45 mg/L for nickel and 1.1 mg/L for copper (21, 22). Similarly, the EC_{50} for the green alga *Selenastrum capricornutum* was only 0.006 mg/L. Although higher tolerances are periodically recorded, it is known that some species can adapt to relatively high cadmium levels. Acute and chronic toxicity to aquatic plants is ameliorated by changing pH and the concomitant effect on the availability of the free cadmium ion. Similarly, marine algae are generally much more tolerant of cadmium than freshwater species, owing to the binding of the free cadmium ion with chloride (23).

4.2. Toxic effect on humans

4.2.1. Intake

Intake of cadmium for a nonoccupationally exposed 70-kg reference man amounts to approximately 0.15 mg/day from food and fluids and <0.001 mg/day through inhalation in nonsmokers. About 0.15 mg are lost per day, mainly though the urine (0.10 mg) and, to a lesser degree, the feces (0.05 mg). The estimated body burden of cadmium is 50 mg, of which 38 mg is located in the soft tissues. These values are doubled for tobacco smokers (5).

4.2.2. Acute toxicity

In humans, acute exposure to cadmium leads to nausea, vomiting, salivation, diarrhea, and muscular cramps. Severe to fatal cases may show

the following symptoms: liver injury, convulsions, shock, renal failure, and cardiopulmonary depression (5).

4.2.3. Chronic toxicity

Renal toxicity, such as proteinuria, is the most common symptom of chronic exposure to cadmium. Renal dysfunction is likely to be displayed in 10% of the population at concentrations of 0.18-0.22 mg Cd/g renal cortex. Any individual with a tissue residue in excess of 0.285 mg/g usually suffers from renal dysfunction. Scott et al. (24), working on human kidneys from the United Kingdom, found that maximum cortex residues, 0.02 mg/g, occurred in 50- to 59-year-olds, followed by those in the 60- to 69- and 40- to 49-year-olds categories. In addition, heavy smokers had about 33% more cadmium in their kidneys than nonsmokers. Nogawa et al. (25) reported that Cd residues in 173 autopsied Japanese reached 0.20 mg/g in the kidney cortex. These individuals came from a highly polluted area and showed kidney damage on autopsy.

B. Chromium (Cr)

Chromium occurs in the earth's crust at an average concentration of 100 mg/kg, principally as minerals in the chromite spinel group. These minerals have the generalized formula (Mg, Fe)O(Cr, Al, $Fe)_2O_3$. Depending on the degree of substitution in the Al, Fe, Cr series, the chromites contain from 13% to 65% Cr_2O_3. Numerous chromium compounds are manufactured from these minerals, most of which contain chromium in the stable 3+ or 6+ state (5).

1. Sources and chemistry of chromium

1.1. Sources

Estimates of the total anthropogenic discharge of chromium to surface waters range from 45×10^3 to 239×10^3 metric tons per year (Table 2-2). The primary sources include domestic waste water from both central and non-central sources, manufacturing processes involving metals, and the dumping of sewage sludge. Abuzkhar et al. (26) showed that the average concentration of chromium in sewage sludge from Tripoli (Libya) was 27.7 mg/kg dry weight, with a range of 0.005-177.4 mg/kg. Similarly, dewatered sludge from a plant in the state of Washington contained 73.1 mg Cr/L(27), while dried sludge from another 25 Washington plants contained residues of 6.610 mg/kg (range 83-137,000 mg/kg) (28). Such values are far higher than those (0.08-8.40 mg/L) found in most landfill leachates (29).

Atmospheric fallout is the seventh most important source of chromium in surface waters, contributing up to 16×10^3 metric tons of chromium per year (Table 2-2). Only about 14% of global atmospheric emissions are from anthropogenic sources, the remainder originating mainly from windborne soil particles and volcanic emissions (15). Chromium is not normally a major contaminant of urban precipitation, even in highly industrialized countries such as Japan (30).

Coastal marine sources are dominated by input from rivers and, to a lesser degree, dredging sludge and dumping of industrial wastes. For example, of the total input of chromium to the Netherland's part of the North Sea (1,600 metric tons per year), 1,520 metric tons come from these three sources annually (31).

Table 2-2. Worldwide anthropogenic input of chromium to freshwater (14).

Source	**Input (thousand metric tons/yr)**
Manufacturing process	
• metals	15-58
• chemicals	2.5-24
• pulp and paper	0.01-1.5
• petroleum products	0-0.2
Domestic wastewater	
• central	8.1-36
• noncentral	6.0-42
Discharge of sewage sludge	5.8-32
Smelting and refining	
• nonferrous metals	3-20
Atmospheric deposition	2.2-16
Base metal mining and dressing	0-0.7

1.2. Chemistry

Although many different oxidation states of chromium exist in the environment, only chromium (III) and (VI) are the most stable (32). The interconversion of chromium (III) and chromium (VI) is controlled by several factors, including the presence and concentrations of chromium species and oxidizing or reducing agents, the electrochemical potentials of the oxidation and reduction

reactions, ambient temperature, light, sorbents, acid-base reactions, complexing agents, and precipitation reactions (33).

Cr^{3+} is classified as a hard acid and forms relatively strong complexes with oxygen donor ligands. These complexes, which are generally stable and kinetically inert, can often be isolated as solids. The principal forms in freshwater include $CrOH^{2+}$, $Cr(OH)_2^{+}$, and $Cr(OH)_4^{-}$. Cr^{6+}, on the other hand, is water soluble, always existing in solution as a component of a complex anion. The anionic species varies with pH, and may be chromate (CrO_4^{2-}), hydroxychromate ($HCrO_4^{-}$), or dichromate ($Cr_2O_7^{2-}$). Dichromate is rare at circumneutral pH, and only becomes common in highly acidic water. At pH>6.5, most Cr^{6+} is present as the chromate ion. Eary and Rai (34) showed that Cr^{3+}could be oxidized to Cr^{6+} by reaction with manganese dioxide. Although the rate of reaction was not appreciably affected by dissolved oxygen, slightly acidic to basic pH water limited the rate of oxidation.

Adsorption of Cr^{6+} by clays, ferric hydroxide, and ferric and manganese oxides is generally a minor fate process, whereas Cr^{3+} is rapidly adsorbed, at least by clays. The rate of adsorption of Cr^{3+} increases with pH to the point where the total amount of bound exceeds that of Cr^{6+} by 30-300 times. This means that although Cr^{6+} is highly mobile in aquatic systems, Cr^{3+} is quickly immobilized in the sediments. Young et al. (35) reported that the adsorption of Cr^{3+} was linearly dependent on soluble metal concentrations, and that subsequent desorption occurred over a period of at least 24 days. That study also showed that more than 50% of Cr^{3+} was reversibly bound, whereas the remainder was tightly bound.

Volatilization, photolysis, and biotransformations do not appear to be important processes in the environmental fate of chromium.

2. Uses of chromium

The principal uses of chromium are in the metallurgical processing of ferrochromium and other metallurgical products, chiefly stainless steel, and to a much lesser extent, in the refractory processing of chrome bricks and chemical processing to make chromic acid and chromate (36).

Chromates are used for the oxidation of various organic materials in the purification of chemicals, in inorganic oxidation, and the production of pigment. In addition, they are also used in photography, photoengraving, and rust and corrosion inhibitors, for example, in diesel engines.

Dichromates are widely used as oxidizing agent, as rust inhibitors on steel, and as wood preservatives. In the last application, they kill fungi, termites, and boring insects. Dichromate is converted to chromic sulfate for leather tanning. In dye industry, dichromate is used as mordant.

A large percentage of chromic acid is used in chrome plating. It is also used in lithography. Because chromite has a high melting point and is chemically inert, it is used in the manufacture of bricks for lining metallurgical furnaces.

Several chromium compounds are used as paint pigment, such as chrome oxide green (Cr_2O_3), as catalysts, as drilling muds, and in photochemical reactions. They are also present as trace in cement.

3. Fate and transport of chromium in environmental media

3.1. Chromium in the atmosphere

Due to the extremely high boiling point of chromium (2676°C), gaseous chromium is rarely encountered. The atmospheric transformation and transport of chromium largely occurs in the liquid phase and solid phase or, more generally, aerosols (37). Thus, chromium emitted into the atmosphere can be particle-bound or dissolved in droplets. Most aerosols generated by physical processes, such as abrasion or bubbling, have a mass median aerodynamic diameter (MMAD) significantly larger than 10 μm. However, emission factors published by the USEPA for particulate matter less than 10 μm (PM_{10}) suggest that mechanical actions on road and construction sites may contribute significant PM_{10} emissions. The droplets formed during electroplating have an MMAD of around 100 μm, while those formed from cooling towers (where chromates have been used as antifouling and antirusting agents) are even larger (38). Bonin et al. (39) found that 90% of the mass of particles released from baths were from 7 and 25 μm in diameter.

Chromium entrained in aerosols may be removed from the atmosphere by both dry deposition and wet deposition, both of which are particle size-dependent processes. In dry deposition, the particles settle and are captured by the soil or surface waters via gravitational sedimentation, impaction, or interception. The overall deposition velocity of atmospheric chromium is a function of the distribution of chromium within the particle phase and the deposition velocities of chromium containing particles that, in turn, are a function of the diameter of the individual particle (40).

Chromium can also be introduced, or reintroduced, into the atmosphere via the wind resuspension of chromium containing soil particles. For particles with a diameter of <50 μm, the wind resuspension process is induced by both mechanical and wind disturbances that provide sufficient energy to overcome gravitational forces and allow particles to be dispersed by the wind. The rate of resuspension of particles due to the wind's action can be estimated by the approach described by Cohen (40).

The reduction of chromium (VI) is far more likely than oxidation in the atmosphere because of the presence and concentrations of reducing agents as well as the acidity of the atmosphere. Estimates of atmospheric half-life for chromium (VI) reduction to chromium (III) range from 16 h to 4.8 days (39). The few materials capable of oxidizing chromium (III) to (VI), such as ozone, occur in concentrations too low to produce measurable conversions in the atmosphere. Chromate may also react with other metallic species, precipitating as lead or zinc chromate (37).

3.2. Chromium in the aquatic environment

Chromium enters natural waters by weathering of chromium containing rocks, direct discharge from industrial operations, wet and dry deposition, and leaching from soils. In waters, all of the transformations described earlier occur: reduction, oxidation, sorption, desorption, dissolution, and precipitation.

Reduction of chromium (VI) can occur under a variety of conditions, even in the presence of oxygen, if a suitable reducing agent is available (41). The most important naturally occurring reducing agents in waters are organic

substances, hydrogen sulfide, sulfur iron sulfide, ammonium, and nitrite (42). Other potential reducing agents include aqueous V^{2+}, Fe^{2+}, and Fe(II) containing mineral (34). The reduction of chromium (VI) is favored under acidic conditions. Experimental studies have shown a wide range of reduction state. For example, complete reduction of chromium (VI) within one day in the presence of dissolved sulfides, while some of the investigated reductive reactions, such as by S^{2-} or Fe^{2+} ions under anaerobic conditions, were particularly instantaneous (43).

In contrast to the atmosphere, many aqueous environments do contain oxidizing agents, such as MnO_2 and Mn^{3+}, in sufficiently high concentrations to produce measurable yields of chromium (VI). Dissolved oxygen by itself did not induce measurable oxidation of chromium (III), spiked into experimental waters, even after 128 days (33). Oxidation of chromium (III) to chromium (VI) was only noted in one of the natural waters and sediments studied, with half-lives ranging from 2 to 9 years. In all cases, the extent of oxidation did not exceed 15% of the initial chromium (III) present. However, the oxidation of chromium (III) can be inhibited by competing substances in natural water (44). Due to kinetic limitations, local heterogeneity, and local catalytic effects of organisms, redox equilibrium conditions may not be attained in many environmental systems, and reduction and oxidation can both occur (42). Except in estuaries, chromium concentrations in seawater are dominated by chromates, probably due to the generally oxidizing conditions in the ocean and low suspended particulate concentration (44).

3.3. Chromium in soils and sediments

The elemental compositions of soils and sediments are influenced by the composition of the parent rock from which they are formed. Thus, the natural concentrations of chromium varies greatly (44). As chromium is weathered from minerals, most will initially be present in the trivalent state, which may be sorbed on hydroxides. Naturally occurring chromates are rare and found only in highly oxidizing environments (45). Thus, the presence of chromate in soils and sediments is almost always the result of human activities.

Chromium input to soils and sediments from anthropogenic sources occur indirectly due to atmospheric deposition (46) but is more commonly due to the dumping of chromium bearing liquid or solid wastes from such sources as chromate by products, ferrochromium slag, or chromium plating baths. These can be any combination of chromium (III) or (VI) of various solubilities. Once in the soil or sediment, chromium can undergo a variety of transformations, such as oxidation, reduction, sorption, precipitation, and dissolution.

Only a few oxidants present in soils and sediments are capable of oxidizing chromium (III) to chromium (VI). The oxidation of chromium (III) by MnO_2 has been shown to occur in soils and oxic sediments but not in anoxic sediments (34). Oxidation of chromium (III) by dissolved oxygen has been found to be insignificant when compared with MnO_2, which is the most likely oxidant of chromium (III) in soils. The rest of the chromium (III) may remain reduced for long periods of time, even in the presence of electron accepting manganese oxides, perhaps because soluble chromium (III) can form complexes with low molecular weight

organic molecules and then be oxidized where redox conditions are optimal. It is also worth noting that the addition of organic residues potentially as a remediation strategy for chromium (VI) contamination to soils containing high levels of oxidized manganese may result in the formation of unstable Mn (III) organic complexes that not only temporarily prevent chromium (III) oxidation but also promote the desired reduction of chromium (VI) (44).

4. Toxic effect of chromium

4.1. Toxic effect on plants

Cr^{6+} is generally only moderately toxic to algae and other aquatic plants. Wang (21) reported that the EC_{50} for duckweed *Lemna minor* was as high as 35 mg/L, giving a maximum permissible concentration of 3.5 mg/L for standard setting purposes. In a related study, Wang (22) showed that these toxic effects were not ameliorated by the presence of particulate matter, either organic and inorganic, under the test conditions. Although it is likely that ligands do affect the toxicity of both Cr^{3+} and Cr^{6+} to aquatic plants under some conditions, no definitive data are available to confirm this point.

4.2. Toxic effect on humans

4.2.1. Intake

Chromium is an essential trace element, forming part of the antidiabetogenic factor, which is essential in the metabolism of insulin. The minimum daily intake is approximately 0.05 mg, through either food or water (5).

Intake of chromium for a nonoccupationally exposed 70-kg reference man comes to 0.03 to 0.1 mg/day from food. Another 0.06 mg/day comes

from drinking water, assuming the daily consumption of water in 2 L with an average chromium concentration of 0.03 mg/L. Intake from inhalation is small,<0.001 mg/day in nonoccupationally exposed population (5).

4.2.2. Acute toxicity

Cr^{6+} compounds are more toxic than Cr^{3+} compounds in humans. Acute exposure to Cr^{6+} produces nausea, diarrhea, liver and kidney damage, internal hemorrhage, dermatitis, and respiratory problems. Cases of acute poisoning by Cr^{3+} compounds are extremely rare, reflecting their low toxicity to the human population (5).

4.2.3. Chronic toxicity

Chronic exposure to Cr^{3+} is often associated with allergic content dermatitis, skin ulcers, nasal membrane and septum irritation, pulmonary congestion and edema, perforated eardrums, and nephritis. No cases of chronic toxicity following ingestion of Cr^{3+} appear to have been reported (5).

C. Duckweed

Duckweed are the smallest flowering plants. They grow as small colonies of plants floating on the surfaces of quiet bodies of water. Growing vegetatively, their multiplication can be extremely rapid, given the proper conditions. These plants are almost all leaf, having essentially no stem tissue, and only one or a few, very fine roots. In nature, duckweed serve as food for many species of fish and aquatic birds. They can tolerate and grow under a wide range of conditions, including on water

polluted with high concentrations of bacteria and some agricultural wastes. These characteristics have brought the duckweed to the attention of environmental engineers and agriculturists alike.

1. Biology of duckweed

Duckweed species are found worldwide and often seen growing in thick, blanket-like mats on still, nutrient rich fresh and brackish waters. They are monocotyledons belonging to the botanical family Lemnaceae and are classified as higher plants, or macrophytes, although they are often mistaken for algae. The family consists of four genera, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*, among which about 40 species have been identified so far.

All species occasionally produce tiny, almost invisible flowers and seeds, but what triggers flowering is unknown. Many studies of duckweed cope with low temperatures by forming a special starchy "survival" frond known as a turion. With cold weather, the turion forms and sinks to the bottom of the pond where it remains dormant until rising temperatures in the spring trigger resumption of normal growth.

1.1. Morphology

Duckweed species are the smallest of all flowering plants. Their structural and functional features have been simplified by natural selection to only those necessary to survive in an aquatic environment. An individual duckweed frond has no leaf, stem, or specialized structures; the entire plant consists of a flat, ovoid

frond as shown in Figure 2-1. Many species may have hair-like rootlets which function as stability organs.

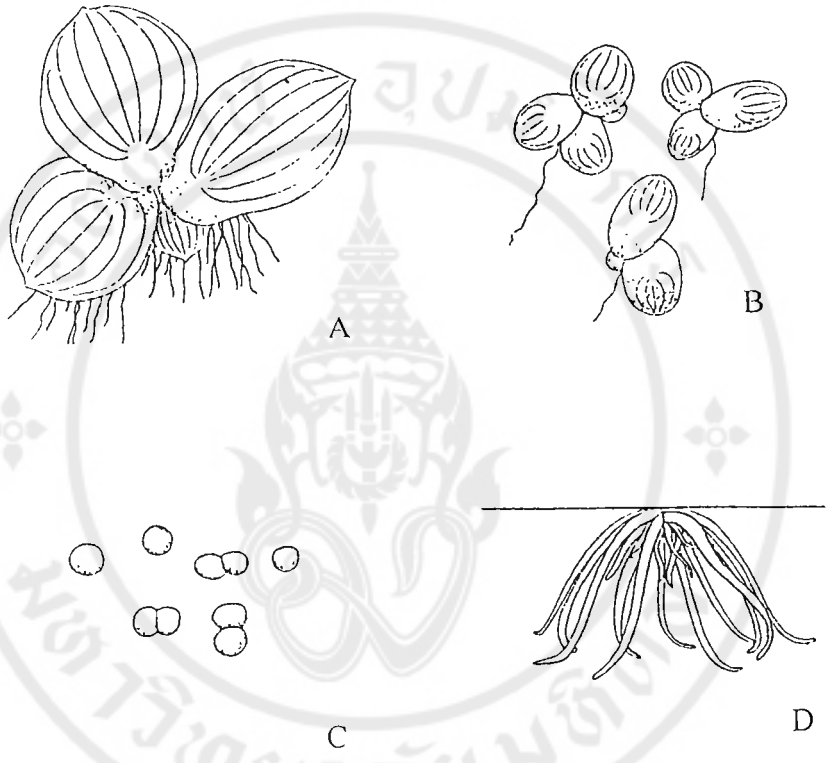

Figure 2-1. Duckweed, the smallest flowering plants genera: A. *Spirodela* × 4; B. *Lemna* × 4; C. *Wolffia* × 4; D. *Wolfiella* × 4 (47).

Species of the genus *Spirodela* have the largest fronds, measuring as much as 20 mm across, while those of *Wolffia* species are 2 mm or less in diameter. *Lemna* species are intermediate size, at 6-8 mm (47). Compared with most plants, duckweed fronds have little fiber, as little as 5 percent, in cultured plants because they do not need structural tissue to support leaves or stems. As a result, virtually all tissue is metabolically active and useful as a feed or food product. This important characteristic contrasts favorably with many terrestrial crops, such as soybeans, rice, or maize, most of whose total biomass is left behind after the useful parts have been harvested.

1.2. Distribution

Duckweed species are adapted to a wide variety of geographic and climatic zones and can be found in all but waterless deserts and permanently frozen polar regions. Most, however, are found in moderate climates of tropical and temperate zones. Many species can survive temperature extremes, but grow fastest under warm, sunny conditions. They are spread by floods and aquatic birds.

Duckweed species have an inherent capability to exploit favorable ecological conditions by growing extremely rapidly. Their wide geographic distribution indicates a high probability of ample genetic diversity and good potential to improve their agronomic characteristics through selective breeding. Native species are almost always available and can be collected and cultivated where water is available, including moderately saline environments.

1.3. Growth conditions

The natural habitat of duckweed is floating freely on the surface of fresh or brackish water sheltered from wind and wave action by surrounding vegetation. The most favorable circumstance is water with decaying organic material to provide duckweed with a steady supply of growth nutrients and trace elements. A dense cover of duckweed shuts out light and inhibits competing submerged aquatic plants, including algae.

Duckweed fronds are not anchored in soil, but float freely on the surface of a body of water. They can be dispersed by fast currents or pushed toward a bank by wind and wave action. If the plants become piled up in deep layers, the lowest layer will be cut off from light and will eventually die. Plants pushed from the water onto a bank will also dry out and die. Disruption of the complete cover on the water's surface permits the growth of algae and other submerged plants that can become dominant and inhibit further growth of a duckweed colony.

Duckweed can grow in full sunlight as well as dense shade. Optimum growth is determined to be between 20 and 30°C, although serious effects will occur with temperatures ranging from between 35 to 40°C. They are more cold tolerant than other aquatic vascular plants, and can sustain temperatures as low as 7°C for normal, practical growth. Under freezing conditions, duckweed will lay dormant on the pond bottom until warmer conditions return. A full, thick mat of duckweed may have a temperature of about 10°C above ambient air conditions due to radiation (48).

Duckweed can tolerate a wide pH range, but survives best between pH values of 4.5 to 7.5. The pH values greater than 10 will have serious effects on growth, and can limit the cover capacity across a pond or water body. A diurnal pH of 10 is possible in ponds only partially covered with duckweed because of algal activity (48).

High metal concentrations will inhibit certain species of duckweed growth, as well as PCB's, and ethylene. Growth will also be inhibited by nitrogen deficient wastewater or by filamentous algae or fungus (48).

To cultivate duckweed, a farmer needs to organize and maintain conditions that mimic the natural environmental niche of duckweed: a sheltered, pond-like culture plot and a constant supply of water and nutrients from organic or mineral fertilizers. Wastewater effluent rich in organic material is a particularly valuable asset for cultivating duckweed because it provides a steady supply of essential nutrients and water.

1.4. Productivity of duckweed

Duckweed reproduction is primarily vegetative. Daughter fronds bud from reproductive pockets on the side of a mature frond. An individual frond may produce as many as 10 generations of progeny over a period of 10 days to several weeks before dying. As the frond ages, its fiber and mineral content increases, and it reproduces at a slower rate.

Duckweed plants can double their mass in less than two days under ideal conditions of nutrient availability, sunlight, and temperature. This is faster than almost any other higher plant. Under experimental conditions, their production rate can approach an extrapolated yield of four metric tons/ha/day of fresh plant biomass, or about 80 metric tons/ha/yr of solid material. This pattern more closely resembles the exponential growth of unicellular algae than that of higher plants and denotes an unusually high biological potential (49).

Sutton and Ornes (50) reported that a mixed population of *Lemna minor* L. and *L. gibba* L. in static sewage effluent resulted in an extrapolated yield of 2.56 to a high of 14.6 tons/ha/yr during an 8 week growth period and maximum yield of *Spirodela polyrhiza* was 6.94 tons/ha/yr.

The relative growth rate of the duckweed *Lemna minor* under conditions of increasing biomass density in manured and unmanured ponds varied. Under unmanured conditions and with a density of up to 1.0 kg fresh weight/m^2, crop yield and relative growth rate were higher for *L. minor* than for *L. gibba*. At higher densities, both clones grew poorly. In the manured pond at very low densities, the growth rate of *L. minor* was considerably higher than that of *L. gibba*, although absolute yield was very low. However, at densities ranging from 1 to 4 kg/m^2 *L. gibba* produced both a higher yield and a higher relative growth rate than *L. minor*. Evidently, *L. gibba* was not only capable of utilizing organic wastes, but was also less affected by increased biomass density of up to 2 kg/m^2 (51).

Duckweed are more productive when grown in nutrient-laden water than most terrestrial agricultural crops. For a small lagoon system and in outdoor tanks, the yield of duckweed was found to be 10 to 12 and 20 tons dry weight/ha/yr, respectively (52).

Sewage effluent is rich in nutrients and therefore can serve as a culture medium for duckweed *Wolffia arrhiza* (L.) Horkel ex Wimmer. Growth rate and total biomass production of *W. arrhiza* at three dilutions of primary treated sewage effluent were investigated. The efficacy of the harvested weed as a food for carps was tested in the confined water of 180 m^2 cement cisterns with 100% sewage effluent. The total extrapolated production of duckweed was 100.5 tons/ha/yr (53).

1.5. Nutritional value

Fresh duckweed fronds contain 92 to 94% water. Fiber and ash content is higher and protein content lower in duckweed colonies with slow growth. The solid fraction of a wild colony of duckweed growing on nutrient-poor water typically ranges from 15 to 25% protein and from 15 to 30% fiber. Duckweed grown under ideal conditions and harvested regularly will have a fiber content of 5 to 15 percent and a protein content of 35 to 45%, depending on the species involved.

Duckweed protein has higher concentrations of the essential amino acids, lysine, and methionine, than most plant proteins and more closely resembles animal protein in that respect. Crude protein content of duckweed, as reported in the literature, varied from 16 to 45% on a dry weight basis. Crude protein contents were 16.1% for *Lemna* sp. under natural condition, 16.2 to 25.7% for

Spirodela polyrhiza (L.) Schleiden, *L. gibba*, *L. minor,* and *Wolffia arrhiza* grown under field conditions (51). An average of 38.2% and a maximum of 43.5% were reported for *S. polyrhiza* cultivated on cattle manure (52).

The crude protein contents of duckweed reported in the literature ranged from <10 to >40% on a dry matter basis (51). Higher crude protein concentration were reported for plants cultivated in nutrient-rich water, but concentrations greater than 30% may not be attainable over extended periods of time in large scale systems. The mean crude protein contents of *Lemna* and *Spirodela* cultivated in septage fed ponds ranged from 24-29% on a dry weight basis.

The essential amino acid level of duckweed was also high. Sewage grown duckweed was reported to have an amino acid profile similar to soybean meal. Duckweed also has fairly high concentrations of the pigments xanthophyll and carotene which deepen the color of the egg yolk of poultry and the skin color of red tilapia (54).

Culley and Epps (55) reported that duckweed contain 6% fat and 7 to 10% fiber. The nutritional value of duckweed is higher than other agricultural crops. Maciejewska et al. (56) compared the crude protein content of *Lemna minor* with cereals, and found that duckweed was the best in terms of protein content which was rather high in albumins but low in globulin. Hillman and Culley (57) compared the crude protein content of duckweed with some agricultural crops (soybean, cotton, seed, peanut, alfalfa hay) and showed that duckweed has the second highest crude protein content (Table 2-3).

Table 2-3. Comparison of annual protein productivity of duckweed and selected crops.

Plants	Percentage					
	Annual tons/acre	**Crude protein**	**Fat**	**Fiber**	**Ash**	**Relative protein production per acre/year**
Duckweed (dry)	7.85	37.0	5.0	7.5	11.0	100.0
Soybeans (dry seed)	0.71	41.7	19.2	5.8	5.4	10.2
Cottonseed (dry)	0.34	24.9	24.7	18.2	3.8	2.9
Peanuts with skin and hulls (dry)	0.70-1.39	23.6	37.9	21.1	3.2	5.7-11.3
Alfalfa hay (sun cured)	1.95-7.00	17.0	1.9	30.6	9.9	11.4-38.3

Cultured duckweed also has high concentrations of trace minerals and pigments, particularly beta carotene and xanthophyll, that make duckweed meal an especially valuable supplement for poultry and other animal feeds. The total content of carotenoids in duckweed meal is 10 times higher than that in

terrestrial plants; xanthophyll concentrations of over 1,000 ppm were documented in poultry feeding trials in Peru. This is economically important because of the relatively high cost of the pigment supplement in poultry feed (57).

2. Importance of duckweed

2.1. Duckweed and wastewater treatment

In comparison with other macrophyte based treatment systems, *e.g.*, those using the floating water hyacinth *Eichhornia crassipes* (L.) Solms or rooted emergent helophytes, sewage treatment systems using duckweed are reported to have a considerable potential for application in pollutant reduction in various types of wastewater (55, 58, 59, 60, 61, 62). Duckweed may have an advantage because of their very high growth rates compared with other herbaceous angiosperm (63). Therefore, duckweed have been advocated and tested repeatedly for the treatment of wastewater (48, 55, 58, 59).

Several species of duckweed are used in wastewater treatment systems. Duckweed based wastewater treatment systems are inexpensive to install as well as to operate and maintain. They are functionally simple, robust in operation, and they can provide tertiary treatment performance equal or superior to conventional wastewater treatment systems now recommended for large scale applications. Duckweed wastewater treatment systems remove, by bioaccumulation, as much as 99% of the nutrients and dissolved solids contained in wastewater (55). Duckweed systems distinguish themselves from other efficient wastewater treatment mechanisms in that they also produce a valuable, protein rich biomass as a by product (64).

Providing accumulated toxin and heavy metals levels are not high, the harvested duckweed biomass may be used as the sole feed input for freshwater pisciculture, and as up to 40% of poultry feed. This biomass also might be useful for a variety of other domestic animals (55).

A significant benefit of duckweed systems over other lagoon based wastewater treatment systems is that they are capable of efficient removal of influent suspended solids, and they prevent formation of algal suspended solids which are the bane of lagoon system effluent (62). This is achieved through the simple mechanism of shading. A dense layer of floating duckweed plants prevents sunlight from reaching algal populations distributed throughout the water column. Unable to photosynthesize they simply die and precipitate to the pond bottom. Systems with enclosed primary treatment units maintain algal inhibition from the outset and will provide marginally better total suspended solids (TSS) removal than systems with open primary lagoons. In either case, duckweed wastewater treatment systems can consistently bring total final effluent TSS to below 5 mg/L (55).

Duckweed plants are remarkably efficient at removing elements which are, for them, growth nutrients. These include some organic compounds, as well as ions of elements such as nitrogen, phosphorus, potassium, magnesium, calcium, sodium, chlorine, boron, and iron, among others. Vermaat and Hanif (65) studied the performance of common duckweed species (Lemnaceae) and the waterfern (*Azolla filiculoides* Lamk.) on different types of wastewater and concluded that duckweed could reduce total-P from 7-9 mg P/L to 2.4±0.2 mg P/L (77% removal) and could reduce Kjeldahl-N from 14-52 mg N/L to 6.2±0.4 mg N/L (94% removal). Duckweed

can directly absorb and metabolize both complex carbohydrates such as sucrose, fructose, and glucose, as well as organic nitrogenous compounds such as urea and most amino acids (58). Because of high efficiency to absorb the element, duckweed also have been widely used to remove heavy metals from wastewater. Srivastav et al. (66) studied the treatment of chromium and nickel in wastewater by using aquatic plants, *Salvinia* and *Spirodela* and reported that the rate of percentage removal of chromium and nickle ions was observed to be 56-96% and 18-72% after the first 2 and 14 days of contact time by both aquatic plants. Wahaab et al. (67) reported that duckweed *Lemna minor* could remove chromium (III) efficiently from wastewater (75-100% in terms of concentration or load reduction), and Cully and Epps (55) concluded that duckweed provides efficiently a mechanism of biological removal of toxicants from wastewater systems.

2.2. Duckweed as an investigative tool

Duckweed also has been widely used as an investigative tool in ecological, biological, and physiological studies (57). This is because duckweed has three characteristics. The first is its vegetative growth habit. There are two meristems, regions of small actively dividing cells, that alternately produce new fronds. Each frond produces new fronds, and so on. Although every frond finally dies, it yields 10 to 20 (or more) others before doing so. The second characteristic is that fronds do not remain attached indefinitely to form increasingly large and complex structure. Instead, they break into colonies representing only a few vegetative generations, thus they maintain a constant relationship to their environment. This situation is analogous to that in an exponentially growing microbial culture, at least until surface crowding

becomes severe. The third characteristic is an almost total lack of woody tissue. Most of the cells are like those of young or maturing leaves, and little photosynthetic energy is devoted to producing or maintaining any other plant structure. From these reasons, it is not surprising that duckweed has a very high relative growth rate (57). Wang (12) reviewed on duckweed toxicity testing and concluded that duckweed plants were fast growing and widely distributed. They were easy to culture, test, and especially suitable for use in complex effluent bioassays, and for testing herbicide pollution in the aquatic environment, lake and river pollution, sediment toxicity, and the like. Duckweed species, *Lemna minor* and *L. gibba* have been recommended as standard test species.

2.3. Duckweed as an animal feed

The composition of duckweed depends on the nutrient content of the water and the prevailing climatic conditions. Harvested duckweed plants contain up to 43% protein on a dry weight and may be used without further processing as a complete feed for fish.

Duckweed protein has a better array of essential amino acids than most vegetable proteins and more closely resembles animal protein (57). It is, therefore, a source of high quality protein to be exploited for domestic animal production. Duckweed, grown on nutrient rich water, has a high concentration of trace minerals, K, P, and pigments, particularly carotene and xanthophyll, that make duckweed meal an especially valuable supplement for poultry and other animals, and it provides a rich source of vitamins A and B for humans. Bhanthumnavin (68) reported that *Wolffia* could serve as a potential source of inexpensive protein in the Southeast

Asian countries. Its protein content was estimated to be several times greater than that of the local crops, and it also produced more dry matter than traditional crops grown in Thailand. In view of its extremely short doubling time (4 days), the prospect of using *Wolffia* to help solving the food problems in developing countries seems very promising.

2.3.1. Use of duckweed in poultry production

The potential nutritional value of duckweed in poultry diets has long been recognized. Dehydrated duckweed has been used to replace alfalfa (lucerne) meal as a protein source in conventional poultry diets. Chickens fed 10% dehydrated duckweed had superior weight gains to those fed conventional protein sources. However, variable responses are often reported depending on the source of the duckweed which can be high protein, low fiber or low protein/high fiber depending on the nutrients in the growth medium.

Many studies have demonstrated that the growth of very young broiler chickens may be retarded with increasing levels of *Lemna gibba* dehydrated meal in the diet, whereas layer hens produced effectively (69, 70), and older broiler birds had excellent growth when fed relatively high levels of *L. gibba* meal. Thus, there is a need to be conservative when using *Lemna* protein meals with young birds.

Undoubtedly duckweed can replace conventional protein and energy sources in chicken diets up to 25% of the total dry matter without jeopardizing a high level of production. This indicates that duckweed of known

chemical analysis can be used in least cost ration formulation for both poultry meat and egg production.

2.3.2. Use of duckweed in fish production

A major limitation to fish farming is that meals high in protein with high biological value are expensive and often locally unavailable. Duckweed grown on water with 10-30 mg NH_3-N/L has a high protein content (around 40%) of high biological value (57). Fresh duckweed is highly suited to intensive fish farming systems with relatively rapid water exchange for waste removal (71), and duckweed is converted efficiently to liveweight by certain fish including carp and tilapia (72, 73).

In recent more detailed studies in Thailand, Hassan and Edwards (73) have grown tilapia in static water concrete tanks and fed them with two species of duckweed, *Lemna perpusilla* L. and *Spirodela polyrrhiza* at levels of 0, 25, 50, or 75 g duckweed DM per kg wet weight of fish. The duckweed was relatively low in protein (approx 24% CP). The *Spirodela* was poorly consumed, whereas *Lemna* was rapidly ingested by fish. Van Dyke and Sutton (72) conducted a feeding trial to study the digestion of duckweed (*Lemna* spp.) by carp and concluded that grass carp digested duckweed surprisingly well. Bhatia (74) found that *L. minor* was consumed by herbivorous fish to the extent of 60 to 70% of the body weight. However, it was observed that advanced fry and fingerlings of Chinese grass carp accepted duckweed, such as *Wolffia arrhiza*, *L. trisulca* L. and *S. polyrhiza* but preferred zooplankton.

Advanced fingerlings, juveniles, and adults were predominantly herbivorous and appeared to relish duckweed and cleared infestations within a few days.

In a study in which *Oreochromis niloticus* was fed *Lemna*, *Hydrilla*, and *Chara* in both ponds and cages at a rate of at least 30% total body weight, *Lemna* rendered a better result than *Hydrilla* and *Chara* in terms of growth rate and protein content of fish flesh. The food conversion rate of *Lemna* ranged from 33 to 39 on a wet weight basis (75).

Hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) were cultured at high densities in an experimental recirculating unit over a period of 89 days with duckweed (*Lemna gibba*) or a combination of duckweed and commercial pellets as feed. When fed duckweed alone, feed intake rate was low, feed conversion ratio good (1:1) and relative growth rate poor (0.6% of body mass/day). Sixty five percent of duckweed consumed was assimilated and 26% converted to fish. When the fish were fed pellets in addition to duckweed, the rate of duckweed consumption decreased and growth rate of fish doubled with a feed conversion ration between 1.2 and 1.8. Fish fed 70% of the mixed diet performed similarly to fish grown on pellets but had a better feed conversion ratio (71).

D. Aquatic plant toxicity test

Toxicity test is a test conducted to obtain information about the adverse effects of an agent on tested organism. Different organisms respond in different ways. The

most extreme responses include death, reduction of reproductive capacity and inhibition of certain enzyme system necessary for normal metabolism.

1. Rationale for aquatic plant toxicity test

Aquatic and terrestrial macrophytes, along with algae, are essential components of aquatic and terrestrial ecosystems. They produce oxygen and organic substances on which most other life forms depend. Plants provide food, shelter, and nesting habitats for other organisms, including insects, invertebrates, fish, amphibians, birds, and mammals. Plants are essential in the processes of nutrient cycling and soil and sediment stabilization. The effect on plants can directly affect structure and function of an ecosystem, resulting in oxygen depletion, decreased primary productivity, increased surface runoff and soil erosion, and degradation of wildlife habitat.

The use of aquatic plants in water quality assessment has been common for years as in-situ biomonitors (sentinel species). Aquatic plants also have been used frequently to remove suspended solids, nutrients, heavy metals, toxic organics, and bacteria from acid mine drainage, agricultural landfill, and urban storm water runoff. In contrast, they have not been commonly used as test species in toxicity tests designed to evaluate the hazard of potential pollutants (76).

Phytotoxicity testing also may be warranted, although not routinely practiced, in the environmental assessment of toxic substances and complex effluents. Effluent components that may be practically nontoxic to fish, crustaceans, and daphnids can injure and kill aquatic vegetation when discharged into receiving waters. In the field, submerged plant species appeared to be more sensitive to effluent toxicity

and less able to compete with more tolerant emergent plant species (77). Thus, effluents could have important implications on the richness and distribution of plant species in an effluent impact zone; the impact can be demonstrated only by appropriate phytotoxicity tests and by extensive field monitoring.

Many reports suggested that lethal effects of toxicants on plants can have significant ecological and economic effects (78). Even sublethal effects can have significant consequences on food production and natural vegetation (79). In addition, plants have a high capacity for bioconcentration and bioconversion, with potentially adverse implications for organisms higher up the food chain.

2. Duckweed toxicity testing

Three base-set tests are currently used for freshwater environmental monitoring, effluent toxicity testing, toxicity assessment of a product. They are tests of *Pimephales promelas*, *Daphnia magna* (or *Daphnia pulex*), and *Selenastrum capricornutum*. These tests are generally applicable to important environmental laws. They are used routinely by federal, industries, and consulting laboratories. Recently the duckweed toxicity test has received much attention. Duckweed is an aquatic plant and is relevant to many aquatic environments, including lakes, streams, effluent, rain, and sediment. Additionally, duckweed is a vascular, flowering plant which provides additional information unlikely to be obtained by the three base-set tests (12).

There are indications that duckweed is tolerant to environmental toxicity, and it is commonly referred to as the carp of plant species. Clark et al. (80) reported that duckweed (*Lemna perpusilla*) was the most abundant macrophyte inhabiting a coal-ash retaining basin of a coal fired power plant, occupying

approximately 95% of the surface area. Seto et al. (81) reported that cadmium caused chlorosis and death to *L. gibba*. Cadmium toxicity, however, was greatly influenced by the nutrient levels in which the specimens were cultured. Duckweed was much more tolerant to cadmium when the plant was cultured in a higher nutrient solution than in a low nutrient solution.

There are other indications, however, suggesting that duckweed is sensitive to toxicity. Wang (22) conducted a series of duckweed toxicity tests on 16 aquatic pollutants. Of special interest is the comparison of the sensitivity to metal toxicity of duckweed and fish species. The results indicated that duckweed was as sensitive or sometimes more sensitive to metal toxicity than fish species.

The preceding discussion appears to be contradictory. On the one hand duckweed plants are described as tolerant to environmental toxicity, while on the other hand the plants are mentioned as sensitive to toxicity. The contradiction can be explained on the basis that the plants may be highly adaptive. Under optimum culture conditions, free of contamination, the plants are responsive to environmental toxicity when they encounter it. At sublethal range, the duckweed plants may adapt and develop resistance quickly due to their fast growth rate. Several reports (82, 83, 84) indicate that fish can develop tolerance. Perhaps the same induced tolerance is the reason that duckweed plants are sometimes insensitive to environmental toxicity.

Many duckweed species have been studied in toxicity test, primarily of the *Lemna* and *Spirodela* genera. *Lemna minor* and *L. gibba* have been recommended as standard test species. Differences in duckweed test methodology occur with regard

to species, test types, test vessels, control tests, nutrient media, end points, and applications.

2.1. Species

Many duckweed species have been studied: *Lemna minor, L. gibba, L. valdiviana, L. polyrrhiza, L. perpusilla, L. paucicostata, Spirodela polyrrhiza, S. punctata,* and *S. oligorhiza*. There are relatively few data for comparative studies on different species. Under low irradiance, Takemoto and Noble (85) found that sulfite toxicity was most pronounced for *L. gibba*, less marked for *S. oligorhiza*, and not observed with *L. valdiviana*. Staves and Knaus (86) reported that *S. polyrrhiza* was least tolerant to chromium, while *S. punctata* and *L. gibba* were more tolerant to concentrations above 10 mg/liter.

The selection of test species is usually based on specimen availability, sensitivity to the toxicant, historical data, and the like. To encourage test standardization and comparability, two species have been recommended. The American Society for Testing and Materials recommends *Lemna gibba*, while Standard Methods for Examination of Water and Wastewater endorses *L. minor*. Although the use of standard species is encouraged, other species should be tested so that new information may help to evolve test methods in the future (12).

2.2. Test types

Duckweed toxicity tests can be used in static, renewal, or flow-through experiments. Static experiments are simple and economical. They are especially useful for screening tests of unknown sample or samples which contain

toxic metals. In general, flow-through and renewal experiments are useful for samples containing volatile or biodegradable compounds (21,22).

2.3. Test vessels

Several different test vessels have been used: glass beakers, flat bottomed test tubes, jars, Erlenmeyer flasks, and culture dishes (21, 22, 87, 88). In general, only one type of test vessel should be used in an experiment. Plastic vessels are not recommended because of strong adherence of duckweed plants to the plastic walls. The test vessel should be covered to avoid excessive evaporation of water and test sample.

2.4. Control tests

There are two types of control samples: negative controls and positive controls. Almost all toxicity tests use a negative control where no test substance is present. The negative control serves as a quality control in an experiment as well as a reference point to which a test sample is compared. Negative control values vary from time to time.

Positive controls are less frequently employed, although they are as important as negative controls. A positive control is a sample to which a reference toxicant is added to determine the degree of response over time. Organic and inorganic compounds have been studied as potential reference toxicants, including pentachlorophenol, phenol, sodium dodecyl sulfate, sodium chloride, cadmium, and chromate (89). Wang (90) found that in an 18-month period, the growth of *Lemna minor* underwent cyclic changes in the negative control, while the duckweed response to chromium toxicity was nearly constant. In general, chromium toxicity to *L. minor*

was not ameliorated by differences in water quality factors such as suspended solids or dissolved fraction (21). On the basis of these and other results, chromium (VI) has been recommended as a universal reference toxicant.

2.5. Nutrient media

Duckweed toxicity tests, which generally rely on growth and multiplication as the test end point, require sufficient plant nutrients for optimum conditions. Plant nutrients are uniformly added to control and test samples.

Several nutrient media have been reported. They included the medium used by Ballard (91), Bristol's medium and Jacob's medium as given by McLay (92), Hoagland's, Hunter's, and Bonner-Devirian's media as given by Nasu and Kugimoto (88), and the medium used by Fekete et al. (87). The composition varied widely among these media.

Nasu and Kugimoto (88) found that the pH of the nutrient medium, the concentration and composition of the nutrients in the medium, and the temperature at which cultures were maintained affected the sensitivity of *Lemna paucicostata* to heavy metals. They recommended the use of Bonner-Devirian's medium at temperatures above 25°C. Nasu et al. (11) reported that the absorption of copper and cadmium in *L. paucicostata* was suppressed by the addition of EDTA to the medium. Only 30 μM of EDTA was sufficient to prevent copper absorption at the concentration of 5-10 μM, whereas 400 μM of EDTA was required to prevent cadmium absorption at the same concentration as copper. They found that the growth inhibition of *Lemna* was proportional to the amount of metal absorption.

2.6. End points

Many end point have been used to express duckweed test results. These end points are generally based on the population of duckweed plants: frond number, plant number, root number, dry or fresh biomass, root length, frond diameter, C-14 uptake, chlorophyll content, and the like (49, 52, 87, 93).

The most commonly used test end point is the frond number. Any visible, protruding bud is included in order to avoid individual bias. The frond count can be made repeatedly until accurate results are obtained. This determination is simple, rapid, and nondestructive.

Nasu and Kugimoto (88) indicated that determinations of dry biomass were the least time consuming and least subject to human error. This method, although an improvement by taking into account change in biomass as a toxic response, does not distinguish between live and dead plants.

Duckweed plants can also exhibit many symptoms when they are under stress. These symptoms include chlorosis (loss of pigment), necrosis (localized dead tissue), colony breakup, root destruction, loss of buoyancy, and gibbosity (hump back or swelling). For example, cadmium is known to cause chlorosis (81).

2.7. Applications

Duckweed toxicity tests are highly versatile in the aquatic environment. The tests are applicable to lake, river, or ground water; single chemical compounds or complex effluents from industrial or municipal sources, organic or inorganic compound, rain samples, and sediment samples (21, 22, 87, 90). Wang and

Williams (94, 95) studied the use of phytotoxicity (common duckweed, cabbage, and millet) for determining the effluent toxicity and reported that of the three industrial sources, the effluent samples from a specialty chemical industry were the most toxic. For two samples from this source, the IC_{50} values for duckweed were less than 1.6% effluent concentration. The samples from an agricultural product utilization plant were the least toxic. For these samples, root-growth test failed to obtain IC_{50} values, while the duckweed tests showed IC_{50} values of 91 and 43% effluent concentration. Among the three types of tests conducted, the duckweed reproduction test showed the greatest sensitivity to effluent toxicity, while root-growth test using cabbage and millet had mixed results.

Duckweed can be used to monitor the effect of river water on phytotoxicity of heavy metals. A 75-L water sample from the Illinois River was collected and used to compare metal toxicity in the river water sample and in deionized water (21). Both waters were spiked with the standard plant nutrients and discovered that there were three different types of results. Barium was moderately toxic in the deionized water and nontoxic in the river water. Cadmium was extremely toxic in the deionized water and also substantially toxic, although somewhat less so, in the river water. Chromium (VI) toxicity was more or less the same in the river water and in the deionized water. These results point out the importance of site specific water quality in the regulation of the metal toxicity.

Chromium (VI) toxicity was found to be least affected by water quality of water samples (90). The chromium ion, consequently, was recommended as a general reference toxicant used in different water samples. Chromium (VI) ion,

furthermore, was reported to be removed in a significant quantity by *Lemna minor*, suggesting important effects on metal dynamics in a polluted water (6). Staves and Knaus (86) demonstrated that the absorption of chromium (VI) by duckweed was directly related to ambient chromium concentration after 8 days of exposure. Among duckweed species test, *Spirodela polyrrhiza* was the least tolerant of chromium, while *S. punctata* and *L. gibba* were more tolerant at concentrations above 10 mg/L.

Wang (94) further expanded the metal toxicity test by using 59 water samples encompassing river and lake waters. Water quality of these samples ranged from very soft (hardness 40 mg/L as $CaCO_3$) to very hard (hardness over 300 mg/L as $CaCO_3$). In this series of experiment, barium, chromium (VI), and nickel were tested and concluded that barium was the least toxic among the three metals and was influenced the most by water quality of the test samples. Chromium was moderately toxic among the three metals and was influenced the least by the water quality of the samples. Nickel was the most toxic and was influenced moderately by the water quality (96).

Cadmium is a toxic metal commonly found in mining, industrial activities, and also from the exhaust gases of automobiles. Cadmium in aquatic ecosystems can affect vegetation directly by being absorbed. Many studies reported about the toxicity test of cadmium by using duckweed. Sigha et al. (97) studied the modulation of cadmium uptake and toxicity in *Spirodela polyrrhiza* due to malathion. They conducted the experiment by treating fronds of *S. polyrrhiza* with different concentrations of cadmium and malathion singly and in combinations. The results showed that cadmium was more toxic than malathion. The chlorophyll content was

more severely affected by cadmium (EC_{50} of > 1.0 μg/mL); however, its toxicity was ameliorated in the presence of malathion (EC_{50} of 2.0 μg/mL). Devi et al. (98) reported that the toxicity of cadmium that reduced frond reproduction of *Lemna gibba* by 50% (EC_{50}) was found to be 800 ppb. Mohan and Hosetti (99) studied the potential phytotoxicity of lead and cadmium to *L. minor* grown in sewage stabilization ponds. The objective of this study was to develop a phytoassay for the heavy metals toxicity in ecotoxicological studies using *L. minor*. The results suggested that the assay was simple and economical. Similar to fish or aquatic bioassay, the plant *Lemna* may also be used as an indicator species in the bioassay evaluation for aquatic system. The sensitivity of *Lemna* plants in terms of biochemical changes and enzyme activities to cadmium (II) and lead (II) was remarkably noticeable. The presence of cadmium and lead drastically decreased the catalase and protease activity and increased the peroxidase activity. The protein, carbohydrate, and chlorophyll pigment content decreased, whereas the proline content increased with the concentration of both metals. Cadmium was proved to be comparatively more toxic than lead. The antagonistic behavior of cadmium and lead was observed in terms of the sensitivity of *Lemna* plants. Duckweed plants grown in the lowest concentration of cadmium and lead in sewage exhibited metal tolerance by budding after prolonged period of exposure. The results suggested that the biochemical changes and the enzyme activity in *Lemna* plants was a promising indicator of heavy metal toxicity. Duckweed assay should be further explored so that its value can be evaluated when more data are available.

The toxicity testing of other metals by using duckweed has been reported. Wang (100) studied the site specific barium toxicity to common duckweed,

Lemna minor. This study was conducted to test barium phytotoxicity in various water samples, encompassing lake and stream water with a wide variation in water quality. A total of 59 samples were collected from 18 stations. Common duckweed, *L. minor*, was used as the test organism. The results showed that barium toxicity to duckweed was highly dependent on site specific water quality. The barium ion was most toxic to duckweed in waters from Hayes Creek and Horseshoe Lake, with IC_{50} 102 and 107 mg/L Ba, and least toxic in Beaucoup Creek, IC_0 to IC_6 at 400 mg/L Ba. The current water quality standard of 5 mg/L Ba (total) was considered adequate to protect common duckweed in all water tested.

Duckweed has many excellent features for toxicological studies of aquatic systems in its small size, rapid growth rate, and submerged habitat. Duckweed test is especially attractive for colored, turbid, and oxygen demanding samples. Duckweed plant is also an indigenous aquatic macrophyte in many waters, and it can be used as a sentinel to aid field observation. It is suggested that duckweed test be further evaluated for its role as a part of battery of tests for effluent toxicity and environmental pollution.

E. Phytoaccumulation of heavy metals

Phytoaccumulation is the use of plants to uptake heavy metals contaminants from the environment. This occurs through the uptake of contaminants by the roots of a plant into its stems and leaves. Certain plants, called hyperaccumulators, absorb unusually large amounts of metals in comparison with other plants (101). There are

about 400 known plants which can serve as hyperaccumulators for metals, such as nickel, cadmium, chromium, and zinc.

Aquatic plants also may be used in phytoaccumulation. Aquatic plants have been shown to substantially reduce the levels of biological oxygen demand (BOD), nutrients, metals, and other pollutants in waters passing them. There is an evidence that aquatic plants can hyperaccumulate trace elements in their tissues when grown in polluted water. Floating aquatic plants, such as common duckweed (*Lemna minor*) and water velvet (*Azolla pinnata*) have been shown to bioconcentrate heavy metals, such as iron and copper up to 78 times their concentration in the wastewater (102). Pinto et al.(8) demonstrated that water hyacinth (*Eichhornia crassipes*) can remove and recover silver from industrial wastewater with high efficiency in a fairly short time. Member of the duckweed family, Lemnaceae, have been found to accumulate cadmium ions to much greater than external concentrations, sometimes producing metal concentration factor (MCF) as high as 25,000 (103). MCF is a useful parameter to evaluate the potential of the accumulating metals, and this value was calculated on a dry weight basis. This MCF is defined as the ratio of the metal concentration in the plants to the initial concentration of metal in the feed solutions. Srivastav et al.(66) found that the MCF value of *Salvinia* was higher when compared with that of *Spirodela* in the single group of metal ion solutions. The higher value of MCF indicates that *Salvinia* is more effective than *Spirodela* for the accumulation of chromium and nickel in the wastewater treatment. The accumulation of some other heavy metals and trace elements, such as zinc, copper, lead, and cadmium by several species of aquatic plants was demonstrated by Dunbabin and Gowmer (104).

Aquatic plants differ in the extent to which they can accumulate different elements. While some plants were reported to be accumulators of specific metals, such as salvinia (*Salvinia natans*) for mercury and giant duckweed (*Lemna polyrrhiza*) for zinc (7, 105), others were found to be unspecific collectors of various metals. For example, Rai et al.(106) found that aquatic plant species, like coontail (*Ceratophyllum demersum*), giant duckweed (*Spirodela polyrrhiza*), bacopa (*Bacopa monnieri*), and wild rice (*Hygrorrhiza aristata*) were able to accumulate, unspecifically, appreciable amounts of various metals including copper, chromium, iron, manganese, cadmium, and lead. In the same study, other aquatic plant species, such as channel grass (*Vallisneria spiralis*) and alligator weed (*Alternathera sessilis*) were found to accumulate these metals to a lesser extent (106). Jana (107) studied the accumulation of mercury and chromium by three aquatic species (*Eichhornia crassipes*, *Hydrilla verticillata*, and *Oedogonium areolatum*) and reported that the accumulation of chromium was highest in *Eichhornia* (10.21 μmol/g dry wt), followed by *Hydrilla* (9.0 μmol/g dry wt), and *Oeodogonium* (6.02 μmol/g dry wt); that of mercury was maximum in *Hydrilla* (4.21 μmol/g dry wt), followed by *Oeodogonium* (3.35 μmol/g dry wt), and *Eichhornia* (2.79 μmol/g dry wt). The accumulation of chromium and mercury in roots of *Eichhornia* was about twelve and two times higher, respectively, than that in shoots of the genus. Higher accumulation of chromium than that of mercury in the aquatic plants was observed.

The accumulation of heavy metals is dependent on the internal concentration of the metal that the toxic effects resulting in a reduction of this process, the time of exposure and the presence of certain co-ions in the solution. Leborans and Novillo (13)

studied toxicity and bioaccumulation of cadmium in *Olisthodiscus luteus* (Raphidophyceae) and concluded that in the 500 μg Cd/L treatment, the cells of *O. luteus* reached an accumulation of cadmium of 0.90-56.42 fg/cell. The concentration bioaccumulated was in clear relation to the concentration of the metal dissolved in the culture. Therefore, when the concentration of metal is higher, cells are able to bioaccumulate more, until reaching a level at which the toxic effects determine a reduction in the uptake of metal. Mondal and Sen (7) found that the plant, *Salvinia natans*, was effective in the removal of mercury (II) from wastewater. Maximum accumulation was noted within a day and maximum removal (about 90%) was recorded below 5 ppm of mercury (II).

Chandra and Sinha (108) studied the removal of copper and cadmium from water by *Bacopa monnieri*. The objective of this experiment was to determine copper and cadmium accumulation by the plants. The results showed that they were capable of accumulating both metals in single and mixed metal treatments, with high concentration factors for both metals. The aquatic vascular plant (*Ceratophyllum demersum*) was investigated as a potential biological filter for the removal of cadmium from wastewater (109). Plants were grown in and harvested weekly from 0.10 M Hoagland nutrient solutions containing concentrations of cadmium from 0.01 to 1.03 μg Cd/mL. Tissue cadmium was positively correlated to increased concentrations of cadmium in solution. Concentration factors (CF) of cadmium in plants after one week were 13.3 for the 0.01 μg Cd/mL treatment 451.4 for plants treated with 0.04 μg Cd/mL, and 506.5 for plants treated with 1.03 μg Cd/mL. Plants treated with 0.01 μg Cd/mL sustained cadmium concentrations almost 9 folds over those at week one.

However, after five weeks, tissue cadmium concentration in plants exposed to 1.03 μg Cd/mL had decreased 97% compared with the week-one concentration. The results suggested that coontail exposed to very low cadmium concentration (0.01 μg Cd/mL) could take up and accumulate cadmium. However, plants exposed to cadmium at 0.04 μg Cd/mL or above did not accumulate cadmium past one week.

The accumulation of lead and zinc by *Azolla pinnata* (water velvet) and *Lemna minor* (common duckweed) was investigated in solutions enriched with 1.0, 2.0, 4.0, and 8.0 mg/L of these two metal ions, which were renewed on alternate days over a 14-day test period (110). The uptake rate of both metal ions was highest when the initial concentration in the test solution was 1.0 mg/L. The concentration of lead or zinc remaining in the residual solutions after treatment with duckweed or water velvet, at 1.0 and 2.0 mg/L levels, increased with the passage of time. At 4.0 and 8.0 mg/L levels, the concentration of lead and zinc remaining in the residual solutions either continuously increased with the passage of time, or first sharply increased (8-10 days) and then remained almost constant. The presence of one metal ion in the solution decreased the uptake rate of the other; such as when water velvet was kept in a solution containing lead alone at 8.0 mg/L level, the value of the concentration factor was 54.5. However, in the presence of equal concentrations of zinc (mixed metal group), the value of the concentration factor for lead decreased to 35.44, indicating that the influence was caused by the presence of the zinc ion.

Phytoaccumulation works most efficiently at contaminated sites with large volumes of water that contain low concentrations of contaminants and cost effective. The harvested plant tissue, rich in accumulated contaminants, is easily and safely

processed by drying, ashing or composting. The volume of toxic waste produced as a result is generally a fraction of that of many current, and the associated costs are much less. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues.

F. Biosorption

1. Biosorption and biosorbents

Biosorption is a process that utilizes dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. Metal sequestration by different parts of the cell can occur via complexation, coordination, chelation of metals, ion exchange, adsorption, and inorganic microprecipitation. Biosoption 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 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, when it is dead. The remaining cell debris, such as cell walls can still represent a potent "biosorbent" (111).

Biosorbent material is basically produced from the raw biomass which features a high metal uptake. When dead and no longer metabolically active, such biomass actually represents a biosorbent material. Biosorbents need to have characteristics suitable for process applications: hardness, porosity, particle size, density, and resistance to a broad spectrum of variable solution parameters, such as

temperature, pH, and solvent content (111). The formulation of biosorbent materials should result in an efficient metal sequestering product which could be effectively and economically used in the biosorption process in its many possible applications. While research in the biosorption field vigorously continues, the formulation of suitable industrial biosorbents represents a typical product development line of efforts which is complementary to the basic exploratory work. A whole new family of biosorbent materials is being developed from the original discoveries of biomass types which possess outstanding metal binding properties.

2. Biosorption isotherms and models

The biosorptive metal uptake can be quantitatively evaluated from experimental biosorption equilibrium isotherms similar to those used for the performance evaluation of activated carbons. Upon contact between the sorbent material and the solution containing the sorbed species, an equilibrium is established at a given temperature whereby a certain amount of the sorbed (metal) species sequestered by the sorbent is in equilibrium with its residue left free in the solution containing then the residual, final, or equilibrium concentration of metal species. The isotherm graphical expression is a plot of the metal uptake by the biosorbent (in weight per weight or mole per weight units) against the residual metal concentration (equilibrium concentration in weight per volume units). The resulting relationship is most often hyperbolic with the biosorbent uptake value leveling off as it approaches the value of complete saturation of the sorbing material at a high concentration of the sorbed species. At least a certain portion of the isotherm curve can often be linearized

by using a log-log plot. Data extrapolation in this case, however, may not be possible (112).

The plot of biosorption isotherms is derived from results of relatively simple contact experiments depicted in Figure 2-2. They are often done using small Erlenmeyer flask (250 mL) with no more than 50 to 100 mL of metal bearing solution (volume, V) of known initial metal concentration (C_i). A series of flasks with the same solution receives different known amounts of biomass (M). The flasks containing this dilute suspension are placed on a shaker, allowing enough time for developing the sorption equilibrium. The contents of each flask are filtered and the filtrate is collected and analyzed for final or residual (equilibrium) metal concentration (C_f). Uptake of the metal (q in milligrams of metal per gram of biomass) is calculated according to the formula:

$$\mathbf{q = V(C_i - C_f)/M}$$

The final concentration measured usually differs according to the amount of biosorbent initially added. The resulting uptake data are plotted against the C_f in a diagram representing the sorption isotherm (112).

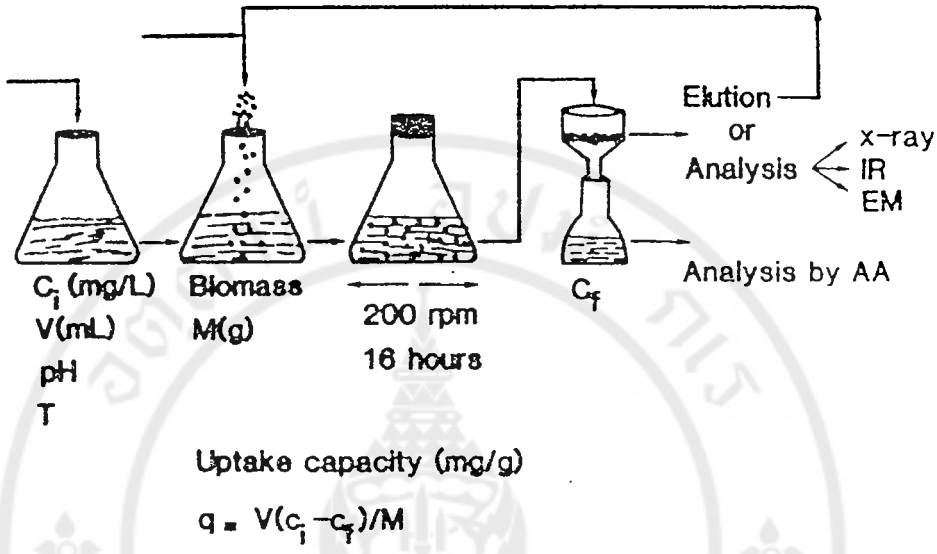

Figure 2-2. Outline of an experimental procedure for determining the equilibrium sorption data for expressing the isotherm relationship (112).

The maximum sorption uptake value is an important feature of the sorbent characterizing its performance at high residual metal concentrations. The shape of the biosorption isotherm is also important from another point of view. An isotherm which is steep from the origin at low residual concentrations of the sorbate is highly desirable because it indicates high affinity of the sorbent for the given sorbed species. Such a sorbent would be performing well at very low concentrations of the sorbed species in the solution. It should be emphasized that the biosorption isotherm reflects an equilibrium process whereby the metal bound to the biosorbent is in a state of equilibrium with its ionic species found still dissolved in the solution. It may take some time for this equilibrium to become established, but when it is assumed, the system theoretically remains in a stable equilibrated condition with a certain amount of

metallic species sequestered, bound, and immobilized by the solid phase of the biosorbent and another portion of the metal remaining dissolved in the solution. The two entities in contact reach the equilibrium state. As a reflection of this basic consideration, there are two implications:

(1) the initial concentration of the sorbate (metal) does not play a significant role in these equilibrium characterizations of the sorbent (isotherm) because it is the final (residual) equilibrium concentration which is of importance, and

(2) the sorption process obviously requires a certain time to reach the equilibrium. In all equilibrium studies required for expressing the sorption isotherm, ample time of contact has to be provided for the system to reach equilibrium (112).

There are two widely accepted and easily linearized adsorption isotherm models used in the literature which were proposed, respectively, by Langmuir and Freundlich.

The Langmuir model has many assumptions, namely,

(1) the surface consists of adsorption sites,

(2) all adsorbed species interact only with a site and not with each other,

(3) adsorption is limited to a monolayer, and

(4) adsorption energy of all sites is identical and independent of the presence of adsorbed species on neighboring sites. The general formula of the Langmuir model equation is

$$q_e = (X_m b C_e)/(1+bC_e)$$

where q_e = uptake of species; X_m = maximum uptake; C_e = equilibrium (final) concentration in solution; and b = constant related to energy of adsorption (111).

The Fruendlich 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 formula of this model is

$$q = kC^{1/n}$$

This can be linearized by taking the natural logarithm of both sides of the equation to give:

$$\ln q = \ln k + 1/n \ln C$$

The intercept ln k gives a measure of the adsorbent capacity and the slope 1/n gives the intensity of adsorption (111).

Both models describe many available biosorption isotherm data equally well. However, they are not models whose terms or parameters would have a convenient and appropriate physical interpretation attached to them. Therefore, the use and significane of these models are highly limited. Fitting the model to the process behavior does not necessarily imply that a pure adsorption phenomenon has taken place.

In addition to equilibrium studies, the kinetics of the biosorption has to be determined in order to establish the time course of the metal uptake. Rapid uptake of the metal by the biosorbent is desirable providing for a short solution biosorbent contact time in the actual process. The contact time necessary eventually determines the size of the contact equipment which, in turn, directly affects both the capital and the operating costs of the process. Both equilibrium and kinetics characterizations of the biosorbent material are crucial for quantitative assessment of its performance and for the process design (112).

The biosorption potentail depends on many aspects of its chemical composition and, at the present state of knowledge, can be assessed only experimentally. It has been established that certain types of biomass could serve as a basis for very potent biosorbents, while others are less suitable. Apparently, different types of biomass may be better for only certain types of metals. The attractive feature of biosorption is a certain specificity of the biosorbent for the divalent and multivalent heavy metal cations. The nutrient status of the organism, its physiological state, the age of cells, and availability of micronutrients during growth, as well as environmental conditions during the actual biosorption process such as pH, temperature, and presence of certain co-ions, are all important parameters affecting the performance of a biosorbent. Ramos et al. (113) studied the adsorption of cadmium (II) from aqueous solution onto activated carbon. The adsorption isotherm of cadmium on coconut shells (activated carbon) was measured in a batch adsorber. Effects of temperature and solution pH on the adsorption isotherm were investigated by determining the adsorption isotherm at temperatures of 10, 25, and 40°C and at initial pH values from 2 to 8. Langmuir isotherm better fitted the experimental data since the average percent deviation was lower than with the Freundlich isotherm. It was noticed that the amount of cadmium (II) adsorbed was reduced about three times by increasing the temperature from 10 to 40°C. It was found that cadmium (II) was not adsorbed on coconut shells at pH of 2 or lower, and that cadmium was precipitated out as $Cd(OH)_2$ at pH values above 9. Maximum adsorption capacity was observed at pH of 8, and the adsorption capacity was decreased about 12 times by reducing the initial pH form 8 to 3. According to the cadmium speciation diagram, the predominant species below pH of 8

is cadmium (II). Thus, cadmium was adsorbed on the coconut shells surface as cadmium (II). It was concluded that the adsorption capacity is a strong function of pH and temperature. The efficiency of the biosorbent metal concentration from an aqueous solution is also greatly influenced by the solution chemistry of the metal.

3. Removal of heavy metals by biosorption

Biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater. Adsorbent materials (biosorbents) derived from suitable biomass can be used for the effective removal and recovery of heavy metal ions from wastewater streams. The major advantages of the biosorption technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials. Biosorption processes are particularly suitable for the treatment of wastewater streams containing dilute heavy metal ion concentrations, or when very low concentrations of heavy metals are required (112). The limitations of the technology include that large-scale production of effective biosorbent materials has not been established, and that the technology has only been tested for limited practical applications.

Biomass of aquatic plants, algae, and plant materials are biological resources which are available in large quantities and can form a good basis for the development of biosorbent materials. *Azolla filiculoides* is a free floating and fast growing symbiotic water fern that is able to accumulate large amounts of heavy metals from aqueous solution. Sanyahumbi et al. (114) studied the removal of lead from solution by the non-viable biomass of the water fern *A. filiculoides* and reported that non-viable biomass of *A. filiculoides* removed up to 93 mg lead/g biomass from the

solution. Lead removal varied from 30% of the initial lead concentration at pH 1.5 to approximately 95% at pH values of 3.5 and 4.5. Lead removal decreased to 30% of the initial lead concentration if the lead concentration was initially over 400 mg/L. Lead removal remained at approximately 90% between 10°C and 50°C. Biomass concentration (4-8 mg/L) had little effect on lead removal. For the chromium (VI) removal studies (115), *A. filiculoides* showed the maximum adsorption capacity as 70.6 mg/g at 18°C and 120.2 mg/g at 32°C, both at pH 2. The metal removal was moderately rapid at low initial chromium (VI) concentration. Zhao and Duncan (116, 117) investigated the removal of nickel and zinc from aqueous solutions and electroplating rinse effluent by *Azolla* biomass in batch experiment. They found that the maximum nickel uptake by *Azolla* in the batch at an optimum pH of 6.5 was 43.3 mg/g and the maximum zinc uptake at an optimum pH 6.0 was 45.2 mg/g. They concluded that the development of an *Azolla*-based biosorbent for wastewater treatment, especially in developing countries, may benefit both environmental problems by removing heavy metals from water using this weed.

The biosorption of heavy metal ions by algal biomass is a promising property with a potential for industrial use. The uptake capacities of the biomass of a group of nine marine macro-algae for heavy metal ions (cadmium, copper, and lead) were evaluated (118). Equilibrium isotherms for each biomass heavy metals system were obtained from batch adsorption experiments. The maximum uptake capacities of the biomass ranged from around 0.8 to 1.6 mmol/g (dry), which were much higher than those of other types of biomass. The results indicated that the biomass of the

marine algae is suitable for the development of efficient biosorbents for the removal and recovery of heavy metals from wastewater.

Matheickal and Yu (119) studied the biosorption of lead from aqueous solutions by marine algae *Ecklonia radiata* and reported that *E. radiata*, can be used for the development of an efficient biosorbent material for heavy metal removal from wastewater. *Ecklonia radiata* exhibited high uptake capacities for lead, cadmium, and copper. In particular, lead sorption was very marked. The equilibrium data fitted well to the Langmuir isotherm model. Within a pH range of 4.5 to 5.5, the uptake capacity of *E. radiata* for lead is 1.36 mM/g (282 mg/g), which is much higher than those of powdered activated carbon and natural zeolite. Studies indicated that sorption of lead increases as pH increases and reaches a plateau at pH 5. The lead uptake process was rapid, with 60% of the sorption completed within 10 minutes. The presence of light metal ions in solution did not affect lead sorption significantly.

Biomass of *Ecklonia radiata* can be used for the development of an efficient biosorbent material for copper removal from wastewater (120). At pH 5.0, the uptake capacity of *E. radiata* for copper is 1.11 mmol/g. The adsorption of copper increases as pH increases and reaches a plateau at pH 5. The copper uptake process was rapid, with 90% of the adsorption completed within 15 minutes. The study suggested that the abundantly and cheaply available *E. radiata* can be used as efficient biosorbent material for copper recovery from wastewater.

Ahuja et al. (121) studied the biosorption by *Oscillatoria auguistissima* and reported that *O. auguistissima* showed a very high capacity for zinc biosorption (641 mg/g dry biomass at a residual concentration of 129.2 ppm) from solution and

was comparable with the commercial ion exchange resin IRA-400 C. Zinc biosorption was rapid, pH dependent, and temperature independent phenomenon. Zinc adsorption followed both Langmuir and Freundlich models. The specific uptake (mg/g dry biomass) of metal decreased with an increase in biomass concentration. They concluded that the native biomass also could efficiently remove zinc from effluents obtained from Indian mining industries.

Pre-treatment of the native biomass considerably improved the swelling properties and physical stability of the biomass granules. Cadmium (II) adsorption properties of pre-treated biomass (PTB) of marine alga *Durvillaea potatorum* were investigated (122). Batch experiment was conducted to determine the adsorption properties of the modified biomass. The adsorption capacity of the biomass strongly depends on equilibrium of pH solution. At pH 5, the maximum adsorption capacity of the pre-treated biomass is 1.1 mmol/g. The kinetics of cadmium adsorption was fast with 90% of adsorption taking place within 30 minutes. The study demonstrated that the pre-treated biomass of *D. potatorum* can be used as an efficient biosorbent for the treatment of cadmium bearing waste streams.

Plant materials, such as sphagnum moss peat, palm pressed fibers, coconut husk, etc. are widely available and abundant natural substances and have been reported to exhibit ion exchange and complexation capacities towards heavy metals (123). It also has been reported that peat has a large specific surface area (>200 m^2/g) and is highly porous (95%) (124). Peat is a complex material containing lignin and cellulose as its major constituents. These constituents, especially lignin, bear polar functional groups, such as alcohols, aldehydes, ketones, acids, and phenolic residues

which can be involved in chemical bonding and complexation roles during the fixation of metal ions from solution (125). Studies have been reported on the use of peat as an adsorbent for the removal of copper, zinc, and cadmium (123, 126, 127). Shama and Forster (128) studied the removal of hexavalent chromium using sphagnum moss peat and reported that sphagnum moss peat, which is essentially oligotrophic, in concentrations ranging from 4 to 40 g/L can be used effectively to remove hexavalent chromium from aqueous solutions. The process is pH dependent, the optimum range being 1.5-3.0.

The removal of chromium (VI) from aqueous solutions by coconut husk fibers (CHF) and palm pressed fibers (PPF) was investigated using batch technique (129). Batch tests showed that pH ranges for effective chromium (VI) removal was between 1.5 and 5 for CHF and between 1.5 and 3 for PPF. The adsorption capacities of CHF and PPF were estimated to be 29 and 14 mg Cr/g substrate at pH 2.0. They concluded that CHF and PPF also could be used as barriers in the landfill to maximize immobilization of toxic metal such as chromium (VI) in leachates.

Low et al. (130) studied the sorption of trivalent chromium from tannery waste by moss. The sorption of chromium (III) from synthetic solution and tannery waste using moss, *Calymperes delessertii*, was conducted under laboratory conditions to assess its potential in removing chromium (III). Parameters investigated include pH, contact time, initial concentration and sorbent dosage. Results obtained from isotherm studies showed that the sorption of chromium (III) by moss, followed the Langmuir isotherm, and the maximum sorption capacities were 15.4 and 13.7 mg/g for synthetic solution and tannery waste, respectively. They concluded that the

biosorbent appears to be suited for treatment of heavy metal waste streams, where extremely low levels of effluent standards were to be met. The biosorbents can be used in packed bed operations, and current facilities used for ion exchange process can be utilized for its application.

Biosorption is an economically feasible and a technically efficient technology for removing and recovering metals from solutions. It can comfortably fit into the metal treatment processes where ion-exchange resins are applied. The biosorption process is competitive in terms of capital and operational costs with the existing treatment technologies.

CHAPTER III

MATERIALS AND METHODS

A. Materials

1. Chemicals

The chemicals used in this study were listed below, as follows:

Chemical name	Chemical formula	Source
Acetone	CH_3COCH_3	Mallinckrodt
Ammonium hydroxide	NH_4OH	Carlo Erba
Boric acid	H_3BO_3	J.T. Baker
Cadmium chloride	$CdCl_2 \cdot H_2O$	Riedel-deHaën
Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	Carlo Erba
Copper sulphate	$CuSO_4$	Merck
1,5-diphenylcarbazide	$C_{13}H_{14}N_4O$	Merck
Ethylenediaminetetraacetate	$Na_2C_{10}H_{14}O_8N_2 \cdot H_2O$	Fluka
Ferric chloride	$FeCl_3 \cdot 6H_2O$	Merck
Magnesium sulphate	$MgSO_4 \cdot 7\ H_2O$	Carlo Erba
Molybdic acid	$H_2MoO_4 \cdot\ H_2O$	Merck
Nitric acid	HNO_3	BDH
Potassium dichromate	$K_2Cr_2O_7$	Carlo Erba
Potassium dihydrogen phosphate	KH_2PO_4	Fluka

Chemical name	Chemical formula	Source
Potassium nitrate	KNO_3	Merck
Sodium hydroxide	NaOH	Merck
Sulfuric acid	H_2SO_4	J.T. Baker
Zinc sulphate	$ZnSO_4$	BDH

2. Instruments

The instruments used in this study were as follows:

2.1. Flame atomic absorption spectrophotometer (FAAS)

FAAS measurements were performed using a Perkin Elmer model 3100 equipped with deuterium background correction (Connecticut, USA), providing a background corrected AA results. The operating parameters for measurement were listed below.

Element : Cd

Wavelength (nm) : 228.8

Operating lamp current (mA) : 5

Slit width (nm) : 0.7 (high)

Flame type : Air acetylene

2.2. Analytical balance

A Precisa balance model 185 AM-FR was used for weighing when accurate amounts were required.

2.3. pH meter

A pH meter of Hanna instruments model 8417, with a glass combination electrode, was used for all pH experiments. Commercial standard buffers

(Merck) of pH 4.00±0.02 (20°C), 7.00±0.02 (20°C), and 10.00±0.05 (20°C) were employed for the instrumental calibration.

2.4. Spectrophotometer

A HACH DR/3 spectrophotometer was used to estimate the absorption spectra of extracted solution in chlorophyll and chromium (VI) determination.

2.5. Centrifuge

Sigma centrifuge, model 301 K was used to separate the residue from the supernatant after extraction.

2.6. Suction pump

Water suction pump, model DOA-U130-BN was used to filter the residue from the solution.

3. Media

3 % Hoagland's nutrient solution used in this study was the Hoagland's nutrient solution of EPA (131) and composed of :

Macronutrients		
$Ca(NO_3)_2 \cdot 4H_2O$	118.00	g/L
KNO_3	50.25	g/L
$MgSO_4 \cdot 7\ H_2O$	24.08	g/L
KH_2PO_4	13.61	g/L
Micronutrients		
H_3BO_3	2.86	g/L
$ZnSO_4$	0.22	g/L

$CuSO_4$	0.08	g/L
$H_2MoO_4 \cdot H_2O$	0.90	g/L
EDTA $FeCl_3 \cdot 6H_2O$	5.00	g/L

Preparation of EDTA $FeCl_3 \cdot 6H_2O$

22.4 g EDTA was dissolved in 500 mL distilled water. This solution was warmed before adding 13.5 g $FeCl_3 \cdot 6H_2O$. Thereafter, the solution was mixed thoroughly to dissolve all salt.

Preparation of 3% Hoagland's nutrient solution

1 mL of micronutrient solution, 1 mL of EDTA $FeCl_3 \cdot 6H_2O$, 1 mL of KH_2PO_4, 5 mL of KNO_3, 5 mL of $Ca(NO_3)_2 \cdot 4H_2O$, and 2 mL of $MgSO_4 \cdot 7\ H_2O$ were pipetted into a 1-L volumetric flask and diluted to 1,000 mL. The final pH of the nutrient solution was 5.6.

B. Methods

1. General methods

1.1. Experimental plant

1.1.1. Classification

Kingdom	Plantae
Phylum	Anthophyta
Class	Monocotyledoneae
Order	Spathioflorae
Family	Lemnaceae
Genus	*Wolffia*

Scientific name *Wolffia globosa*

Common name Tropical watermeal (khai-nam)

1.1.2. Characteristics

The characteristics of *Wolffia globosa* were as follows:

Root : None.

Shape of plant body : The shape of *Wolffia globosa* is ovoid or ellipsoid-cylindrical (longer than wide) and transparent green throughout (some populations may contain individuals with darker green dorsal surface). The dorsal surface is rounded on edges with upper central portion flattened, floating with only the central portion of dorsal surface above water and without brown epidermal pigment cells.

Size : The size of *Wolffia globosa* is only 0.4-0.9 mm long, the smallest of all known flowering plants. It rivaled in minuteness only by the Australian sp. *W. angusta* Landolt.

Veins : None.

Budding pouch position : *Wolffia globosa* has one funnel-shaped pouch at basal end and budding pouch often with distinct collar of elongate cells at junction with daughter plant.

Flower (fruit) position : *Wolffia globosa* has flower position within floral cavity on dorsal surface and is not enclosed by a spathe.

Arrangement of clonal clusters : The arrangement of clonal clusters are solitary or two connected.

Habitat : The natural habitat of *Wolffia globosa* is floating freely at surface of quiet streams and ponds, and often mixed with other Lemnaceae and aquatic plants (Figure 3-1).

Range : Generally, *Wolffia globosa* is confined to ponds and ditches of hot interior valleys of central and southern California at low elevations, and in sloughs along rivers draining the western slopes of the Sierra Nevada. It may has been introduced into California, perhaps in rice fields of Central Valley; outside of California. The only other location for this species in the United States is Pinellas County, Florida and worldwide distribution in tropical regions including southern Florida (Pinellas County), Africa, India, Southeast Asia, Malaysia, Philippines and Hawaiian Islands. This species is the edible "khai-nam" or "water eggs" of Thailand (Figure 3-2).

1.2. Culture of *Wolffia globosa*

Wolffia globosa were collected from an unpolluted water body at Nong-Chock district in Bangkok. The plants were cleaned thoroughly under gentle running water to remove other duckweed species, adhering algae, and insect larvae. The plants were transferred to plastic aquaria of 2-L capacity containing 3 % Hoagland's nutrient solution (pH 5.6), and were provided with a light intensity of 45 μmoles $m^{-2}s^{-1}$ at 12/12, L/D cycle by cool white fluorescent tube light (light intervals were controlled by a time clock) at 26±2°C (Figure 3-3). The culture solutions were replaced every 7 days and newly grown fronds were transferred to new plastic aquaria containing nutrient solution. Purified cultures from the stock culture were selected for use in the experimental work.

Figure 3-1. Showing the duckweed *Wolffia globosa* mixed with other Lemnaceae and aquatic plants.

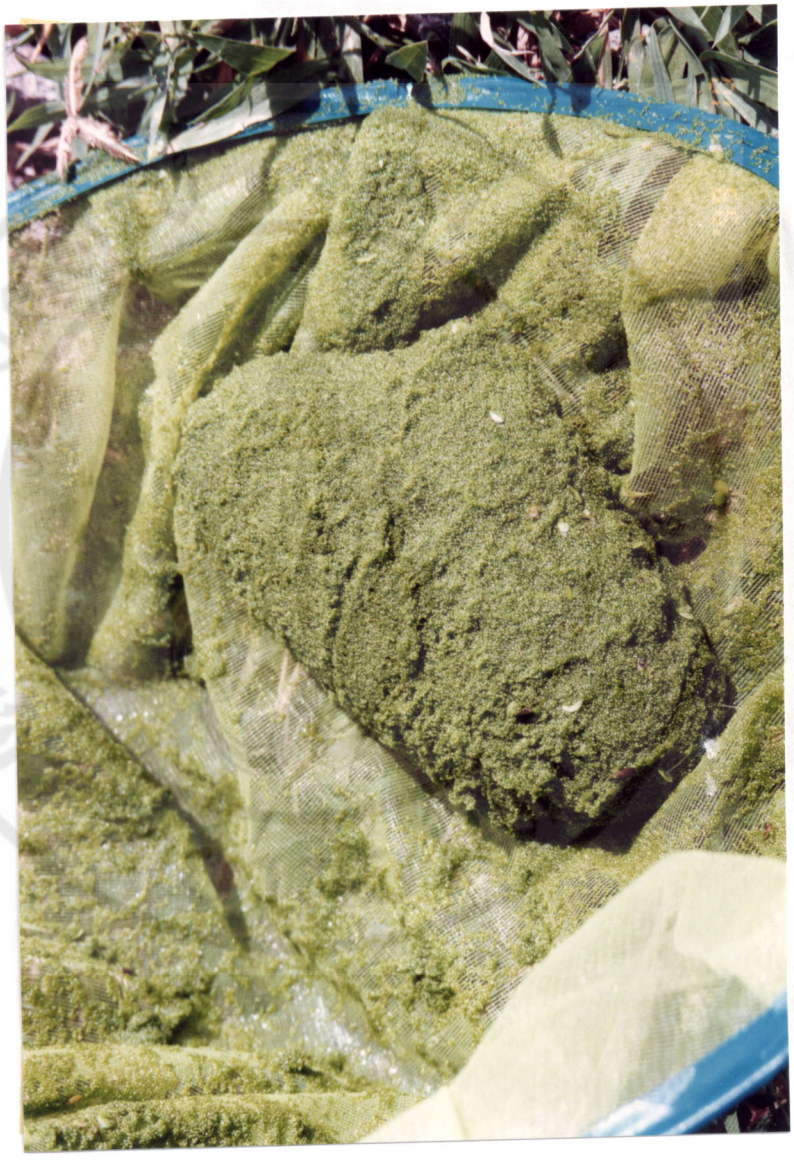

Figure 3-2. Showing the edible *Wolffia globosa.* ("khai-nam" or "water eggs" in Thailand).

Figure 3-3. Showing the culture of *Wolffia globosa* in the controlled condition (light intensity of 45 μmoles $m^{-2}s^{-1}$ at 12/12, L/D cycle by cool white fluorescent tube light at 26±2°C).

1.3. Preparation of experimental glassware

The glasswares used in all experiments were cleaned by washing with tap water and detergent, rinsing with cleaning solution of 10 % HNO_3, and then washing thoroughly with tap water and distilled water, respectively.

1.4. Preparation of toxicants and stock solution

Stock solutions of heavy metals were freshly prepared by dissolving the salts of heavy metals in distilled water. Stock solutions were acidified by adding a few drops of concentrated nitric acid in order to reduce precipitation and absorption of the metal ions.

1.4.1. Cadmium (II)

The stock solution of cadmium (1,000 mg/L) was prepared by dissolving analytical grade of cadmium chloride ($CdCl_2 \cdot H_2O$) 1.792 g in distilled water 1,000 mL. The concentration was expressed in term of cadmium ion (Cd^{2+}) in milligrams per liter (mg/L) of solution.

1.4.2. Chromium (VI)

The stock solution of chromium (1,000 mg/L) was prepared by dissolving analytical grade of potassium dichromate ($K_2Cr_2O_7$) 2.829 g in distilled water 1,000 mL. The concentration was expressed in term of chromium ion (Cr^{6+}) in milligrams per liter (mg/L) of solution.

2. Experimental methods

2.1. Toxicity test and accumulation of metal

2.1.1. Growth measurement

One gram of newly grown fronds from the stock culture was stocked into plastic aquaria which allowed ample space for growth during the 42 days experimental period. The plastic aquaria were filled with 3 % Hoagland's nutrient solution, and the pH was adjusted to 5.6. The experiment was set up in triplicate.

The old solution were replaced once a week with a fresh medium containing macronutrients and micronutrients, and the pH was adjusted to ensure normal growth. At weekly intervals, the duckweed was skimmed from the plastic aquaria and its fresh weights were recorded. Thereafter, it was carefully returned to its plastic aquaria, in which its growth was allowed to continue. The process was repeated until no further change in fresh weight was obtained.

2.1.2. Toxicity procedures

The 3 % Hoagland's nutrient medium (150 mL), kept in 250 mL conical flasks, was supplemented with four nominal concentrations (1, 2, 4, 8 mg/L) of cadmium (II) and chromium (VI) individually prepared from their salts $CdCl_2 \cdot H_2O$ and $K_2Cr_2O_7$, respectively. Fronds cultured in the nutrient medium without metals were treated as control. The experiment was set up in triplicate for each concentration and duration. *Wolffia globosa* (2 g fresh wt) were inoculated into each flask and covered with clear plastic film to prevent air contamination under controlled conditions (illuminated for 12 h/day light period, photon flux intensity of 45 μmoles $m^{-2}s^{-1}$ at 26±2°C) (Figure 3-4). Plants from each container were separately harvested after 3, 6, 9, and 12 days and were processed for biomass, chlorophyll, and metal content.

Figure 3-4. Showing the flasks used in the toxicity and accumulation studies.

2.1.3. Biomass estimation

Treated and control *Wolffia globosa* were gently blotted and allowed to stand for one hour to drain excess water and weighed after each harvest on day 3, 6, 9, and 12. The biomass was expressed as percentage of control and EC_{50} of each exposure time was determined.

2.1.4. Chlorophyll determination

Treated and control *Wolffia globosa* were used for chlorophyll estimation by the absorption spectra of fronds extracts in a spectrophotometer according to Arnon (132) and MacKinney (133).

After treatment, the fronds of *Wolffia globosa* were weighed and chlorophyll were extracted by homogenizing fronds twice in a tissue grinder in ammoniacal acetone (81.8 $(CH_3)_2CO$/18 H_2O/0.2 NH_4OH v/v). The homogenate was quantitatively transferred to a 5 mL centrifugal tube and centrifuged at 3,000 g for three minutes. The filtered extract was placed in the dark immediately following centrifugation. Immediately before recording the spectrum, the extract was diluted to a 50 mL volumetric flask and made up to volume with ammoniacal acetone.

The absorbance of the extract was measured at both 663 nm (A663) and 645 nm (A645). These are positions in the spectrum where maximum absorption by chlorophyll a and b occur. The instrument was set at zero absorbance with a tube containing ammoniacal acetone before readings were made with the solution of pigments. The cuvette was rinsed with ammoniacal acetone after each use of the solution of pigments.

The EC_{50} of each exposure time was determined and concentration of chlorophyll a and b in mg/L was calculated by the formula :

Chlorophyll a in mg/L $= 12.7 \times A663 - 2.69 \times A645$

Chlorophyll b in mg/L $= 22.9 \times A645 - 4.68 \times A663$

Total chlorophyll in mg/L $= 8.02 \times A663 + 20.2 \times A645$

2.1.5. Accumulated toxicants analysis

1) Digestion of plant samples

Digestion of plant samples for this study was done by using the nitric acid digestion method described in the Standard Methods by APHA, AWWA, and WEF (134).

After the exposure of *Wolffia globosa* to cadmium (II) and chromium (VI) concentration, the plants were removed at each selected time (3, 6, 9, 12 days), rinsed with distilled water, dried at 105°C for 24 h, and weighed. The dried samples were homogenized in a tissue grinder and transferred into a 15 mL test tube containing a few glass beads. Ten mL of concentrated nitric acid was added to the test tube. Finally, the solution was heated slowly until digestion was completed as shown by a lighted-yellowish in color. The test tube wall was washed down with distilled water, and the solution was filtered through Whatman 0.45 μm membrane filter. The filtrate was transferred to a 50-mL volumetric flask with two 5-mL portions of water, and the rinsings were added to the volumetric flask. The filtrate was cooled, diluted to mark, and mixed thoroughly. A few drops of nitric acid were added to preserve the sample and were kept in a refrigerator at 4°C before use.

2) Heavy metal determination

Cadmium (II)

After digestion, the cadmium (II) concentrations were determined by using the flame atomic absorption spectrophotometer (Figure 3-5). The results of the accumulation experiments were reported as concentration of cadmium (II) in plants (μg Cd^{2+}/g dry weight of *Wolffia*).

Chromium (VI)

After digestion, the chromium (VI) concentrations were determined by colorimeter using the 1,5-diphenylcarbazide method described in the Standard Methods by APHA, AWWA, and WEF (4).

A pH meter and 0.2 N H_2SO_4 were used for adjusting the digested solution to pH 1.0±0.3. 250 mg. 1,5-diphenylcarbazide (1,5-diphenylcarbohydrazide) was dissolved in 50 mL acetone and 2.0 mL of diphenylcarbazide solution was added to the digested solution, and were mixed and allowed to stand for 5 to 10 minutes until a full red-violet color developed. An appropriate portion was transferred to a cuvette and its absorbance at 540 nm was measured. Distilled water was used as a reference. The results of the accumulation experiments were reported as concentration of chromium (VI) in plants (μg Cr^{6+}/g dry weight of *Wolffia*).

2.1.6. Statistical analysis

The mean number of biomass, chlorophyll, and metal content were calculated and subjected to analysis of variance (ANOVA) using randomized block design and Least Significant Difference method (LSD) on the SPSS

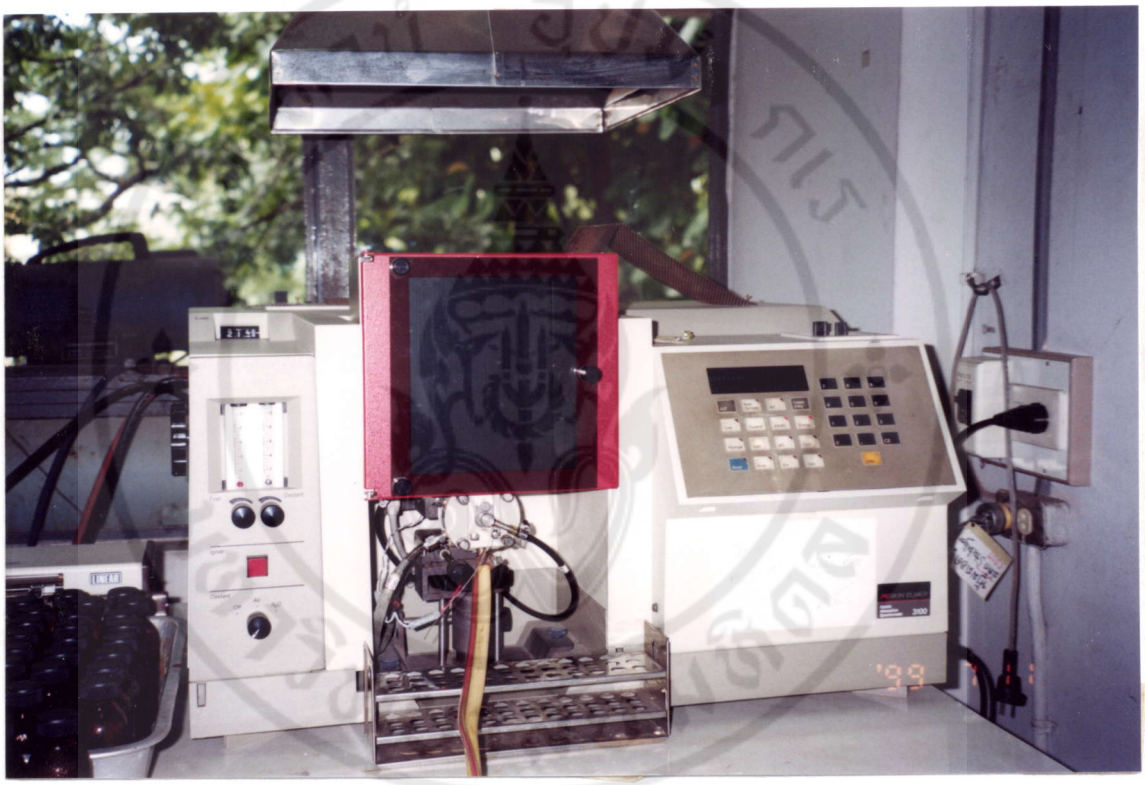

Figure 3-5. Showing the flame atomic absorption spectrophotometer (FAAS) used in this study.

for window program (SPSS, Inc.), after analysis of the homogeneity of variance according to Cochran's test (135).

Effective concentration at 50 % (EC_{50}) of metal on biomass and chlorophyll content in *Wolffia globosa* for each metal concentration and exposure times were expressed as percentage biomass and chlorophyll content relative to controls. Biomass and chlorophyll content were plotted against log 10 metal concentration, and the concentrations at which 50 % biomass and chlorophyll content decreased were calculated from regression equations generated by SPSS-Probit for window (SPSS, Inc.).

Correlation analysis was used to see if there was a relationship between variables.

2.2. Biosorption of dried *Wolffia globosa* biomass

2.2.1. Preparation of dried *Wolffia globosa* biomass

Fresh *Wolffia globosa* was collected from the stock culture. Prior to use, it was washed with distilled water, dried in an oven for six hours at 80°C and ground in an electrical blender to a gritty consistency. The biomass was sieved to remove any particles over 2 mm in size before use and was stored in a desiccator (Figure 3-6).

2.2.2. Preparation of heavy metal solutions

Analytical grade reagents were used in all cases. Stock cadmium (II) and chromium (VI) solutions (1,000 mg/L) were prepared in distilled water as $CdCl_2 \cdot H_2O$ and $K_2Cr_2O_7$, respectively. All working solutions were prepared by diluting stock solutions with distilled water.

Figure 3-6. Showing the dried *Wolffia globosa* biomass.

2.2.3. Adsorption isotherm studies

Batch sorption tests were carried out at 26±2°C on a rotary shaker with eccentricity of 0.5 cm at 200 rpm, for five hours using 250 mL conical flasks. The cadmium (II) and chromium (VI) solutions (100 mL) were adjusted to required initial pH values before the addition of the dried *Wolffia globosa* biomass (4 g/L) (Figure 3-7). The pH values of the solution was checked and adjusted every hour during the incubation by the addition of either H_2SO_4 or NaOH. Isotherm studies were performed by varying the initial cadmium (II) and chromium (VI) concentrations from 10 to 400 mg/L and the pH between 1.5 and 6 for chromium (VI) and between 4 and 7 for cadmium (II). After shaking the flasks for five hours, the reaction mixtures were filtered through Whatman 0.45 μm membrane filter, and the filtrate was analyzed for residual cadmium (II) and chromium (VI) concentrations. A Langmuir sorption model was adopted for the estimation of the maximum metal uptake (X_m) where this could not be reached in the experiment (136,137). The equation was as follows :

$$C_e / q_e = 1 / (X_m b) + (C_e / X_m)$$

where X_m is indicative of maximum adsorption capacity, b is Langmuir constant, the ratio of the adsorption/desorption rates related to energy of adsorption through the Arrhenius equation (138), q_e is the metal uptake (mg of metal/g of biomass) at equilibrium, and C_e is the equilibrium concentration of metal (mg/L). From a plot of C_e / q_e vs. C_e, the slope ($S = 1/X_m$) gives X_m and the intercept ($I = 1/bX_m$) gives b (139).

Figure 3-7. Showing the shaker used for adsorption isotherm studies.

2.2.4. Effect of contact times

Dried *Wolffia globosa* biomass of 1.2 g was thoroughly mixed with 300 mL of cadmium (II) and chromium (VI) solutions of varying concentrations ranging from 10 to 400 mg/L. The suspensions were shaken for 24 hours at 26±2°C using 500 mL conical flasks. Samples (1 mL) were collected at required times and metal contents were analyzed.

2.2.5. Heavy metals determination

Cadmium (II) concentrations were determined by the flame atomic absorption spectrophotometer.

Chromium (VI) concentrations were determined by colorimeter using the 1,5-diphenylcarbazide method.

2.2.6. Statistical analysis

The mean number of equilibrium concentration in adsorption isotherms and effect of contact time studies were calculated and subjected to analysis of variance (ANOVA) using randomized block design and Least Significant Difference method (LSD) on the SPSS for window program (SPSS, Inc.), after analysis of the homogeneity of variance according to Cochran's test (135).

Correlation analysis was used to see if there was a relationship between variables.

CHAPTER IV

RESULTS

A. Toxicity test and accumulation of heavy metals

1. *Wolffia globosa* growth measurement

Wolffia globosa was cultured in 3% Hoagland's nutrient solution under the controlled condition (light intensity of 45 μmole $m^{-2}s^{-1}$ at 12/12, L/D cycle by cool white fluorescent tube light at 26±2°C). The biomass of *W. globosa* was measured for six weeks as shown in Table 4-1, and its growth curve is shown in Figure 4-1. The three-week-old *W. globosa* was used for toxicity test procedures.

Table 4-1. The biomass of *Wolffia globosa* (g dry wt) which was cultured in 3% Hoagland's nutrient solution under the control condition for six weeks.

Time (week)	Biomass of *W. globosa* (g dry wt)
0	0.0400 ± 0.0002
1	0.0404 ± 0.0003
2	0.0440 ± 0.0004
3	0.0600 ± 0.0001
4	0.0900 ± 0.0003
5	0.0730 ± 0.0004
6	0.0700 ± 0.0004

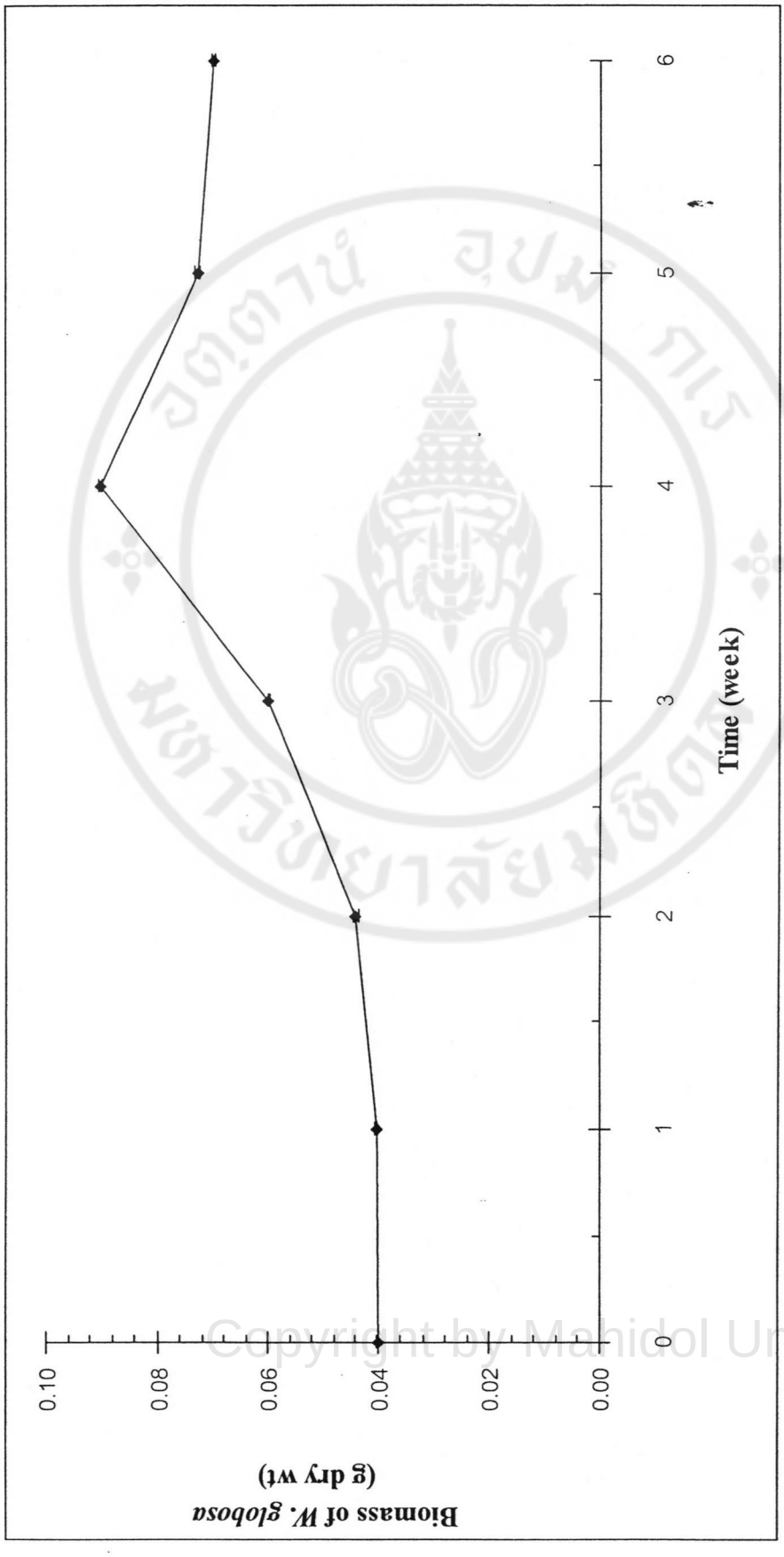

Figure 4-1. The growth curve of *Wolffia globosa* cultured in 3% Hoagland's nutrient solution under the controlled condition for six weeks.

2. The biomass productivity

Biomass productivities (% of control) in relation to different exposure times and levels of Cd (II) and Cr (VI) are shown in Table 4-2, Figure 4-2, and Figure 4-3. The statistical analysis of the effects of Cd (II) and Cr (VI) on the biomass productivity in *Wolffia globosa* indicated that there were significant decreases ($P<0.05$) when the exposure times and concentrations were increased. *Wolffia globosa* obtained at 1, 2, 4, and 8 mg/L on each exposure time decreased significantly ($P<0.05$) in biomass productivities from control, and biomass productivities at 6, 9, and 12 days decreased significantly ($P<0.05$) from the first three days for all concentrations. However, at low concentrations, the results showed stimulation in growth of *W. globosa* at 1 mg/L Cr (VI) on day 3, whereas in the case of Cd (II), a decrease in biomass productivity on day 3 was observed. At very high concentrations, the fronds of *W. globosa* on day 12 survived upto 8 mg/L Cr (VI), whereas in the case of Cd (II), this concentration was lethal to the plants. When the effects of these metals on biomass productivity were compared, *W. globosa* which were exposed in Cr (VI) solutions showed significantly higher rates in biomass productivity ($P<0.05$) than *W. globosa* which were exposed in Cd (II) solutions. This suggested that Cd (II) showed more severe effect on biomass productivity than Cr (VI).

Table 4-2. The effects of Cd (II) and Cr (VI) on the biomass productivity (% of control) of *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

C_i (mg/L)	Biomass productivity (%)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
Control	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
1	71.69±0.21	103.88±0.12	64.30±0.21	93.52±0.09	52.61±0.25	75.84±0.03	43.50±0.01	66.89±0.04
2	69.37±0.15	94.33±0.19	56.65±0.27	82.02±0.08	49.39±0.15	70.56±0.18	38.85±0.01	59.98±0.02
4	64.73±0.17	92.84±0.13	48.67±0.39	75.51±0.15	40.73±0.36	65.76±0.14	31.61±0.01	54.93±0.03
8	60.55±0.15	86.62±0.07	37.69±0.25	70.20±0.12	26.54±0.24	57.18±0.03	LD	45.13±0.02

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

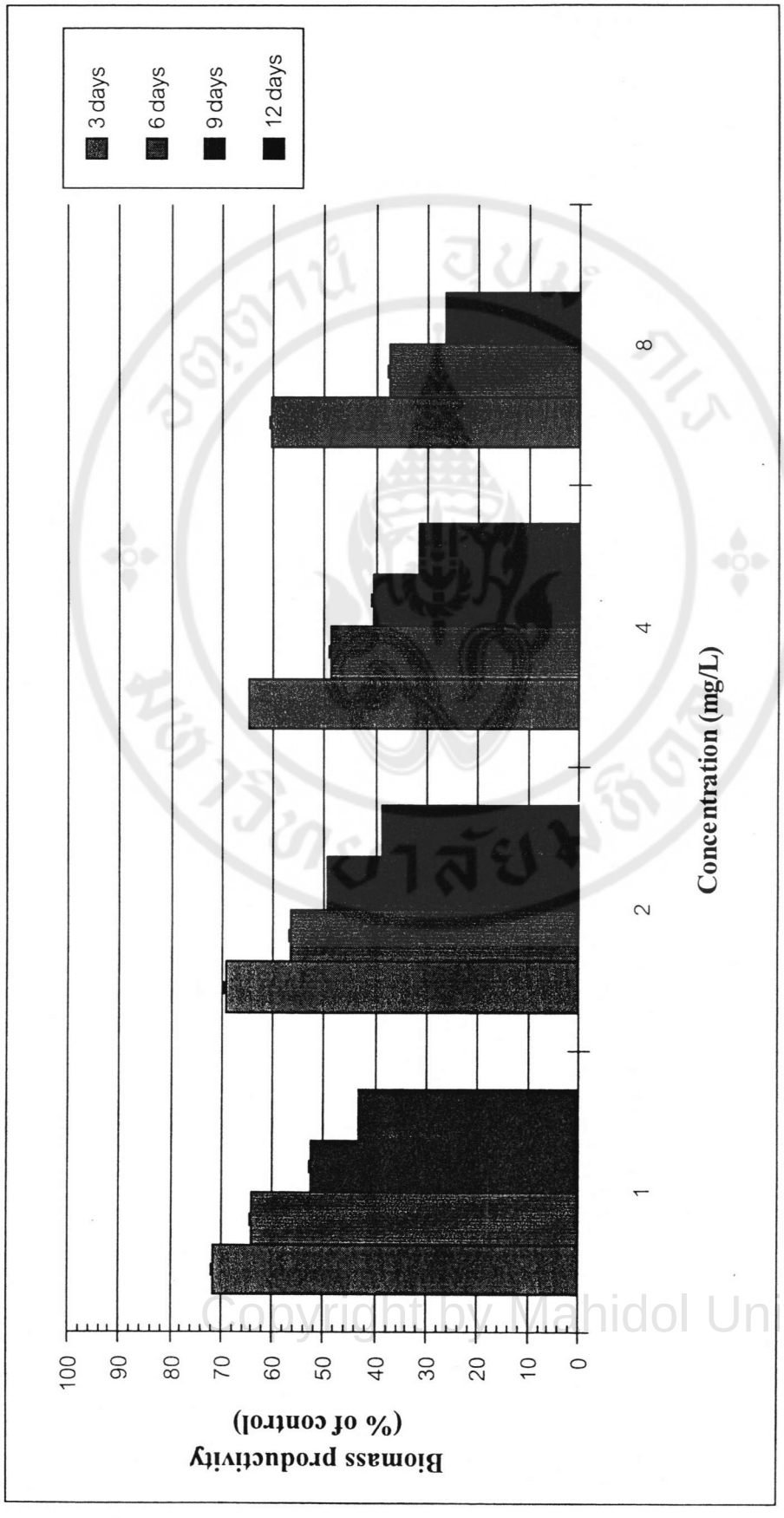

Figure 4-2. The effects of Cd (II) on biomass productivity (% of control) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

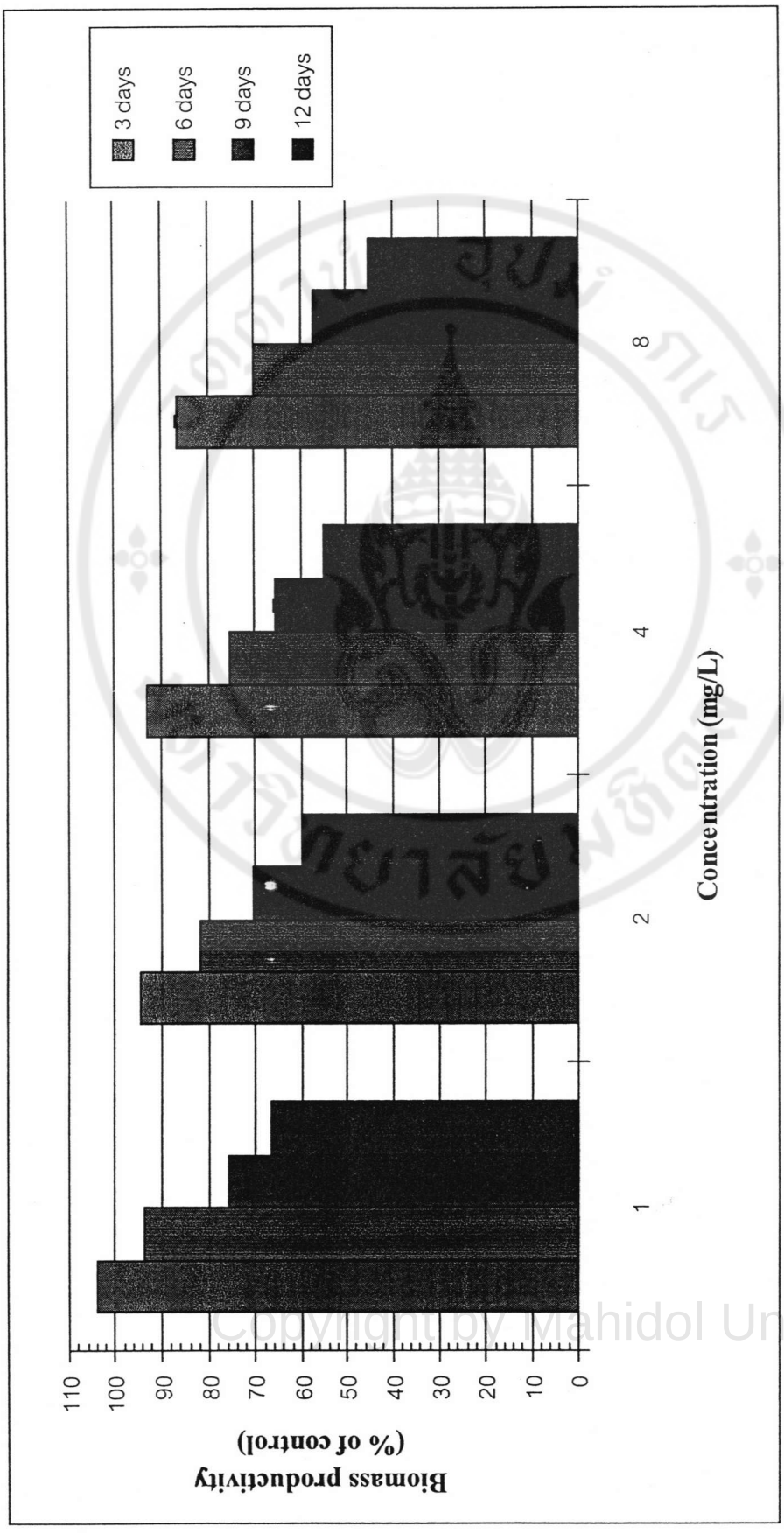

Figure 4-3. The effects of Cr (VI) on biomass productivity (% of control) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

The effective concentrations at 50% (EC_{50}) of Cd (II) and Cr (VI) on the biomass productivity in *Wolffia globosa* showed a progressive decrease when exposure times were increased. The EC_{50} of Cd (II) at 3, 6, and 9 days were 48.88 mg/L, 3.27 mg/L, and 1.53 mg/L, respectively, and for Cr (VI) at 3, 6, 9, and 12 days were 194.72 mg/L, 23.60 mg/L, 18.21 mg/L, and 5.49 mg/L, respectively (Table 4-3). The EC_{50} of Cd (II) was lower than that of Cr (VI) at the same duration, suggesting that Cd (II) was comparatively more toxic than Cr (VI).

Table 4-3. The effective concentrations at 50% (EC_{50}) of Cd (II) and Cr (VI) on the biomass productivity in *Wolffia globosa* at different exposure times (3, 6, 9, and 12 days).

Exposure time (days)	**EC_{50} (mg/L)**	
	Cd^{2+}	**Cr^{6+}**
3	48.88	194.72
6	3.27	23.60
9	1.53	18.21
12	LD	5.49

LD = Lethal dose

3. The chlorophyll content

The effects of Cd (II) and Cr (VI) on the chlorophyll content (mg/g fresh wt.) in *Wolffia globosa* at different concentrations and exposure times are shown in Table 4-4, Table 4-5, Table 4-6, Figure 4-4, Figure 4-5 Figure 4-6, Figure 4-7, Figure 4-8, and Figure 4-9.

The statistical analysis of the effects of Cd (II) and Cr (VI) on the chlorophyll content in *Wolffia globosa* indicated that there were significant decreases ($P<0.05$) when the exposure times and concentrations were increased. Chlorophyll contents of *W. globosa* obtained at 1, 2, 4, and 8 mg/L of each exposure time decreased significantly ($P<0.05$) when compared with those of control. Also, chlorophyll contents at 6, 9, and 12 days decreased significantly ($P<0.05$) during the first three days for all solution concentrations. At higher metal concentrations, chlorophyll decreased considerably during the long exposure period. A maximum of 0.56 mg/g of total chlorophyll was observed in control experiments; a minimum of 0.20 mg/g, was obtained from the plants grown at 8 mg/L Cr (VI) for 12 days and 0.12 mg/g was obtained from the plants grown at 8 mg/L Cd (II) for 9 days after treatment. Plants exposed to ≥ 4 mg/L Cd (II) after 6 days and to 8 mg/L Cr (VI) after 9 days exhibited a slight chlorosis (fronds became yellow) but showed a complete chlorosis after 12 days. Plants exposed to 8 mg/L Cd (II) were dead. When the effects of these metals on chlorophyll contents were compared, *W. globosa* exposed in Cr (VI) solutions showed a significantly higher chlorophyll content ($P<0.05$) than *W. globosa* exposed in Cd (II) solutions. This suggested that Cd (II) exhibited a more severe effect on chlorophyll content than Cr (VI).

Table 4-4. The effects of Cd (II) and Cr (VI) on chlorophyll a (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

C_i (mg/L)	Chlorophyll a (mg/g fresh wt)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
Control	0.2812±0.0002	0.2812±0.0002	0.2901±0.0002	0.2901±0.0002	0.3001±0.0002	0.3001±0.0002	0.3001±0.0002	0.3001±0.0002
1	0.2421±0.0001	0.2912±0.0002	0.1503±0.0002	0.2514±0.0002	0.1316±0.0002	0.1868±0.0001	0.1212±0.0002	0.1615±0.0001
2	0.2241±0.0002	0.2725±0.0001	0.1313±0.0002	0.2205±0.0001	0.1112±0.0002	0.1768±0.0002	0.1007±0.0002	0.1517±0.0002
4	0.2003±0.0003	0.2508±0.0002	0.1001±0.0001	0.1918±0.0002	0.0841±0.0001	0.1586±0.0001	0.0702±0.0002	0.1203±0.0002
8	0.1952±0.0002	0.2208±0.0002	0.0807±0.0002	0.1641±0.0001	0.0605±0.0002	0.1345±0.0001	LD	0.1106±0.0002

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

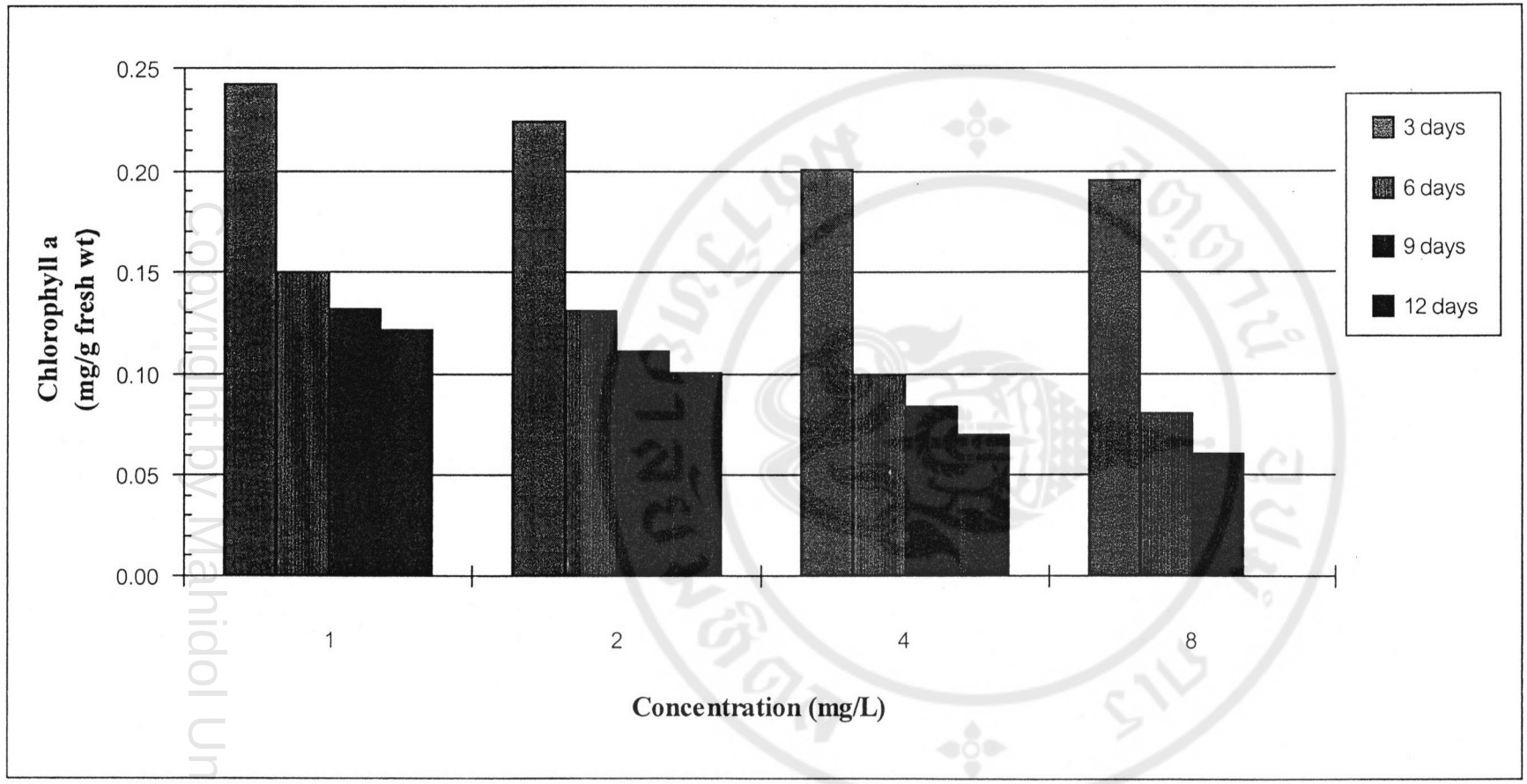

Figure 4-4. The effects of Cd (II) on chlorophyll a (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

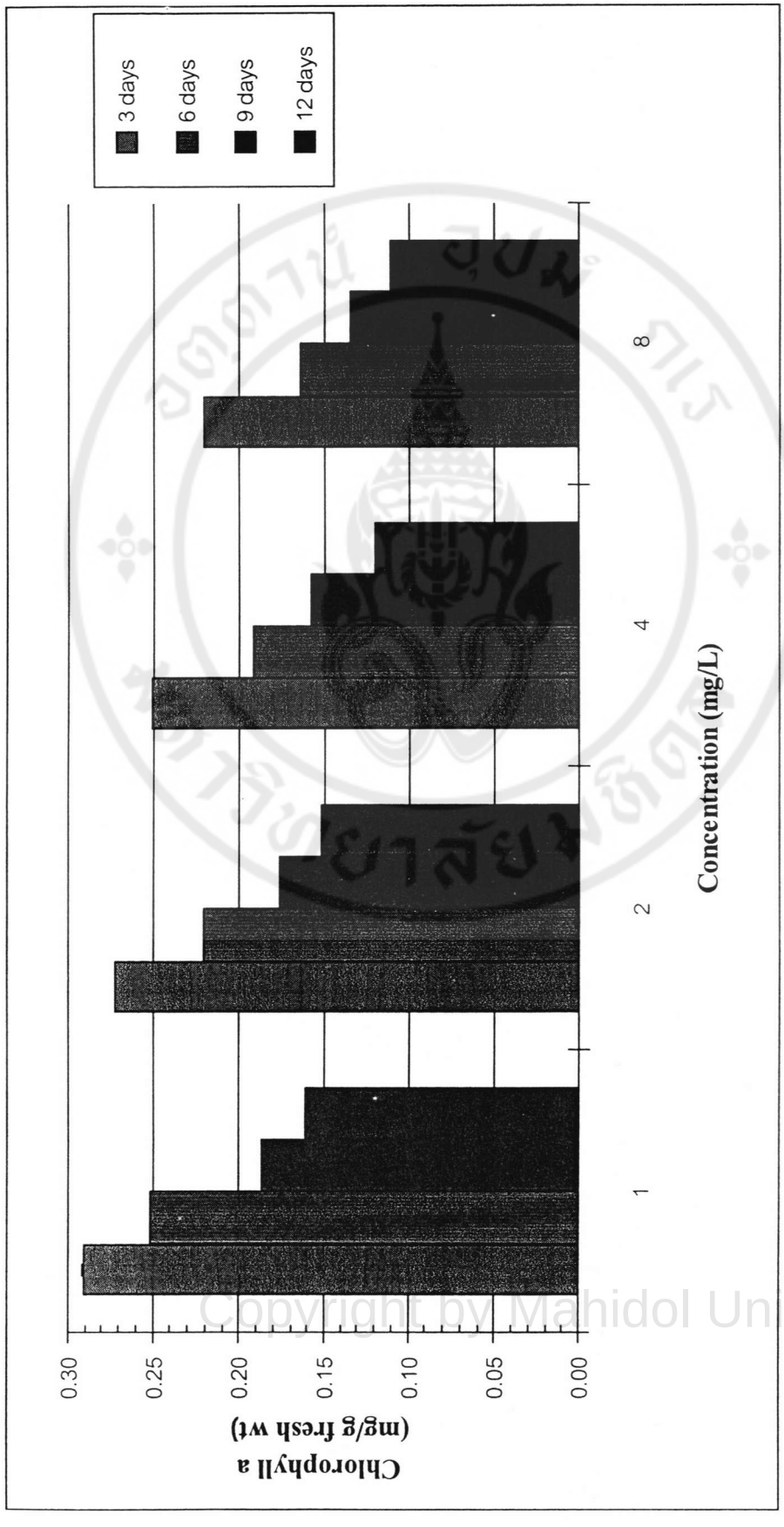

Figure 4-5. The effects of Cr (VI) on chlorophyll a (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

Table 4-5. The effects of Cd (II) and Cr (VI) on chlorophyll b (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

C_i (mg/L)	Chlorophyll b (mg/g fresh wt)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
Control	0.2586±0.0001	0.2586±0.0001	0.2591±0.0001	0.2591±0.0001	0.2594±0.0001	0.2594±0.0001	0.2595±0.0001	0.2595±0.0001
1	0.2242±0.0003	0.2608±0.0001	0.1212±0.0002	0.2242±0.0002	0.1001±0.0002	0.1658±0.0002	0.0902±0.0002	0.1411±0.0001
2	0.1922±0.0002	0.2406±0.0002	0.1001±0.0002	0.1946±0.0001	0.0907±0.0001	0.1566±0.0001	0.0842±0.0002	0.1302±0.0001
4	0.1832±0.0002	0.2287±0.0002	0.0812±0.0002	0.1622±0.0002	0.0653±0.0002	0.1343±0.0002	0.0542±0.0001	0.1001±0.0002
8	0.1764±0.0002	0.1903±0.0001	0.0620±0.0001	0.1413±0.0002	0.0521±0.0002	0.1182±0.0002	LD	0.0908±0.0002

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

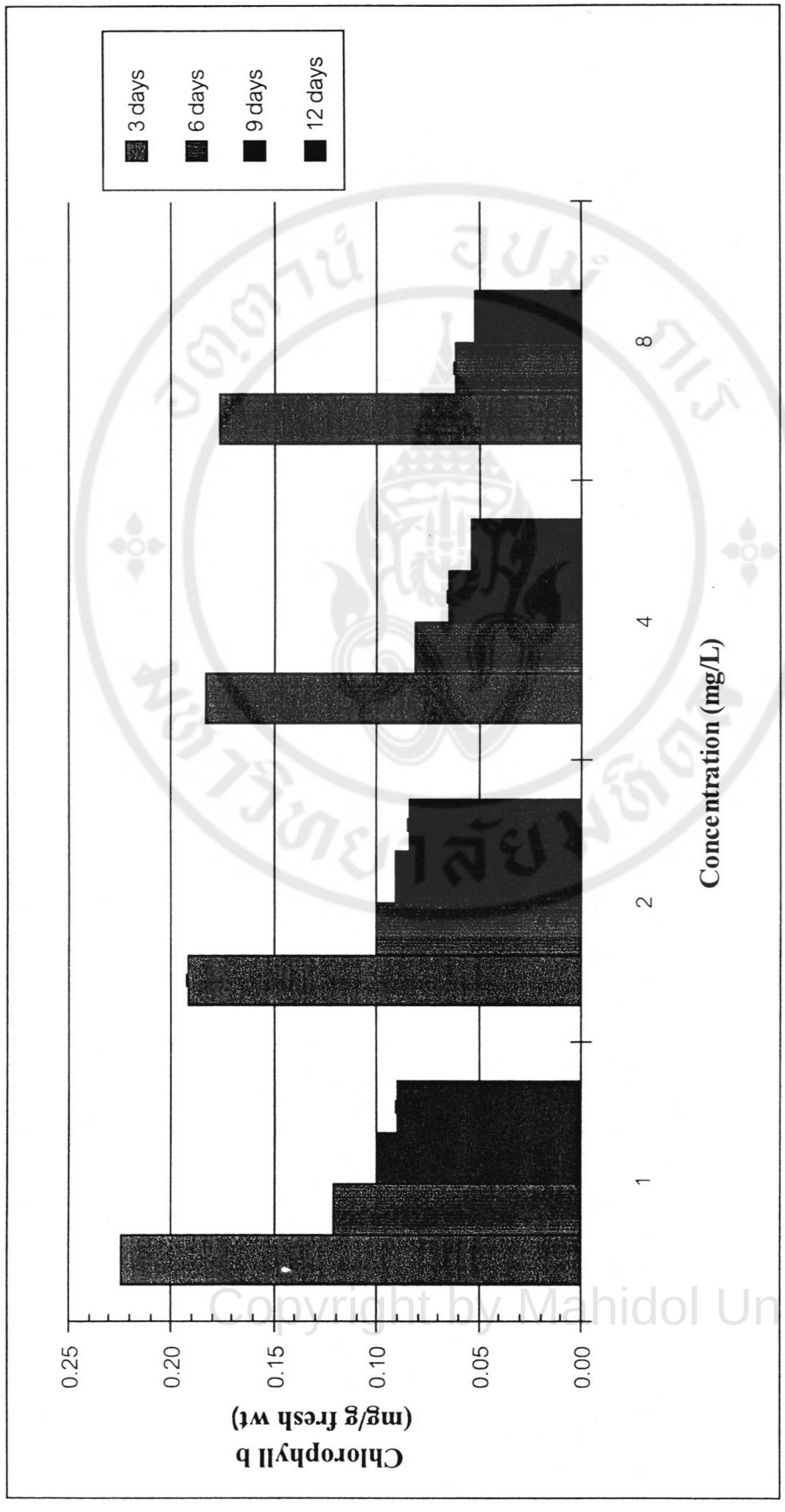

Figure 4-6. The effects of Cd (II) on chlorophyll b (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

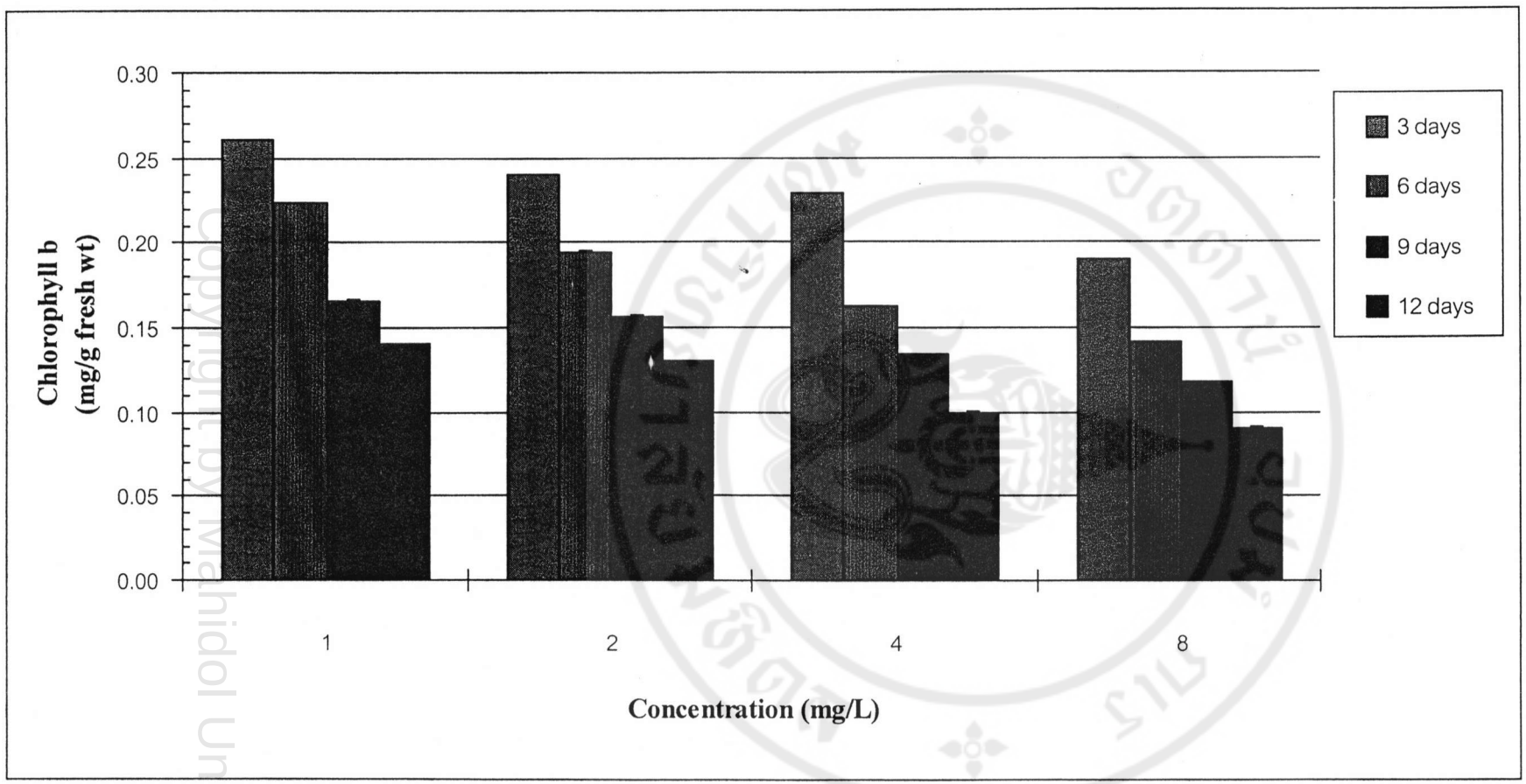

Figure 4-7. The effects of Cr (VI) on chlorophyll b (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

Table 4-6. The effects of Cd (II) and Cr (VI) on total chlorophyll (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

C_i (mg/L)	Total chlorophyll (mg/g fresh wt)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
Control	0.5398±0.0002	0.5398±0.0002	0.5492±0.0002	0.5492±0.0002	0.5595±0.0001	0.5595±0.0001	0.5596±0.0001	0.5596±0.0001
1	0.4663±0.0004	0.5520±0.0002	0.2715±0.0001	0.4756±0.0002	0.2318±0.0001	0.3525±0.0001	0.2114±0.0002	0.3026±0.0001
2	0.4163±0.0001	0.5131±0.0001	0.2314±0.0003	0.4151±0.0002	0.2019±0.0001	0.3334±0.0002	0.1849±0.0002	0.2819±0.0001
4	0.3835±0.0003	0.4795±0.0001	0.1813±0.0001	0.3540±0.0002	0.1494±0.0001	0.2929±0.0001	0.1244±0.0001	0.2204±0.0000
8	0.3716±0.0002	0.4111±0.0001	0.1427±0.0001	0.3053±0.0001	0.1127±0.0003	0.2527±0.0001	LD	0.2013±0.0002

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

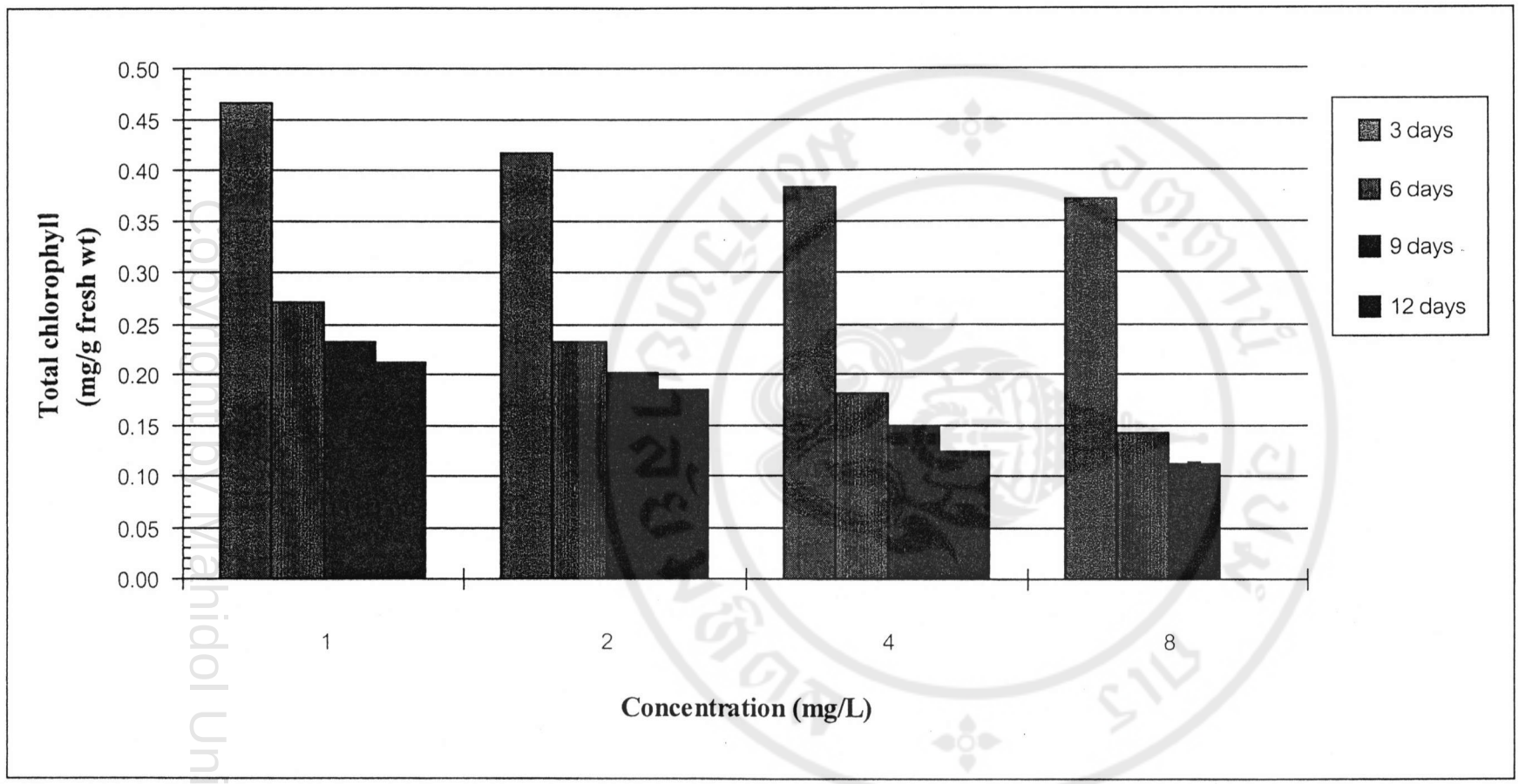

Figure 4-8. The effects of Cd (II) on total chlorophyll (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

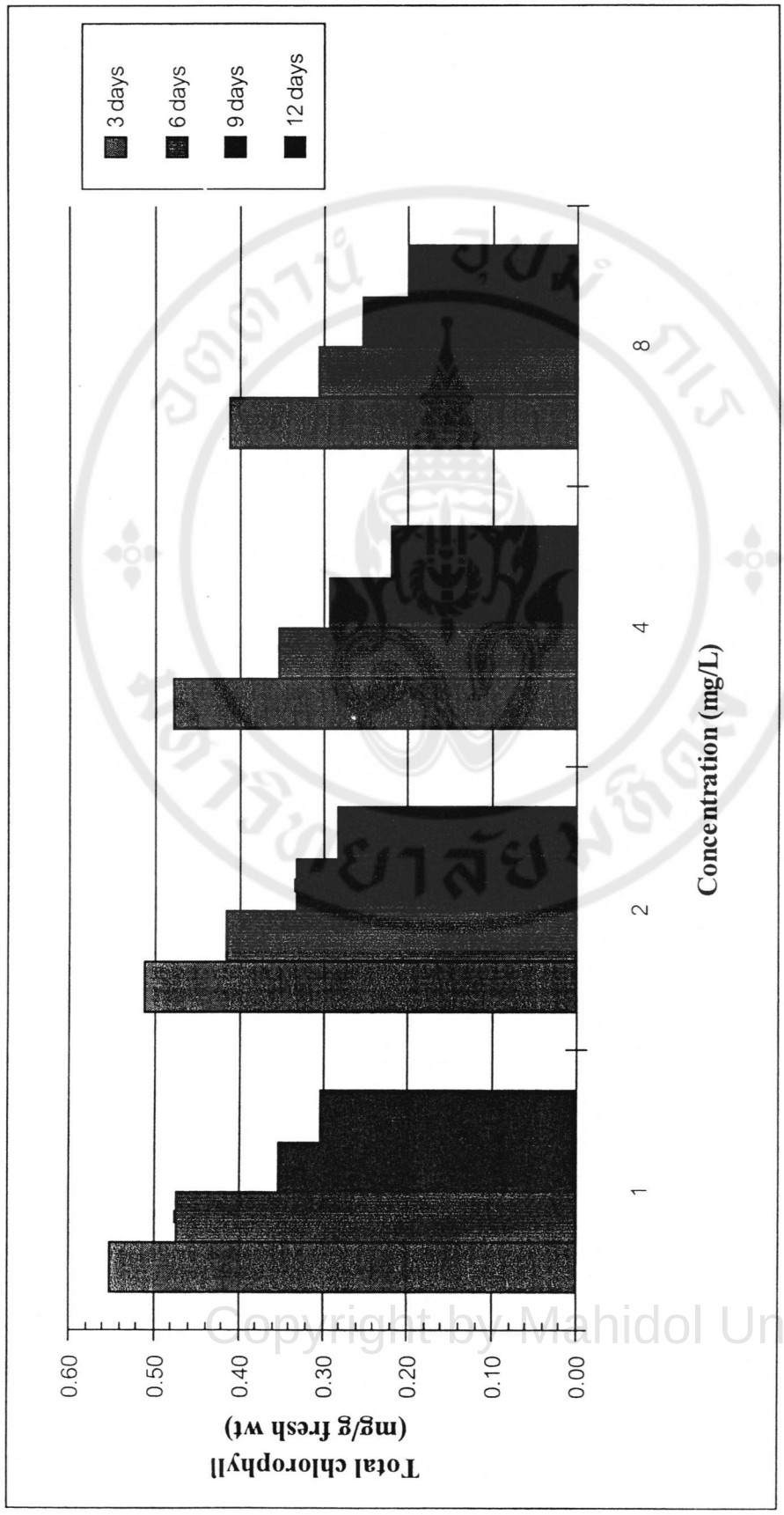

Figure 4-9. The effects of Cr (VI) on total chlorophyll (mg/g fresh wt) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

The effective concentrations at 50% (EC_{50}) of Cd (II) and Cr (VI) on the chlorophyll contents in *Wolffia globosa* at different exposure times are shown in Table 4-7. The EC_{50} of Cd (II) on total chlorophyll at 3, 6, and 9 days were 37.00 mg/L, 0.99 mg/L, and 0.54 mg/L, respectively, and for Cr (VI) at 3, 6, 9, and 12 days were 22.86 mg/L, 9.96 mg/L, 5.02 mg/L, and 1.65 mg/L, respectively. The EC_{50} of Cd (II) was lower than that of Cr (VI) during the same duration, suggesting that Cd (II) was comparatively more toxic than Cr (VI).

Table 4-7. The effective concentrations at 50% (EC_{50}) of Cd (II) and Cr (VI) on the chlorophyll content in *Wolffia globosa* at different exposure times (3, 6, 9, and 12 days).

Exposure time (days)	**EC_{50} (mg/L)**					
	Chl a		**Chl b**		**Total chl**	
	Cd^{2+}	**Cr^{6+}**	**Cd^{2+}**	**Cr^{6+}**	**Cd^{2+}**	**Cr^{6+}**
3	39.28	22.38	34.59	22.69	37.00	22.86
6	1.22	10.86	0.77	9.11	0.99	9.96
9	0.67	4.95	0.39	5.10	0.54	5.02
12	LD	1.67	LD	1.64	LD	1.65

LD = Lethal dose
Chl a = Chlorophyll a (mg/g fresh wt)
Chl b = Chlorophyll b (mg/g fresh wt)
Total chl = Total chlorophyll (mg/g fresh wt)

4. The accumulation of Cd (II) and Cr (VI)

The effects of Cd (II) and Cr (VI) on the accumulation (μg/g dry wt) of *Wolffia globosa* at different concentrations and exposure times are shown in Table 4-8, Figure 4-10, and Figure 4-11. The statistical analysis of the effects of Cd (II) and Cr (VI) on the accumulation in *W. globosa* indicated that there were significant increases ($P<0.05$) in tissue levels when the exposure times and concentrations were increased. *Wolffia globosa* showed significantly higher accumulations of Cd (II) and Cr (VI) ($P<0.05$) at 1, 2, 4, and 8 mg/L than those of control solutions at each exposure time, indicating that *W. globosa* might attain higher accumulation at still higher solution levels. The accumulation of metals increased significantly ($P<0.05$) with the passage of times (3-6 days). However, when exposure times were increased from 9 days to 12 days, *W. globosa* showed no significant increases ($P>0.05$) in tissue levels for all solution concentrations, suggesting that these plants approached their maximum accumulation in 12 days. At the lowest background Cr (VI) concentration (1 mg/L), the accumulation was 700.90 μg/g dry wt after 12 days. At the highest concentration of the metal (8 mg/L), the accumulation was found to be 3,500.77 μg/g dry wt for the same duration. In Cd (II) treatment, the fronds accumulated 907.57 μg/g dry wt at 1 mg/L, whereas at 4 mg/L, the fronds showed 3,373.30 μg/g dry wt accumulation after 12 days.

Table 4-8. The effects of Cd (II) and Cr (VI) on the accumulation (μg/g dry wt) of *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

C_i (mg/L)	Metal accumulation (μg/g dry wt)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
Control	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
1	484.67±1.56	288.00±1.57	686.33±1.20	493.53±1.65	856.00±1.61	608.23±0.68	907.57±1.25	700.90±0.36
2	890.73±1.76	494.53±1.15	1,295.13±2.75	898.10±1.13	1,656.13±1.12	1,152.00±1.05	1,750.17±1.07	1,302.03±0.65
4	1,605.70±1.30	854.97±0.91	2,403.60±2.61	1,605.93±1.40	3,112.90±2.46	2,141.20±1.11	3,373.30±1.21	2,441.23±0.49
8	3,053.33±2.81	1,282.57±1.60	4,567.90±2.94	2,274.17±1.93	5,931.87±0.78	2,951.47±0.55	LD	3,500.77±0.71

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

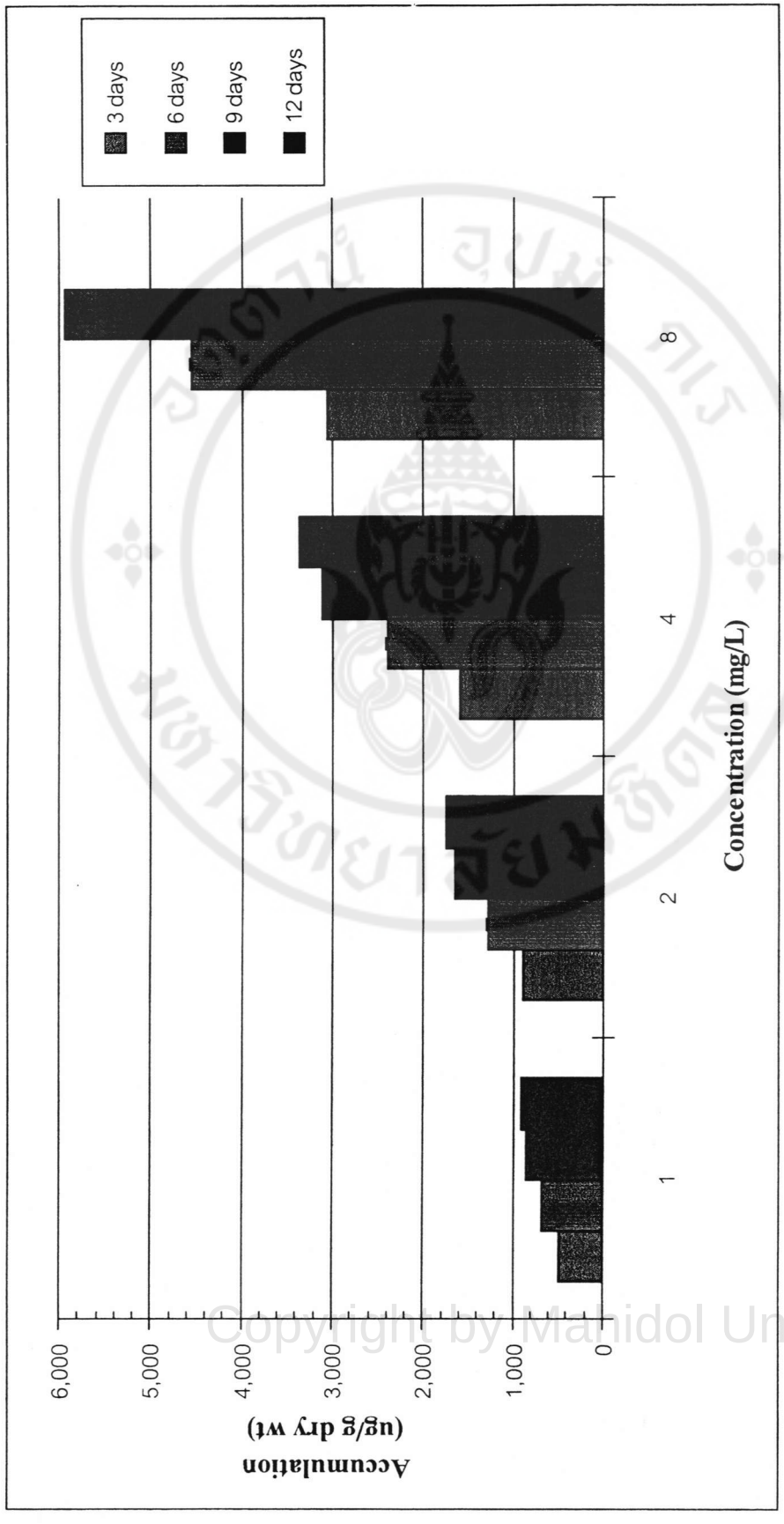

Figure 4-10. The accumulation of Cd (II) by *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

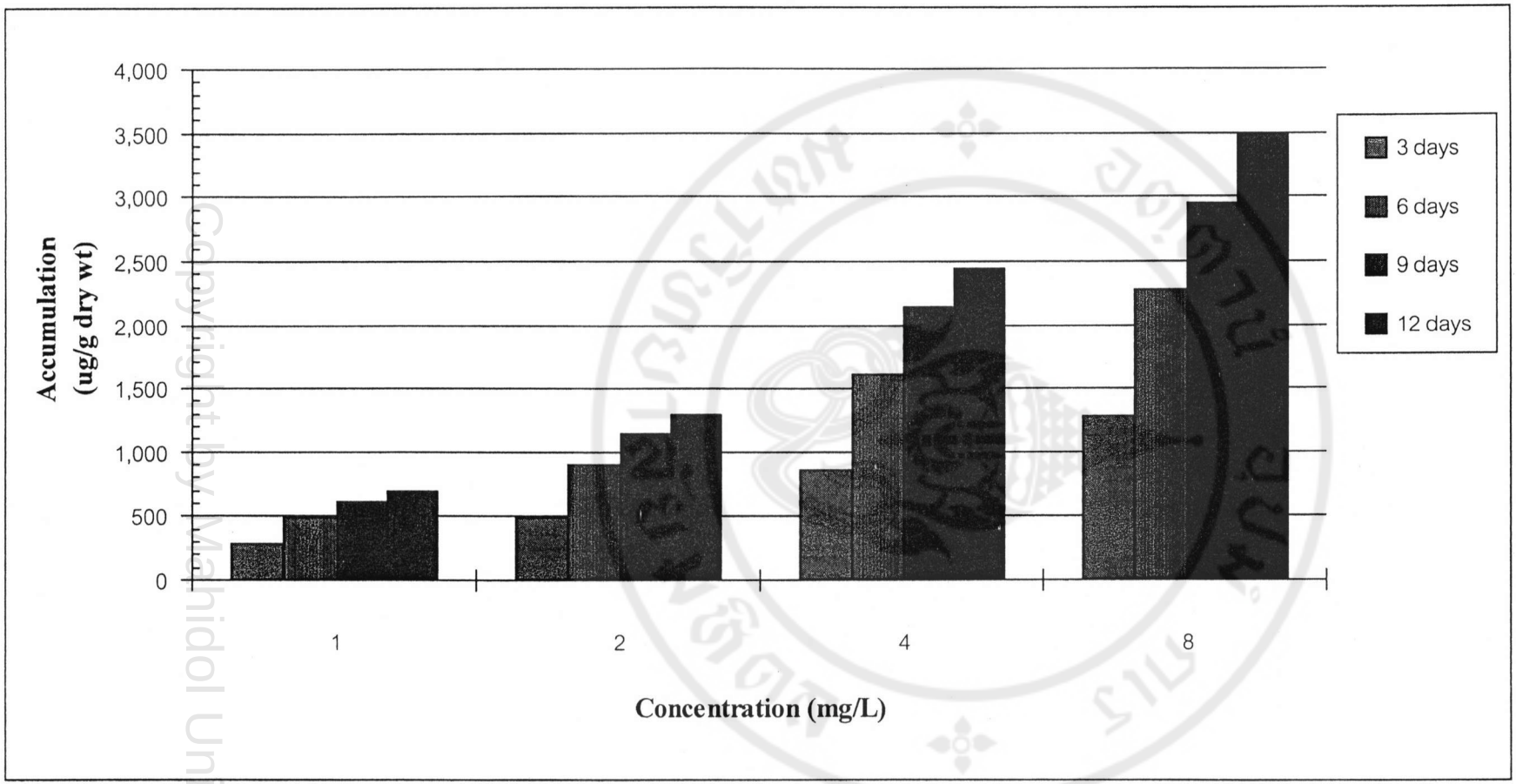

Figure 4-11. The accumulation of Cr (VI) by *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

The effects of Cd (II) and Cr (VI) on the rates of accumulation (μg/g dry wt/day) of *Wolffia globosa* at different concentrations and exposure times are shown in Table 4-9, Figure 4-12, and Figure 4-13. The rates of accumulation of both metals decreased rapidly on the first 6 days and then slowly decreased on day 9 and day 12. At the lowest Cr (VI) concentration (1 mg/L), the rates of accumulation were 288.00 and 92.67 μg/g dry wt/day after 3 days and 12 days, respectively. At the highest concentration of metal (8 mg/L), the rates of accumulation were 1,282.57 and 549.30 μg/g dry wt/day after 3 days and 12 days, respectively. In Cd (II) treatment (1 mg/L), the rates of accumulation were 484.67 and 51.57 μg/g dry wt/day after 3 days and 12 days, respectively, whereas at 4 mg/L, the rates of accumulation were 1,605.70 and 260.40 μg/g dry wt/day after 3 days and 12 days, respectively.

Table 4-9. The effects of Cd (II) and Cr (VI) on the rates of accumulation (μg/g dry wt./day) of *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9 and 12 days).

C_i (mg/L)	Rates of metals accumulation (μg/g dry wt/day)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
Control	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
1	484.67±1.56	288.00±1.57	201.67±1.89	205.53±1.56	169.67±1.21	114.70±1.14	51.57±1.65	92.67±1.54
2	890.73±1.76	494.53±1.15	404.40±1.50	403.57±1.97	361.00±1.54	253.90±2.36	94.03±1.48	150.03±1.69
4	1,605.70±1.30	854.97±0.91	797.30±1.33	750.97±1.46	709.30±2.36	535.27±2.10	260.40±1.69	300.03±1.47
8	3,053.33±2.81	1,282.57±1.60	1,514.57±2.69	991.60±2.30	1,363.97±1.29	677.30±1.56	LD	549.30±1.87

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

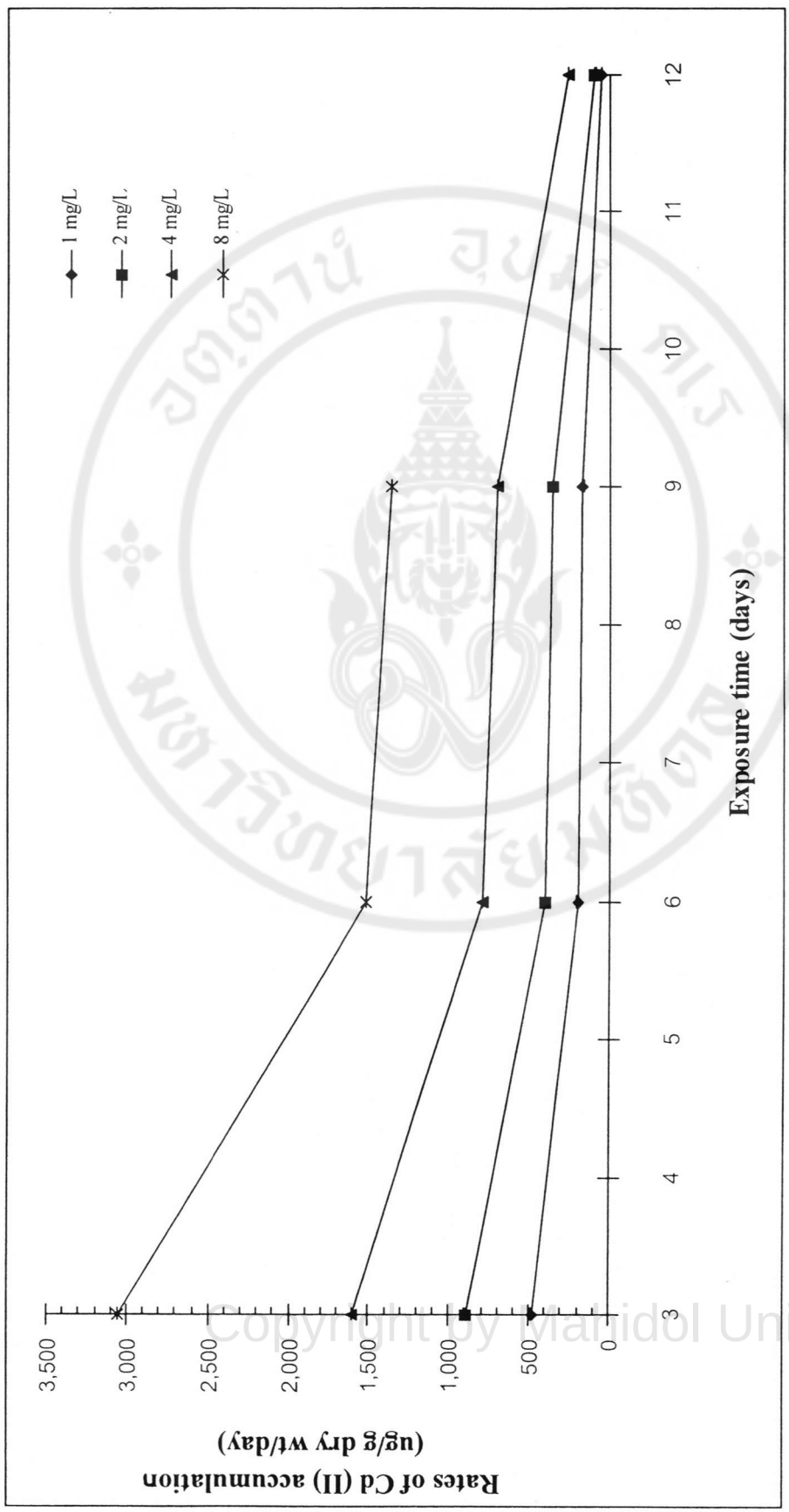

Figure 4-12. The rates of Cd (II) accumulation (µg/g dry wt/day) of *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

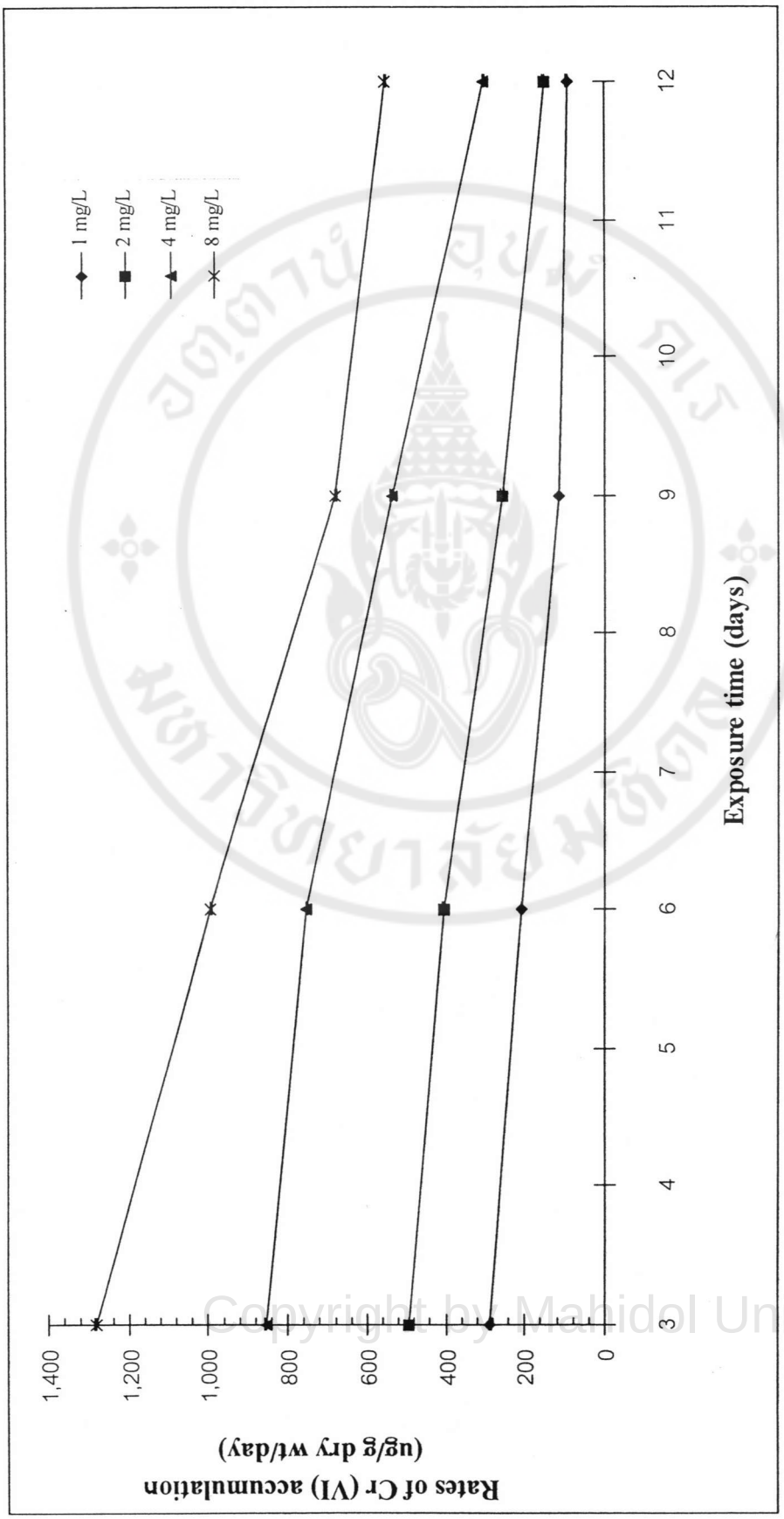

Figure 4-13. The rates of Cr (VI) accumulation (µg/g dry wt/day) of *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

5. The bioconcentration factor (BCF)

The bioconcentration factor (BCF) provides an index of the ability of the plant to accumulate the trace element with respect to the trace element concentration in the substrate. This factor is defined as the ratio of metal concentration in the biomass to the initial concentration of metal ion in the feed solution. The BCF for Cd (II) and Cr (VI) in *Wolffia globosa* at different concentrations and exposure times are shown in Table 4-10, Figure 4-14, and Figure 4-15. From these data, it would appear that there is a gradual decrease in Cd (II) and Cr (VI) accumulate potential in plant with an increase in Cd (II) and Cr (VI) concentration in feed solutions at each exposure time. In Cd (II) and Cr (VI) treatments, *W. globosa* obtained at 2, 4, and 8 mg/L of each exposure time decreased significantly ($P<0.05$) in BCF from 1 mg/L, indicating that the ability of the plant to accumulate Cd (II) and Cr (VI) was decreased when Cd (II) and Cr (VI) concentrations were increased. The value of the BCF for Cd (II) is higher than that for Cr (VI), indicating that the accumulation potential of Cd (II) is higher than that of Cr (VI) in *W. globosa*.

Table 4-10. The bioconcentration factor (BCF) for Cd (II) and Cr (VI) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

C_i (mg/L)	Bioconcentration factor (BCF)							
	3 days		6 days		9 days		12 days	
	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}	Cd^{2+}	Cr^{6+}
1	484.67±1.56	288.00±1.57	686.33±1.20	493.53±1.65	856.00±1.61	608.23±0.68	907.57±1.25	700.90±0.36
2	445.37±0.88	247.27±0.58	647.57±1.38	449.05±0.56	828.07±0.56	576.00±0.53	875.08±0.53	651.02±0.33
4	401.43±0.32	213.74±0.23	600.90±0.65	401.48±0.35	778.23±0.61	535.30±0.28	843.33±0.30	610.31±0.12
8	381.67±0.35	160.32±0.20	570.99±0.37	284.27±0.24	741.48±0.10	368.93±0.07	LD	437.60±0.09

C_i = Initial concentration (mg/L)
LD = Lethal dose
Each value is the mean of triplicate ± S.D.

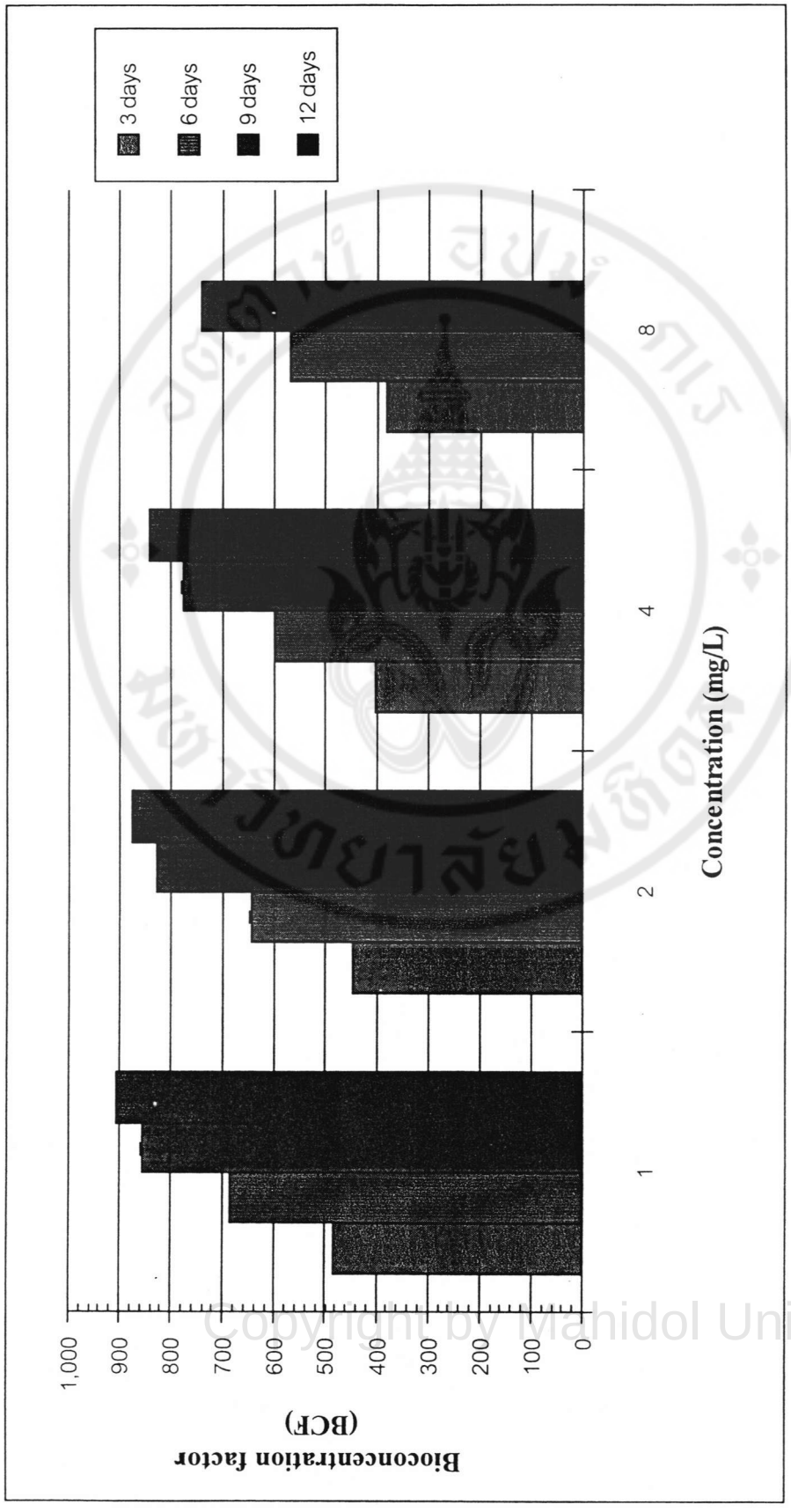

Figure 4-14. The bioconcentration factor (BCF) for Cd (II) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

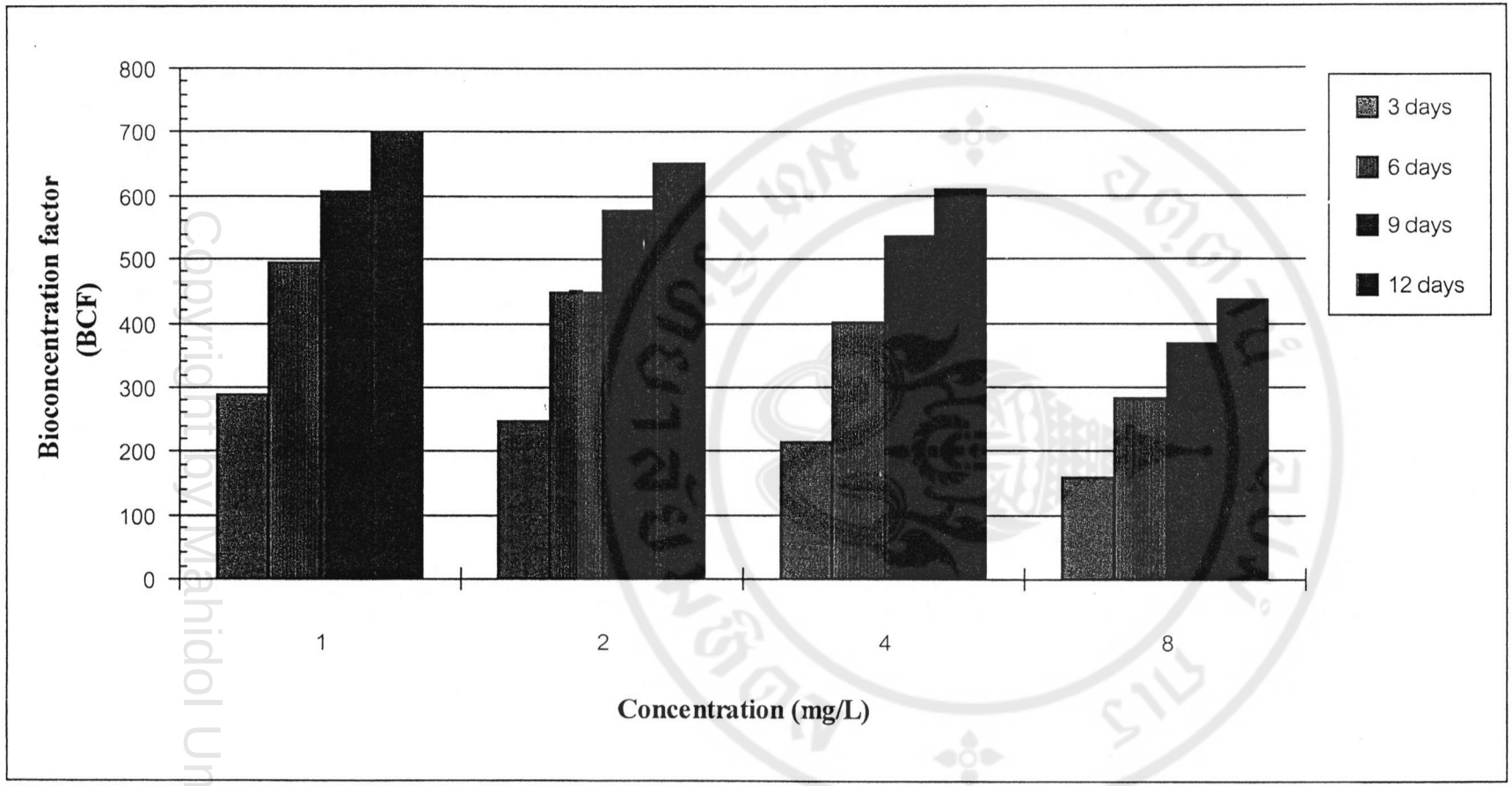

Figure 4-15. The bioconcentration factor (BCF) for Cr (VI) in *Wolffia globosa* at different concentrations (1, 2, 4, and 8 mg/L) and exposure times (3, 6, 9, and 12 days).

B. Biosorption of dried *Wolffia globosa* biomass

1. The adsorption isotherm studies

Sorption equilibrium was established when most of the cadmium and chromium ions were adsorbed on to *Wolffia globosa* after five hours of incubation. The typical sorption isotherms for the *W. globosa*-Cd (II) and *W. globosa*-Cr (VI) systems at different pH values at 25°C are shown in Table 4-11, Table 4-12, Figure 4-16, and Figure 4-17.

Table 4-11. The equilibrium concentration (mg/L) of Cd (II) and Cr (VI) at different concentrations and pH values.

C_i (mg/L)	C_e (mg/L)							
	Cadmium (II)				Chromium (VI)			
	pH 4	pH 5	pH 6	pH 7	pH 1.5	pH 3	pH 5	pH 6
10	0.90±0.01	0.60±0.01	0.46±0.01	0.14±0.01	0.46±0.01	1.27±0.11	3.00±0.16	6.15±0.27
20	2.00±0.23	1.56±0.16	1.02±0.15	0.41±0.05	1.04±0.12	4.05±0.19	7.02±0.25	13.48±0.23
40	5.00±0.46	4.51±0.40	3.04±0.34	1.03±0.13	3.46±0.26	9.25±0.25	14.91±0.54	30.01±0.33
60	10.45±0.98	8.02±0.76	6.04±0.51	2.25±0.24	6.02±0.42	15.15±0.36	25.16±0.25	47.00±0.35
80	15.16±0.45	12.45±0.34	9.46±0.30	3.51±0.29	9.15±0.56	23.44±0.44	35.00±0.52	64.84±0.49
100	25.15±0.76	21.09±0.66	14.21±0.54	5.03±0.41	13.15±0.74	31.15±0.54	47.16±0.45	81.02±0.46
200	100.44±1.54	60.11±1.01	45.60±0.97	20.75±0.26	35.00±0.65	90.15±0.98	120.46±0.78	170.49±0.87
400	265.16±2.56	220.15±2.46	164.55±1.45	101.38±1.30	144.94±1.56	237.15±1.78	290.15±2.16	360.15±2.42

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-12. The uptake (mg/g) of Cd (II) and Cr (VI) at different concentrations and pH values.

C_i (mg/L)	Uptake of metals (mg/g)							
	Cadmium (II)				Chromium (VI)			
	pH 4	pH 5	pH 6	pH 7	pH 1.5	pH 3	pH 5	pH 6
10	2.28±0.97	2.35±0.92	2.39±0.89	2.46±0.91	2.39±0.87	2.18±0.76	1.75±0.65	0.96±0.09
20	4.50±1.15	4.61±1.11	4.75±1.26	4.90±1.37	4.74±1.54	3.99±1.03	3.25±1.11	1.63±0.51
40	8.75±1.27	8.87±1.29	9.24±1.36	9.74±1.41	9.13±1.39	7.69±1.01	6.27±1.23	2.50±0.84
60	12.39±1.99	12.99±1.87	13.49±2.01	14.44±2.33	13.50±2.38	11.21±1.87	8.71±1.39	3.25±1.01
80	16.21±2.34	16.89±2.31	17.64±2.01	19.12±2.36	17.71±2.24	14.14±2.10	11.25±1.96	3.79±1.21
100	18.71±2.13	19.73±2.21	21.45±2.25	23.74±2.30	21.71±2.21	17.21±2.15	13.21±2.03	4.75±1.36
200	24.89±2.65	34.97±2.35	38.60±2.54	44.81±2.74	41.25±2.63	27.46±2.34	19.89±2.15	7.38±1.78
400	33.71±2.74	44.96±2.84	58.86±2.86	74.65±2.94	63.76±2.68	40.71±2.52	27.46±2.10	9.96±1.96

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

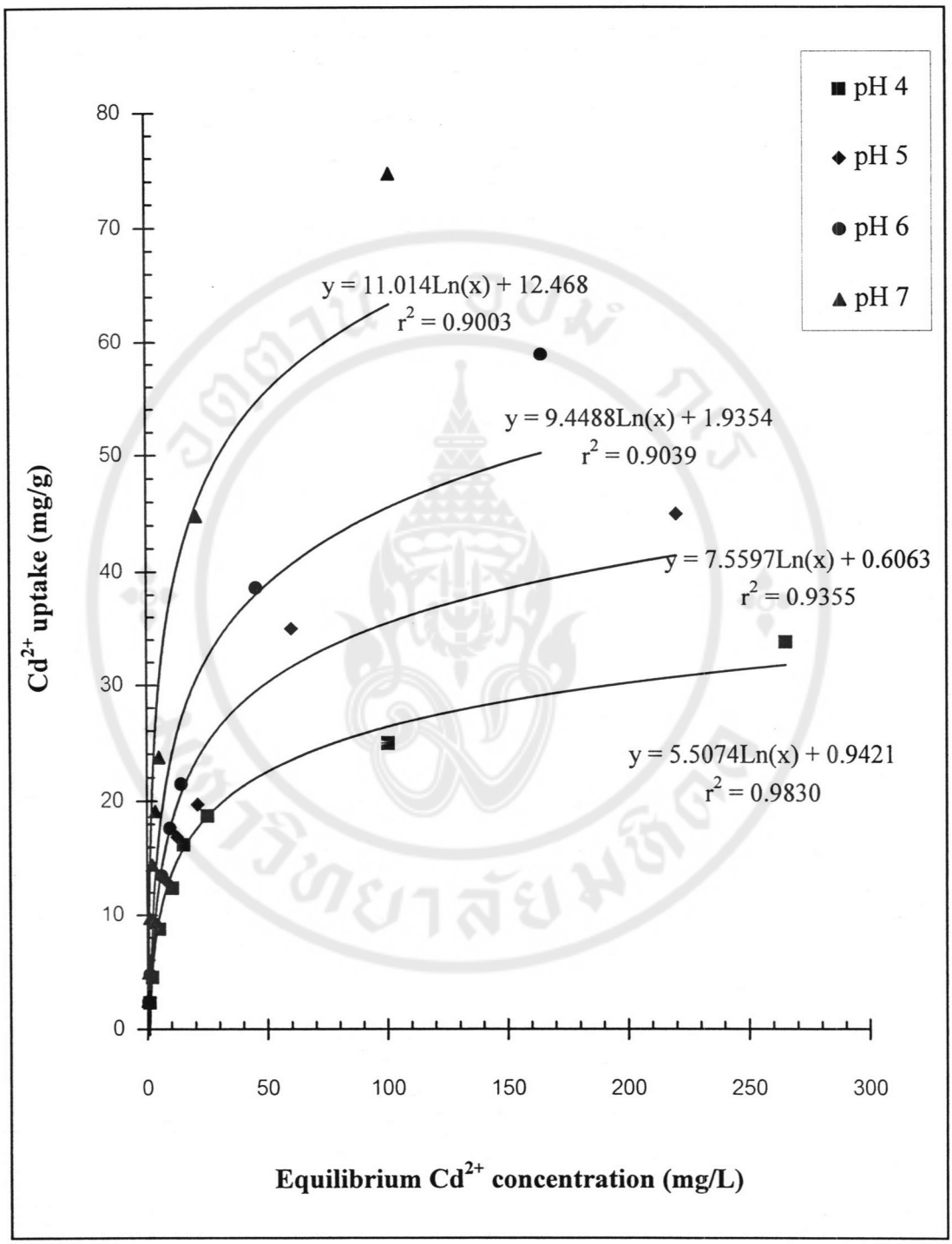

Figure 4-16. The adsorption isotherms of *Wolffia globosa*-Cd (II) system at 25°C after five hours of incubation (*Wolffia globosa* biomass; 4 g/L; stirring rate 200 rpm).

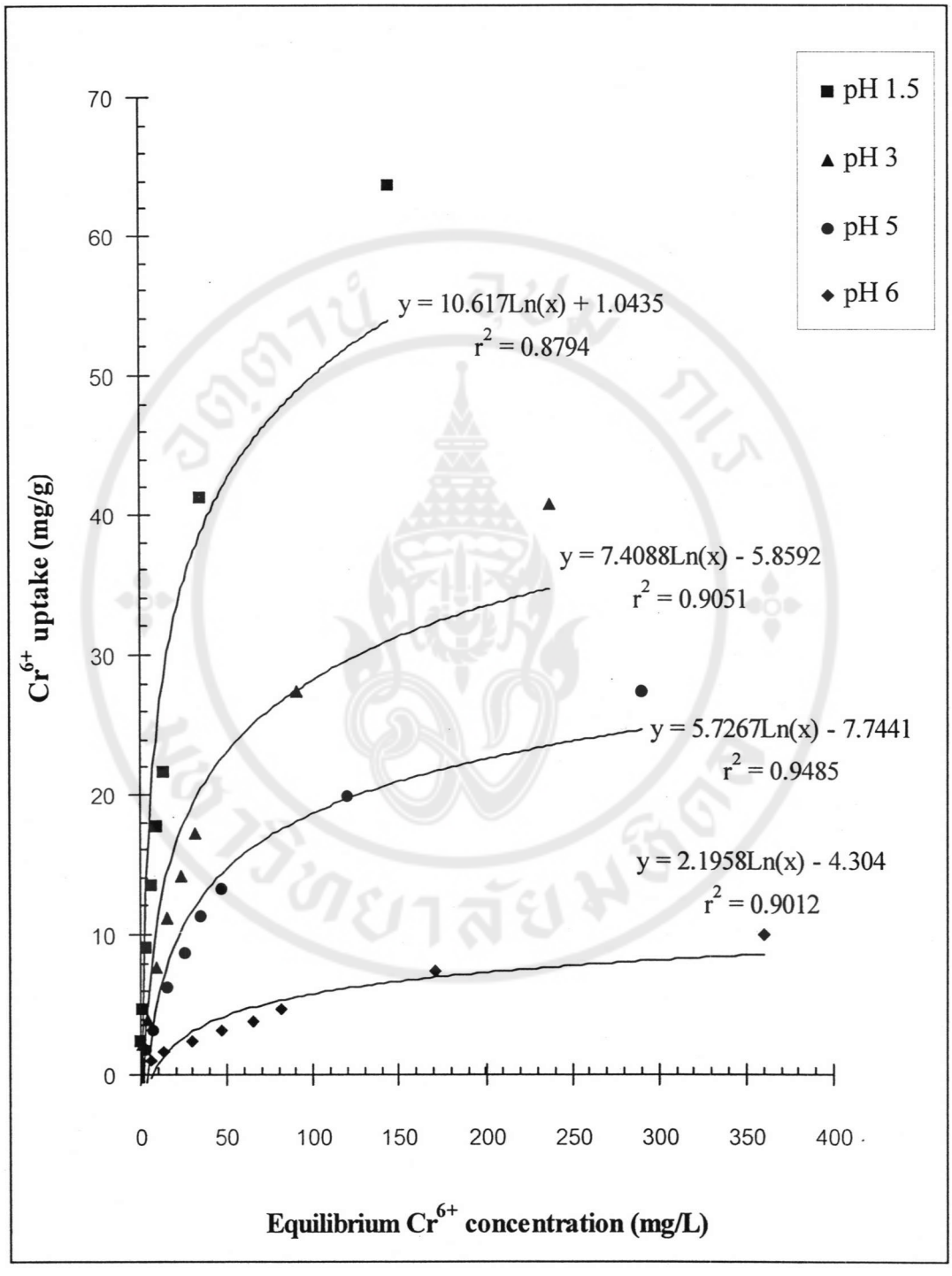

Figure 4-17. The adsorption isotherms of *Wolffia globosa*-Cr (VI) system at 25°C after five hours of incubation (*Wolffia globosa* biomass; 4 g/L; stirring rate 200 rpm).

In *Wolffia globosa*-Cd (II) system, the metal uptake showed a significantly increase ($P<0.05$) with increasing equilibrium metal concentrations at each pH value. The isotherms were rather steep at lower initial concentration (10-100 mg/L), implying the suitability of *W. globosa* to treat dilute Cd (II) solutions. When the pH values were increased, there were slight increases in the metal uptake at pH 4 to pH 6, and at pH 7 the metal uptake increased significantly ($P<0.05$), indicating that pH 7 was optimum for the adsorption in a buffered system. The adsorption isotherms were found to follow the typical Langmuir adsorption pattern, as shown by the linear transformation (Figure 4-18). The Langmuir model used for this linearization is valid for monolayer adsorption onto a surface, containing a finite number of identical sites. The model also assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of the surface. From the linearized Langmuir plots, the isotherm constants were derived by performing linear regression on each set of data and are presented in Table 4-13. The correlation coefficients, r^2, for pH values ranging from 4 to 7 were found to be > 0.97, which indicated that there was a strong positive relationship between C_e and q_e. Isotherm data were used to calculate the maximum adsorption capacity of *W. globosa* by substituting the required equilibrium concentrations in the Langmuir equation. The highest experimentally observed Cd (II) uptake value of 74.65 mg/g (at C_e = 101.38 mg/L) still did not represent the full saturation value in the isotherm for pH 7, and the calculated X_m was as high as 80.65 mg/g while the removal achieved at pH4, pH 5, and pH 6 were lower (35.09, 48.78, and 65.36 mg/g). The Langmuir constant, b, can serve as an indicator of the isotherm rise in the region of lower residual metal concentrations, which reflects the strength or

affinity of the sorbent for the solute. It was found to increase as the incubation pH was raised, which implies that the removal of Cd (II) ions at high pH could be more complete than that at low pH. The pH of the initial incubation is therefore an important factor in the uptake of Cd (II) by *W. globosa*. The 4-6 pH range not only resulted in a low Cd (II) uptake, but also affected the b value of the isotherm, decreasing the capacity of *W. globosa* for adsorbing metal from a dilute Cd (II) solution.

Table 4-13. Showing the Langmuir constants of *Wolffia globosa*-Cd (II) binding.

pH	Temperature (°C)	Xm (mg/g)	b	r^2
4	25	35.09	5.26×10^{-2}	0.99
5	25	48.78	4.80×10^{-2}	0.99
6	25	65.36	4.69×10^{-2}	0.98
7	25	80.65	1.04×10^{-1}	0.99

Xm = Maximum adsorption capacity
b = Langmuir constant
r^2 = Correlation coefficient

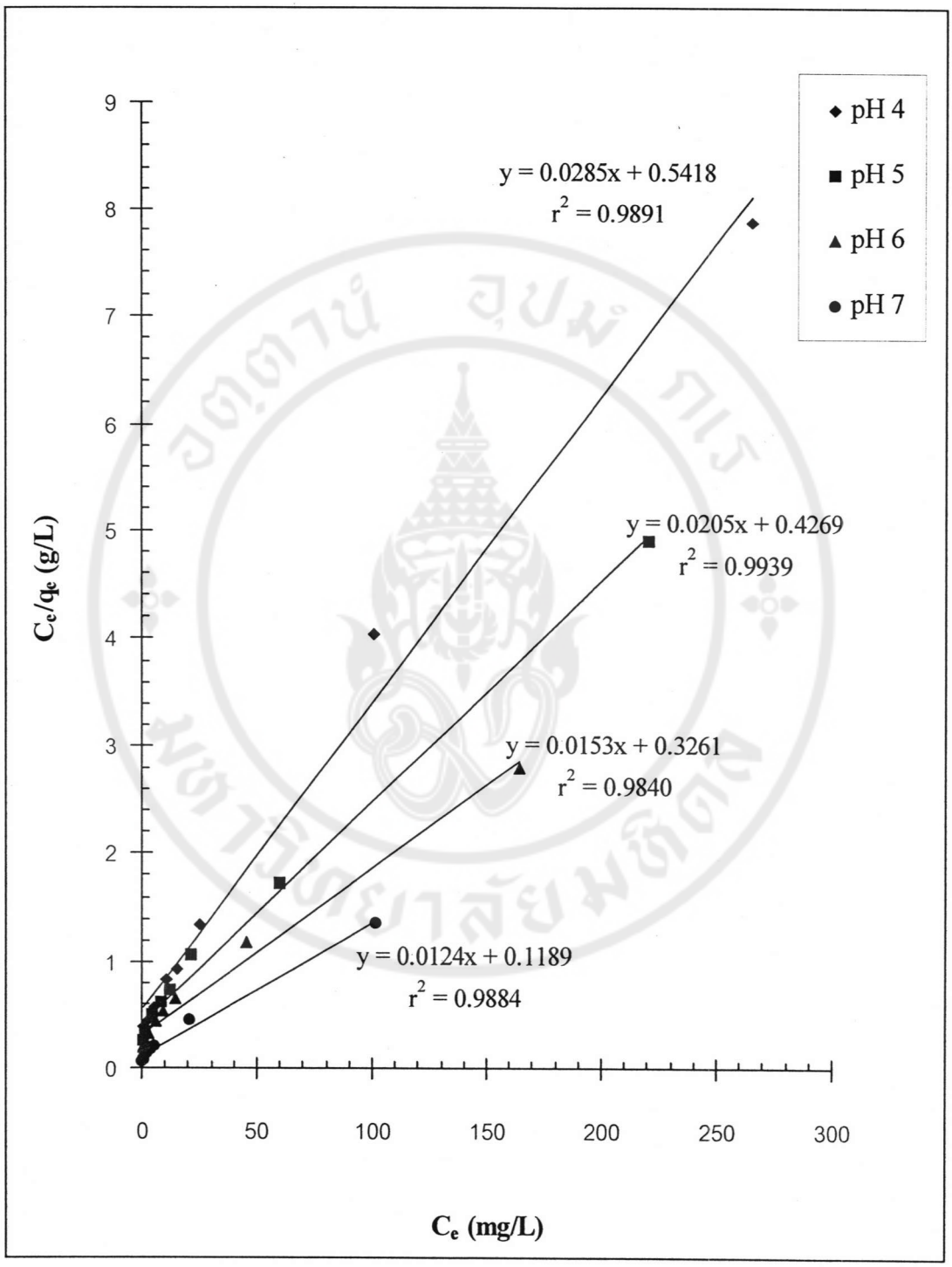

Figure 4-18. Showing the linearized plots of Langmuir isotherms for Cd (II) sorption obtained at different pH values at 25°C (*Wolffia globosa* biomass; 4 g/L; stirring rate 200 rpm).

In *Wolffia globosa*-Cr (VI) system, the metal uptake showed significantly increase ($P<0.05$) with increasing equilibrium metal concentrations at each pH value. The isotherms were rather steep at lower initial concentration (10-100 mg/L), implying the suitability of *W. globosa* to treat dilute Cr (VI) solutions. When the pH values were decreased from 6 to 1.5, there were slight increases in the metal uptake. When the pH values were decrease from 3 to 1.5, the metal uptake increased significantly ($P<0.05$), suggesting that Cr (VI) was removed most effectively in an acidic environment. The isotherm data showed a good compliance with the Langmuir equation (Table 4-14 and Figure 4-19). The correlation coefficients, r^2, for the 1.5-6 pH range were found to be > 0.94, which indicated that there was a strong positive relationship between C_e and q_e. The calculated X_m for Cr (VI) at pH 1.5 was as high as 73.53 mg/g, while the removal achieved at pH 3, pH 5, and pH 6 were lower (47.39, 33.11, and 12.85 mg/g). The values of b, related to the energy of adsorption, appeared to decrease with increasing pH.

Table 4-14. Showing the Langmuir constants of *Wolffia globosa*-Cr (VI) binding.

pH	Temperature (°C)	Xm (mg/g)	b	r^2
1.5	25	73.53	4.23×10^{-2}	0.98
3	25	47.39	2.09×10^{-2}	0.98
5	25	33.11	1.50×10^{-2}	0.99
6	25	12.85	8.36×10^{-3}	0.95

Xm = Maximum adsorption capacity
b = Langmuir constant
r^2 = Correlation coefficient

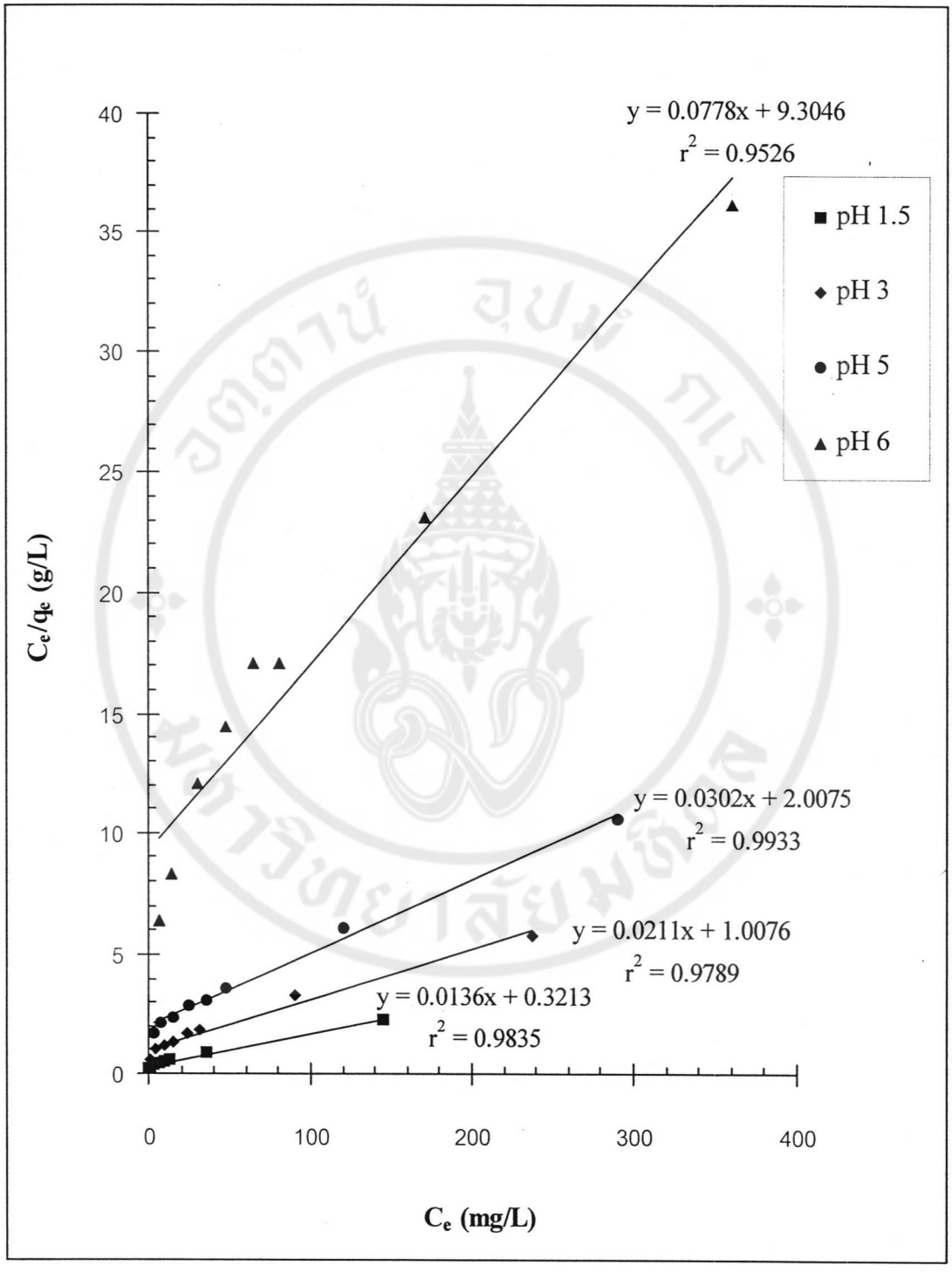

Figure 4-19. Showing the linearized plots of Langmuir isotherms for Cr (VI) sorption obtained at different pH values at 25°C (*Wolffia globosa* biomass; 4 g/L; stirring rate 200 rpm).

2. The effects of contact times on Cd (II) and Cr (VI) sorption

The effects of contact times of Cd (II) sorption were studied by varying Cd (II) concentrations ranging from 10 to 400 mg/L at pH 4 to pH 7. It is clear from this that most of the Cd (II) were adsorbed during the first five hours. The Cd (II) concentrations showed a significantly decrease ($P<0.05$) when the contact times were increased at all pH values, and the Cd (II) uptake was relatively fast for all the concentrations studied. At pH 4, 5, 6, and 7, there were sharp decreases in the Cd (II) concentrations in the first hour and a small decrease was observed when the contact times were increased to five hours. At the contact times of more than five hours, the residual Cd (II) concentrations were stable, suggesting that the maximum adsorption was reached (Table 4-15, Table 4-17, Table 4-19, Table 4-21, Figure 4-20, Figure 4-22, Figure 4-24, and Figure 4-26). The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 4 are shown in Table 4-16, Table 4-18, Table 4-20, Table 4-22, Figure 4-21, Figure 4-23, Figure 4-25, and Figure 4-27.

Table 4-15. The effects of contact times on the equilibrium Cd (II) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 4.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	**1**	**1.5**	**2**	**3**	**4**	**5**	**7**	**9**	**24**
10	5.03±0.15	3.10±0.11	2.58±0.16	2.00±0.10	1.51±0.12	1.22±0.11	0.90±0.01	0.90±0.01	0.90±0.01	0.90±0.01
20	13.00±0.21	10.00±0.26	7.00±0.18	5.00±0.15	3.50±0.16	3.00±0.13	2.00±0.23	2.00±0.23	2.00±0.23	2.00±0.23
40	28.00±0.26	21.00±0.15	15.00±0.12	8.51±0.13	7.50±0.15	5.52±0.12	5.00±0.46	5.00±0.46	5.00±0.46	5.00±0.46
60	45.00±0.19	32.00±0.26	24.60±0.22	16.04±0.26	14.02±0.18	12.00±0.15	10.45±0.98	10.45±0.98	10.45±0.98	10.45±0.98
80	60.01±0.25	43.60±0.24	34.02±0.31	25.11±0.27	20.06±0.20	17.02±0.20	15.16±0.45	15.16±0.45	15.16±0.45	15.16±0.45
100	76.53±0.36	56.70±0.46	45.17±0.36	38.02±0.25	31.11±0.26	27.14±0.19	25.15±0.76	25.15±0.76	25.15±0.76	25.15±0.76
200	172.30±1.01	145.02±1.10	130.11±1.02	122.20±1.14	109.00±1.02	103.50±1.19	100.44±1.54	100.44±1.54	100.44±1.54	100.44±1.54
400	346.00±2.36	300.05±2.01	290.09±2.03	280.10±2.01	273.00±2.02	268.00±2.09	265.16±2.56	265.16±2.56	265.16±2.56	265.16±2.56

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-16. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 4.

C_i (mg/L)	Cd (II) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	49.70±1.50	69.00±1.10	74.20±1.60	80.00±1.00	84.90±1.20	87.80±1.10	91.00±0.10	91.00±0.10	91.00±0.10	91.00±0.10
20	35.00±1.05	50.00±1.30	65.00±0.90	75.00±0.75	82.50±0.80	85.00±0.65	90.00±1.15	90.00±1.15	90.00±1.15	90.00±1.15
40	30.00±0.65	47.50±0.38	62.50±0.30	74.73±0.33	81.25±0.38	86.20±0.30	87.50±1.15	87.50±1.15	87.50±1.15	87.50±1.15
60	25.00±0.32	46.67±0.43	59.00±0.37	73.27±0.43	76.63±0.30	80.00±0.25	82.58±1.63	82.58±1.63	82.58±1.63	82.58±1.63
80	24.99±0.31	45.50±0.30	57.48±0.39	68.61±0.34	74.93±0.25	78.73±0.25	81.05±0.56	81.05±0.56	81.05±0.56	81.05±0.56
100	23.47±0.36	43.30±0.46	54.83±0.36	61.98±0.25	68.89±0.26	72.86±0.19	74.85±0.76	74.85±0.76	74.85±0.76	74.85±0.76
200	13.85±0.50	27.49±0.55	34.95±0.51	38.90±0.57	45.50±0.51	48.25±0.60	49.78±0.77	49.78±0.77	49.78±0.77	49.78±0.77
400	13.50±0.64	24.99±0.52	27.48±0.50	29.98±0.50	31.75±0.51	33.00±0.50	33.71±0.59	33.71±0.59	33.71±0.59	33.71±0.59

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

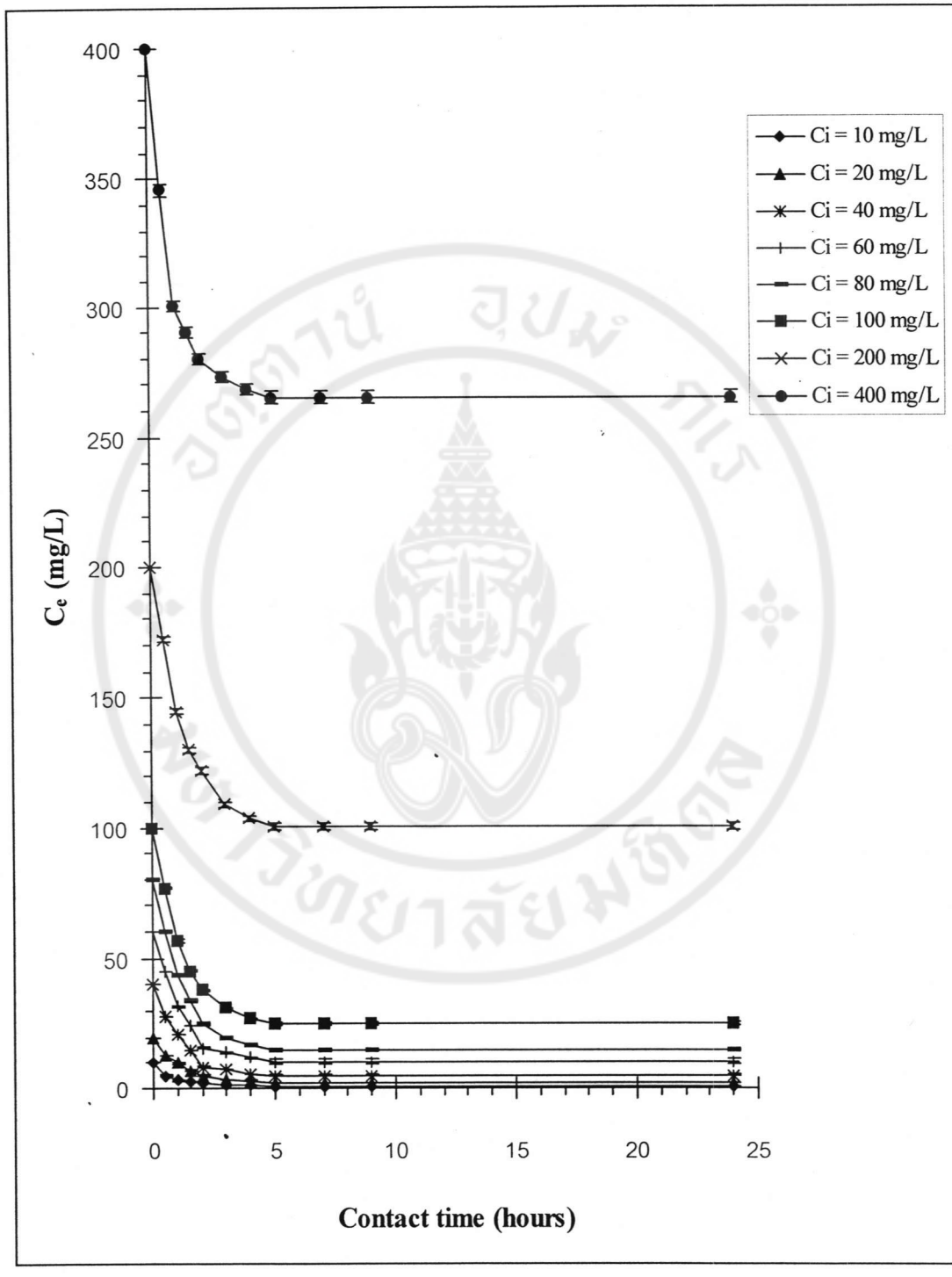

Figure 4-20. The effects of contact times on the equilibrium Cd (II) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 4.

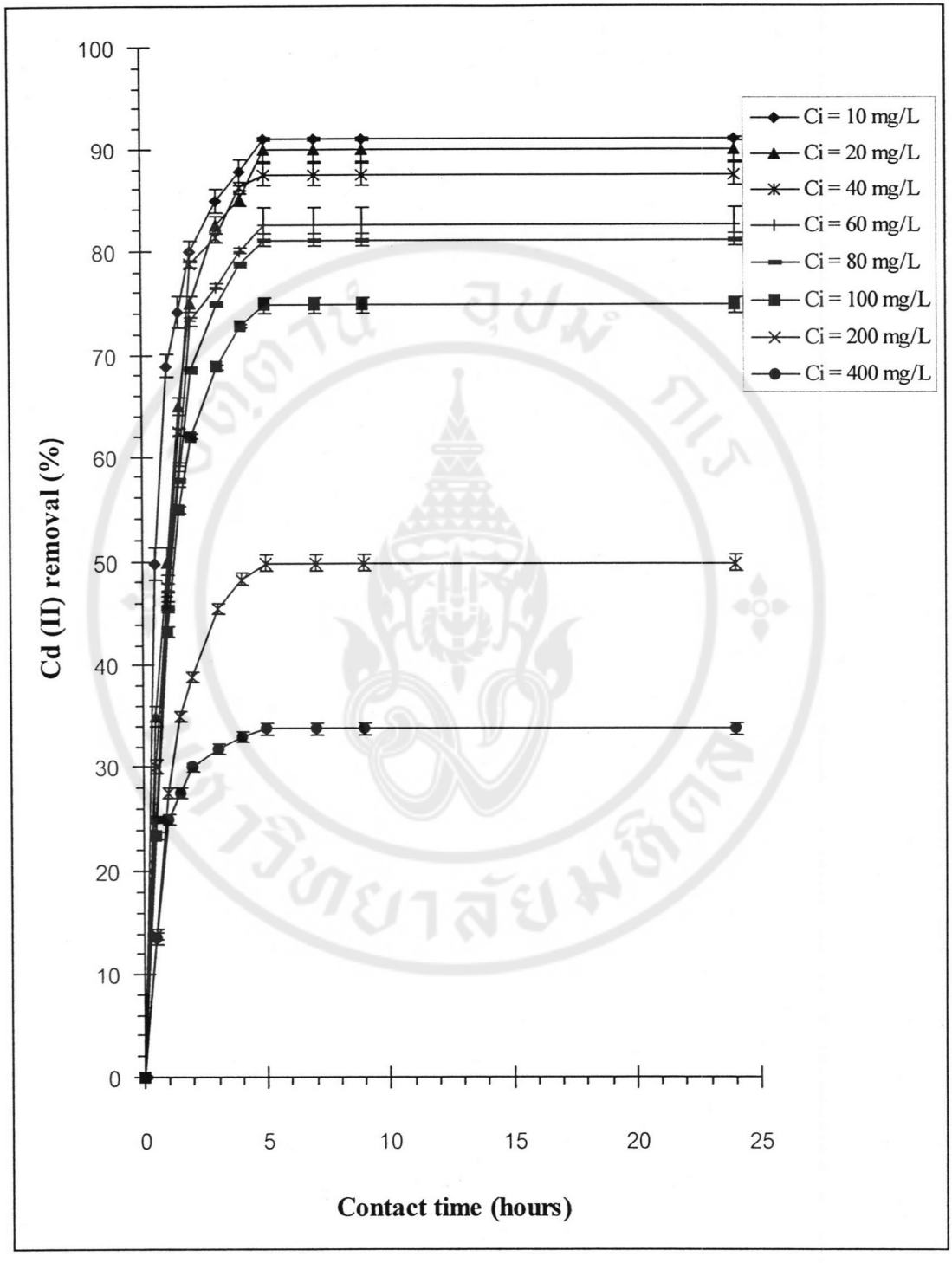

Figure 4-21. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 4.

Table 4-17. The effects of contact times on the equilibrium Cd (II) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 5.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	**1**	**1.5**	**2**	**3**	**4**	**5**	**7**	**9**	**24**
10	4.05±0.15	2.05±0.15	1.47±0.11	0.97±0.12	0.81±0.09	0.73±0.07	0.60±0.01	0.60±0.01	0.60±0.01	0.60±0.01
20	10.81±0.23	8.56±0.65	7.42±0.24	5.42±0.19	3.10±0.15	1.80±0.15	1.56±0.16	1.56±0.16	1.56±0.16	1.56±0.16
40	25.60±0.41	18.23±0.56	15.60±0.42	11.63±0.64	7.01±0.36	5.00±0.19	4.51±0.40	4.51±0.40	4.51±0.40	4.51±0.40
60	40.18±0.36	28.40±0.46	25.60±0.36	19.70±0.98	14.56±0.54	8.50±0.16	8.02±0.76	8.02±0.76	8.02±0.76	8.02±0.76
80	54.15±0.26	42.60±0.25	35.60±0.56	28.40±0.44	19.80±0.15	13.70±0.29	12.45±0.34	12.45±0.34	12.45±0.34	12.45±0.34
100	70.20±0.59	55.02±0.67	45.03±0.49	36.90±0.42	25.12±0.51	22.70±0.24	21.09±0.66	21.09±0.66	21.09±0.66	21.09±0.66
200	165.15±1.25	134.15±1.23	110.50±1.65	95.15±1.69	75.55±0.98	65.45±0.97	60.11±1.01	60.11±1.01	60.11±1.01	60.11±1.01
400	330.02±2.98	291.13±2.54	270.10±2.57	255.00±2.45	240.15±2.36	227.15±2.64	220.15±2.46	220.15±2.46	220.15±2.46	220.15±2.46

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-18. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 5.

C_i (mg/L)	Cd (II) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	59.50±1.50	79.50±1.50	85.30±1.10	90.30±1.20	91.90±0.90	93.50±0.70	94.00±0.10	94.00±2.02	94.00±2.02	94.00±2.02
20	45.94±1.15	57.20±3.25	62.90±1.20	72.90±0.95	84.50±0.75	91.00±0.75	92.20±0.80	92.20±2.03	92.20±2.03	92.20±2.03
40	36.00±1.02	54.43±1.40	61.00±1.05	70.93±1.60	82.48±0.90	87.50±0.47	88.73±1.00	88.73±1.87	88.73±1.87	88.73±1.87
60	33.03±0.60	52.67±0.77	57.33±0.60	67.17±1.63	75.73±0.90	85.83±0.27	86.63±1.27	86.63±1.86	86.63±1.86	86.63±1.86
80	32.31±0.32	46.75±0.31	55.50±0.70	64.50±0.55	75.25±0.19	82.88±0.36	84.44±0.43	84.44±1.59	84.44±1.59	84.44±1.59
100	29.80±0.59	44.98±0.67	54.97±0.49	63.10±0.42	74.88±0.51	77.30±0.24	78.91±0.66	78.91±1.58	78.91±1.58	78.91±1.58
200	17.43±0.63	32.93±0.61	44.75±0.83	52.43±0.85	62.23±0.49	67.28±0.49	69.95±0.51	69.95±1.59	69.95±1.59	69.95±1.59
400	17.50±0.75	27.22±0.64	32.48±0.64	36.25±0.61	39.96±0.59	43.21±0.66	44.96±0.61	44.96±1.52	44.96±1.52	44.96±1.52

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

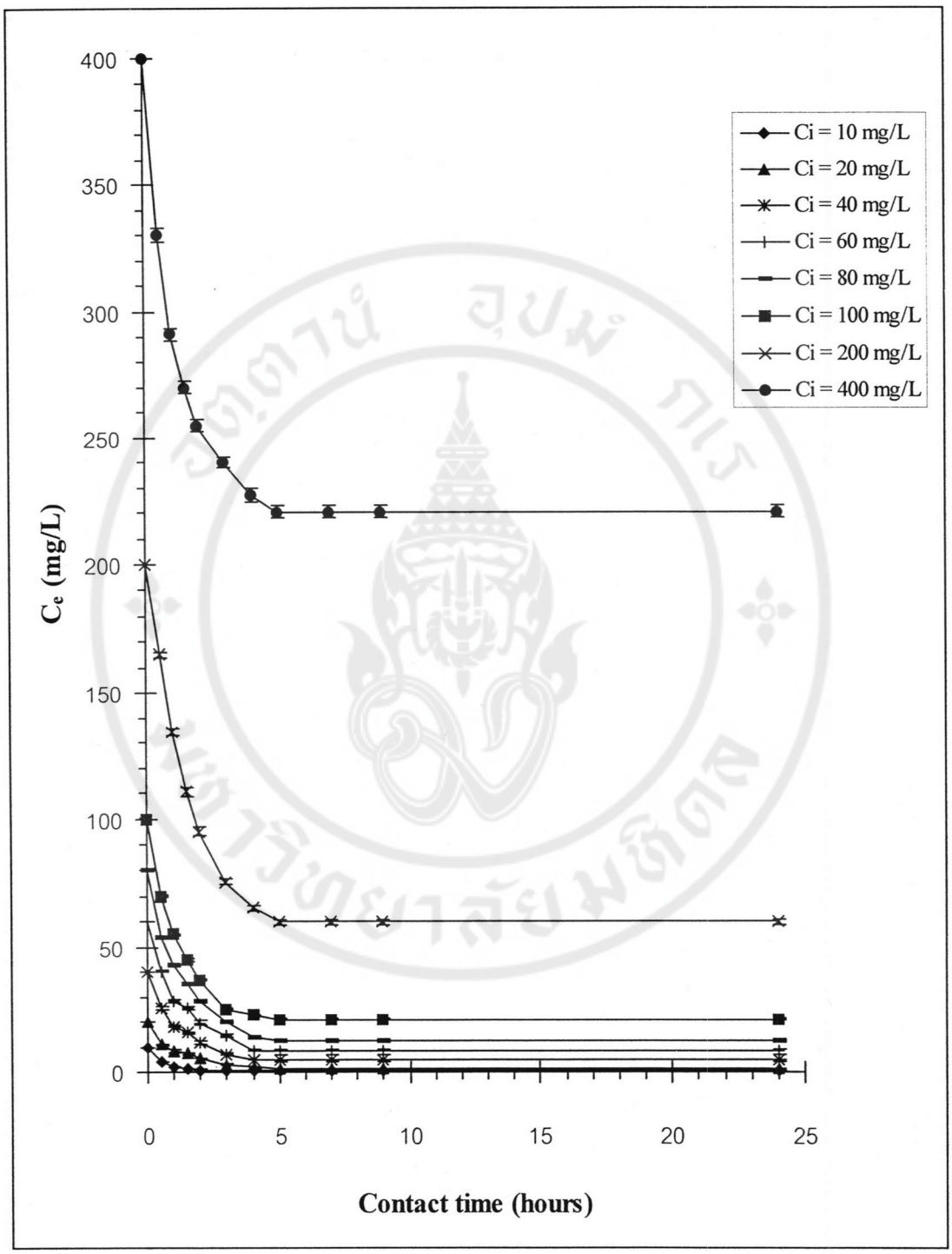

Figure 4-22. The effects of contact times on the equilibrium Cd (II) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 5.

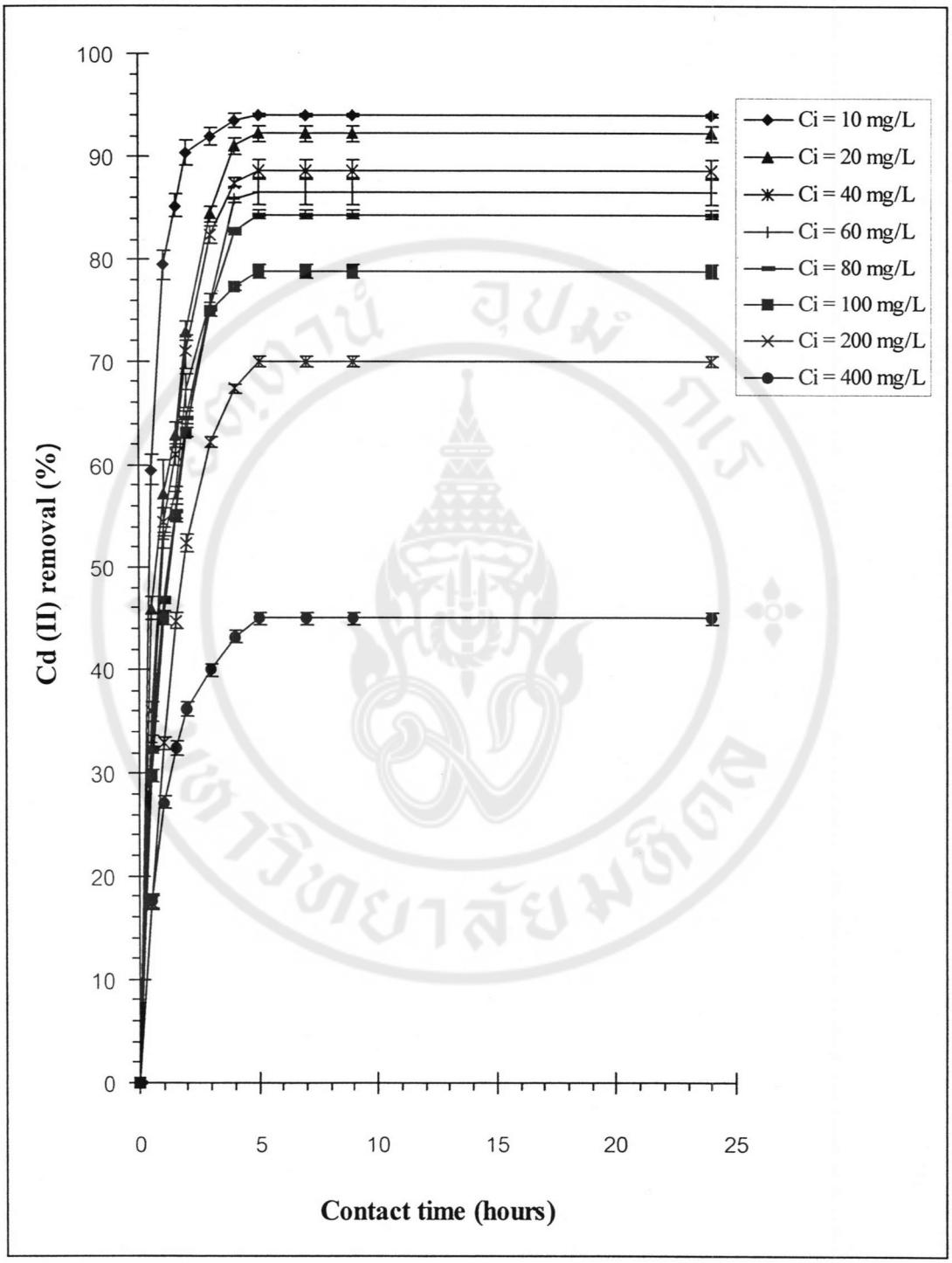

Figure 4-23. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 5.

Table 4-19. The effects of contact times on the equilibrium Cd (II) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 6.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	3.87±0.19	1.89±0.16	1.26±0.20	0.84±0.10	0.67±0.08	0.55±0.03	0.46±0.01	0.46±0.01	0.46±0.01	0.46±0.01
20	7.89±0.24	6.12±0.44	4.56±0.24	3.26±0.21	2.47±0.25	1.32±0.15	1.02±0.15	1.02±0.15	1.02±0.15	1.02±0.15
40	16.30±0.51	13.02±0.15	11.00±0.46	8.05±0.36	6.03±0.13	3.55±0.19	3.04±0.34	3.04±0.34	3.04±0.34	3.04±0.34
60	37.26±0.16	21.30±0.64	16.90±0.15	13.46±0.44	10.02±0.46	6.89±0.45	6.04±0.51	6.04±0.51	6.04±0.51	6.04±0.51
80	51.23±0.64	32.15±0.46	24.16±0.18	19.45±0.45	14.63±0.40	11.01±0.49	9.46±0.30	9.46±0.30	9.46±0.30	9.46±0.30
100	67.98±0.45	48.45±0.54	39.45±0.67	29.82±0.39	20.15±0.66	15.99±0.40	14.21±0.54	14.21±0.54	14.21±0.54	14.21±0.54
200	150.15±1.98	125.26±1.56	105.15±1.38	84.15±0.78	65.81±0.47	55.12±0.37	45.60±0.97	45.60±0.97	45.60±0.97	45.60±0.97
400	300.15±2.68	265.22±2.64	235.25±2.65	211.05±2.16	189.15±1.26	170.15±1.18	164.55±1.45	164.55±1.45	164.55±1.45	164.55±1.45

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-20. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 6.

C_i (mg/L)	Cd (II) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	61.30±1.90	81.10±1.60	87.40±2.00	91.60±1.00	93.30±0.80	94.50±0.30	95.40±0.10	95.40±0.10	95.40±0.10	95.40±0.10
20	60.55±1.20	69.40±2.20	77.20±1.20	83.70±1.05	87.65±1.25	93.40±0.75	94.90±0.75	94.90±0.75	94.90±0.75	94.90±0.75
40	59.25±1.27	67.45±0.38	72.50±1.15	79.88±0.90	84.93±0.33	91.13±0.47	92.40±0.85	92.40±0.85	92.40±0.85	92.40±0.85
60	37.90±0.27	64.50±1.07	71.83±0.25	77.57±0.73	83.30±0.77	88.52±0.75	89.93±0.85	89.93±0.85	89.93±0.85	89.93±0.85
80	35.96±0.80	59.81±0.57	69.80±0.22	75.69±0.56	81.71±0.50	86.24±0.61	88.18±0.38	88.18±0.38	88.18±0.38	88.18±0.38
100	32.02±0.45	51.55±0.54	60.55±0.67	70.18±0.39	79.85±0.66	84.01±0.40	85.79±0.54	85.79±0.54	85.79±0.54	85.79±0.54
200	24.93±0.99	37.37±0.78	47.43±0.69	57.93±0.39	67.10±0.24	72.44±0.19	77.20±0.48	77.20±0.48	77.20±0.48	77.20±0.48
400	24.96±0.67	33.70±0.66	41.19±0.66	47.24±0.54	52.71±0.31	57.46±0.30	58.86±0.36	58.86±0.36	58.86±0.36	58.86±0.36

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

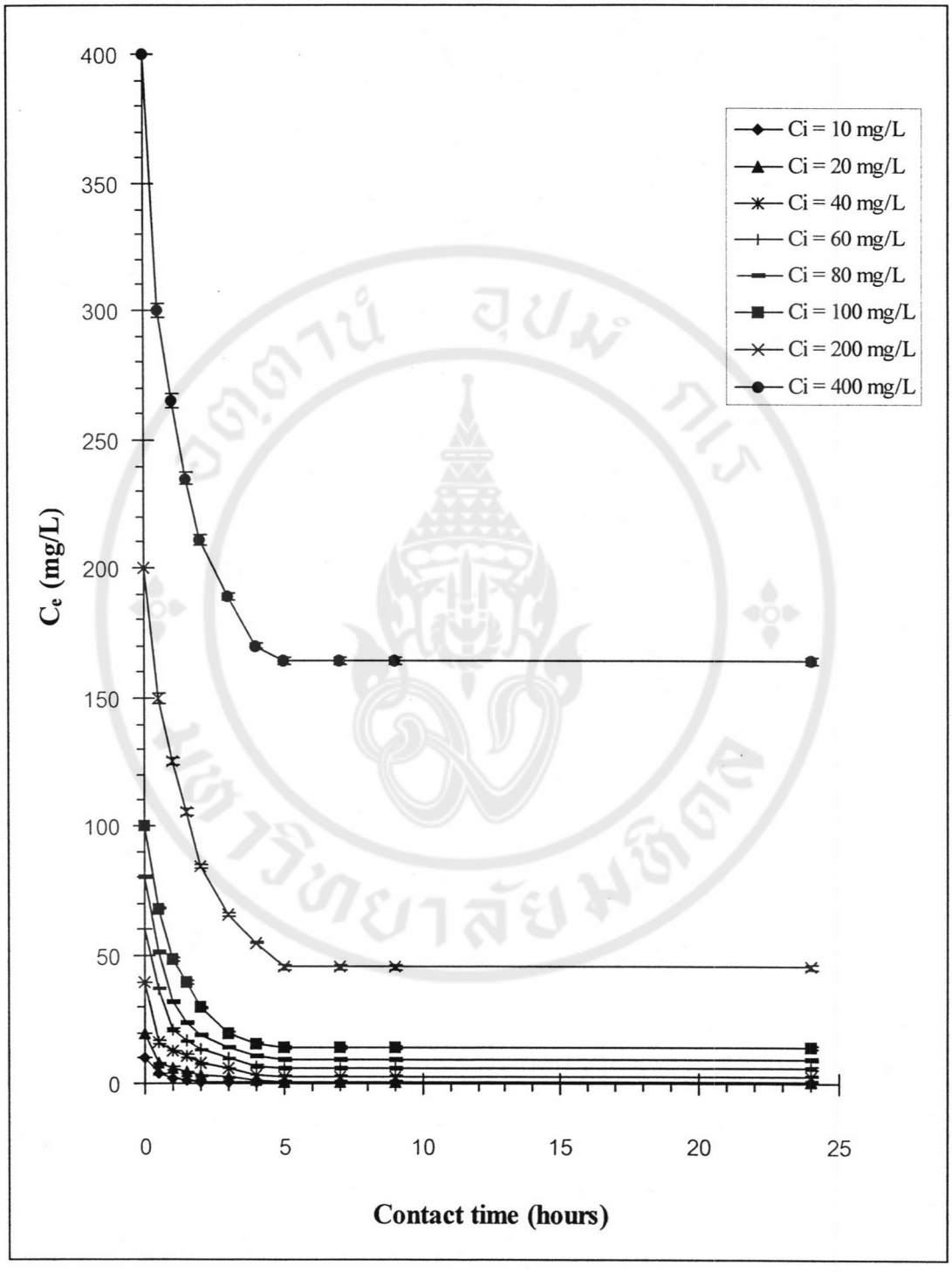

Figure 4-24. The effects of contact times on the equilibrium Cd (II) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 6.

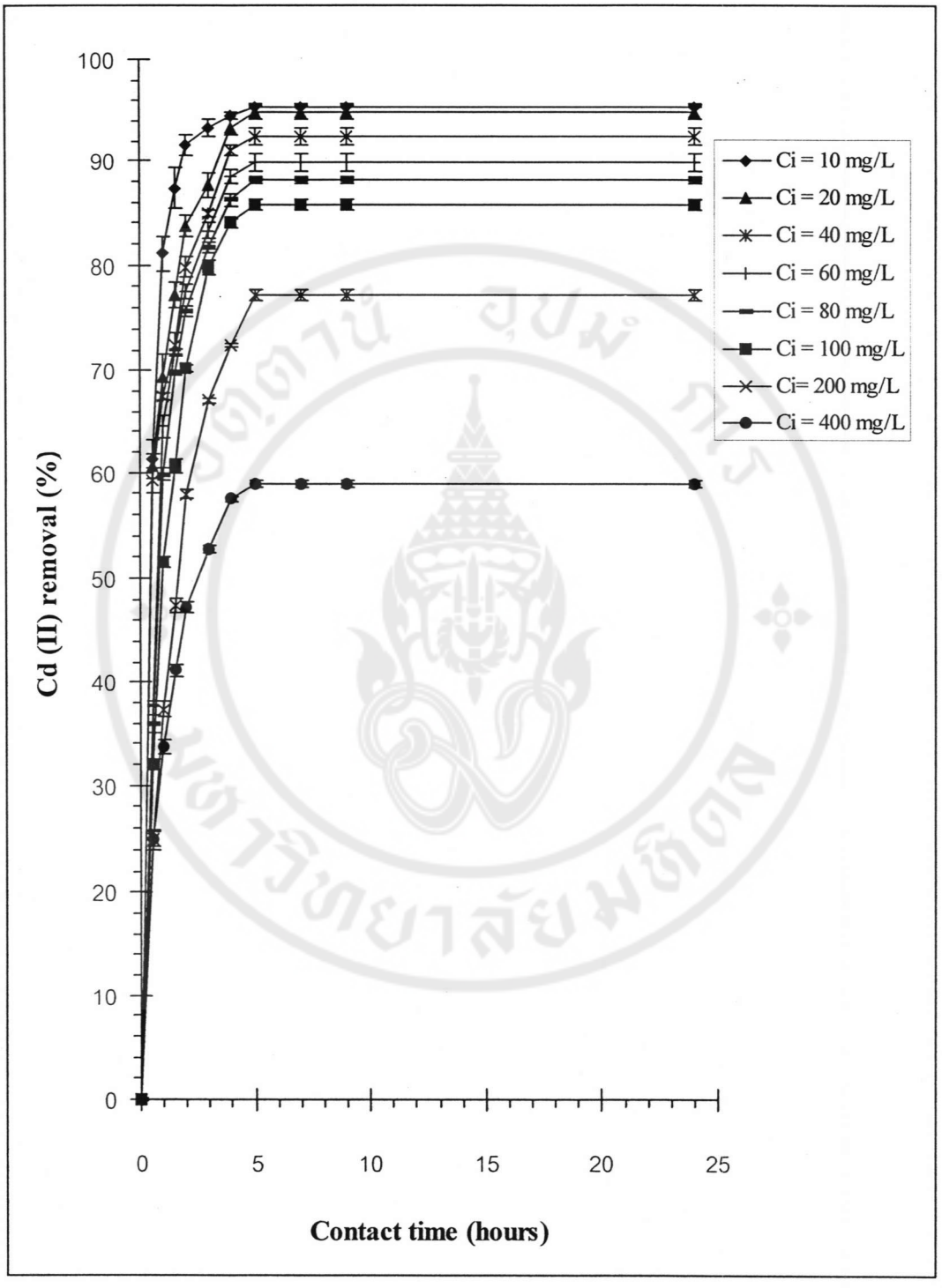

Figure 4-25. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 6.

Table 4-21. The effects of contact times on the equilibrium Cd (II) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 7.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	3.10±0.10	1.46±0.15	0.98±0.09	0.65±0.05	0.35±0.02	0.20±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.14±0.01
20	7.01±0.15	6.00±0.26	4.00±0.15	2.65±0.19	0.99±0.09	0.58±0.02	0.41±0.05	0.41±0.05	0.41±0.05	0.41±0.05
40	15.25±0.19	12.60±0.34	10.52±0.24	6.30±0.21	3.26±0.11	1.56±0.09	1.03±0.13	1.03±0.13	1.03±0.13	1.03±0.13
60	26.31±0.24	20.01±0.38	16.50±0.46	10.36±0.30	5.69±0.15	3.01±0.11	2.25±0.24	2.25±0.24	2.25±0.24	2.25±0.24
80	40.89±0.54	27.50±0.69	22.50±0.64	15.90±0.24	8.89±0.19	5.52±0.15	3.51±0.29	3.51±0.29	3.51±0.29	3.51±0.29
100	53.60±0.48	35.69±0.70	30.20±0.45	21.36±0.46	13.65±0.22	8.95±0.23	5.03±0.41	5.03±0.41	5.03±0.41	5.03±0.41
200	135.60±1.54	98.56±1.01	65.04±0.61	48.50±0.50	35.15±0.35	25.65±0.31	20.75±0.26	20.75±0.26	20.75±0.26	20.75±0.26
400	283.02±2.69	230.50±2.74	199.60±1.78	175.00±1.69	145.25±1.29	120.25±1.36	101.38±1.30	101.38±1.30	101.38±1.30	101.38±1.30

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-22. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 7.

C_i (mg/L)	Cd (II) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	69.00±1.00	85.40±1.50	90.20±0.90	93.50±0.50	96.50±0.20	98.00±0.10	98.60±0.10	98.60±0.10	98.60±0.10	98.60±0.10
20	64.95±0.75	70.00±1.30	80.00±0.75	86.75±0.95	95.05±0.45	97.10±0.10	97.95±0.25	97.95±0.25	97.95±0.25	97.95±0.25
40	61.88±0.48	68.50±0.85	73.70±0.60	84.25±0.53	91.85±0.27	96.10±0.22	97.43±0.33	97.43±0.33	97.43±0.33	97.43±0.33
60	56.15±0.40	66.65±0.63	72.50±0.77	82.73±0.50	90.52±0.25	94.98±0.18	96.25±0.40	96.25±0.40	96.25±0.40	96.25±0.40
80	48.89±0.68	65.63±0.86	71.88±0.80	80.13±0.30	88.89±0.24	93.10±0.19	95.61±0.36	95.61±0.36	95.61±0.36	95.61±0.36
100	46.40±0.48	64.31±0.70	69.80±0.45	78.64±0.46	86.35±0.22	91.05±0.23	94.97±0.41	94.97±0.41	94.97±0.41	94.97±0.41
200	32.20±0.77	50.72±0.50	67.48±0.31	75.75±0.25	82.43±0.17	87.18±0.16	89.63±0.13	89.63±0.13	89.63±0.13	89.63±0.13
400	29.25±0.67	42.38±0.69	50.10±0.44	56.25±0.42	63.69±0.32	69.94±0.34	74.66±0.32	74.66±0.32	74.66±0.32	74.66±0.32

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

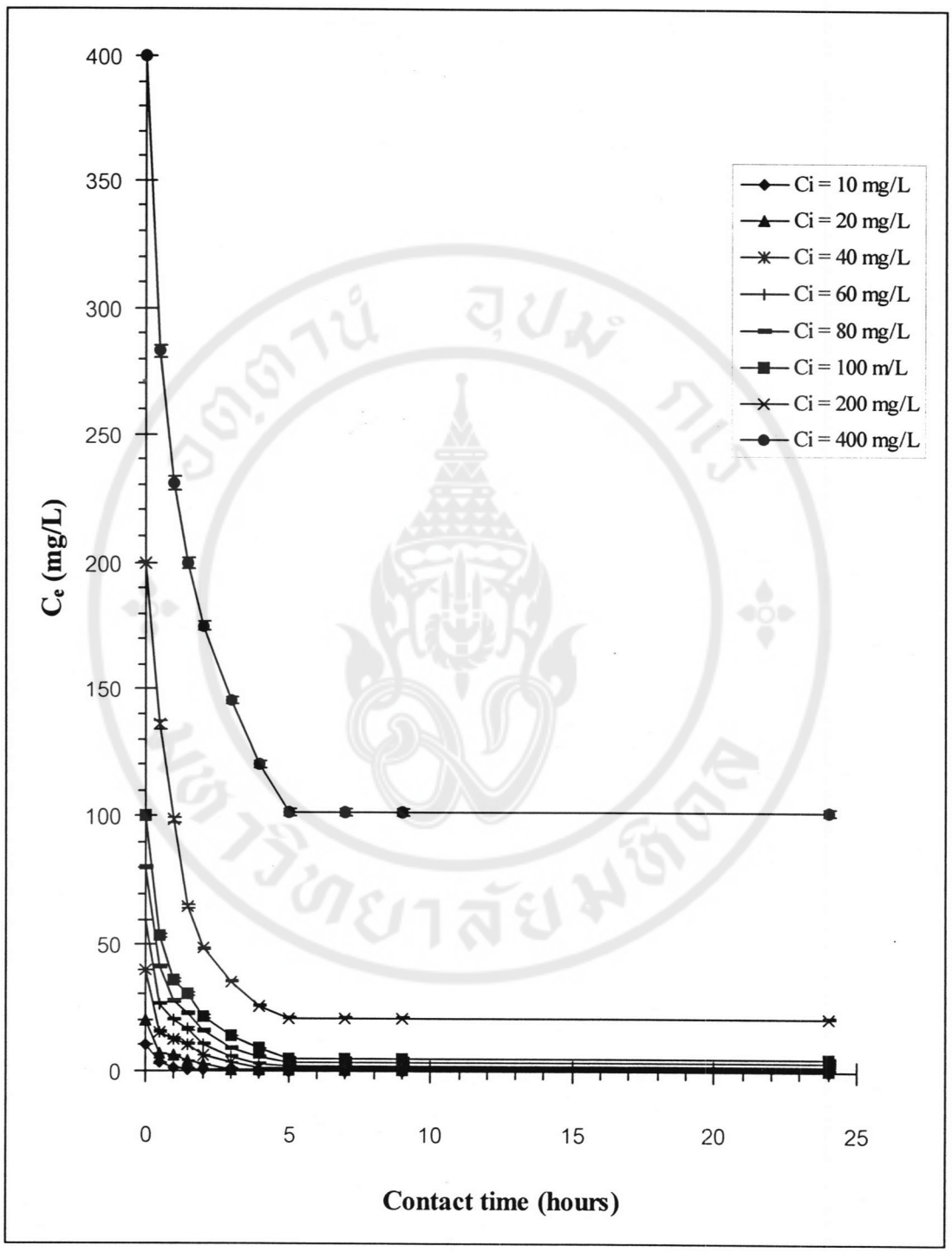

Figure 4-26. The effects of contact times on the equilibrium Cd (II) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 7.

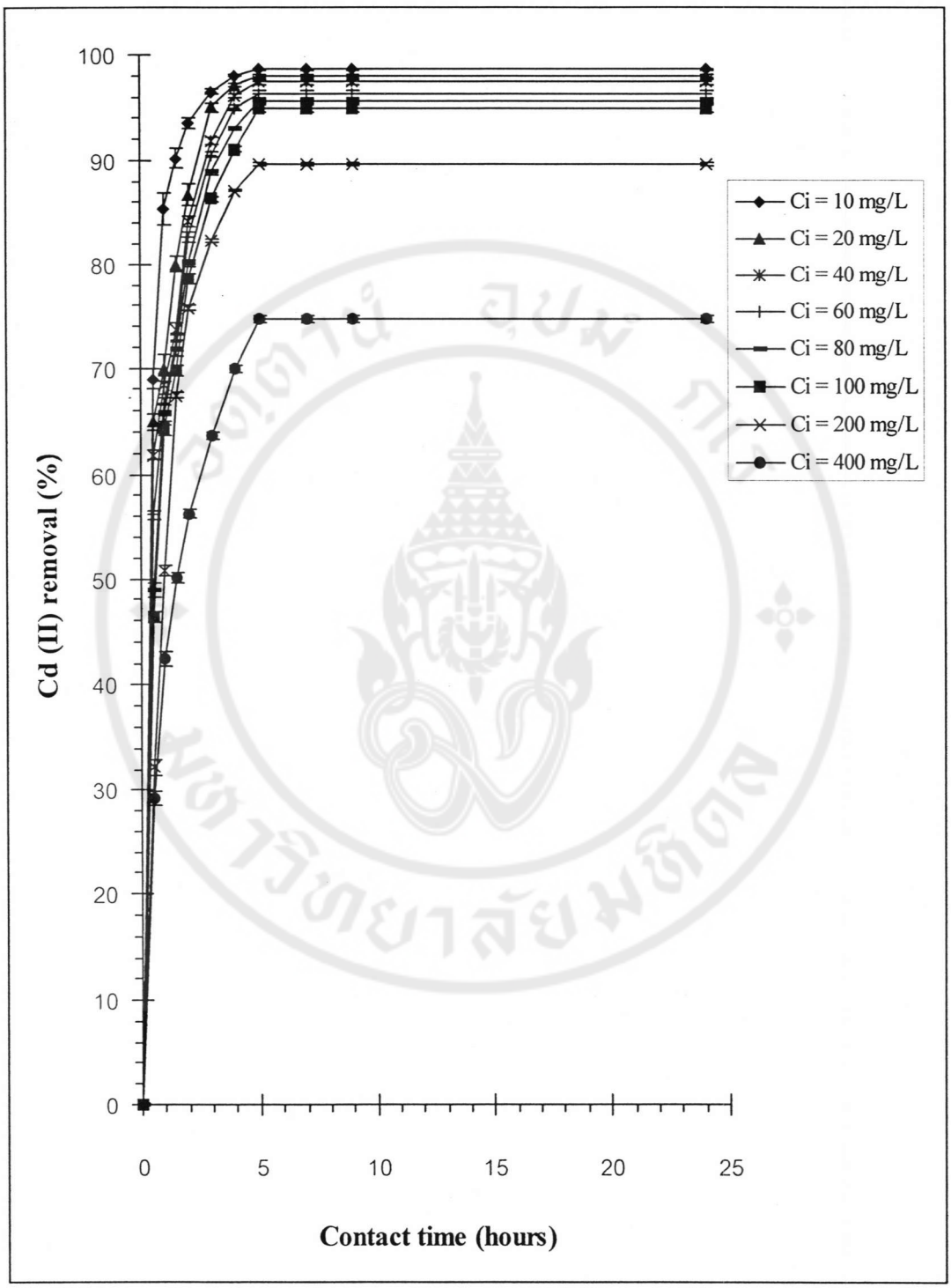

Figure 4-27. The effects of contact times on the Cd (II) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 7.

In *Wolffia globosa*-Cr (VI) system, the effect of contact times to Cr (VI) sorption were studied by varying Cr (VI) concentrations ranging from 10 to 400 mg/L at pH 1.5 - pH 6. The Cr (VI) concentrations decreased significantly ($P<0.05$) when the contact times were increased at all pH values, and the Cr (VI) uptake was relatively fast for all the concentrations studied. At pH 1.5, 3, 5, and 6, *W. globosa*-Cr (VI) system decreased sharply in the Cr (VI) concentrations in the first hour, and when the contact times were increased to five hours, a small decreased was observed. At the contact times of more than five hours, a residual Cr (VI) concentrations were stable, suggesting that the maximum adsorption was completed within five hours (Table 4-23, Table 4-25, Table 4-27, Table 4-29, Figure 4-28, Figure 4-30, Figure 4-32, and Figure 4-34). The effect of contact times on the Cr (VI) removal (%) by *W. globosa* biomass at different concentrations at pH 1.5, 3, 5 and 6 are shown in Table 4-24, Table 4-26, Table 4-28, Table 4-30, Figure 4-29, Figure 4-31, Figure 4-33, and Figure 4-35.

Table 4-23. The effects of contact times on the equilibrium Cr (VI) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 1.5.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	**1**	**1.5**	**2**	**3**	**4**	**5**	**7**	**9**	**24**
10	6.00±0.11	3.46±0.10	1.57±0.14	1.10±0.15	0.71±0.09	0.56±0.02	0.46±0.01	0.46±0.01	0.46±0.01	0.46±0.01
20	15.46±0.19	10.45±0.19	7.45±0.21	4.70±0.21	2.78±0.19	1.50±0.15	1.04±0.12	1.04±0.12	1.04±0.12	1.04±0.12
40	30.42±0.24	20.45±0.21	15.50±0.28	11.45±0.19	6.45±0.25	4.68±0.18	3.46±0.26	3.46±0.26	3.46±0.26	3.46±0.26
60	45.16±0.32	30.56±0.35	20.46±0.25	15.46±0.34	10.01±0.34	7.65±0.31	6.02±0.42	6.02±0.42	6.02±0.42	6.02±0.42
80	60.30±0.36	45.62±0.39	32.65±0.36	21.56±0.45	13.46±0.26	10.64±0.34	9.15±0.56	9.15±0.56	9.15±0.56	9.15±0.56
100	75.89±0.41	54.45±0.38	39.85±0.45	27.96±0.24	18.69±0.41	14.65±0.27	13.15±0.74	13.15±0.74	13.15±0.74	13.15±0.74
200	150.36±1.56	103.50±1.28	70.96±0.65	55.60±0.68	45.20±0.67	38.45±0.49	35.00±0.65	35.00±0.65	35.00±0.65	35.00±0.65
400	305.62±2.89	232.50±2.64	200.69±2.11	178.56±1.97	159.60±1.54	150.69±1.43	144.94±1.56	144.94±1.56	144.94±1.56	144.94±1.56

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-24. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 1.5.

C_i (mg/L)	Cr (VI) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	40.00±1.10	65.40±1.00	84.30±1.40	89.00±1.50	92.90±0.90	94.37±0.20	95.40±0.10	95.40±0.10	95.40±0.10	95.40±0.10
20	22.70±0.95	47.75±0.95	62.75±1.05	76.50±1.05	86.10±0.95	92.50±0.75	94.80±0.60	94.80±0.60	94.80±0.60	94.80±0.60
40	23.96±0.60	48.88±0.52	61.25±0.70	71.38±0.48	83.88±0.63	88.30±0.45	91.35±0.65	91.35±0.65	91.35±0.65	91.35±0.65
60	24.73±0.53	49.07±0.58	65.90±0.42	74.23±0.57	83.32±0.57	87.25±0.52	89.97±0.70	89.97±0.70	89.97±0.70	89.97±0.70
80	24.63±0.45	42.98±0.49	59.19±0.45	73.05±0.56	83.18±0.32	86.70±0.42	88.56±0.70	88.56±0.70	88.56±0.70	88.56±0.70
100	24.11±0.41	45.55±0.38	60.15±0.45	72.04±0.24	81.31±0.41	85.35±0.27	86.85±0.74	86.85±0.74	86.85±0.74	86.85±0.74
200	24.82±0.78	48.25±0.64	64.52±0.33	72.20±0.34	77.40±0.34	80.78±0.25	82.50±0.33	82.50±0.33	82.50±0.33	82.50±0.33
400	23.60±0.72	41.88±0.66	49.83±0.53	55.36±0.49	60.10±0.38	62.33±0.36	63.77±0.39	63.77±0.39	63.77±0.39	63.77±0.39

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

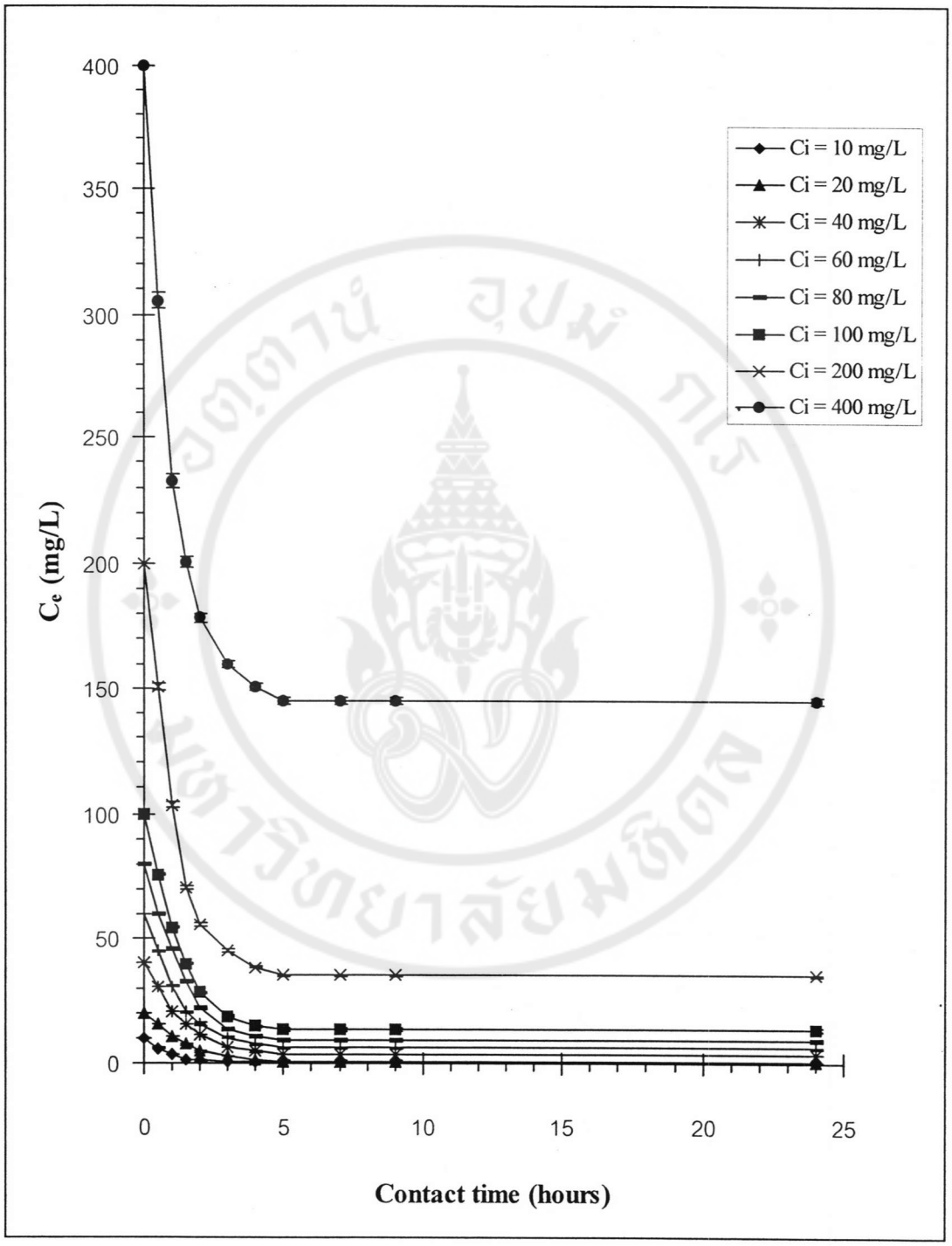

Figure 4-28. The effects of contact times on the equilibrium Cr (VI) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 1.5.

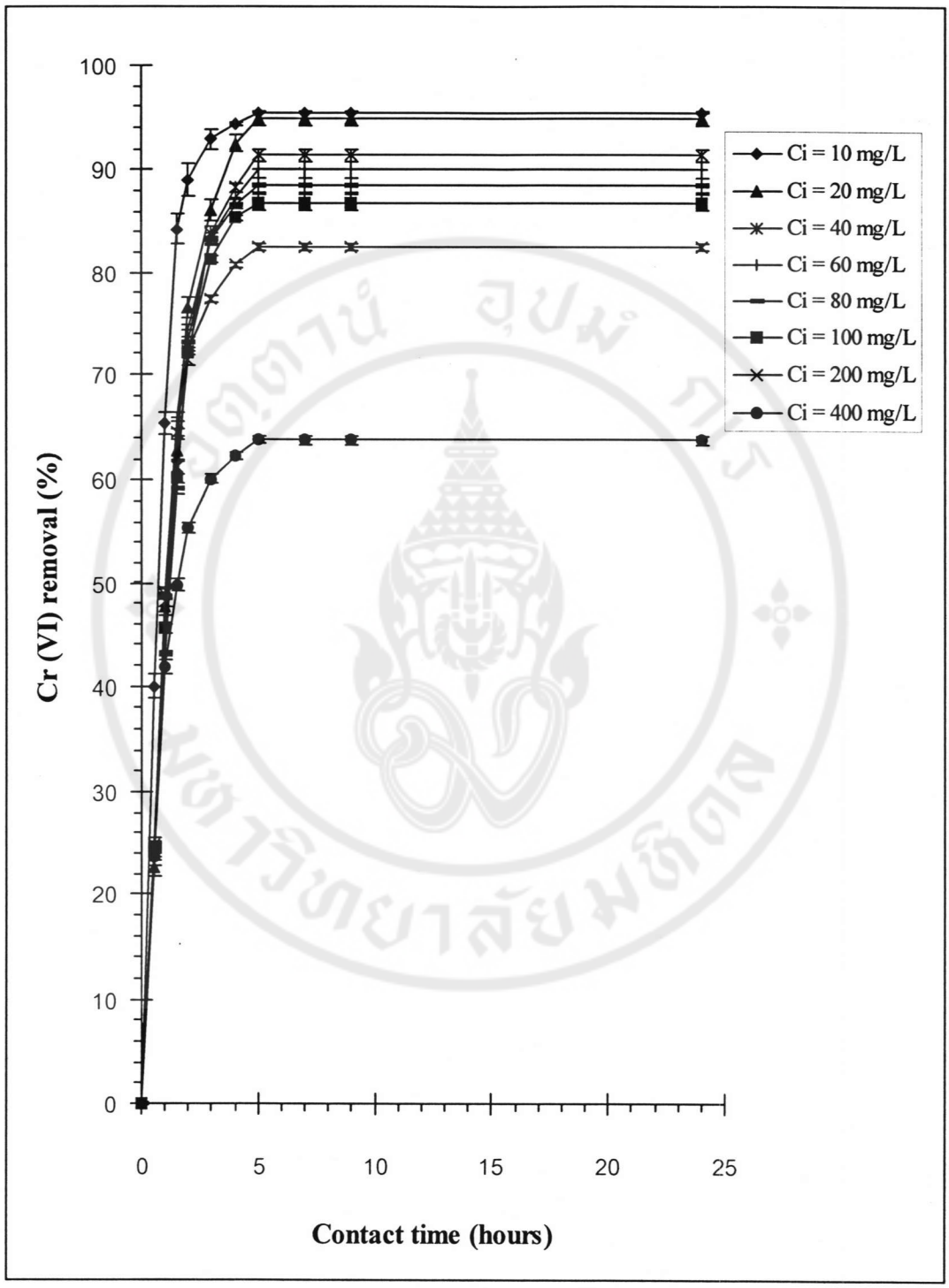

Figure 4-29. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 1.5.

Table 4-25. The effects of contact times on the equilibrium Cr (VI) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 3.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	**1**	**1.5**	**2**	**3**	**4**	**5**	**7**	**9**	**24**
10	7.80±0.13	5.60±0.16	3.50±0.19	2.00±0.12	1.58±0.10	1.36±0.12	1.27±0.11	1.27±0.11	1.27±0.11	1.27±0.11
20	16.59±0.19	12.36±0.26	8.45±0.21	5.90±0.19	4.90±0.29	4.50±0.21	4.05±0.19	4.05±0.19	4.05±0.19	4.05±0.19
40	33.50±0.25	25.69±0.25	17.89±0.36	13.69±0.36	10.36±0.26	9.51±0.25	9.25±0.25	9.25±0.25	9.25±0.25	9.25±0.25
60	48.26±0.46	35.67±0.58	22.65±0.45	19.68±0.41	16.89±0.30	15.50±0.31	15.15±0.36	15.15±0.36	15.15±0.36	15.15±0.36
80	67.89±0.64	55.69±0.46	45.69±0.42	34.65±0.34	27.98±0.37	24.69±0.36	23.44±0.44	23.44±0.44	23.44±0.44	23.44±0.44
100	85.65±0.41	68.09±0.59	50.60±0.46	40.39±0.40	35.67±0.45	32.69±0.41	31.15±0.54	31.15±0.54	31.15±0.54	31.15±0.54
200	165.00±1.59	140.20±1.54	126.60±1.11	112.60±1.36	100.30±1.22	92.36±0.98	90.15±0.98	90.15±0.98	90.15±0.98	90.15±0.98
400	351.20±2.39	300.25±2.48	280.45±2.36	265.90±2.42	249.30±2.37	240.10±2.17	237.15±1.78	237.15±1.78	237.15±1.78	237.15±1.78

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-26. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 3.

C_i (mg/L)	Cr (VI) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	22.00±1.30	44.00±1.60	65.00±1.90	80.00±1.20	84.20±1.00	86.40±1.20	87.30±1.10	87.30±1.10	87.30±1.10	87.30±1.10
20	17.05±0.95	38.20±1.30	57.75±1.05	70.50±0.95	75.50±1.45	77.50±1.05	79.75±0.95	79.75±0.95	79.75±0.95	79.75±0.95
40	16.25±0.63	35.78±0.63	55.28±0.90	65.78±0.90	74.10±0.65	76.23±0.62	76.88±0.63	76.88±0.63	76.88±0.63	76.88±0.63
60	19.57±0.77	40.55±0.97	62.25±0.75	67.20±0.68	71.85±0.50	74.17±0.52	74.75±0.60	74.75±0.60	74.75±0.60	74.75±0.60
80	15.14±0.80	30.39±0.57	42.89±0.52	56.69±0.43	65.03±0.46	69.14±0.45	70.70±0.55	70.70±0.55	70.70±0.55	70.70±0.55
100	14.35±0.41	31.91±0.59	49.40±0.46	59.61±0.40	64.33±0.45	67.31±0.41	68.85±0.54	68.85±0.54	68.85±0.54	68.85±0.54
200	17.50±0.79	29.90±0.77	36.70±0.56	43.70±0.68	49.85±0.61	53.82±0.49	54.93±0.49	54.93±0.49	54.93±0.49	54.93±0.49
400	12.20±0.60	24.94±0.62	29.89±0.59	33.53±0.60	37.68±0.59	39.98±0.54	40.71±0.45	40.71±0.45	40.71±0.45	40.71±0.45

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

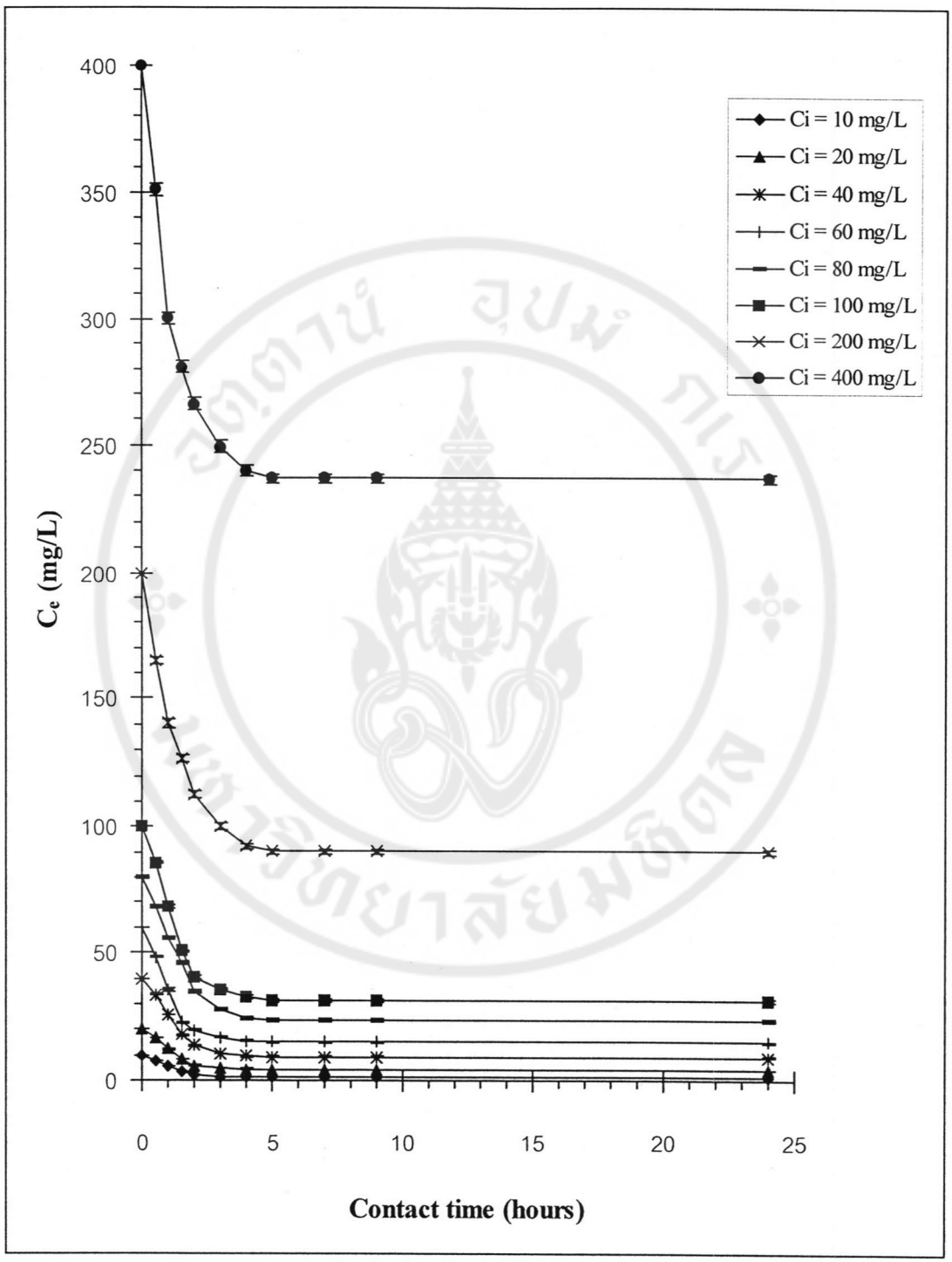

Figure 4-30. The effects of contact times on the equilibrium Cr (VI) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 3.

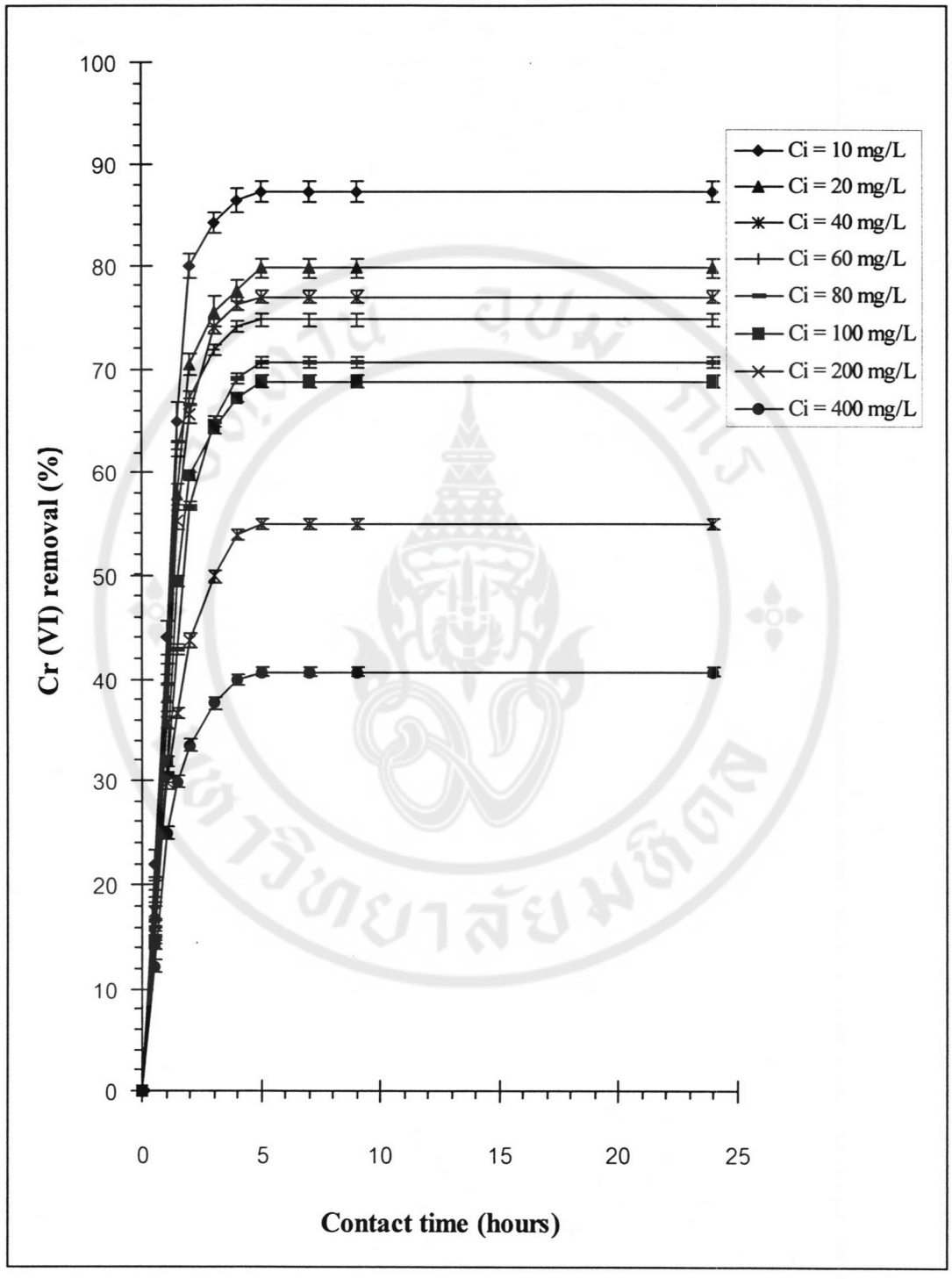

Figure 4-31. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 3.

Table 4-27. The effects of contact times on the equilibrium Cr (VI) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 5.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	**1**	**1.5**	**2**	**3**	**4**	**5**	**7**	**9**	**24**
10	8.50±0.19	7.02±0.17	5.68±0.15	4.58±0.17	3.56±0.16	3.25±0.15	3.00±0.16	3.00±0.16	3.00±0.16	3.00±0.16
20	17.05±0.21	13.56±0.24	10.02±0.19	8.50±0.26	7.65±0.22	7.26±0.25	7.02±0.25	7.02±0.25	7.02±0.25	7.02±0.25
40	34.66±0.31	26.90±0.31	22.36±0.24	18.97±0.34	16.90±0.38	15.42±0.31	14.91±0.54	14.91±0.54	14.91±0.54	14.91±0.54
60	50.36±0.39	37.89±0.38	30.69±0.37	28.10±0.40	26.10±0.41	25.50±0.36	25.16±0.25	25.16±0.25	25.16±0.25	25.16±0.25
80	69.99±0.46	58.96±0.46	47.98±0.48	39.00±0.42	37.11±0.34	36.02±0.37	35.00±0.52	35.00±0.52	35.00±0.52	35.00±0.52
100	88.96±0.45	70.45±0.49	56.36±0.55	52.36±0.64	50.16±0.51	48.26±0.44	47.16±0.45	47.16±0.45	47.16±0.45	47.16±0.45
200	170.36±1.19	145.62±1.64	135.60±1.33	130.50±1.17	125.60±1.23	122.56±1.69	120.46±0.78	120.46±0.78	120.46±0.78	120.46±0.78
400	355.69±2.67	330.60±2.39	315.90±2.15	305.60±2.39	295.90±2.97	293.65±2.38	290.15±2.16	290.15±2.16	290.15±2.16	290.15±2.16

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-28. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 5.

C_i (mg/L)	Cr (VI) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	15.00±1.90	29.80±1.70	43.20±1.50	54.20±1.70	64.40±1.60	67.50±1.50	70.00±1.60	70.00±1.60	70.00±1.60	70.00±1.60
20	14.75±1.05	32.20±1.20	49.90±0.95	57.50±1.30	61.75±1.10	63.70±1.25	64.90±1.25	64.90±1.25	64.90±1.25	64.90±1.25
40	13.35±0.78	32.75±0.77	44.10±0.60	52.58±0.85	57.75±0.95	61.45±0.78	62.73±1.35	62.73±1.35	62.73±1.35	62.73±1.35
60	16.07±0.65	36.85±0.63	48.85±0.62	53.17±0.67	56.50±0.68	57.50±0.60	58.07±0.42	58.07±0.42	58.07±0.42	58.07±0.42
80	12.51±0.57	26.30±0.58	40.03±0.60	51.25±0.53	53.61±0.42	54.98±0.46	56.25±0.65	56.25±0.65	56.25±0.65	56.25±0.65
100	11.04±0.45	29.55±0.49	43.64±0.55	47.64±0.64	49.84±0.51	51.74±0.44	52.84±0.45	52.84±0.45	52.84±0.45	52.84±0.45
200	14.82±0.59	27.19±0.82	32.20±0.67	34.75±0.59	37.20±0.62	38.72±0.85	39.77±0.39	39.77±0.39	39.77±0.39	39.77±0.39
400	11.08±0.67	17.35±0.60	21.03±0.54	23.60±0.60	26.03±0.74	26.59±0.60	27.46±0.54	27.46±0.54	27.46±0.54	27.46±0.54

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

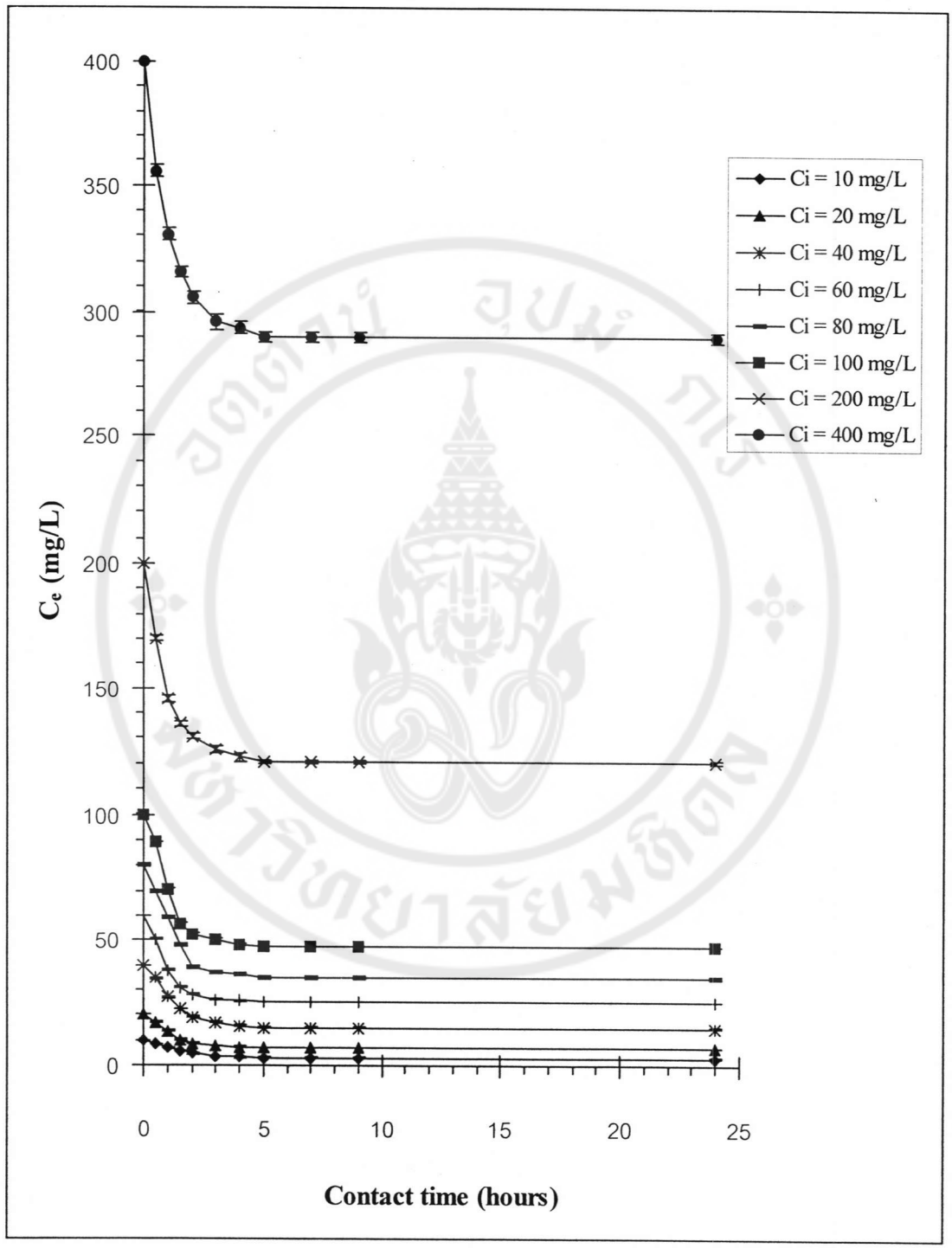

Figure 4-32. The effects of contact times on the equilibrium Cr (VI) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 5.

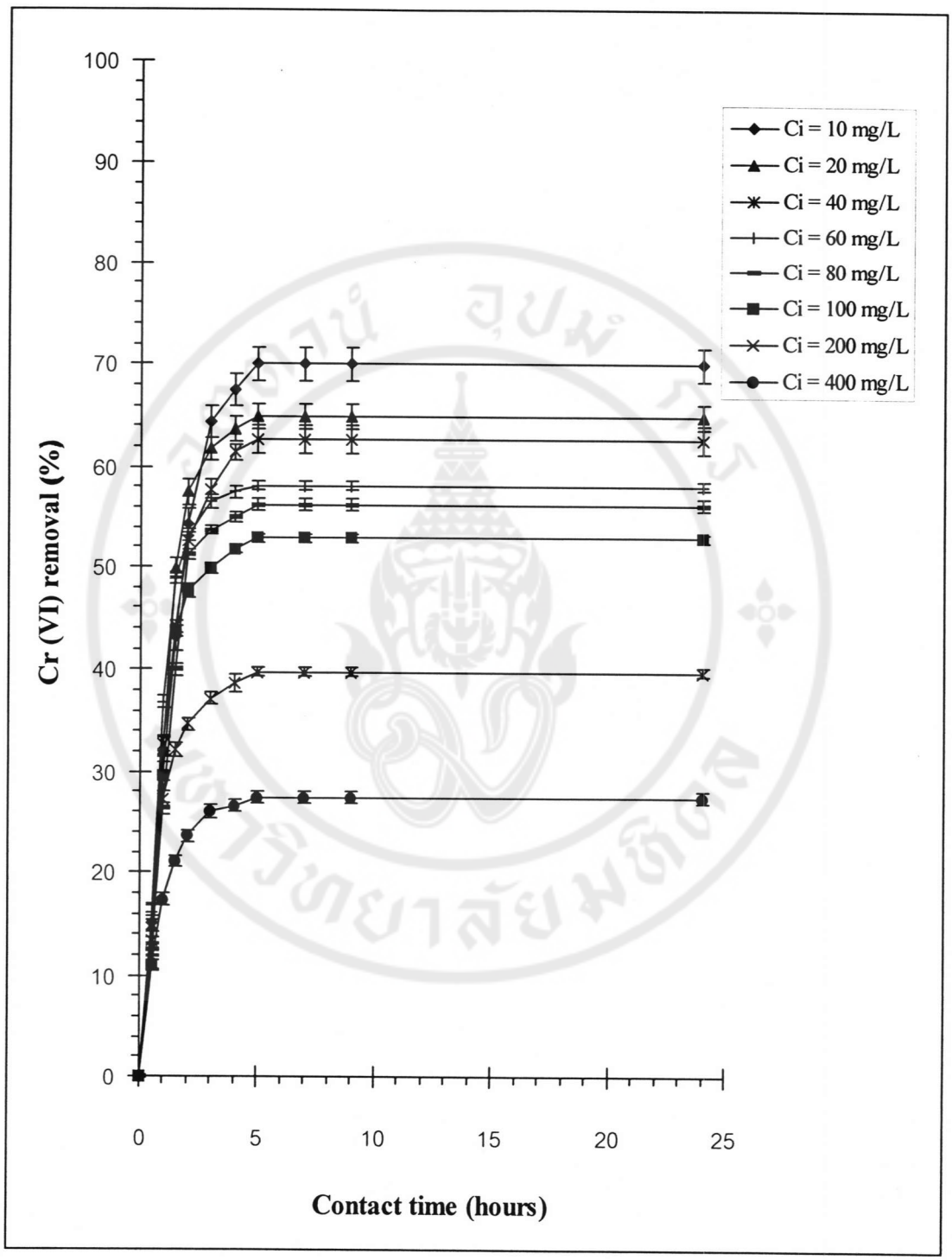

Figure 4-33. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 5.

Table 4-29. The effects of contact times on the equilibrium Cr (VI) concentration (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 6.

C_i (mg/L)	C_e (mg/L)									
	Time (hours)									
	0.5	**1**	**1.5**	**2**	**3**	**4**	**5**	**7**	**9**	**24**
10	8.89±0.19	7.54±0.20	7.15±0.18	6.80±0.15	6.40±0.16	6.22±0.24	6.15±0.27	6.15±0.27	6.15±0.27	6.15±0.27
20	17.59±0.16	16.23±0.26	15.00±0.32	14.36±0.31	13.99±0.31	13.65±0.32	13.48±0.23	13.48±0.23	13.48±0.23	13.48±0.23
40	36.90±0.23	34.60±0.34	32.55±0.26	31.26±0.28	30.55±0.24	30.25±0.36	30.01±0.33	30.01±0.33	30.01±0.33	30.01±0.33
60	55.99±0.34	52.01±0.51	50.12±0.54	48.75±0.37	47.99±0.39	47.23±0.48	47.00±0.35	47.00±0.35	47.00±0.35	47.00±0.35
80	75.69±0.49	70.13±0.66	67.02±0.49	66.11±0.40	65.45±0.48	65.12±0.67	64.84±0.49	64.84±0.49	64.84±0.49	64.84±0.49
100	90.26±0.61	88.00±0.68	85.00±0.61	84.00±0.69	82.50±0.71	81.50±0.75	81.02±0.46	81.02±0.46	81.02±0.46	81.02±0.46
200	190.53±1.36	180.00±1.23	176.30±1.94	174.00±1.28	172.43±1.67	171.15±1.45	170.49±0.87	170.49±0.87	170.49±0.87	170.49±0.87
400	385.40±2.81	375.96±2.94	370.12±2.67	365.98±2.69	362.89±2.48	361.50±2.33	360.15±2.42	360.15±2.42	360.15±2.42	360.15±2.42

C_i = Initial concentration (mg/L)
C_e = Equilibrium concentration (mg/L)
Each value is the mean of triplicate ± S.D.

Table 4-30. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 6.

C_i (mg/L)	Cr (VI) removal (%)									
	Time (hours)									
	0.5	1	1.5	2	3	4	5	7	9	24
10	11.10±1.90	24.60±2.00	28.50±1.80	32.00±1.50	36.00±1.60	37.80±2.40	38.50±2.70	38.50±2.70	38.50±2.70	38.50±2.70
20	12.05±0.80	18.85±1.30	25.00±1.60	28.20±1.55	30.05±1.55	31.75±1.60	32.60±1.15	32.60±1.15	32.60±1.15	32.60±1.15
40	7.75±0.57	13.50±0.85	18.63±0.65	21.85±0.70	23.63±0.60	24.38±0.90	24.98±0.82	24.98±0.82	24.98±0.82	24.98±0.82
60	6.68±0.57	13.32±0.85	16.47±0.90	18.75±0.62	20.02±0.65	21.28±0.80	21.67±0.58	21.67±0.58	21.67±0.58	21.67±0.58
80	5.39±0.61	12.34±0.82	16.23±0.61	17.36±0.50	18.19±0.60	18.60±0.84	18.95±0.61	18.95±0.61	18.95±0.61	18.95±0.61
100	9.74±0.61	12.00±0.68	15.00±0.61	16.00±0.69	17.50±0.71	18.50±0.75	18.98±0.46	18.98±0.46	18.98±0.46	18.98±0.46
200	4.74±0.68	10.00±0.61	11.85±0.97	13.00±0.64	13.79±0.83	14.43±0.72	14.76±0.44	14.76±0.44	14.76±0.44	14.76±0.44
400	3.65±0.70	6.01±0.74	7.47±0.67	8.51±0.67	9.28±0.62	9.63±0.58	9.96±0.61	9.96±0.61	9.96±0.61	9.96±0.61

C_i = Initial concentration (mg/L)
Each value is the mean of triplicate ± S.D.

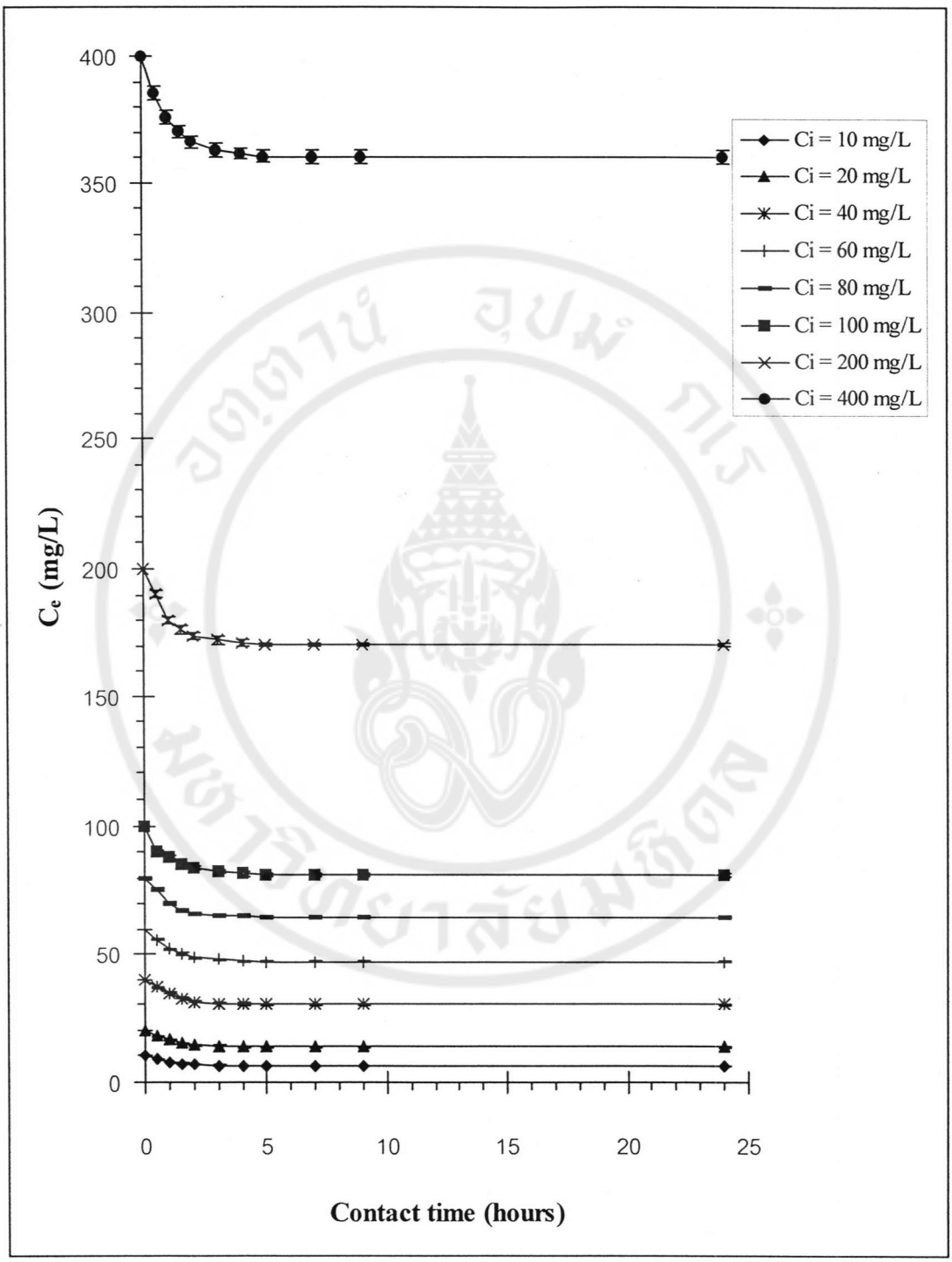

Figure 4-34. The effects of contact times on the equilibrium Cr (VI) concentrations (mg/L) by *Wolffia globosa* biomass at different concentrations at pH 6.

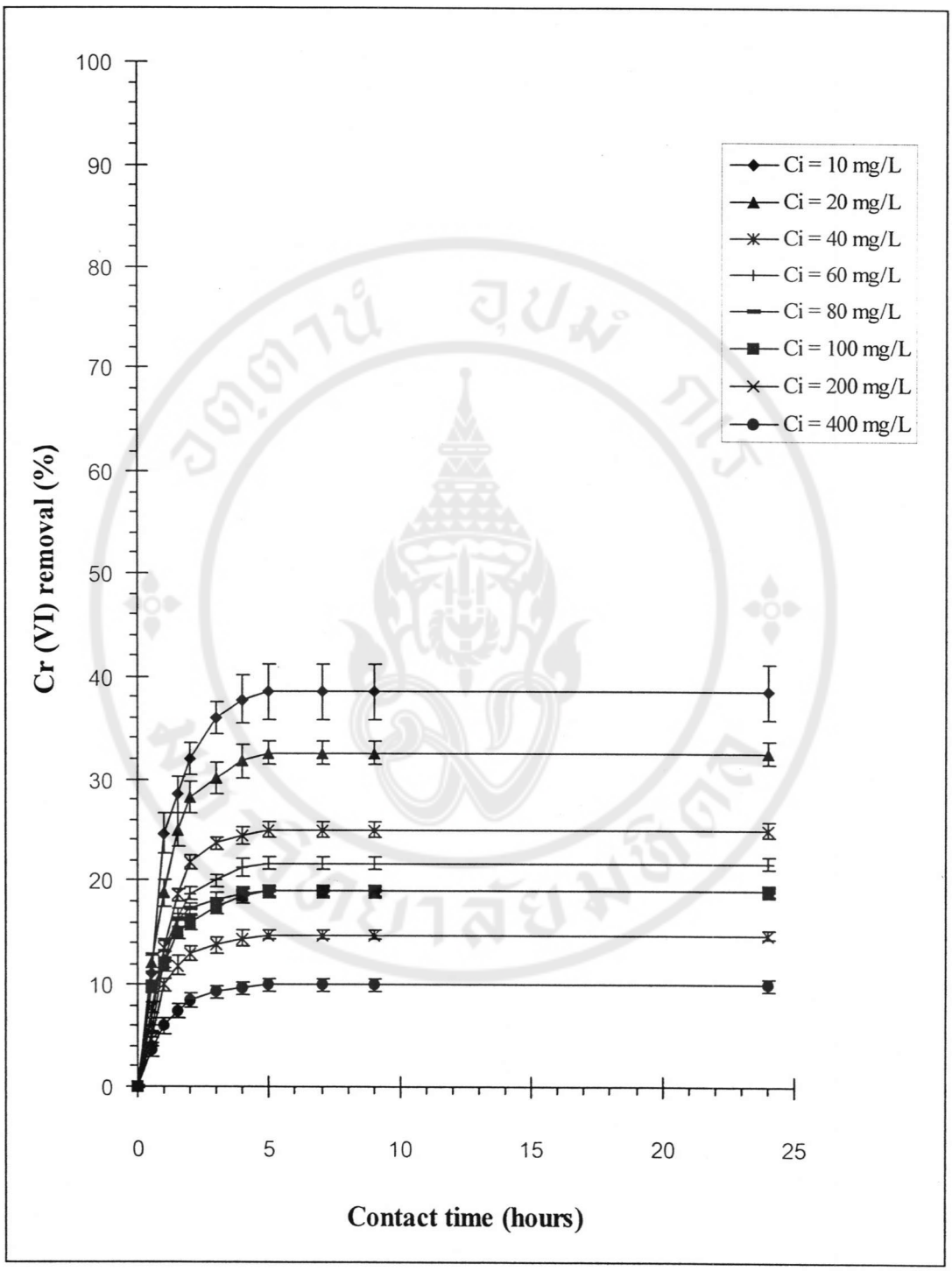

Figure 4-35. The effects of contact times on the Cr (VI) removal (%) by *Wolffia globosa* biomass at different concentrations at pH 6.

CHAPTER V

DISCUSSION

A. Toxicity test and accumulation of heavy metals

Water pollution by heavy metals in industrial waste effluents is now a global problem. The precipitation method for the removal of Cd (II) and Cr(VI) from wastewater is not adequate. Recently, aquatic plants such as *Pistia stratiotes*, *Eichhornia crassipes*, duckweed family, have drawn much attention of workers as a tool for the removal of heavy metals (102, 103, 104, 105, 106, 107). The present study was undertaken in order to evaluate the Cd (II) and Cr(VI) accumulating capacity and toxicity of these metals to duckweed, *Wolffia globosa.*

From the results of *Wolffia globosa* growth measurement, growth curves of *W. globosa* showed the typical three phases: (1) initial lag phase, (2) linear growth phase where exponential growth occurred, and (3) declining phase where the growth rate declined. Linear growth began after three weeks and the highest biomass of *W. globosa* cultured in 3% Hoagland's nutrient solution grew up from 0.0400 to 0.0900 g dry wt for four weeks under the controlled condition (light intensity of 45 μmole $m^{-2}s^{-1}$ at 12/12, L/D cycle by cool white fluorescent tube light at 26±2°C). Space should not be a problem because of its microscopic size, but it still exhibited the normal sigmoid curve. *Wolffia* is usually found in any available space between *Lemna* and *Spirodela*, and it adheres closely to them. So (148) studied the growth characteristics of duckweeds and their potential use as organic fertilizers in

Hong Kong and reported that the growth rate of *Lemna*, *Spirodela*, and *Wolffia* were 2.78, 1.58, and 0.0038 g (dry wt) $m^{-2}d^{-1}$. *Lemna* showed the highest growth rate when comparing the three species in single cultures.

In the present study, it is clear that the biomass productivity in *Wolffia globosa* significantly decreased when the exposure times and metals concentrations were increased. A decline in biomass productivity may be due to increased tissue permeability and loss of membrane integrity of plant tissue (7). Cadmium (II) and chromium (VI) pose deleterious effects on much of the biochemical machinery required for cell survival and have numerous sites of action within the plant (99). However, at low concentration, the result showed a stimulation in biomass productivity of *W. globosa* at 1 mg/L Cr (VI) on day 3 which may be attributed to the fact that the plant utilizes chromium at low concentration as a micronutrient for their survival. Mangi et al. (6) carried out similar experiments using *Lemna* and *Spirodela* and observed similar results. In the present study, when the effects of these metals on the biomass productivity were compared, *W. globosa* exposed in Cr (VI) solutions showed higher rates in biomass productivity than *W. globosa* exposed in Cd (II) solutions. This suggested that Cd (II) exhibited a more toxic effect on biomass productivity than Cr (VI).

The results on the effects of Cd (II) and Cr (VI) on the chlorophyll content in *Wolffia globosa* revealed that chlorophyll a, b, and total chlorophyll significantly decreased when the exposure times and metal concentrations were increased. This decline in chlorophyll concentrations might be caused by a reduction in the synthesis of chlorophyll by possibly increasing chlorophyllase activity, disorderness of

chloroplast membrane and inactivation of electron transport of photosystem II (7,149). The chlorosis may appear to be Fe deficiency and the interaction of toxic metals and iron have been studied for many years. Chlorosis from excess metals appear to be due to a direct or an indirect interaction with foliar iron (4). Haghiri (150) reported that high Cd (II) content in the growing medium suppresses the iron uptake by the plants. Root et al. (151) felt that Cd (II)-induced chlorosis in corn leaves could be due to changes in Fe:Zn ratios. In others, Cd (II) toxicity appeared to induce phosphorus deficiency or reduce manganese transport problems (152). In the present study, when the effects of these metals on chlorophyll contents were compared, *W. globosa* exposed in Cr (VI) solutions showed higher chlorophyll content than that of *W. globosa* exposed in Cd (II) solutions. This suggested that Cd (II) exhibited a more toxic effect on chlorophyll content than Cr (VI).

Wolffia globosa possesses the potential to accumulate metals in its tissue. The tissue metal concentration depends on the concentration of the metal in the ambient water and time of exposure. The data revealed that, under experimental conditions, the accumulation of Cd (II) and Cr (VI) by *W. globosa* were increased when the exposure times and metal concentrations were increased. *Wolffia globosa* showed a higher accumulation of Cd (II) than that of Cr (VI) which suggested that this plant species has more selectivity towards Cd (II). This might be due to its cation-selective abilities (107). In the present study, *W. globosa* accumulated Cd (II) to the highest concentrations of 5.9 mg Cd(II)/g with a BCF ranging from 381 to 908 at various Cd (II) supply levels and exposure times. The maximal limit of accumulation of a trace element that must be achieved by a plant species to be classified as a hyperaccumulator

and hence a good phytoremediator of that element is not yet well defined (153, 154). The following concentrations in the dry matter of any aboveground tissues have been suggested as thresholds to define hyperaccumulation in terrestrial plants: 10,000 μg/g for Zn and Mn; 1,000 μg/g for Ni, Co, Cu,Cr, and Pb; and 100 μg/g for Cd and Se (155). However, the use of element concentration in dry plant tissues as a criterion for identifying plant species that can be good phytoremediators does not take into account the trace element concentration in the root substrate. The bioconcentration factor may sometimes be a better indicator if the external trace element level is too low (153, 154). Thus, in this treatment of what to be considered a good accumulator, we will base our discussion on arbitrary criteria of Zayed et al. (153) and Zhu et al. (154): its ability to bioconcentrate the element in its tissues, *e.g.*, a BCF of over 1,000 (a 100 fold compared on a fresh weight).

Based on the above criteria, our results showed that *Wolffia globosa* was a good accumulator of Cd (II) (BCF = 907.57). However, some other aquatic plant species have been shown to exhibit higher accumulation of Cd (II) and, therefore, are considered excellent Cd (II) accumulators. In two separate studies, Muramoto and Oki (156) and Muramoto et al. (157) showed that water hyacinth accumulated 36 and 10.6 mg Cd(II)/g, respectively. Several aquatic plant species attained higher BCF values than *W. globosa*. Rai et al. (106) reported Cd (II) BCF values ranging from 2,125 to 29,000 for six aquatic plant species (coontail, giant duckweed, bacopa, wild rice, channel grass, and alligator weed) and two algae (*Hydrodictyon reticulatum* and *Chara corallina*). Other species of duckweed were also shown to be even better accumulators of Cd (II) than *W. globosa*. Several studies have found high levels of Cd (II)

accumulation in inflated duckweed (*Lemna gibba*) and ivy duckweed (*L. trisulca*). These species accumulated 12.8 and 2.3 mg Cd(II)/g with a Cd (II) BCF of 5,953 and 3,594, respectively (98, 158).

In comparison, other aquatic plant species were proven to be poor accumulators of Cd (II). Bulrush (*Scirpus robustus*) and saltmeadow cordgrass (*Spartina patens*) accumulated 0.2 and 0.25 mg Cd(II)/g when exposed to 0.5 and 1.0 mg/L Cd (II), respectively (161). Very low Cd (II) BCF were observed for many aquatic plant species, *e.g.*, 73 for cattail (*Typha latifolia*) (160), 400 for bacopa and bulrush (108,159), and 250 for saltmeadow cordgrass (159).

In the case of Cr (VI) accumulation by *Wolffia globosa*, the results showed that Cr (VI) concentration in *W. globosa* tissues ranged from 0.29 to 3.50 mg Cr(VI)/g, depending on supply concentrations and exposure times. In comparison, coontail accumulated 0.16 to 0.87 mg Cr(VI)/g (161), musk grass (*Chara*) and hydrilla (*Hydrilla*) accumulated 0.29 and 0.59 mg Cr(VI)/g, respectively (162). Water hyacinth accumulation capacity ranged from 0.05 to 0.15 mg Cr(VI)/g in various treatments (107, 163), while soft stem bulrush (*Scirpus validus*) and yellow nut grass (*Cyperus esculentus*) accumulated 0.55 and 0.73 mg Cr(VI)/g, respectively (159). Greatest Cr BCF values were reported for coontail (15,330-31,400) (106, 161) and for water net (*Hydrodictyon reticulatum*) (11,394) (106). Chromium BCF values in this study ranged from 160-701. Thus, *W. globosa* may be considered as a moderate accumulator of Cr (VI).

It is clear from this study that, *Wolffia globosa* is generally regarded as a poor people's food and has attracted little attention as a potentially significant source of

human food. Because of having high accumulation potential of Cd (II) and Cr (VI) by *W. globosa*, the harvest of *W. globosa* for consumption must be carefully considered. From our studies, the results on the accumulation of Cd (II) and Cr (VI) in *W. globosa* were 484.67 and 288.00 μg/g , respectively, at 1 mg/L 3 days after treatment, whereas the toxicity of Cd (II) and Cr (VI) such as renal dysfunction is likely to be displayed in humans at concentrations of 150-220 μg/g, indicating that accumulated *W. globosa* can cause the toxic effect to the human health even if it was exposed at low concentration and duration. Therefore, before harvesting, the surrounding environment and characteristics of the plant should be observed, such as nearby factory, chlorosis of fronds and abnormal morphology.

B. Biosorption of dried *Wolffia globosa* biomass

From the results of the present study, sorption equilibrium was established when most of the cadmium and chromium ions were adsorbed on to *Wolffia globosa* after five hours of incubation. In *W. globosa*-Cd (II) system, the metal uptake increased when the initial pH values were increased. The maximum adsorption of Cd (II) occurred at an initial pH of 7. In general, at an initial pH between 4 and 7, the amount of Cd (II) adsorbed diminished when the pH was decreased, indicating that Cd (II) and H^+ ions are competing for the active sites on the *W. globosa*. Ramos et al. (113) investigated the adsorption of Cd (II) from aqueous solution onto activated carbon, and they found similar results that at an initial pH below 2, Cd (II) did not adsorb onto the carbon; however, varying the initial pH from 3 to 4 resulted in an 8 to 9 fold increase in Cd (II) adsorption.

Depending on the pH of the solution, the Cd (II) in aqueous solution can form various species or hydrocomplexes. Reed and Matsumoto (164) reported that the principal species of Cd (II) are formed according to the following reactions:

$$Cd^{2+} + H_2O \rightleftharpoons Cd(OH)^{+} + H^{+} \qquad pK_1 = 9.0$$

$$Cd^{2+} + 2H_2O \rightleftharpoons Cd(OH)_2 + 2H^{+} \qquad pK_2 = 19.1$$

$$Cd^{2+} + 3H_2O \rightleftharpoons Cd(OH)_3^{-} + 3H^{+} \qquad pK_3 = 30.3$$

$$Cd^{2+} + 2H_2O \rightleftharpoons Cd(OH)_2(s) + 2H^{+} \qquad pK_4 = 30.3$$

Based on these equilibrium constants, a speciation diagram was constructed and is depicted in Figure 5-1. The diagram shows that at pH below 7, the Cd (II) ion predominates, and at pH values just above 9, cadmium begins to precipitate out as $Cd(OH)_2$. At pH 8, the species distribution is approximately 90% Cd (II) and 10% $Cd(OH)^{+}$. This means that all the species occurring at pH values of 8 and below carry a positive charge either as Cd (II) or $Cd(OH)^{+}$.

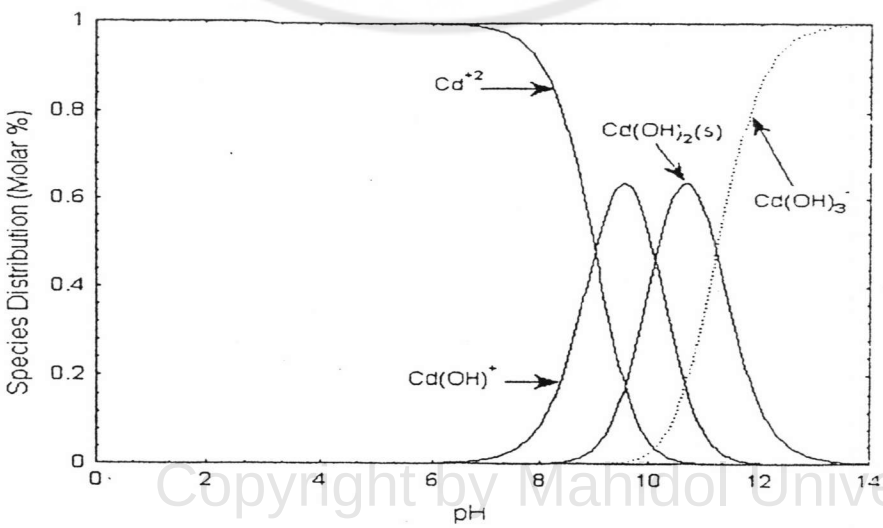

Figure 5-1. Speciation diagram for cadmium complexes present in aqueous solutions.

In *Wolffia globosa*-Cr (VI) system, Cr (VI) is removed most effectively in an acidic environment. The presence and state of chromium in natural waters depend on the possibilities of the reduction of Cr (VI) and the oxidation of Cr (III) in the water. Cr (VI) ions are normally present in soluble form, while Cr (III) ions are normally adsorbed by solid or accumulated in the sediments (165).

Chromium (VI) is readily hydrolyzed in water. The dominant Cr (VI) species at total chromium concentrations below 500 mg/L are the oxyanions $HCrO_4^-$ and CrO_4^{2-}. The equilibrium reaction between the two species is highly dependent on pH:

$$HCrO_4^- \rightleftharpoons H^+ + CrO_4^{2-} \qquad \log k = -6.51$$

At low pH, $HCrO_4^-$ is the dominant species, while CrO_4^{2-} predominates in a higher pH environment such as in natural groundwaters. The dichromate (VI) ions ($Cr_2O_7^{2-}$) predominate in acidic environment at Cr (VI) concentrations higher than 500 mg/L (165).

Chromium undergoes redox reactions in certain conditions:

At low pH : $Cr_2O_7^{2-} + 14H^+ + 6e^- \rightleftharpoons 2Cr^{3+} + 7H_2O$

At moderate pH : $CrO_4^{2-} + 8H^+ + 3e^- \rightleftharpoons Cr^{3+} + 4H_2O$

The reduction reaction require H^+ ions to drive the reaction (165).

In the present study, it is clear that the maximal adsorption of Cr (VI) occurred at an initial pH of 1.5. The metal uptake decreased with increasing initial pH values. This is due to the surface of the *Wolffia globosa* becoming more negative with more OH^- ions in the solution. The increase on pH would favor the formation of chromate ions (CrO_4^{2-}) that are not readily adsorbed by *W. globosa*, since they have very low

affinity for the *W. globosa* surface. Sharma and Forster (128) studied the removal of Cr (VI) using sphagnum moss peat and showed the similar results.

The effects of contact times to Cd (II) and Cr (VI) sorption were studied and indicated that Cd (II) and Cr (VI) were adsorbed rapidly and more efficiently when lower concentrations were tested. This rapid kinetics has a significant practical importance as it will facilitate smaller reactor volumes ensuring efficiency and economy. Similar rapid metal uptake has been reported by several researchers (115, 116, 117). They have suggested that ion exchange is the key to the rapid establishment of uptake equilibrium. However, the mechanism of Cd (II) and Cr (VI) uptake by *W. globosa* has not been elucidated and should be investigated in the future study.

C. The comparison with other sorbents

Table 5-1 and Table 5-2 show a summary of various adsorbents used for the adsorption of Cd (II) and Cr (VI). The comparison has been made in terms of X_m (mg/g). Although a direct comparison of *Wolffia globosa* with other reported adsorbents is difficult due to the varying experimental conditions employed in those studies, but in general, the adsorption capacity of *W. globosa* is considerably higher than many of these adsorbents. However, the potential of any material as an adsorbent will have to be evaluated from an economic perspective. Among the adsorbents, activated carbon has to be purchased commercially and the cost varies from place to place; *W. globosa* can either be collected from dams, rivers and wetlands in large quantity or cultivated easily where it has not been introduced. The *W. globosa* biomass

could be the lowest cost candidate for effective removal of Cd (II) and Cr (VI) in treatment of heavy-metal-laden wastewater.

Table 5-1. Showing the Cd (II) adsorption potential of various sorbents.

Sorbent	X_m (mg/g)	Reference
Wolffia globosa	80.65	Present study
Duloite GT-73	66.32	Volesky et al., 1993 (140)
Fe (III)/Cr (III) hydroxide	47.21	Nomasivayam and Ranganathan, 1995 (141)
Penicillium chrysogenum	43.84	Fourest et al., 1994 (142)
Pseudomonas aeruginosa	42.72	Chang et al., 1997 (143)
Saccharomyces cerevisiae	38.22	Volesky et al., 1993 (140)
Rhizopus arrhizus	30.35	Tobin et al., 1984 (144)
Clinoptiloite	23.61	Curkovie et al., 1997 (145)
Peat moss	22.48	Gosset et al., 1986 (137)
Granulated activated carbon	7.87	Ramos et al., 1997 (113)

Table 5-2. Showing the Cr (VI) adsorption potential of various sorbents.

Sorbent	X_m (mg/g)	Reference
Wolffia globosa	73.53	Present study
Azolla filiculoides	70.60	Zhao and Duncan, 1997 (115)
Activated carbon (Filtrasorb-400)	57.70	Huang and Wu, 1977 (138)
Leaf mould	43.00	Sharma and Forster, 1994 (146)
Coconut-husk fibers	29.00	Tan et al., 1993 (129)
Coconut-shell-based activated carbon	20.00	Alaerts et al., 1989 (147)
Palm-pressed fibers	14.00	Tan et al., 1993 (129)

CHAPTER VI

CONCLUSION

In *Wolffia globosa* growth measurement, linear growth began after three weeks and the highest biomass of *W. globosa* cultured in 3% Hoagland's nutrient solution grew up from 0.0400 to 0.0900 g dry wt for four weeks under the controlled condition. The three-week-old *W. globosa* was used for toxicity test procedures.

The effects of Cd (II) and Cr (VI) on the biomass productivity in *Wolffia globosa* indicated that there were significant decreases ($P<0.05$) when the exposure times and concentrations were increased. However, at low concentrations, the results showed a stimulation in growth of *W. globosa* at 1 mg/L Cr (VI) on day 3. The effective concentrations at 50% (EC_{50}) of Cd (II) and Cr (VI) on the biomass productivity in *W. globosa* showed a progressive decrease when exposure times were increased. The EC_{50} of Cd (II) was lower than Cr (VI) in the same duration, suggesting that Cd (II) was comparatively more toxic than Cr (VI).

The effects of Cd (II) and Cr (VI) on the chlorophyll content in *Wolffia globosa* indicated that there were significant decreases ($P<0.05$) when the exposure times and concentrations were increased. The effective concentration at 50% (EC_{50}) of Cd (II) and Cr (VI) on the chlorophyll content in *W. globosa* showed a progressive decrease when exposure times were increased. The EC_{50} of Cd (II) was lower than Cr (VI) in the same duration, suggesting that Cd (II) was comparatively more toxic than Cr (VI).

The tissue metal concentration depends on the concentration of the metal in the ambient water and time of exposure. The effects of Cd (II) and Cr (VI) on the accumulation in *Wolffia globosa* indicated that there were significant increases ($P<0.05$) in tissue levels when the exposure times and concentrations were increased. *Wolffia globosa* showed higher accumulation (higher in BCF) of Cd (II) than Cr (VI), suggesting that this plant specie has more selectivity towards Cd (II).

Sorption equilibrium was established when most of the cadmium and chromium ions were adsorbed on to *Wolffia globosa* after five hours of incubation. In *W. globosa*-Cd (II) system, when the pH values were increased, there were slight increases in the metal uptake at pH 4 to pH 6, and at pH 7 the metal uptake increased significantly ($P<0.05$), indicating that pH 7 was optimum for the adsorption in a buffered system. In *W. globosa*-Cr (VI) system, when the pH values were decreased from 6 to 3, there were slight increases in the metal uptake. When the pH values were decreased from 3 to 1.5, the metal uptake increased significantly ($P<0.05$), suggesting that Cr (VI) was removed most effectively in an acidic environment.

The effects of contact times to Cd (II) and Cr (VI) sorption were studied and indicated that Cd (II) and Cr (VI) were adsorbed rapidly and more efficiently when lower concentrations were tested.

From the results of the present study, concerning the toxicity test and biosorption of Cd (II) and Cr (VI) by using duckweed *Wolffia globosa*, it may be suggested that the data obtained could be used to set up the pretreated industrial water toxicity standards, and that the plants may also be useful in the toxicity reduction schemes. Since *W. globosa* is very sensitive to heavy metal when compared to other

plant species, it can be used as an indicator species for the assessment of ecotoxicological effects of wastewater and for effluent monitoring. The results obtained from this kind of experiments can provide information for the rational regulation and control of industrial and municipal effluents.

REFERENCES

1. Alloway BJ, Ayres DC. Chemical principles of environmental pollution. 2nd ed. London: Blackie Academic and Professional, 1997.

2. Nriagu JO. A silent epidemic of environmental metal poisoning. Environ Pollut 1988; 50: 139-61.

3. Raven PH, Berg LR, Johnson GB. Environment. USA: Saunders College, 1995.

4. Das P, Samantaray S, Rout GR. Studies on cadmium toxicity in plants : a review. Environ Pollut 1997; 98(1): 29-36.

5. Moore JW. Inorganic contaminants of surface water. USA: Springer-Verlag, 1991.

6. Mangi J, Schmidt K, Pankow J, Gaines L, Turner P. Effects of chromium on some aquatic plants. Environ Pollut 1978; 16: 285-91.

7. Sen AK, Mondal NG. *Salvinia natans* - As the scavenger of Hg (II). Water Air and Soil Pollut 1987; 34: 439-46.

8. Pinto CLR, Caconia A, Souza MM. Utilization of water hyacinth for removal and recovery of silver from industrial wastewater. Wat Sci Tech 1987; 19(10): 89-101.

9. Baker A. Accumulators and excluders - strategies in the response of plants to heavy metal. J Plant Nutr 1981; 3(1-4): 643-54.

10. Rosenfeld I, Beath OA. Selenium, geobotany, biochemistry, toxicity, and nutrition. New York: Academic Press, 1964.

11. Nasu Y, Kugimoto M. Comparative studies on the adsorption of cadmium and copper in *Lemna paucicostata*. Environ Pollut (Ser A) 1983; 32: 201-9.

12. Wang W. Literature review on duckweed toxicity testing. Environ Res 1990; 52: 7-22.

13. Leborans GF, Novillo A. Toxicity and bioaccumulation of cadmium in *Olisthodiscus luteus* (Raphidophyceae). Wat Res 1996; 30(1): 201-9.

14. Nriagu JO, Pacyna JM. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 1988; 333: 134-9.

15. Nriagu JO. A global assessment of natural sources of atmospheric trace metals. Nature 1989; 338: 47-9.

16. Yeats PA, Bewers JM. Evidence for anthopogenic modification of global transport of cadmium. In: Nriagu JO, Sprague JB, editors. Cadmium in the aquatic environment. New York: Wiley, 1987: 19-34.

17. Hutton M, Symon C. The quantities of cadmium, lead, mercury, and arsenic entering the U.K. environment from human activities. Sci Total Environ 1986; 57: 129-50.

18. Samanidou V, Fytianos K. Partitioning of heavy metals into selective chemical fractions in sediments from rivers in northern Greece. Sci Total Environ 1987; 67: 279-85.

19. Merian E. Metals and their compounds in the environment. New York: Verlagsgesells Chaft, 1991.

20. Ravera O. Cadmium in freshwater ecosystem. Experientia 1986; 40: 2-14.

21. Wang W. The effect of river water on phytotoxicity of Ba, Cd, and Cr. Environ Pollut 1986; 11: 193-204.

22. Wang W. Toxicity tests of aquatic pollutants by using common duckweed. Environ Pollut 1986; 11: 1-14.

23. Klerks PL, Weis JS. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ Pollut 1987; 45: 173-205.

24. Scott R, Aughey E, Fell GS, Quinn MJ. Cadmium concentrations in human kidneys from the UK. Human Toxicol 1987; 6: 111-9.

25. Nogawa K, Honda R, Yamada Y, Kido T, Tsuritani I, Ishizaki M. Critical concentration of cadmium in kidney cortex of humans exposed to environmental cadmium. Environ Res 1986; 40: 251-60.

26. Abuzkhar AA, Gibali AS, Elmehrik YI, Ahmatullah R. Chemical monitoring of sewage wastes for their use in crop production. I. Solid sludge as a soil amendment. Environ Monitor Assess 1987; 8: 127-33.

27. Nevissi AE, DeWalle FB, Sung JFC, Mayer K, Dalsey R. Heavy metal variability of different municipal sludge as measured by atomic absorption and inductively coupled plasma emission spectroscopy. J Environ Sci Health 1988; A23: 823-41.

28. Sung JFC, Nevissi AE, DeWalle FB. Concentration and removal efficiency of major and trace elements in municipal wastewater. J Environ Sci Health 1986; 21: 435-48.

29. Lema JM, Mendez R, Blazquez R. Characteristics of landfill leachates and alternatives for their treatment: a review. Water Air and Soil Pollut 1988; 40: 223-50.

30. Sakai H, Sasaki T, Saito T. Heavy metal concentrations in urban snow as an indicator of air pollution. Sci Total Environ 1988; 77: 163-74.

31. Beukema AA, Hekstra GP, Venema C. The Netherland's environmental policy for the North Sea and Wadden Sea. Environ Monitor Assess 1986; 7: 117-65.

32. Shupack IS. The chemistry of chromium and some resulting analytical problems. Environ Health Perspect 1991; 92: 7-11.

33. Saleh FY, Parkerton TF, Lewis RV, Huang JH, Dickson KL. Kinetics of chromium transformations in the environment. Sci Total Environ 1989; 86: 25-41.

34. Eary LE, Rai D. Kinetics of chromium (III) oxidation to chromium (VI) by reaction with manganese dioxide. Environ Sci Technol 1987; 21:1187-93.

35. Young TC, DePinto JV, Kipp TW. Adsorption and desorption of Zn, Cu, and Cr by sediments from the Raisin River (Michigan). J Great Lakes Res 1987; 13: 353-66.

36. Waldron HA. Metal in the environment. London: Academic Press, 1980.

37. Seigneur C, Constantinou E. Chemical kinetic mechanism for atmospheric chromium. Environ Sci Technol 1995; 29: 222-31.

38. Stern RM. Chromium compounds: production and occupational exposure. In: Langrad S, editor. Biological and environment aspects of chromium. Amsterdam: Elsevier Biomedical Press, 1982: 16-47.

39. Bonin MP, Flower WL, Renzi RF, Peng LW. Size and concentration measurement of particles produced in commercial chromium plating processes. J Air Waste Manag Assoc 1995; 45: 902-7.

40. Cohen Y. Intermedia transport modeling in a multimedia environment. In: Cohen Y, editor. Pollutants in a multimedia environment. New York: Plenum Press, 1986: 38-50.

41. Anderson LD, Kent DB, Davis JA. Batch experiments characterizing the reduction of Cr (VI) using suboxic material from a mildly reducing sand and gravel aquifer. Environ Sci Technol 1994; 28: 178-85.

42. Bodex I, Lyman WF, Reehl WF, Rosenblatt DH. Environmental inorganic chemistry properties, processes, and estimation methods. New York: Pergamon Press, 1988..

43. Schroeder RL, Lee GF. Potential transformation of chromium in natural waters. Water Air and Soil Pollut 1995; 4: 335-65.

44. Cary EE. Chromium in air, soils, and natural waters, in biological and environment aspects of chromium. New York: Elsevier Biomedical Press, 1982.

45. Richard FC, Bourg ACM. Aqueous geochemistry of chromium: a review. Water Res 1991; 25(7): 807-16.

46. Rosas I, Belmont R, Baez A, Villalobos-Pietrini R. Some aspects of the environmental exposure to chromium residues in Mexico. Water Air and Soil Pollut 1989; 48: 463-75.

47. Hillman WS. The Lemnaceae, or duckweed: a review of the descriptive and experimental literature. Botan Rev 1961; 27: 221-87.

48. Zirschky J, Reed SC. The use of duckweed for waste water treatment. J Wat Poll Control Fed 1988; 60: 1253-8.

49. Culley DD, Rejmankova E, Kvet J, Frye JB. Production, chemical, quality, and use of duckweed (Lemnaceae) in aquaculture, waste management, and animal feeds. J World Maric Soc 1981; 12: 27-49.

50. Sutton DL, Ornes WH. Phosphorus removal from static sewage effluent using duckweed. J Environ Qual 1975; 4: 367-70.

51. Porath D, Hepner B, Koton A. Duckweed as an aquatic crop: an evaluation of clones for aquaculture. Aqua Bot 1979; 7: 273-8.

52. Said MZM, Culley DD, Standifer LC, Epps EA, Myers RW, Boney SA. Effect of harvest rate, waste loading and stocking density on the yield of duckweeds. Proc World Maricult Soc 1979; 10: 769-80.

53. Naskar KR, Banerjee AC, Chakraborty NM, Ghosh A. Yield of *Wolffia arrhiza* (L). Horkel ex Wimmer from cement cisterms with different sewage concentrations, and its efficacy as a carp feed. Aquaculture 1986; 51: 211-6.

54. Truax RE, Culley DD, Griffith M, Johnson WA, Wood GP. Duckweed for chicken feed. Louisiana Agrc 1972; 16: 8-9.

55. Culley DD, Epps EA. Use of duckweeds for wastewater treatment and animal feed. J Wat Pollut Control Fed 1973; 45: 337-47.

56. Maciejewska PW, Konopska L, Narzymska E. Protein in duckweed (*Lemna minor* L.). Acta Soc Bot Pel 1970; 39(2): 212-20.

57. Hillman WS, Culley DD. The uses of duckweed. Amer Sci 1978; 66: 442-51.

58. Harvey RM, Fox JL. Nutrient removal using *Lemna minor*. J Wat Poll Control Fed 1973; 45: 1928-38.

59. Reddy KR, DeBusk WF. Nutrient removal potential of selected aquatic macrophytes. J Environ Qual 1985; 14: 459-62.

60. Brix H, Schierup HH. The use of aquatic macrophytes in water pollution control. Ambio 1989; 18: 101-7.

61. Edwards P, Hassan M, Chao CH, Pacharaprakiti C. Cultivation of duckweed in septage-loaden earthen ponds. Biores Technol 1992; 40: 109-17.

62. Alaerts GJ, Mahbular Rahman M, Kelderman P. Performance analysis of a full-scale duckweed covered sewage lagoon. Wat Res 1996; 30: 843-52.

63. Lambers H, Poorter H. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv Ecol Res 1992; 23: 187-95.

64. Oron G, Porath Dan, Jansen H. Performance of the duckweed species *Lemna gibba* on municipal wastewater for effluent renovation and protein production. Biotech Bioen 1987; 29: 258-68.

65. Vermaat JE, Hanif MK. Performance of common duckweed species (Lemnaceae) and the waterfern *Azolla filiculoides* on different types of wastewater. Wat Res 1998; 32(9): 2569-76.

66. Srivastav RK, Gupta SK, Nigam KDP, Vasudevan P. Treatment of chromium and nickel in wastewater by using aquatic plants. Wat Res 1994; 28(7): 1631-8.

67. Wahaab RA, Lubberding HJ, Alaerts GJ. Copper and chromium (III) uptake by duckweed. Wat Sci Tech 1995; 32(11): 105-10.

68. Bhanthumnavin K. *Wolffia arrhiza* as a possible source of inexpensive protein. Nature 1971; 232: 495.

69. Haustein AT, Gillman RH, Skillicorn PW, Vergara V, Guevara V, Gastanaduy A. Duckweed, a useful strategy for feeding chickens: performance of layers fed with sewage-grown Lemnaceae species. Poult Sci 1988; 69: 1835-44.

70. Haustein AT, Gillman RH, Skillicorn PW, Guevara V, Diaz F, Vergara V. Compensatory growth in broiler chicks fed on *Lemna gibba*. Brit J Nutri 1992; 68: 329-35.

71. Gaigher IG, Porath D, Granoth G. Evaluation of duckweed (*Lemna gibba*) as feed for tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) in a recirculating unit. Aquaculture 1984; 41: 235-44.

72. Van Dyke JM, Sutton DL. Digestion of duckweed (*Lemna* spp.) by the grass carp (*Ctenopharyhgolon edella*). J Fish Biol 1977; 11: 273-8.

73. Hassan MS, Edwards P. Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrhiza*) as feed for Nile Tilapia (*Oreochromis niloticus*). Aquaculture 1992; 104: 315-26.

74. Bhatia HL. Grass carp can control aquatic weeds. Indi Farming 1970; 20(2): 36-7.

75. Rifai SA. Control of reproduction of *Tilapia nilotica* using cage culture. Aquaculture 1980; 20: 177-85.

76. Lewis MA. Use of feshwater plants for phytotoxicity testing: a review. Environ Pollut 1995; 87: 319-36.

77. Dickman M, Prescott C, Kaiser KL. Variations in the aquatic vegetation of the Welland River (Ontario, Canada) above and below an industrial waste discharge. Great Lakes Res 1983; 9: 317-25.

78. Altieri MA, Letourneau DK. Vegetation management and biological control in agroecosystems. Crop Protect 1982; 1: 405-30.

79. Hunsaker CT, Graham RL, Suter GW, O'Neill RV, Barnthouse LW, Gardner RH. Assessing ecological risk on a regional scale. Environ Manage 1990; 14: 325-32.

80. Clark JR, Van Hassel VH, Nicholson RB, Cherry DS, Cairos J. Accumulation and depuration of metals by duckweed (*Lemna perpusilla*). Ecotoxicol Environ Saf 1981; 5: 87-96.

81. Seto M, Takahashi Y, Ushijima T, Tazaki T. Chlorotic death of *Lemna gibba* by cadmium in different concentrations of nutritional minerals. Japan J Limnol 1979; 40: 61-5.

82. Duncan DA, Klaverkamp JF. Tolerance and resistance to cadmium in white sucker (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc or selenium. Canad J Fish Aquat Sci 1983; 40: 128-38.

83. Benson WH, Birge WJ. Heavy metals tolerance and metallothionein induction in fathead minnows: result from field an laboratory experiments. Environ Toxicol Chem 1985; 4: 209-17.

84. Dixon DG, Sprague JB. Acclimation to copper by rainbow trout (*Salmo gairdneri*): a modifying factor in toxicity. Canad J Fish Aquat Sci 1981; 38: 880-8.

85. Takemoto BK, Noble D. Differential sensitivity of duckweeds (Lemnaceae) to sulfite. 1. Carbon assimilation and frond replication rate as factors influencing sulfite phytotoxicity under low and high irradiance. New Phytol 1986; 103: 525-39.

86. Staves RP, Knaus RM. Chromium removal from water by three species of duckweed. Aquat Bot 1985; 23: 261-73.

87. Fekete A, Riemer DN, Motto HL. A bioassay using common duckweed to evaluate the release of available phosphorus from pond sediments. J Aquat Plant Manag 1976; 14: 19-25.

88. Nasu Y, Kugimoto M. Lemna (duckweed) as an indicator of water pollution. I. The sensitivity of *Lemna pancicostata* to heavy metals. Arch Environ Contam Toxicol 1981; 10: 159-69.

89. Fogels A, Sprague JB. Comparative short term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants. Wat Res 1977; 11: 811-7.

90. Wang W. Chromate ion as a reference toxicant in aquatic phytotoxicity test. Environ Toxical Chem 1987; 6: 409-14.

91. Ballard EG. A comparison study of organic nitrogenous compounds to serve as sole sources of nitrogen for growth of plants. Plant Soil 1966; 25: 153-66.

92. McLay CL. The effect of pH on the population growth of three species of duckweed: *Spirodela oligorrhiza*, *Lemna minor*, and *Wolffia arrhiza*. Freshwater Biol 1976; 6: 125-36.

93. Glandon RP, McNabb CD. The uptake of boron by *Lemna minor*. Aquat Bot 1978; 4: 53-64.

94. Wang W, Williams JM. Screening and biomonitoring of industrial effluents using phytotoxicity test. Environ Toxicol Chem 1988; 7: 645-52.

95. Wang W, Williams JM. The use of phytotoxicity tests (common duckweed, cabbage, and millet) for determining effluent toxicity. Environ Monit Assess 1990; 14: 59-69.

96. Wang W. Toxicity of nickel to common duckweed (*Lemna minor*). Environ Toxicol Chem 1987; 6: 961-7.

97. Sigha S, Rai UN, Chandra P. Modulation of cadmium uptake and toxicity in *Spirodela polyrrhiza* (L.) Schleiden due to malathion. Environ Monit Assess 1995; 38: 67-73.

98. Devi M, Thomas DA, Barber JT, Fingerman M. Accumulation and physiological and biochemical effects of cadmium in a simple aquatic food chain. Ecotoxicol Environ Saf 1996; 33: 38-43.

99. Mohan BS, Hosetti BB. Potential phytotoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization ponds. Environ Pollut 1997; 98(2): 233-8.

100. Wang W. Site-specific barium toxicity to common duckweed, *Lemna minor*. Aquat Toxicol 1988; 12: 203-12.

101. Raskin I, Kumar PBAN, Dushenkov S, Salt D. Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 1994; 5: 285-90.

102. Jain SK, Vasudevan P, Jha NK. Removal of some heavy metals from polluted water by aquatic plants: studies on duckweed and water velvet. Biol Wastes 1989; 28: 115-26.

103. Huebert DB, Shay JM. The effect of cadmium and its interaction with external calcium in the submerged aquatic macrophyte *Lemna trisulca* L. Aquat Toxicol 1991; 20: 57-72.

104. Dunbabain JS, Bowmer KH. Potential use of constructed wetlands for treatment of industrial wastewaters containing metals. Sci Total Environ 1992; 111: 151-68.

105. Sharma SS, Gaur JP. Potential of *Lemna polyrrhiza* for removal of heavy metals. Ecol Eng 1995; 4: 37-43.

106. Rai UN, Sinha S, Tripathi RD, Chandra P. Wastewater treatability potential of some aquatic macrophytes: removal of heavy metals. Ecol Eng 1995; 5: 5-12.

107. Jana S. Accumulation of Hg and Cr by three aquatic species and subsequent changes in several physiological and biochemical plants parameters. Water Air and Soil Pollut 1988; 38: 105-9.

108. Sinha S, Chandra P. Removal of Cu and Cd from water by *Bacopa monnieri* L.. Water Air and Soil Pollut 1990; 40: 271-6.

109. Ornes WH, Sajwan KS. Cadmium accumulation and bioavailability in coontail (*Ceratophyllum demersum* L.) plants. Water Air and Soil Pollut 1993; 69: 291-300.

110. Jain SK, Vasudevan P, Jha NK. *Azolla pinnata* R.Br. and *Lemna minor* L. for removal of lead and zinc from polluted water. Wat Res 1990; 24(2): 177-83.

111. Volesky B, Kratochvil D. Advances in the biosorption of heavy metals. Trends Biotech 1998; 16: 291-300.

112. Volesky B. Biosorption of heavy metals. USA: CRC Press, 1990.

113. Ramos RL, Mendez JRR, Barron JM, Rubin LF, Coronado RMG. Adsorption of cadmium (II) from aqueous solution onto activated carbon. Wat Sci Technol 1997; 35(7): 205-11.

114. Sanyahumbi D, Duncan JR, Zhao M, Hille RV. Removal of lead from solution by the non-viable biomass of the water fern *Azolla filiculoides*. Biotech Let 1998; 20(8): 745-7.

115. Zhao M, Duncan JR. Batch removal of sexivalent chromium by *Azolla filiculoides*. Biotechnol Appl Biochem 1997; 26: 179-82.

116. Zhao M, Duncan JR. Removal and recovery of nickel from aqueous solution and electroplating rise effluent using *Azolla filiculoides*. Proc Biochem 1998; 33(3): 249-55.

117. Zhao M, Duncan JR, Hille RPV. Removal and recovery of zinc from solution and electroplating effluent using *Azolla filiculoides*. Wat Res 1999; 33(6): 1516-22.

118. Yu Q, Matheickal JT, Yin P, Kaewsarn P. Heavy metal uptake capacities of common marine macro algal biomass. Wat Res 1999; 33(6): 1534-7.

119. Matheickal JT, Yu Q. Biosorption of lead from aqueous solutions by marine algae *Ecklonia radiata*. Wat Sci Technol 1996; 34(9): 1-7.

120. Matheickal JT, Yu Q, Feltham J. Cu (II) binding by *Ecklonia radiata* biomaterial. Environ Technol 1997; 18: 25-34.

121. Ahuja P, Gupta R, Saxena RK. Zn (II) biosorption by *Oscillatoria auguistissina*. Proc Biochem 1999; 34: 77-85.

122. Matheickal JT, Yu Q, Woodburn GM. Biosorption of cadmium (II) from aqueous solutions by pre-treated biomass of marine alga *Durvillaea potatorum*. Wat Res 1999; 33(2): 335-42.

123. Chen HX, Gosset T, Thevenot DR. Batch copper ion binding an exchange properties of peat. Wat Res 1990; 24: 1463-71.

124. McLellan JK, Rock CA. Pretreating landfill leachate with peat to remove metals. Water Air and Soil Pollut 1988; 37: 203-15.

125. Coupal B, Lalancetted JM. The treatment of wastewaters with peat moss. Wat Res 1976; 10: 1071-6.

126. Benchikh LM. Zinc removal using peat adsorption. Environ Technol Lett 1989; 10: 101-8.

127. Chaney RL, Hundermann PT. Use of peat moss columns to remove cadmium from wastewaters. J Wat Pollut Control Fed 1979; 51: 17-21.

128. Sharma DC, Forster CF. Removal of hexavalent chromium using sphagnum moss peat. Wat Res 1993; 27(7): 1201-8.

129. Tan WT, Ooi ST, Lee CK. Removal of chromium (VI) from solution by coconut husk and palm pressed fibres. Environ Technol 1993; 14: 277-82.

130. Low KS, Lee CK, Tan SG. Sorption of trivalent chromium from tannery waste by moss. Environ Technol 1997; 18: 449-54.

131. Environmental Pretection Agency. Test methods for assessing the effects of chemicals on plants. In: Rubinstien R, Smith J, editors. EPA-560-17-008 Final Report. Washington DC: U.S. Environmental Protection Agency, 1975: 175-82.

132. Arnon DI. Copper enzyme in isolated chloroplasts polyphenol oxidases in *Beta vulgaris*. Plant Physiol 1949; 24: 1-14.

133. MacKinney G. Absorption of light by chlorophyll solutions. J Biol Chem 1941; 144: 315-23.

134. APHA, AWWA, WEF. Standard methods for the examination of water and wastewater. 20th ed. Washington DC: n.p., 1998.

135. Winer BJ. Statistical principles in experimental design: international student edition. London: n.p., 1981.

136. Holan ZR, Volesky B, Prasetyo I. Biosorption of cadmium by biomass of marine algae. Biotech Bioeng 1993; 41: 819-25.

137. Gosset T, Trancart JL, Thevenot DR. Batch metal removal by peat: kinetics and thermodynamics. Water Res 1986; 20: 21-6.

138. Huang CP, Wu HM. The removal of chromium (VI) from dilute aqueous solution by activated carbon. Wat Res 1977; 11: 673-9.

139. Wang TC, Weissman JC, Ramesh G, Varadarajan R, Benemann JR. Bioremoval of toxic elements with aquatic plants and algae. In: Hinchee RE, Means JL, Burris DR, editors. Bioremediation of Inorganics. Columbus (OH): Battelle Press, 1995: 65-9.

140. Volesky B, May H, Holan Z. Cadmium biosorption by *S. cerevisiae*. Biotechnol Bioeng 1993; 41: 826-9.

141. Namasivayam C, Ranganathan K. Removal of Cd (II) from wastewater by adsorption on "waste" Fe(III) Cr(III) hydroxide. Wat Res 1995; 29: 1737-44.

142. Fourest E, Canal C, Roux JC. Improvement of heavy metal biosorption by mycelial dead biomasses (*Rhizopus arrhizus*, *Muchor miehei*, and *Pencillium chrysogenum*): pH control and cationic activation. FEMS Microbiol Rev 1994; 14: 325-32.

143. Chang JS, Law R, Chang CC. Biosorption of lead, copper and cadmium by biomass of *Pseudomona aeruginosa* PU21. Wat Res 1997; 31: 1651-8.

144. Tobin JM, Cooper DG, Newfeld RJ. Uptake of metal ions by *Rhizopus arrhizus* biomass. Appl Environ Microbiol 1984; 47: 821-4.

145. Curkovic L, Stefanovic SC, Filipan T. Metal ion exchange by natural and modified zeolites. Wat Res 1997; 31: 1379-82.

146. Sharma DC, Froster CF. Treatment of chromium(VI) wastewaters using the sorptive potential of leaf mould. Biores Technol 1994; 49: 31-40.

147. Alaerts GJ, Jitjaturunt V, Kelderman P. Use of coconut-shell-based activated carbon from chromium(VI) removal. Wat Sci Technol 1989; 21: 1707-4.

148. So ML. Growth characteristics of duckweeds and their potential use as organic fertilizers in Hong Kong. In: Reddy KR, Smith WH, editors. Aquatic plants for water treatment and resource recovery. USA: Magnolia, 1987: 755-62.

149. Sen AK, Mondal NG, Mandal S. Studies of uptake and toxic effects of Cr(VI) on *Pistia stratiotes*. Wat Sci Tech 1987; 19: 119-27.

150. Haghiri F. Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc and soil temperature. J Environ Qual 1974; 3: 180-3.

151. Root RA, Miller RJ, Koeppe DE. Uptake of cadmium-its toxicity and effect on the iron-to-zinc ratio in hydroponically grown corn. J Environ Qual 1975; 4: 473-6.

152. Goldbold DL, Huttermann A. Effect of zinc, cadmium and mercury on root elongation of *P. abies* (Karst) seedlings and the significance of these metals to forest die-back. Environ Pollut 1985; 38: 375-81.

153. Zayed AM, Gowthaman S, Terry N. Phytoaccumulation of trace elements by wetland plants: I. Duckweed. J Environ Qual 1998; 27: 715-21.

154. Zhu YL, Zayed AM, Qian JH, Souza M, Terry N. Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. J Environ Qual 1999; 28: 339-44.

155. Reeves RD, Baker AJM, Brooks RR. Abnormal accumulation of trace metals by plants. Mining Environ Manage 1996; 3(3): 4-8.

156. Muramoto S, Oki Y. Removal of some heavy metals from polluted water by water hyacinth (*Eichhornia crassipes*). Bull Environ Contam Toxicol 1983; 30: 170-7.

157. Muramoto S, Oki Y, Mishizaki H, Aoyama I. Variation in some element contents of water hyacinth due to cadmium or nickel treatment with or without anionic surface active agents. J Environ Sci Health 1989; A24(8): 925-34.

158. Huebert DB, Shay M. The response of *Lemna trisulca* L. to cadmium. Environ Pollut 1993; 80: 247-53.

159. Lee CR, Sturgis TC, Landin MC. Heavy metal uptake by marsh plants in hydroponic solution cultures. J Plant Nutr 1981; 3(1-4): 139-51.

160. McNaughton SJ, Folsom TC, Lee T, Park F, Price C, Roeder D. Heavy metal tolerance in *Typha latifolia* without the evolution of tolerant races. Ecology 1974; 55: 1163-5.

161. Garg P, Chandra P. Toxicity and accumulation of chromium in *Ceratorphyllum demersum* L. Bull Environ Contam Toxicol 1990; 44: 473-8.

162. Rai UN, Tripathi RD, Sinha S, Chandra P. Chromium and cadmium bioaccumulation and toxicity in *Hydrilla verticillata* Royle and *Chara corallina* Wildenow. J Environ Sci Health 1995; A30(3): 537-51.

163. Zaranyika MF, Ndapwadza T. Uptake of Ni, Zn, Fe, Co, Cr, Pb, Cu, and Cd by water hyacinth (*Eichhornia crassipes*) in Mukuvisi and many rivers, Zimbabwe. J Environ Sci Health 1995; A30(1): 157-69.

164. Reed BE and Matsumoto MR. Modeling cadmium adsorption by activated carbon using the Langmuir and Freundlich isotherm expressions. Sep Sci Technol 1993; 28(13/14): 2179-95.

165. Selomulya C, Meeyoo V, Amal R. Mechanisms of Cr(VI) removal from water by various types of activated carbons. J Chem Technol Biotechnol 1999; 74: 111-22.

BIOGRAPHY

NAME Miss Benjaporn Boonyapookana

DATE OF BIRTH 30 May 1975

PLACE OF BIRTH Bangkok, Thailand

INSTITUTIONS ATTENDED Thammasat University, 1992-1995:
Bachelor of Science (Environmental Science)
Mahidol University, 1997-1999:
Master of Science (Environmental Biology)