การสะสมของแคดเมียมในผักบุ้งที่ปลูกในน้ำที่มีการปนเปื้อนแคดเมียม

นางสาวแวววลี ประมูล

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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CADMIUM ACCUMULATION IN WATER SPINACH GROWN IN CADMIUM

CONTAMINATED WATER

Miss Weawvalee Pramoon

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for the Degree of Master of Science Program in Environmental Management
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แววลี ประมูล : การสะสมของแคดเมียมในผักบุ้งที่ปลูกในน้ำที่มีการปนเปื้อนของแคดเมียม (CADMIUM ACCUMULATION IN WATER SPINACH GROWN IN CADMIUM CONTAMINATED WATER) อ. ที่ปรึกษา : รศ. ดร. เสนีย์ กาญจนวงศ์, 135 หน้า. ISBN 974-14-1967-8.

การศึกษานี้มีวัตถุประสงค์เพื่อหาความเป็นพิษของแคดเมียมต่อการเจริญเติบโตของผักบุ้ง การสะสมของแคดเมียมในผักบุ้งที่ปลูกในน้ำ ที่มีการปนเปื้อนของแคดเมียมในระยะยาวที่ความเข้มข้นต่างๆ และการสะสมของแคดเมียมในผักบุ้งซึ่งปลูกในแหล่งน้ำที่ปนเปื้อนด้วยแคดเมียมปริมาณสูงอย่างกะทันหันในระยะเวลาสั้น การทดลองทำในภาชนะพลาสติกขนาด 45 ลิตร (เส้นผ่าศูนย์กลาง 60 ซม. x สูง 24 ซม.) โดยมีหลังคาพลาสติกใสคลุมป้องกันน้ำฝน ทดลอง4 ซ้ำต่อความเข้มข้นแคดเมียม น้ำที่เพาะปลูกเตรียมจากน้ำทิ้งของระบบบำบัดน้ำเสียชุมชนที่มีสารอาหารพอเพียงในการเจริญเติบโต และเติมแคดเมียมคลอไรด์ให้ได้ความเข้มข้นต่างๆ ผลการศึกษาความเป็นพิษในการเพาะปลูก 30 วัน พบว่าแคดเมียมความเข้มข้น 7.6 มก/ล. ปลูกผักบุ้งได้ แต่ที่ความเข้มข้น 59.1 มก/ล. เกิดอาการใบเหลือง และผักตาย ในการปลูกระยะยาว 130 วัน พบว่า ผักบุ้งมีผลผลิตลดลงตามธรรมชาติแม้ในแปลงควบคุมที่ไม่เติมแคดเมียมเลย ที่ความเข้มข้นไม่เกิน 0.053 มก/ล. มีการเจริญเติบโตปกติ ขณะที่ความเข้มข้น 0.446 มก/ล. พืชจะเริ่มตายหลังจากปลูกไปแล้ว 40 วัน ที่ความเข้มข้นแคดเมียมต่ำ ไม่เกิน 0.053 มก/ล. มีแคดเมียมสะสมมากในราก ขณะที่ในใบและลำต้นมีค่าใกล้เคียงกัน และเกินมาตรฐานความปลอดภัยในการบริโภคของ European Commission (EU) แต่ถ้าคิดตามDietary Intake ขององค์การอนามัยโลก (WHO) ผักบุ้งที่ปลูกในน้ำที่มีแคดเมียมความเข้มข้น 0.005 มก/ล. สามารถบริโภคได้ไม่เกิน 1,700 กรัม/สัปดาห์ใน กรณีความเข้มข้นแคดเมียมในน้ำสูงช่วง 0.135-0.446 มก/ล. การปนเปื้อนมีสูงสุดในราก, ลำต้น และใบตามลำดับและมีค่าเกินระดับความปลอดภัยหลายเท่า ในการทดลองที่ผักบุ้งถูกปนเปื้อนในน้ำ 0.148 มก/ล. ระยะสั้น พบว่า ถ้าไม่เกิน 1 วัน การสะสมของแคดเมียมจะไม่เกินค่าความปลอดภัยในการบริโภคของ EU

สาขาวิชาการจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต..............................

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา..............................

4789488120: MAJOR ENVIRONMENTAL MANAGEMENT
KEY WORD: CADMIUM / ACCUMULATED / WATER SPINACH

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The objectives of this study were to assess toxicity effects of cadmium on water spinach (*Ipomoea aquatica*), evaluate the cadmium accumulation in cadmium contaminated water at various concentrations under long-term cultivation and assess cadmium accumulation under shock loading contamination. The cultivation units were 45-L plastic bowls (diameter 60 cm x height 24 cm). Installed under transparent plastic roof to prevent rain. There were 4 replications in each cadmium concentration. The effluent of domestic wastewater treatment plant which contained sufficient nutrients for plant growth was mixed with cadmium chloride at various cadmium concentrations. The toxicity test conducted for 30 days revealed that cadmium concentration 7.6 mg/L had no toxicity effects on water spinach while concentration of 59.1 mg/L had chlorosis and plant die-off. The long-term cultivation showed that there was natural die-off and reduction of yield, as observed in control plots without cadmium addition. At cadmium concentration within 0.053 mg/L, normal yields were found while concentration of 0.446 mg/L had plant die-off since 40^{th} day of cultivation. For low cadmium in water, i.e. within 0.053 mg/L, cadmium accumulated in roots higher than in leaves and stems, which were not much different. The accumulated level exceed European Commission (EU) standard for vegetable. Based on dietary intake rate of World Health Organization, water spinach grown in cadmium concentration of 0.005 mg/L can be consumed at the rate of 1,700 g/week. For high cadmium in water, i.e. 0.135-0.446 mg/L, cadmium accumulated highest in roots, stems and leaves respectively, and much higher than EU standard. Under shock loading of plant in cadmium concentration 0.148 mg/L for a short period, it was found that cultivation duration of 1 day reduction in cadmium contamination in plant to be within EU standard.

Field of study Environmental Management Student's signature Weawvalee Pramoon

Academic year 2005 Advisor's signature Seni Karnchanawong

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LIST OF ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
ANZFA	Australia New Zealand Food Authority
APHA	American Public Health Association
AWWA	American Water Works Association
cm	Centimeter
^{o}C	Degree Celsius
CEC	Cation Exchange Capacity
d	Day
Cd	Cadmium
$CdCl_2.2.5H_2O$	Cadmium Chloride
Con.	Concentration
e.g.	For example
EC_{50}	The molar concentration of an agonist, which produces 50% of the maximum possible response for that agonist
EPA	Environmental Protection Agency
EU	European Commission
EDTA	Ethylene diamine tetraacetic acid
EGTA	Ethylene diamine tetraacetic acid
ft	foot
FAO	Food and Agriculture Organization
g/cm^3	Gram per cubic centimeter
ha	hectare
H_2S	Hydrogen sulfite
i.e.	That is
in	inch
L	Liter
LC	Lethal Concentration
LD	Lethal Dose
m	Meter
mm	Millimeter

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mg/L	Milligram per liter
mg/kg	Milligram per kilogram
M	Molar
MCL	Maximum contaminant level
MCLG	Maximum contaminant level goal
OSHA	Occupational Safety and Health Administration
ppm	part per million
RfD	Reference Dose
RfC	Reference Concentration
TN	Total Nitrogen
TP	Total phosphorus
µm	Micrometer
UNESCAP	United Nation Economic and Social Commission for Asia and the Pacific
WHO	World Health Organization
WEF	World Economic Forum

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CHAPTER 1

INTRODUCTION

1.1 Motivation

The increasing of the population and development results in the community expanding. The development in agriculture and industry manufacturing, which in turn increases the amount of products, is a cause of much environmental pollution. An obvious topic that has resulted from this is water pollution; examples of which include the discharge of dirty water from factories, agricultural facilities and animal farms to the rivers. Toxic chemical contamination in water resources comes from wastewater from industry or the water from agricultural areas that apply insecticides and herbicides. The most significant problem is the accumulation of heavy metals in natural water resources. The main sources of heavy metals are from industrial wastewater, the chemicals from agriculture that have heavy metal composition and households such as garage which used petrol in the operation.

It should be realized that when heavy metals enter water resource they do not break down into the environment, but absorb or adsorb into the soil, sediment or aquatic life and can change to be the temporarily safe forms.

Thailand is a fertile country, and agriculture, which broadly includes crop cultivation, is the Thai economy's largest and most important sector. The expansion of industries, which are not sufficiently managed, commits aggression to agricultural areas. This especially refers to the industries along the river that discharge their wastewater into the rivers which are the main water source used for cultivation. For example, Samut Prakarn province, which is located southeast of Bangkok, is the most heavily industrialized and polluted province in Thailand. Straddling the Chao Phraya River, the province has more than 5,000 factories and one million people. The sanitation and wastewater management facilities in the province are ineffective in dealing with the large wastewater flows from industrial, commercial, and residential sources. The results include the severe degradation of the water quality and lead to the deterioration of public health, as evidenced by the incidences of water-and sanitation-related diseases. Many of the waterways have been ecologically weakened, and most of the beneficial uses of the water from the Chao Phraya River have been lost.

Recent industrialization activities along the river in Thailand have increased the discharge of industrial wastes into marine waters. Many aquatic plants like water spinach and water hyacinth are the first to be exposed to this pollution.

The accumulation of elements like heavy metals in the water will increase the risk of their uptake and movement in the food chain which makes it a current topic of great concern. The cultivation areas located near contamination sources, such as mining, factory and large cities, are most heavily affected. In polluted areas, the transfer of toxic elements from water to plants is of great concern. It would be advantageous to predict before cultivation whether the concentration of a given contaminant in plants was within the acceptable level.

Water spinach (*Ipomoea aquatica*) is an aggressive water plant native to China and naturalized throughout many tropical and semitropical regions. Although it is an invasive weed, it is also an important green vegetable in Asian cuisine. The creeping stems are hollow and float on the water surface. In Thailand it is a popular vegetable that is cultivated in fresh water courses. (http://www.iisgcp.org/EXOTICSP/waterspinach.htm#descrip).

These often serve as recipients for domestic and other sorts of wastewater that often contain a variety of pollutants, such as heavy metals, especially cadmium which has increased conspicuously as a result of industry operations such as mining industry. Although industrial wastewater is treated according to the standard before being discharged into the natural water, some pollutants still remain, and accumulate in natural waters where they are available for uptake by plants (phytoremediation). Based on a scenario of a river receiving industrial effluent containing cadmium at low concentrations and water spinach cultivation along river banks, cadmium uptake under long–term cultivation should be investigated. There are possible fluctuations in the cadmium concentration in the river water if the wastewater treatment plant is not well operated and cadmium uptake during that period is also observed.

1.2 Objectives

The objectives of this study are:

1.2.1 To investigate cadmium transport from cadmium contaminated water at various concentrations to water spinach. The permissible concentration of cadmium in water for water spinach cultivation, as food, evaluated.

1.2.2 To evaluate the responses and tolerance of water spinach to various cadmium concentrations in water.

1.3 Hypotheses

The hypotheses of this study are listed as follows:

1.3.1 Various cadmium concentrations may differently affect water spinach growth.

1.3.2 Water spinach grown in cadmium contaminated water at concentrations equal to the Class-3 water quality standard, drinking water standard, industrial effluent standard and deep well standard may absorb cadmium and the contamination level may be within that of the food standard.

1.3.3 Water spinach grown in cadmium contaminated water, in excess of the industrial effluent standard may absorb cadmium to a level that is unsuitable for consumption.

1.3.4 Water spinach which is exposed to a shock load concentration for a short period may accumulate cadmium.

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1.4 Scope of this study

1.4.1 The experiments were conducted in 24 and 32 laboratory-scale cultivation units made from 45-L plastic bowls (diameter 60 cm. x height 24 cm.) and placed outdoors. All studies took place in four replicates.

1.4.2 A translucent pavilion was used to exclude the rain from the experiment plots.

1.4.3 The contaminated water was synthesized by varying the cadmium concentrations in the treated domestic wastewater (before chlorination) from the Chiang Mai University wastewater treatment plant.

1.4.4 There were three major studies, i.e. cadmium toxicity test on water spinach growth, cadmium uptake under long-term cultivation and cadmium uptake under shock load conditions.

1.4.5 The cadmium concentrations investigated in the toxicity tests were 0 (control), 10, 50, 100, 250 and 500 mg/L.

1.4.6 The cadmium concentrations used for long-term cultivation were 0.005, 0.01, 0.03 and 0.1 mg/L, representing the Class-3 water quality standard, drinking water standard, industrial effluent standard and deep well standard, respectively; and 0.05, 0.2 and 0.5 mg/L, representing the industrial effluent, which do not comply with the standard.

1.4.7 For the shock load study, a cadmium concentration baseline of 0 mg/L and cadmium concentration shock load of 0.2 mg/L were used with shock load periods of 1, 3, 5, 7 and 9 days, respectively.

1.4.8 The plant grown was water spinach (*Ipomoea aquatica*). Initial measurements of the cadmium content in the water spinach were done before the experiment.

1.1 Benefits of this Study

1.5.1 To obtain the responses and tolerance level of water spinach to various cadmium concentrations in water.

1.5.2 To obtain the fate and transport of cadmium in various parts of water spinach that were cultivated in various cadmium contaminated water concentrations under long- term cultivation.

1.5.3 To assess the transport of cadmium in water spinach under the shock load condition of high cadmium.

1.5.4 To assess whether present water standards on cadmium are suitable for water spinach cultivation.

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CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Cadmium

2.1.1 Physiochemical properties

Cadmium is an element that occurs naturally in the earth's crust. Cadmium is not usually present in the environment as a pure metal, but as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide) (ATSDR, 1999). In the air, cadmium is rapidly oxidized into cadmium oxide. However, when reactive gases or vapor such as carbon dioxide, water vapor, sulfur dioxide, sulfur trioxide or hydrogen chloride are present, cadmium vapor reacts to produce cadmium carbonate, hydroxide, sulfite, sulfate or chloride, respectively. Several inorganic cadmium compounds are quite soluble in water, e.g. acetate, chloride and sulfate; whereas cadmium oxide, carbonate and sulfide are almost insoluble. It reacts slowly with hot hydrochloric acid and does not react with alkalies. It is slowly oxidized by moist air to form cadmium oxide. Cadmium occurs in nature in ores, and is obtained as a by-product from the extraction, separation and recovery of those metals in refinery plants (OSHA,1993). The physical and chemical properties of cadmium and compounds are presented in Table 2.1.

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Table 2.1: Physical and chemical properties of cadmium and cadmium chloride (Adapted from http://www.atsdr.cdc.gov/toxprofiles/tp5-c3.pdf)

Properties	**Characteristics**	
	Cadmium	**Cadmium chloride**
Molecular weight	112.41	183.32
Valence	2	2
Color	Silver-white	Colorless
Physical state	Lustrous solid	Rhombohedral crystal
Melting point	321 ^{o}C	563 ^{o}C
Boiling point	765 ^{o}C	960 ^{o}C
Density	8.65 g/cm^3 at 25 ^{o}C	3.33 g/cm^3 at 20 ^{o}C
Odor	Odorless	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water	Insoluble	Soluble
Organic solvent	Acid, NH_4NO_3, hot H_2SO_4	Acetone, Slightly soluble in Methanol and Ethanol.
Vapor pressure	1 mm Hg at 394 ^{o}C	10 mm Hg at 656 ^{o}C; 40 mm Hg at 736 ^{o}C; 760 mm Hg at 967 ^{o}C

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2.1.2 Uses of cadmium

Cadmium is a by-product of the primary non-ferrous metal industry. Rather than disposing of it as a waste, engineers have been able to utilize its unique properties for many important industrial applications.

The most remarkable characteristics of cadmium are its great resistance to corrosion, its low melting-point and excellent electrical conduction. Cadmium compounds exhibit excellent resistance to chemicals and high temperatures. Finally, cadmium pigments produce intense colorings such as yellow, orange and red, and are well known pigments in artists' colors, plastics, glasses, ceramics and enamels (http://www.cadmium.org/introduction.html).

A primary use for cadmium metal is as an anticorrosive, and it is electroplated onto steel. Cadmium may serve as an electrode component in alkaline batteries and may be used in alloys, silver solders, and welding. Cadmium exposures in general occur in refining and smelting operations. Relative to the metals with which it is found, cadmium volatilizes readily during these processes because of its low boiling point (765 ^{o}C) and high vapor pressure. The cadmium then condenses to form fine airborne particles that react almost immediately with oxygen to form respirable cadmium oxide. Other general industry groups where exposure to cadmium may occur include electroplating, battery manufacturing, and pigment and plastics manufacturing. In addition, cadmium exposure is associated with welding, brazing, and painting operations in many other industries. (OSHA,1993)

In recent years, the consumption pattern of cadmium in its various end use applications has increasingly shifted away from the traditional market areas of pigments, coatings stabilizers to rapidly growing applications in rechargeable nickel-cadmium batteries (almost 70% of its use) as shown in Figure 2.1 (http://www.cadmium.org/introduction.html).

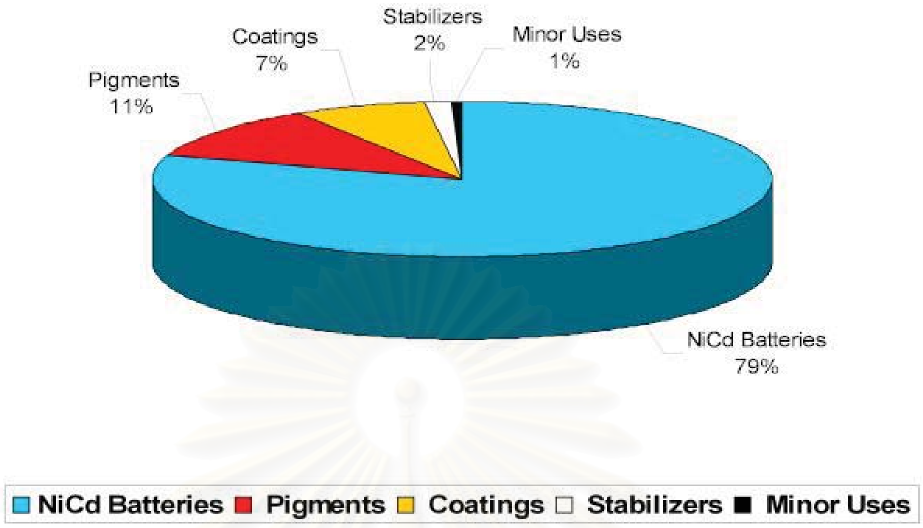

Figure 2.1: Western World Cadmium Consumption Pattern**,** 2003

(Adapted from http://www.cadmium.org/introduction.html)

Cadmium chloride ($CdCl_2$) is also widely used in industry. It is used in fungicides, the manufacturing of cadmium yellow pigments, galvanoplasty, laboratory analyses of sulfides to absorb H_2S, and electroplating, and as a test for pyridine bases and as an ice-nucleating agent. It is also used as an addition to tinning solutions, as a mordant in the dyeing and printing of textiles, as a component of metal finishing baths and aerosols, as an agent in photocopying, as an agent in the manufacture of coatings for electronic vacuum tubes, as an agent in the manufacture of special mirrors, as a solid film lubricant, as a catalyst, as a fog inhibitor in photographic film emulsions, as a chemical intermediary for cadmium sulfide, as a colorant for pyrotechnics, and in pesticides. Workers can be exposed to cadmium chloride dusts and aerosols in battery manufacturing, electroplating, alloy and solder production, ceramics and vapor lamps production, and welding. (OSHA,1993)

The United Nation Economic and Social Commission for Asia and the Pacific (UNESCAP) reported that the rapid industrialization of much of South-East Asia has led to the potential for heavy metal contamination of soil in a variety of ways and on a variety of scales. The principal ways can be classified into four sources: mining activity, industrial activity, wastewater reuse, and fertilizers.

In Thailand, mining activities spread mine spoil and tailings from ore processing. Much mining activity in the region is artisanal and unregulated, resulting in pollution over quite a large area. For example, it is estimated that some 5,000–10,000 ha are contaminated with cadmium in the region. (www.unescap.org/esd/water/publications/CD/escap-iwmi/keynote.pdf).

2.1.3 Source and environmental fate

Cadmium is a relatively rare element (0.2 mg/kg in the earth crust) and is not found in its pure state in the nature. It occurs mainly in association with the sulfide ores of zinc, lead and copper. Cadmium has only been produced commercially since the twentieth century. It is a by-product of the zinc industry; its production is thus determined essentially by that of zinc. Before the First World War cadmium was not usually recovered from zinc plants or other nonferrous metals plants, which resulted in an uncontrolled contamination of the environment for decades.
(www.euro.who.intdocumentaiq6_3cadmium.pdf.)

Cadmium distribution arises from two major source categories, natural sources and man-made or anthropogenic sources.

2.1.3.1 Natural sources

Even though the average cadmium concentration in the earth's crust is generally placed between 0.1 and 0.5 ppm, much higher levels may accumulate in sedimentary rocks, and marine phosphates. In addition, weathering and the erosion of parent rocks result in the transport by rivers of large quantities of cadmium to the world's oceans. Moreover, volcanic activity is also a major natural source of cadmium release to the atmosphere. Furthermore, forest fires have also been reported as a natural source of cadmium air emissions. (http://www.cadmium.org/introduction.html)

2.1.3.2 Anthropogenic sources

Anthropogenic activities are associated with industrialization and agricultural activities such as atmospheric deposition, waste disposal, waste incineration, urban effluent, vehicle exhausts, fertilizer application and the long-term application of sewage sludge in agricultural land (Vig et al, 2003).

a) Mining activity

Wu (2001) reported that Thailand's Zinc silicate ore produced mainly from the Mae Sot Mine by PDI totaled 167,024 t in 2004 compared with 249,539 t in 2003. The average metal content of zinc silicate ore produced was 26% zinc in 2004 compared with 25% zinc in 2003. According to PDI, a full-scale flotation plant, which upgraded the low-grade ores to concentrates, was commissioned next to the existing flotation plant. The new flotation plant would increase production of concentrate by as much as 97,000 t. The Mae Sot Mine had been in operation for 21 years. The remaining ore reserves were estimated to be 3.6 Mt at a grade of 12.7% zinc (Padaeng Industry Public Company Ltd., 2005).

Mining activity spread mine spoil and tailings and in some cases, by the use of heavy metals in ore processing.

Theerapunsatien (1995) studied the heavy metal contamination from the zinc refinery near the Ping River (Figure 2.2), which received heavy metals, especially cadmium and zinc both in direct and indirect ways, i.e. the community and industry situated in the Ping Basin. Some heavy metals dispersed through river, precipitated and accumulated in sediment, and entered the food web system. The last consumers who received high concentrations of heavy metals were humans.

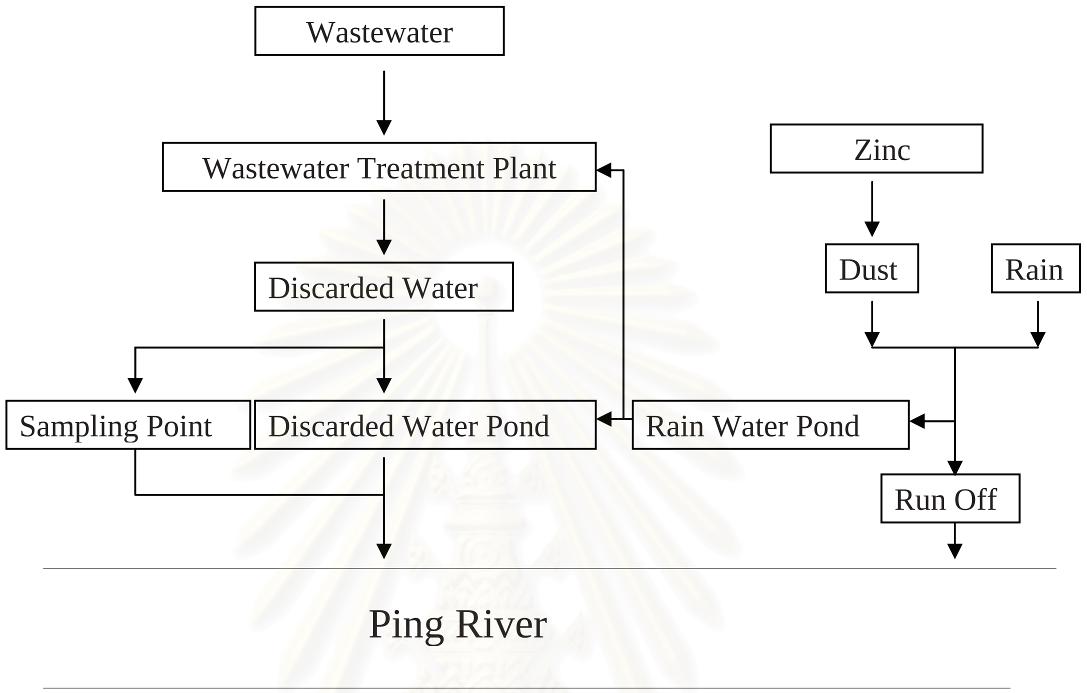

Figure 2.2: Distribution of Heavy Metals from the Zinc Refinery

(Adapted from Theerapunsatien, 1995)

Prayut (1999) studied the distribution of cadmium and zinc in soil from zinc mines. An analysis of the soil contamination from cadmium and zinc was done in 3 areas: the upstream and downstream areas, located in the same watershed as the zinc mine, and the adjacent area outside the watershed boundary. The results showed that the total cadmium and zinc contamination in the downstream area was higher than that of the upstream area and the adjacent area.

b) Industrial activity

The processing and reclamation of metals by industry has led to the widespread contamination of soils in urban and peri-urban areas. It can even happen in more rural areas where cottage industries are processing metals.

c) Wastewater reuse

Wastewater is potentially a valuable source of both water and nutrients and there is a long history of the use of untreated wastewater for irrigation. Some of the remaining wastewater is inadvertently used because it is mixed with surface water in canals carrying irrigation water. The remaining wastewater can result in contamination traveling large distances largely through the transport of contaminated sediments. While the use of treated wastewater would be much better both for human health and for the environment, this seems unlikely to be adopted on a large scale in the near future.

Land application of raw sewage and industrial wastewater in combination with fertilizers is being used extensively to raise the vegetable around the sewer disposal sites in India. The amounts of cadmium presented in the industrial wastewater were quite higher as compared to the maximum permissible limit of cadmium prescribed by the FAO. The amount of cadmium present in soil irrigated with raw sewage water industrial wastewater was higher as compared to the maximum permissible limit established by the EU for toxic metals (Antil et al., 2001).

d) Fertilizers

Aside from the indirect effect of nitrogen fertilizers on soil acidification, phosphate fertilizers can increase the soil loads of various trace metals, most notably cadmium and uranium. The extent of trace metal contamination very much depends on the geological source of the phosphate rocks used for making the fertilizers

In New Zealand, cadmium levels had increased in the topsoil of New Zealand soil. The cadmium level increase is associated with the application of phosphate fertilizer. Over 80% of the cadmium added along with phosphate fertilizer remained in the topsoil (Taylor, 1997).Fertilizers commonly used in Argentina were analyzed to determine concentrations of chromium, cadmium, copper, zinc, nickel and lead. Rock phosphate contained the highest levels of cadmium and zinc. The levels of cadmium and lead were significantly relative to those naturally present in soils. Continuous fertilization of soils could increase the heavy metal contents exceeding natural abundances in soils; and therefore, thetransfer of these metals to the human food chain must not be overlooked (Carmelo, 1997).

2.1.4 Health effects

Cadmium is a highly cumulative poison with a biologic half-life estimated at about 20-30 years in humans. About half of the body burden of cadmium is found in the liver and kidney. The total body burden reaches a plateau in humans at around age 50. Cigarette smoke is a source of cadmium, and the body burden of cadmium is about 1.5 to 2 times greater in smokers than in nonsmoker of the same age.

Health effects from acute and chronic exposure to cadmium in both humans and animals are summarized as follows.

Acute — Metal fume fever may result from acute exposure with flu-like symptoms of weakness, fever, headache, chills, sweating and muscular pain. Acute pulmonary edema usually develops within 24 hours and reaches its maximum in three days. If death from asphyxia does not occur, symptoms may resolve within a week.

Chronic — The most serious consequence of chronic cadmium poisoning is cancer (lung and prostate). The first observed chronic effect is generally kidney damage, manifested by the excretion of excessive (low molecular weight) protein in the urine. Cadmium also is believed to cause pulmonary emphysema and bone disease (osteomalcia and osteoporosis). Cadmium may also cause anemia, teeth discoloration (Cd forms CdS) and the loss of smell (anosmia). (OSHA, 1993)

The most spectacular and publicized occurrence of cadmium poisoning resulted from dietary intake of cadmium by people in the Jintsu River Valley near Fuchu, Japan The victims were afflicted by *itai, itai* disease. The symptoms were the result of painful osteomalacia (bone disease) combined with kidney malfunction. Cadmium poisoning in the Jintsu River Valley was attributed to irrigated rice contaminated from an upstream mine producing lead, zinc, and cadmium. (Stanley, 1999)

In the biological systems, the interrelationships of zinc and cadmium are very important. While they may often be considered together in terms of their ratios, it should be borne in mind that zinc is an essential element while cadmium is not. In animals, cadmium binds to the protein metallothionen. The binding of cadmium to this protein is less than the bond with zinc. The role this protein plays is not well understood, but it probably is important in the transport of cadmium.(Louis, 2003)

The form of cadmium that is of most interest with regard to its health effects from inhalation exposure is cadmium oxide because it is the main form of airborne cadmium. For oral exposures, cadmium chloride is most often tested in animal studies because of its high water solubility and the resulting high concentrations of cadmium delivered to target sites.

The routes for cadmium intake are listed as follow:

- Breathing contaminated workplace air (battery manufacturing, metal soldering or welding).
- Eating foods containing it; low levels in all foods (highest in shellfish, liver, and kidney meats).
- Breathing cadmium in cigarette smoke (doubles the average daily intake).
- Drinking contaminated water.
- Breathing contaminated air near the burning of fossil fuels or municipal waste

The major route for cadmium intake is ingestion (for non-smokers). This is largely due to the presence of trace levels of cadmium in foodstuffs of natural origin or of the use of phosphate fertilizers and sludge on agricultural soils. (http://www.cadmium.org/introduction.html)

A report from epidemiology office, Department of Communicable Disease Control, Thailand found that victims from toxic chemicals totaled 2,717 persons; they were categorized to industry, with 853 persons, and pesticide or herbicide, 1,864 persons. Moreover, there was 1 dead person from industry and 9 dead persons from pesticide or herbicide in 2004.

The amount of victims due to toxic chemicals from industry in 2003 compared to 2004 increased more than five times. This may be due to increased cadmium contamination in the environment and agriculture products, especially in Hoy Nam Tao Catchments, Mae Sot District, Tak Province. (http://infofile.pcd.go.th/mgt/pollution2547_5Hzard.pdf)

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Table 2.2: Victims and dead persons from toxic chemical in 1995-2004.

Toxic	Year	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
From agriculture	sick	3,398	3,175	2,844	4,305	4,171	3,109	2,653	2,571	2,342	1,864
	dead	21	32	29	18	33	21	15	11	9	9
From industry	sick	162	201	211	287	365	1,177	280	180	157	853
	dead	2	-	1	1	1	4	-	-	-	1
Total	sick	3,560	3,376	3,055	4,592	4,536	4,286	2,933	2,751	2,499	2,717
	dead	23	32	30	19	34	25	15	11	9	10

Source : Epidemiology office Department of Communicable Disease Control Ministry of Public Health.

In 2004, toxic chemicals which affected human health were categorized as petroleum with 131 persons, gas and volatile with118 persons, heavy metals (manganese, mercury, arsenic and cadmium) with 556 persons and lead with 48 persons. There was 1 dead person from petroleum. (http://infofile.pcd.go.th/mgt/pollution2547_5Hzard.pdf)

THAILAND—2004. In response to concerns about the cadmium contamination problem in the vicinity of Mae Tao Creek in the Mae Sot District of Tak Province and to subsequent news reports in the media, Padaeng Industry Public Co. Ltd. (PDI) reportedly commissioned the National Research Center for Environmental and Hazardous Waste Management at Chulaongkorn University to conduct a research study to find sustainable and systematic ways to solve the problem. The company expected findings of the study to be completed by early 2005, and the result of the study would be presented to relevant Government agencies as their recommendation for resolving the problems. In addition, the company set aside a budget of $74,400 (3 million baht) per year to assist the health, standard of living, and livelihood of the local citizens in the area (Padaeng Industry Public Co. Ltd., 2005).

2.1.5 Bioavailability of cadmium

It should be mentioned that not all Cd in soil is available for plant uptake. The degree of availability for uptake is called bioavailability, and is affected by numerous factors which may be manipulated to improve Cd phytoextraction.

2.1.5.1 Cation exchange capacity (CEC)

The most important factor regulating Cd bioavailability is the cation exchange capacity (CEC) of the soil. Clay particles, called micelles, are negatively charged and reversibly bind (adsorb) positively charged particles (cations) to their surface. Cations such as Cd may be exchanged for H^+ on the micelle surface. Furthermore, cation exchange capacity varies among soil types, regulated by the relative amount of clay present

2.1.5.2 pH

pH refers to the concentration of hydrogen ions (H^+). pH dramatically effects the CEC of soil by limiting the available exchange sites at a low pH. H^+ binds to soil particles tighter than other cations, thus, any metal bound to a soil particle will get booted off in the presence of excess H^+ (Garcia-Miragaya and Page, 1978). At low pH values (<6), H^+ is in excess and replaces all other cations on the micelle, thus making them bioavailable. At high pH values (>7), cations are less bioavailable because they have less competition from H^+ for available binding sites. Many cations bind to free hydroxyl groups (OH^-) and form insoluble hydrous metal oxides which are unavailable for uptake. Decreasing the pH of soil will increase Cd bioavailability, and will usually increase the plant uptake of Cd, unless the Cd elicits a toxic response in the plant. Optimum Cd mobility is achieved at pH = 4.5 - 5.5 (Bingham, 1980).

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Other factors include soil amendments, competitive cations, fertilizer, mycorrhizae, chelation, phytochelations, role of sulfyr, oxidative stress, translocation, metallothioneins, organic acids and EDTA/EGTA. (Bingham, 1980).

2.1.6 Regulations

The U.S. Environmental Protection Agency (EPA) classifies cadmium as a probable human carcinogen (Group B1) and has established a reference dose (RfD) of 0.0005 mg/kg/day in water and 0.001 mg/kg/day in food. The reference concentration (RfC) is undergoing review by an EPA Workgroup. Cadmium is generally subjected to specific regulatory limits. The EPA has set the maximum contaminant level goal (MCLG) for cadmium in drinking water at 0.005 mg/L; the maximum contaminant level (MCL) at 0.005 mg/L (ATSDR, 1999).

In Thailand, according to the Enhancement and Conservation of National Environment Quality Act B.E. 2535 (1992), the cadmium level in surface water Class-3 water quality standard should not exceed 0.005 mg/L. No more than 0.01 mg/L and 0.03 mg/L cadmium should be found in drinking water and industrial effluent, respectively.

The Australia New Zealand Food Authority (ANZFA) and European Commission have set the cadmium level in vegetables at 0.1 mg Cd/ kg wet weight (ANZFA, 2006). The European Commission has set the maximum permissible limit in leafly vegetables at 0.2 mg Cd /kg wet weight and 0.1 mg Cd /kg wet weight in roots.(Antil et.al, 2001).

The pollution control department Ministry of Natural Resources and Environment have set the Class-3 quality standard of cadmium concentration at 0.005 mg/L, the drinking water standard of cadmium concentration at 0.01 mg/L, the industrial effluent standard of cadmium at 0.03 mg/L and the deep well standard of cadmium concentration at 0.1 mg/L. There are the cadmium concentration that used in topic Cadmium uptake under long-term cultivation.

Class 3 is Medium clean fresh surface water resources used for: consumption, but passing through an ordinary treatment process before using and agriculture

2.2 Fate of cadmium in crop

Cadmium has no beneficial effects in plants, but is considered as a contaminant of food and feed. Cadmium can be retained in the body for many years, leading to a risk of chronic toxicity with excessive intake (Jackson and Alloway, 1992)

Not only is it non-essential for life, it is highly toxic to most organisms, having a toxicity 2-20 times higher than many other heavy metals (Vassilev, 1998). It is therefore considered a very serious pollutant. Cd content in soil has been dramatically increased from anthropogenic sources including smelters and agricultural applications of fertilizer and sewage sludge. Since Cd in soil is available for plant uptake and subsequent human uptake, Cd in the environment poses a significant health risk.(Cox, 2000)

Cadmium can also enter the soil or water from spills or leaks at hazardous waste sites if large amounts of dissolved cadmium are present at the site. The form of cadmium at these sites is important since many forms do not easily dissolve in water. Fertilizers often contain some cadmium that will enter the soil when fertilizers are applied to crops.

Minimizing the Cd content of crops is desirable, as dietary ingestion is a major form of cadmium entry into the body. However, efforts to minimize Cd concentration in foodstuffs must also take into consideration the food supply requirements for an ever-expanding world population. Therefore, it is necessary to develop management practices which can produce an acceptable level of Cd in food, both in the short and long-term, while maintaining stable and affordable crop production. (Mclaughlin, 1994)

2.3 Water spinach cultivation

This perennial aquatic vine is confined to the tropics and subtropics zones because it is susceptible to frosts and does not grow well when temperatures are below 23.9 ^{o}C. Water spinach can reproduce sexually by producing one to four seeds in fruiting capsules or vegetative by stem fragmentation. It is a member of the "morning-glory" family. The common names include water spinach, chinese water spinach or morning glory or water bindweed. The scientific name is *Ipomoea aquatic*, *Ipomoea repens* Roth, *I. reptans* Poiret, *Convolvulus repens* Vahl. The plant's origin is central to south China.

A botanical description is listed as follows:

Flowers: Funnel shaped, solitary or in few flowered clusters at leaf axils, two inches wide, pink to white in color, and darker in the throat (rarely nearly white).

Leaves: Alternate, simple, with smooth petioles 3-14 cm long; leaf blades generally arrowhead shaped but variable, smooth (rarely hairy), to 17 cm long, with tips pointed; blades held above water when stems floating.

Stems: Vine like, trailing, with milky sap and roots at the nodes; usually up to 2.7 m. long but can be much longer.

Fruit: An oval or spherical capsule, woody at maturity, 1 cm long, holding 1 to 4 grayish seeds.

Pictures of each of the parts are shown in Figure 2.3

(http://www.iisgcp.org/EXOTICSP/waterspinach.htm#descrip)

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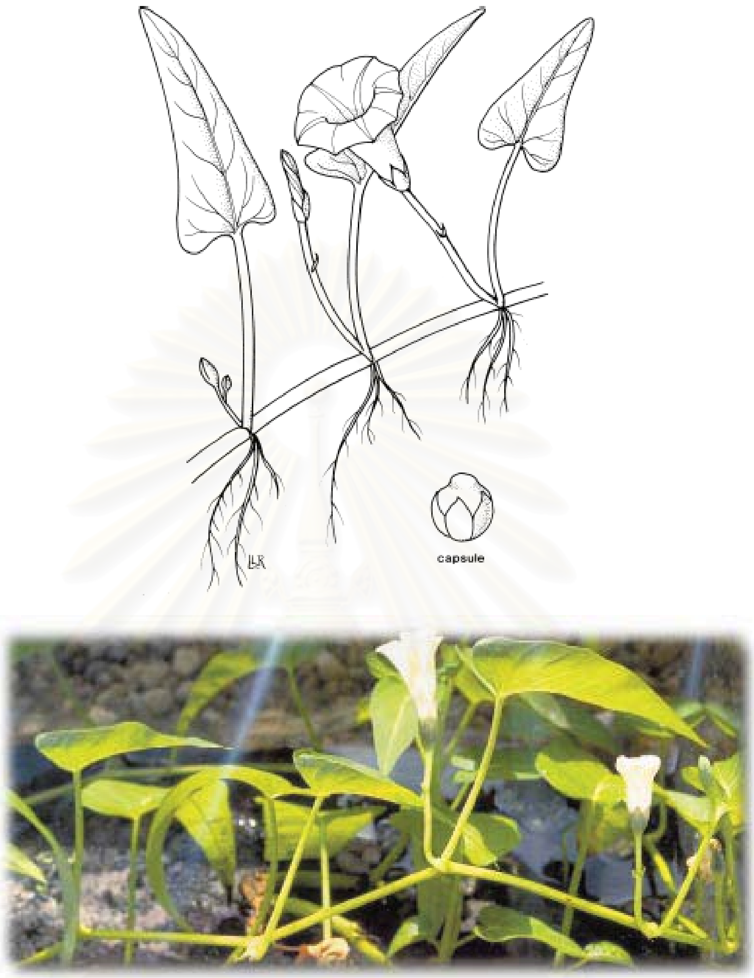

Figure 2.3: Picture of water spinach

(Adapted from http://www.iisgcp.org/EXOTICSP/waterspinach.htm#descrip)

Water spinach (*Ipomoea aquatica*) is an aquatic morning glory native to China. It is a popular cultivated green vegetable in China, India, Malaysia, Africa, Brazil, the West Indies, and Central America. It is sold in Asian markets in southern California under the name of "on-choy" or "ong-chow" and is also served in some Asian restaurants. Water spinach is a creeping, herbaceous vine with alternate leaves arising from buoyant, hollow stems that root at the nodes. A single plant can branch profusely with floating stems that form dense mats on the water surface.

Although it is a tasty vegetable, it has become a prolific naturalized weed in Africa, Australia, the Pacific Islands and South America. Due to its aggressive growth rate, water spinach has the potential to invade moist cultivated areas, such as rice and sugar cane fields, and wetlands such as the Everglades, natural lakes and rivers, drainage canals and ditches.

The water spinach in Thailand can be classified into three types: Water Convolvulus, Thai water Convolvulus, and Chinese water Convolvulus (Jaipakdee, 2001).

Water convolvulus: Water convolvulus is an annual aquatic plant that grows on the water surface or the ground. The stem is small and tapering, greenish red in colour and segmented. The young shoots are crisp while the mature shoots are rather tough. The leaves are single leaves, heart-shaped and can be eaten with chili paste (nam prik) or papaya salad (som tam). The plant is productive all year round and grows in all regions of the country. (Approximate size of the leaf is 1.5 cm. wide and 3 to 4 cm. long).

Thai water convolvulus: Thai water convolvulus is an annual aquatic plant that spreads its stems on the water surface. The stem is succulent, crisp, green in colour and segmented. The leaves are heart-shaped and commonly used in Thai curry “kaeng tepo”, sour spicy soups, fried with pork, used in noodle soup and eaten as a fresh vegetable with chili paste (nam prik). The plant is most productive during the rainy season. It thrives in general water sources. (Approximate size of the leaf is 5 cm. wide and 7 cm. long as shown in Figure 2.4).

Chinese water convolvulus: Chinese water convolvulus is an annual plant with straight, segmented stems that does not creep along the ground. The leaves are long and tapering. They are usually fried and used in making “suki yaki”. The plant is productive all year round and grows well in all types of soil. Chinese water convolvulus is rich in vitamin A. (Approximate size of the leaf is 3.5 cm. wide and 8-10 cm. long).

Figure 2.4: Picture of Thai water convolvulus.

(Adapted from http:// waynesword.palomar.edu/ ww0804.htm)

2.4 Literature review

2.4.1 Accumulation of heavy metal in plants

Karnchanawong et al. (2002) studied the heavy metal contamination levels in Chinese kale, cabbage, turnip and rice in laboratory-scale plots and full-scale plots that were irrigated by effluent from domestic wastewater treatment plants. Zinc and copper were found at higher levels than cadmium and lead in all crops while rice absorbed the highest amounts of heavy metals. However, the contamination levels were far below permissible standards and not much different from plots grown by natural water. It can be concluded that crops grown by the effluent of domestic wastewater treatment plants are safe for consumption.

Jiang et al.(2004) investigated the effect of Cd^{2+}concentrations on the root, bulk and root growth of garlic and the uptake and accumulation of Cd^{2+} by garlic roots, bulbs and shoots. The range of cadmium chloride concentration was 10^{-6}-10^{-2} M. The results indicated that Cd^{2+} could significantly inhibit the root growth of garlic only above 10^{-3} M, and it had a stimulatory effect on root length at lower concentrations of 10^{-6}-10^{-5} M. The cadmium content in the roots of garlic increased as the solution concentration of Cd^{2+} increased. However, the plants transported only a small amount of cadmium to their bulbs and shoots and concentrations in these tissues were low.

Jain et al. (1987) studied the potential utilization of water spinach since it grew without much effort, as a result of which it was favored by farmers. The plant had high protein content and could be used as animal feed. This aquatic weed could also scavenge organic and inorganic components from aqueous media, rendering it useful in wastewater treatment systems. In test results, it had shown a high absorption capacity for nitrates and heavy metals.

Hu et al.(1996) studied cadmium accumulation in five species of seaweeds with particular attention given to Gradaria tenuistipitata, which grew well in coastal regions with fresh water influx. These species accumulated Cd in the range of 223-496 μg/g dry weight within 6 days of exposure. The lethal concentration, of Cd in a culture medium of G. tenuistipitata was determined to be 0.3 g/l. Cadmium uptake by G. tenuistipitata followed biphasic kinetics. A rapid phase of accumulation occurred within 2 days followed by a slow phase during prolonged treatment. Greater than 50% of the accumulated Cd was bound firmly to the algae 10 days after the algae were placed into Cd-free seawater, following previous exposure to Cd. Cadmium accumulation was proportional to the external Cd concentration under sublethal Cd concentrations. G. tenuistipitata accumulated much less Cd in darkness than in the presence of light, implying that the Cd uptake process is at least partially energy dependent.

Suppradid (2005) investigated cadmium transport in kale cultivation when irrigated with cadmium contaminated water at different concentrations. It was found that the cadmium added was mainly adsorbed in the soil while very small proportions were absorbed in the kale and leached out into infiltrated water. The infiltrated water had relatively low cadmium concentrations as compared to irrigated water. The cadmium contamination in kale was found in a descending order: roots, leaves, stems. Based on the infiltrated water's quality and safety of kale's consumption, cadmium in irrigated water should not exceed 0.023 mg/l.

2.4.2 Toxicity of cadmium

En et al. (1998) investigated the toxicity on water spinach caused by nickel in a water culture. Water spinach grown in aerated nutrient solution in a greenhouse was treated with various levels of nickel, copper, zinc, chromium, cadmium, manganese, arsenic, aluminum, and lead. The injury symptoms caused by these metals were compared. Among these metals, only nickel produced specific symptoms of long-vein necrosis on the leaves and stems. Leaf yellowing or chlorosis also appeared on the plants treated with nickel, zinc, chromium, cadmium, and manganese, but not on those treated with copper, arsenic, aluminum, and lead. The copper caused only stunting and small leaf symptoms, while the arsenic and aluminum caused only wilting symptoms. Some of these nine elements produced browning or necrotic symptoms on roots, but the symptoms were not specific. All of tested metals except Cd resulted in visible symptoms on leaves or roots for the tested concentrations. The toxicity threshold concentrations for Ni, Cu, Zn, Cr, Cd, Mn, As, and Al were 2.5, 5, 5, 1.25, 1, 20, 2, and 50 ppm, respectively. These results indicate that water spinach has the potential for use as an indicator plant for Ni in the environment.

An (2004) investigated the differential toxicity and bioavailability of cadmium in sweet corn (*Zea mays*), wheat (*Triticum aestivum*), cucumber (*Cucumis sativus*) and sorghum (*Sorghum bicolor*) in laboratory soil microcosms, in order to recommend some sensitive plant species to assess the ecotoxicity of soil contaminated by cadmium. The results illustrated that the presence of cadmium decreased seeding growth, EC_{50} (the molar concentration of an agonist, which produces 50% of the maximum possible response for that agonist) values for shoot or root growth, was calculated by the Trimmed Spearman–Karber method. Root growth was a more sensitive endpoint than shoot growth because of the greater accumulation of cadmium in the root. Moreover, the bioavailability and transport of Cadmium within plants were related to its concentration and the plant species.

Zaman et al. (1998) evaluated the responses and tolerance of radish plants to various levels of Cd and Pb in soil, and assessed whether the radish could be used as a biomonitor of Cd and Pb contamination in the environment. These experiments also indicated that Cd and Pb affected the development of radish plants to different degrees. Plant growth was more inhibited in the Cd contaminated soils than in the Pb contaminated soils probably for the following reasons: (1) Pb is more bound to the soil particles, and therefore, less available to plant tissue, or (2) Cd is more mobile than Pb within the plant tissues, and therefore, is capable of exerting more toxic effects. Since the radish demonstrated different levels of sensitivity in response to various concentrations of Cd or Pb in soils, it is suggested that these plants may be used as biomonitors/bioindicators for Cd and Pb assessments in metal polluted soils. Moreover, since this variety of radish had the potential to survive in Cd and Pb contaminated soils, it was possible that these plants might be able to absorb such metals from soil and thus might be used in phytoremediation.

Gothberg et al. (2004) estimated the importance of ambient nutrient concentrations for the accumulation of mercury, cadmium and lead in water spinach. The plants were exposed to nutrient solutions of different strengths and with varying metal concentrations. Metal-induced toxic effects, which might possibly affected the yield of the plants, were also studied. The lower the nutrient strength in the medium, the higher the metal concentrations that accumulated in the different plant parts and the lower the metal concentration in the medium at which metal-induced toxic effects occurred. Accordingly, internal metal concentrations in the plants were correlated to toxic effects. Plants exposed to metals retained a major proportion of the metals in the roots, which had a higher tolerance than shoots for high internal metal concentrations.

Greger et al. (1994) studied the influence of salinity on Cd uptake in submerged macrophytes using *Potamogeton pectinatus* as a model species and examined the partitioning of Cd between sediment and water. They found cadmium uptake in *P. pectinatus* from water decreased with increasing salinity, most likely due to Cd forming complexes with chloride and sulphate. In the presence of sediment, Cd uptake increased with increasing salinity, because Na^{+} and Mg^{2+} replaced Cd^{2+} in the colloids of the sediment, thereby increasing the Cd concentration of the water and subsequent uptake by plant shoots. Osmotic potential did not influence the release of Cd from the sediment or Cd uptake by the plant. Cadmium uptake by roots and the plant-available fraction of Cd in the sediment were not affected by salinity. Cadmium bound firmly to the sediment decreased with increased salinity.

2.4.3 Transport of heavy metal

Rai et al. (2001) investigated toxic metal accumulation in edible parts of some aquatic crops growing in various contaminated waters. The sites were located near highways having high anthropogenic activities in the catchments area. The edible parts of aquatic plants were the fruits of *T. natans* and the leaves of water spinach. The accumulation of metals in different fruit parts, varied in order: Fe> Pb>Cr>Mn>Cu. In spite of the high metal accumulation values in raw fruits, the toxic metal contents were reduced after boiling. For water spinach, the metal accumulated values were found to be highest for Fe followed by Pb and Cr with traces of Cd. Metals accumulation were found in leaves; however the metal accumulating potential varied considerably depending upon the level of metal contamination in the water bodies in which all plants were grown.

Peralta-Videaa et al. (2002) evaluated the combined effects of cadmium, copper, nickel, and zinc applied to montmorillonite containing soils at three pHs, on seed germination and plant growth**.** Alfalfa plants were grown in soil-pots contaminated with a mixture of Cd^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}, at 50 mg/kg each, at pHs of 4.5, 5.8, and 7.1. Under the effects of the heavy metal mixture, nickel was the most accumulated element in the shoot tissue, with 437, 333, and 308 ppm at pH 7.1, 5.8, and 4.5, respectively.

Cadmium was found to be second in accumulated concentrations with 202 ppm, 124 ppm, and 132 ppm at pH 7.1, 5.8, and 4.5, respectively, while zinc was third, followed by copper.

Sawidis et al. (1995) monitored heavy metals and other pollutants of water and submerged soils by using aquatic plants as indicator of the amounts of pollution. They found that the lake was less contaminated than the rivers, and the contamination of which was dependent on the metal species. Sediment had greater Cu, Pb, and Ni contamination than the aquatic plants, whereas the reverse was the case for Zn, Cd and Mn. The mean heavy metal contents of the sediments and aquatic plants were in the descending order of Mn>Zn>Ni>Cu>Pb; whereas in the accumulation of metals by plant tissues, the corresponding order was root>rhizomes>leaf>flower>stem>seed.

Anita et al. (1999) investigated information about the interaction of heavy metals with inorganic and organic molecules of plants. They grew cucumber plants in contaminated – free or contaminated (10^{-5} M Cd, Ni, Pb or V) nutrient solutions The analysis of the xylem saps and the digested root samples were carried out by an EXTRA IIA total reflection X-ray fluorescence spectrometer. The effects of Cd, Pb, Ni, and V on the accumulation and transport processes of Ca, K, Fe, Mn and Zn in cucumber plants were established. It could be stated that water uptake is strongly hampered by cadmium. Comparing the investigated element contents in the root and in the xylem fluids it was suggested that the main part Cd and Pb accumulated in was the root and only a very small amount of these two elements were transported to the shoots. On the other hand, the mobility of the elements investigated was different and increased in order of V<<Pb<Cd<Ni .The influence of heavy metals on the accumulation and transport of the essential elements depended on the variable parameters (pollutants and essential elements). From the data obtained was concluded that the general effects of heavy metals investigated changed in the following sequence: Cd>Pb~Ni>>V.

CHAPTER III

METHODOLOGY

3.1 Cadmium contaminated water preparation

There were three major studies, i.e. toxicity tests on water spinach growth, cadmium uptake under long-term cultivation and cadmium uptake under shock load conditions.

Cadmium chloride ($CdCl_2.2.5H_2O$, Univar, reagent grade, 99.0%) was used to prepare 100 and 1000 mg Cd /L stock solutions. The contaminated water was prepared with treated domestic effluent (before chlorination) from the Chiang Mai University wastewater treatment plant and stock solution to the required concentrations as follows:

Toxicity test on water spinach growth: Cadmium in water was expected to be at concentrations of 0, 10, 50, 100, 250, and 500 mg/L, respectively. However, due to discrepancies in preparation, the average results were 0, 7.6, 59.0, 118.9, 242.6 and 553.7 mg/L, respectively.

Cadmium uptake under long-term cultivation: Cadmium concentrations in water was expected to be at concentrations of 0, 0.005, 0.01, 0.03, 0.05, 0.1, 0.2, and 0.5 mg/L, respectively however average values were 0.00047, 0.0047, 0.017, 0.035, 0.053, 0.135, 0.201 and 0.446 mg/L, respectively. The concentrations that used in this study were followed by the cadmium concentration standard which set by the pollution control department, Ministry of Natural Resources and Environment as mentioned in topic 2.1.6.

Additional investigations at a cadmium concentration of 0.141 mg/L were conducted during the later part of the study.

Cadmium uptake under shock load conditions: Cadmium in water at 0.14 mg/L was initially prepared to grow water spinach for a short period 1, 3, 5, 7 and 9 days, out of 20 days of cultivation.

3.2 Laboratory-scale plot

The laboratory-scale plots were made from 45-L plastic bowls (diameter 60 cm x height 24 cm) and placed outdoors on the deck at the Department of Environmental Engineering, Chiang Mai University. There were covered with a transparent plastic sheet to prevent rain, as shown in Figure 3.1

A total of 24 plots were used in toxicity tests and the cadmium uptake under shock load condition while 32 plots were used in the cadmium uptake under long-term cultivation. The plan of the plots is shown in Figure 3.2


a)


b)


c)

Figure 3.1: Pictures of the laboratory-scale plots

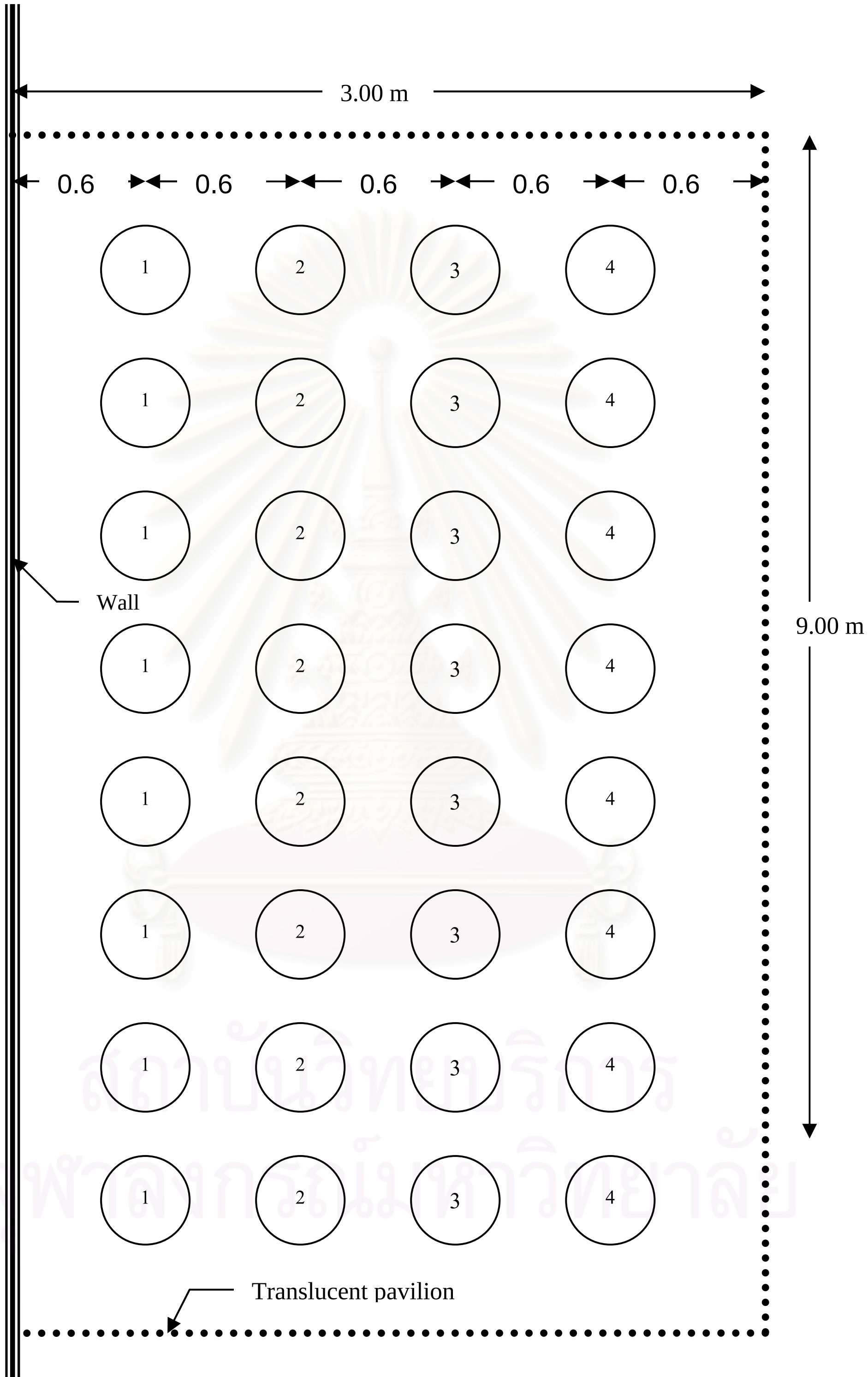

Figure 3.2: Plan of laboratory-scale plot

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3.3 Water spinach cultivation

The water spinach in Thailand can be classified into three types: Water Convolvulus, Thai water Convolvulus, and Chinese water Convolvulus.

For this experiment Thai water convolvulus was chosen as the testing plant because it could spread its stems on the water surface and the size of leaf is relatively big.

Water spinaches (*Ipomoea aquatica*) were collected from the Natural River in Chiang Mai province.

The water spinach was grown on the cadmium contaminated water without fertilizer and pesticide applications in the following rotation.

Toxicity test on water spinach growth: Water spinach was harvested after growing for 30 days.

Cadmium uptake under long-term cultivation: Water spinach was harvested after growing for 20-30 days with 5 total cultivations (130 consecutive days).

Cadmium uptake under shock load condition: Water spinach was harvested after growing for 20 days.

Starting stems were used in each study. They were initially stocked in two concrete tanks (diameter 1.0 m. × 0.4 m. height). The healthy stems with roots were selected and leaves were cut off. The starting stem length was 10-12 cm as shown in Figure 3.3

Figure 3.3: Starting stem

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3.4 Plant sample collection and analysis

In the toxicity test and cadmium uptake under shock load condition, all parts of the plants were removed. They were separated into leaves, stems and leaves. For cadmium uptake under long- term cultivation, all leaves and stems above water were removed during each harvest. Plant samples (leaves and stem) were collected from part 1 in the first harvest and the remaining harvests were done according to part number. The leaves and stems were removed from plots according to their location. The plots were divided into 6 parts, and separated by plastic strips as shown in Figure 3.4. These methods ensured that roots leaves and stems in each part would be exposed to cadmium for the required cultivation periods.

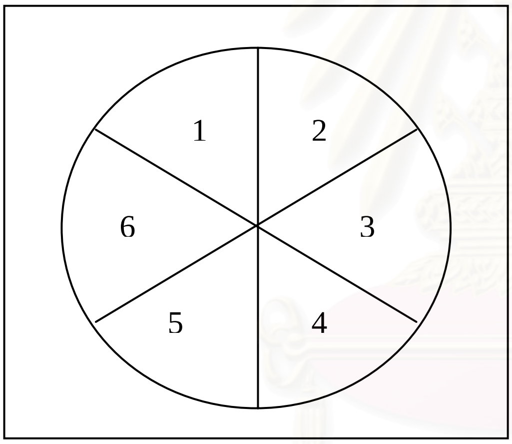

Figure 3.4: Plant sampling points

The water spinach samples were separated into roots, stems, and leaves. They were dried in an oven at 75˚C for about 72 hours and ground using a grinding mill. All water spinach samples were kept in the desiccators prior to acid digestion (Appendix A) and analysis for cadmium content by flame atomic absorption or graphite furnace atomic absorption (Appendix B).

3.5 Water sample collection and analysis

Water samples were collected at the beginning of the cultivation and periodically during the growing period, from 1 cultivation plot out of 4 replications. The samples were filtered through a 0.45 µm membrane filter, acidified with nitric acid to a pH of 2 prior to storage in a refrigerator at approximately 4 ^{o}C (APHA, AWWA and WEF, 1998). They were analyzed for cadmium content by flame atomic absorption or graphite furnace atomic absorption (Appendix B).

3.6 Experimental procedures

3.6.1 Toxicity test on water spinach growth

- Healthy and equal-sized water spinach plants were selected and measured for their cadmium content before the experiment.
- The tested bunches including roots were cut around 12 cm in length, weighed, and grown in 24 cultivation units with cadmium contaminated water at 0, 7.6, 59.0, 118.9, 242.6, and 553.7 mg/L, respectively. There were four replicates at each cadmium concentration.
- During the water spinach cultivation, water samples were taken and analyzed for cadmium concentrations at 7, 15, 21 and 30 days intervals during the plants' cultivation.
- After plants grew to maturing sizes, all plants were removed, measured in length, and the wet and dry weight of each of the parts (roots, stems including shoots, leaves) was determined.
- Cadmium contents in roots, stems and leaves were analyzed.
- Those parameters mentioned above were compared to ascertain the indicator of cadmium inhibition.

3.6.2 Cadmium uptake under long-term cultivation

- Healthy and equal-sized stem of water spinach including roots were measured for their cadmium content before the experiment.

- The tested bunches including their roots were cut in equal-size, weighed, and grown in 32 cultivation units with cadmium contaminated water at cadmium concentrations of 0.000, 0.005, 0.017, 0.035, 0.053, 0.135, 0.201, and 0.446 mg/L, respectively. An additional study at a cadmium concentration of 0.141 mg/L was added during the later part of study.

- Each study was conducted in four replicates. During water spinach cultivation, water samples were taken to be sampled and analyzed for their cadmium concentration at the 3 –day interval during cultivation. When the cadmium concentration and water volume decreased, additional water and cadmium were added to maintain a required concentration.

- After plants grew to marketable size, 20-30 days, they were harvested. All edible parts (leaves and stems including shoots) above water were cut. Some roots and stems, including shoots, in the designated sampling part (as described in 3.5) were taken.

- The edible parts were measured for their wet weight and dry weight. The cadmium content in each part of the water spinach (roots, stems including shoots and leaves) were analyzed.

- Additional cadmium contaminated water was added to the cultivation units for the consecutive experiments.

- There were 6 total cultivations, which took place over a period of 130 days to investigate long-term cadmium uptake and accumulation.

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3.6.3 Cadmium uptake under shock load condition.

- Healthy water spinach including roots were measured for their cadmium content before the experiment.

- The tested bunches, including roots, were cut in equal-size, weighed, and grown in 24 units, which were divided into 6 groups. Group 1 was grown at the baseline condition (0 mg Cd/L). Groups 2, 3, 4, 5, and 6 were grown at the baseline condition and then exposed to shock load concentrations for 1, 3, 5, 7, and 9 days respectively. Each study was conducted in four replicates.

- The plants exposed to shock load conditions were removed and put in new plots with cadmium concentrations of 0.148 mg/L for a certain period.

- Tap water was used to rinse the water spinach bunches before they were put back to the baseline condition.

- After 20 days, all plants (stems including shoot, leaves and roots) were removed and measured for wet and dry weigh.

- The cadmium content in each part of the water spinach (roots, stems including shoots and leaves) was analyzed.

3.7 Statistical analysis

Differences in the weight increase of the reference plants grown at different cadmium concentrations were calculated using analysis of variance (one-way ANOVA) in Appendix H.

3.8 Wastewater treatment

In all experiments, the contaminated water discharged from the experimental units will be treated with lime for cadmium precipitation as cadmium hydroxide in sludge form and toxic sludge was separately collected for solidification by using Portland cement in Appendix G.

CHAPTER IV

RESULTS AND DISCUSSIONS

All experiments used treated water (before chlorination) from the Chiang Mai University waste water treatment plant. The characteristics of the grab samples (taken on June, 1 2005) and analyzed by the Department of Environmental Engineering, Chiang Mai University were as follows: BOD 5.75 mg/L, total nitrogen 4.4 mg/L, total phosphorus 1.42 mg/L, pH 7.23, and cadmium content 0.00 mg/L. The treated water contained sufficient nutrients for plant growth and fertilizers were not added throughout the study. Cadmium chloride was added to prepare the cadmium contaminated water to the required concentration in each experiment. It was found that algae had grown in cultivation plots. Since algae under sun light cause photosynthesis resulting in CO_2 uptake, O_2 production and OH^- releasing. The pH and dissolved oxygen diurnal variations are common in the facultative pond (http://www.leeds.ac.uk/civil/ceri/water/tphe/publicat/pdm/med/treatwsp.pdf).

pH also affects the bioavailability of cadmium on plants since cadmium will shift to insoluble cadmium oxide as presented in equation 1 with solubility constant (Ksp) equal to 2×10^{-14}. As with soil, soil solution pH will influence diffusion rates greatly because of the strong effect pH has on Cd solubility in soils (Lindsay, 1979)

$$Cd^{++} + 2OH^{-} \rightleftharpoons Cd(OH)_2 \qquad (1)$$

The diurnal variation of pH was monitored in the control cultivation plot (0 mg/L) and pH variations occurred as shown in Figure 4.1

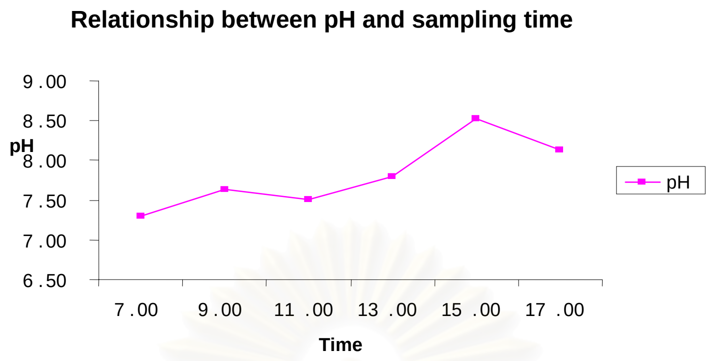

Figure 4.1: Diurnal variations of pH in control plot on June 12, 2005

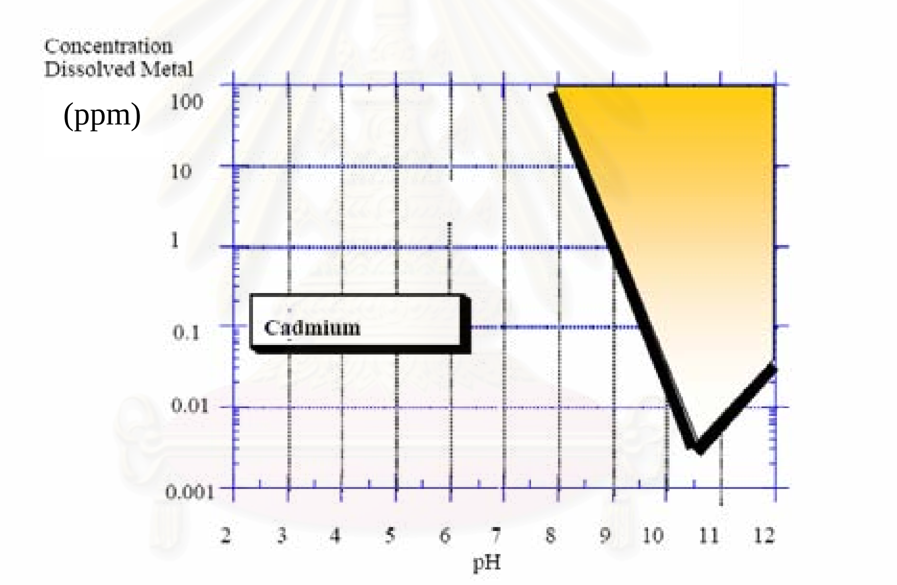

Figure 4.2: Theoretical solubility of cadmium vs. pH

It was found that pH increased to alkaline range (pH>8) in the afternoon and this phenomenon obvious reduced the soluble cadmium ion. To minimize the effect of variations in pH, water samplings were done in the morning time only. pH dramatically effects the cadmium uptake of plant by limiting the available exchange sites at low pH. H+ bind to root's surface tighter than other cations, thus, any metal bound to root 's surface will get booted off in the presence of excess H+ (Garcia-Miragaya and Page, 1978). At low pH (<6), H+ is in excess and replaces all other cations on the root's surface, thus making them bioavailable. At high pH (>7), cations are less bioavailable because they have less competition from H+ for available binding sites same as the theoretical solubility of cadmium which shown in Figure 4.2.

Decreasing the pH of water will increase Cd bioavailability, and will usually increase plant uptake of Cd unless the Cd elicits a toxic response in the plant.

4.1 Toxicity test on water spinach growth

4.1.1 Characteristics of the water

Cadmium concentration in the water

The cadmium concentration in the water that was used in this experiment is presented in Table 4.1

Table 4.1: Actual cadmium concentration of the water in the toxicity test on water spinach growth

Sampling time (days)	Target concentration in water (mg/L)					
	0.00 mg/L	**10.00 mg/L**	**50.00 mg/L**	**100.00 mg/L**	**250.00 mg/L**	**500.00 mg/L**
0	0.0	14.7	62.9	111.1	282.5	552.0
7	0.0	9.1	74.3	141.6	223.3	529.5
15	0.0	8.2	38.9	95.2	225.8	560.3
21	0.0	2.2	76.2	151.1	225.3	668.2
30	0.0	3.6	42.9	95.6	256.4	458.6
Average	0.0	7.6	59.0	118.9	242.6	553.7
SD.	0.0	4.9	17.4	26.1	26.2	75.5

The cadmium concentrations in the water were expected to be 0, 10, 50, 100, 250 and 500 mg/L, respectively. However, a discrepancy in the preparation of the cadmium resulted in some variations and actual concentrations were different from the targeted concentrations as presented in Table 4.1 and Figure 4.2 shows the actual cadmium concentrations of the water which were still close to target concentrations (R^2= 0.9951).

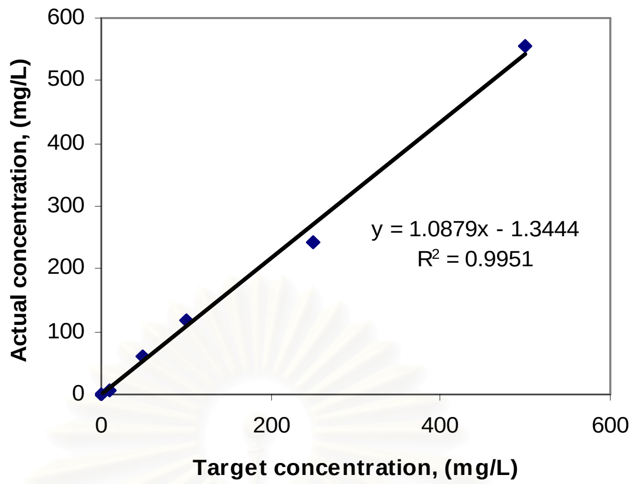

Figure 4.3: Relationship between target and actual cadmium concentration.

The pH of the water was in the alkaline range with pH values starting from 7.5-8.3, as shown in Appendix C Table C.1. The tolerance limit of pH for water spinach cultivation ranged from 6.5 to 9.0 (Rattan et al., 2005 and Patel et al., 2004) and the water had pH values within the permissible limit.

According to the rather high pH, some parts of the cadmium were in their oxidized form and not bioavailable to the water spinach. It should be reminded that cadmium in the water was measured in its filterable (0.45µm) form.

4.1.2 Water spinach

4.1.2.1 Toxicity effect

The water spinach cultivation period was in June 2005. It can be classified as rainy season. Meteorology reports from this month were as follows: the average temperature was 29.1± 0.8 ^{o}C, the humidity was 72 %, and the rain intensity was 193.8± 74.1 mm. (http://www.tmd.go.th/program/ngr_n.php). It was found that water spinach which had been grown in cadmium contaminated water with concentrations higher than 7.6 mg/L died within 6 days as presented in Table 4.2, Figure 4.3 and Figure 4.4. The leaves of the water spinach grown in cadmium contaminated water with concentrations over than 7.6 mg/L did not develop, and the terminal parts of stem rotted and died.

a) Cadmium concentration 0.0 mg/L b) Cadmium concentration 7.6 mg/L

Figure 4.4: Photographs of water spinach after 30 days of cultivation

Figure 4.5: Photographs of water spinach after 6 and 9 days of cultivation at the cadmium concentration of 118.9 mg/L

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Table 4.2: Observations on growth of water spinach at various cadmium concentrations.

Plot growth (Cd conc.) / Time (days)	Plot 1 (0 mg/L)	Plot 2 (7.6mg/L)	Plot 3 (59.1mg/L)	Plot 4 (118.9mg/L)	Plot 5 (242.6mg/L)	Plot 6 (553.mg/L)
0	Starting stem length 12 cm, some roots about 2 cm					
3	Root growing, no leaves	Roots slightly growing, no leaves	No leaves. The stems slightly declining	No leaves. The stems declining	No leaves. The stems declining	No leaves. The stems declining
6**	New buds, leaves size 2.5-4.0 cm	New buds, leaves size 1.2-2.5 cm	No leaves. The stems slightly declining	Some stems were rotten	Some stems were rotten	Some stems were rotten
9	It had 3-4 leaves/ stem and every knot has a bud	It had 2-3 leaves/ stem	New buds, No leaves	Some stems were rotten	Some stems died	Some stems died
12	Every knot had leaves	Some knots had leaves	Leaves size very small	20% died	60% died	70% died
15	Every knot had leaves	Some knots had leaves	Leaves size very small	100% died	100% died	100% died
18	Every knot had leaves	Some knots had leaves	Leaves size very small	100% died	100% died	100% died
24	Every knot had leaves	Some knots had leaves	60 % died	100% died	100% died	100% died
30	Every knot had leaves	Some knots had leaves	90 % died	100% died	100% died	100% died

Remark ** The time that water spinach started declining.

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The presence of cadmium decreased the seeding growth of cadmium in sweet corn (*Zea mays*), wheat (*Triticum aestivum*), cucumber (*Cucumis sativus*) and sorghum (*Sorghum bicolor*) (An, 2004). Chen et al. (2003) reported the germination rate and growth of carrot and radish plants were inhibited at the cadmium concentration of 20 mg/L, and the inhibition increased with the increasing concentrations of cadmium, both in the liquid culture and in the pot experiment.

The mechanisms that control the uptake of cadmium by plant root and its accumulation in edible plant foods are not well understood. Cadmium absorption across the plasma membrane of root cells is controlled by the electrochemical potential difference between the activity of Cd^{2+} in the cytosol and on root apoplassms. (Costa and Morel, 1993). The toxicity of cadmium in plants can be studied in the presence of proline. Proline has been shown to accumulate under conditions of water shortage, high salinity, chilling, heat and heavy metal exposure. It is also considered to have important protective roles against heavy metal stresses (Alia-Saradhi, 1991; Sharma et al., 1998). Thus free proline is considered to be one of the indicators of environmental stress. It is an important detoxifying mechanism that the free proline chelated Cd ions in plants and formed a nontoxic Cd–proline complex (Sharma et al., 1998).

The addition of Cd resulted in drastic reductions of the xylem fluid. The explanation for this great hindrance is that the cadmium disturbs the water uptake through the roots and influences the transpiration. This phenomena has already been observed in the case of other plants, too (Broyer et al, 1972).

From 30 days cultivation, it can be concluded that the growth threshold concentration of water spinach for Cd was 7.6 mg/L. This is in agreement with Sun and Wu (1998) who reported the toxicity threshold concentration of water spinach for Cd was at 1 ppm because in this study, the water spinach was grown in 7.6 mg/L cadmium contaminated water but it had leaf yellowing or chlorosis which was unusual naturally (Figure 4.5). That symptom also appeared on the water spinach treated with nickel, zinc, chromium, cadmium, and manganese by Sun and Wu (1998).

a) 0.0 mg/L b) 7.6 mg/L

Figure 4.6: Photographs of leaf yellowing.

4.1.2.2 Yield of water spinach

The yield of water spinach grown in the toxicity test is presented in Table 4.3 and total weight of water spinach is shown in Figure 4.6. The water spinach grown in control plots (0 mg/L) had weight increases of 21.3-29.4% In the plots with a contaminated water concentration of 7.6 mg/L, the weight increase were lower and varied from 6.5-16.1%. The yields of the edible part (stem and leaf) in the control were 332.4 - 470.1 kg/ha while those grown in 7.6 mg/L cadmium concentration were 109.6 - 293.6 kg/ha. The lengths of the stems in both, groups were not different. They were generally 12 cm long while expanding occurred at the bud of water spinach as shown in Figure 4.4. The yield of water spinach grown in the cadmium contaminated water concentrations of 0 mg/L and 7.6 mg/L were not statistically different (P>95%).

Table 4.3: Yields of water spinach grown in the toxicity test on water spinach growth cultivation.

Actual concentration in water (mg/L)	Replication 1				Replication 2				Replication 3				Replication 4			
	B (g)	A (g)	In %	Edible yield kg/(ha.crop)	B (g)	A (g)	In %	Edible yield kg/(ha.crop)	B (g)	A (g)	In %	Edible yield kg/(ha.crop)	B (g)	A (g)	In %	Edible yield kg/(ha.crop)
0.0 mg/L	50.4	61.3	21.6	385	46.0	59.1	28.4	463	45.1	58.4	29.4	470	44.2	53.6	21.3	332
7.6 mg/L	52.1	60.4	16.1	293	46.9	50.4	7.4	124	47.8	50.9	6.5	109	46.0	51.9	12.9	209
59.1 mg/L	45.7	Die	Die	ND	47.9	Die	Die	ND	42.7	Die	Die	ND	45.2	Die	Die	ND
118.9 mg/L	47.8	Die	Die	ND	43.2	Die	Die	ND	49.5	Die	Die	ND	45.3	Die	Die	ND
242.6 mg/L	42.7	Die	Die	ND	50.5	Die	Die	ND	48.2	Die	Die	ND	51.9	Die	Die	ND
553.7 mg/L	43.9	Die	Die	ND	42.9	Die	Die	ND	45.8	Die	Die	ND	39.8	Die	Die	ND

B = Before cultivation weight

A= After 30 day cultivation weight

In = Increase

ND = No data

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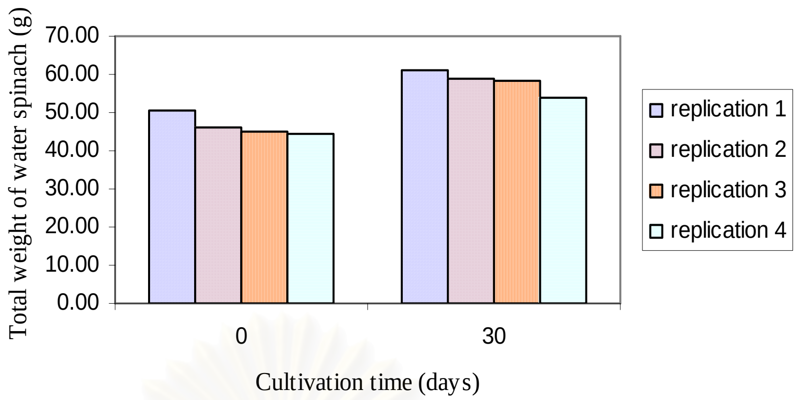
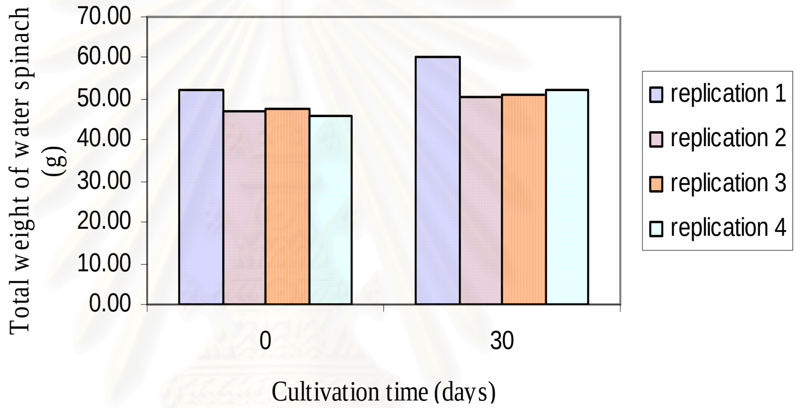


a) Cadmium concentration 0.0 mg/L



b) Cadmium concentration 7.6 mg/L

Figure 4.7: Total weight of water spinach grown in toxicity test

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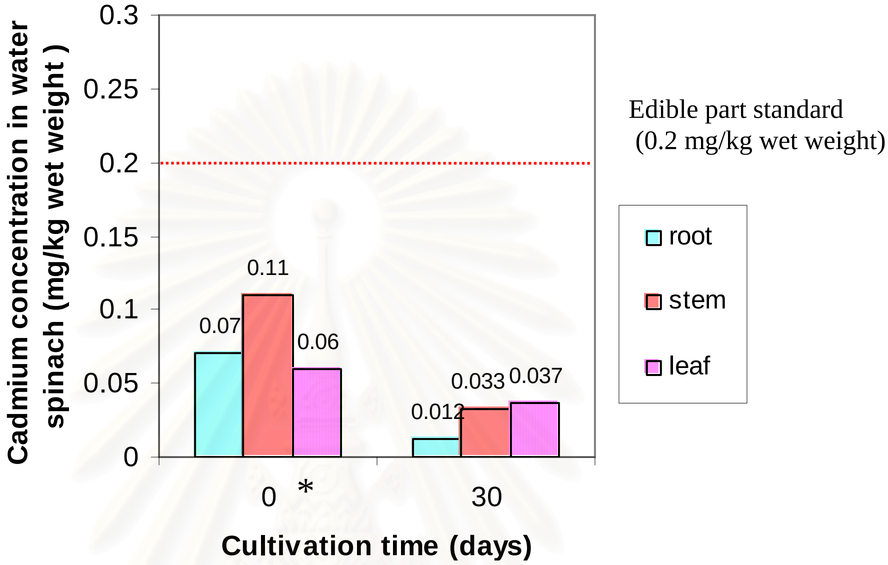
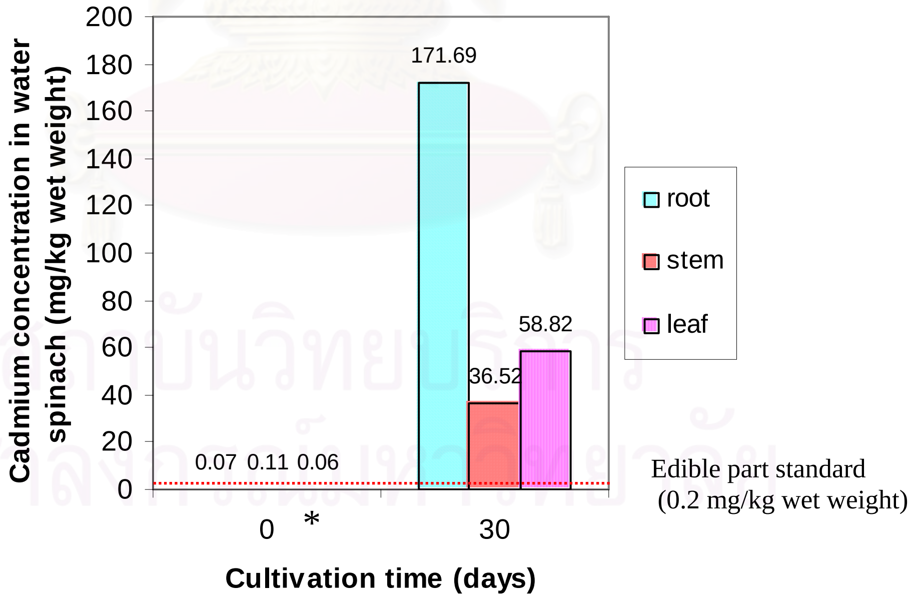
4.1.2.3 Cadmium accumulation in water spinach

The cadmium accumulation in parts of water spinach can be separated into three parts: roots, stems (including shoot) and leaves. The variations of cadmium contamination in each part of the water spinach are shown in Figure 4.7



a) Cadmium concentration 0.0 mg/L



b) Cadmium concentration 7.6 mg/L

Remark 0 * Initial concentration of cadmium in water spinach

Figure 4.8: Cadmium accumulation in the parts of the water spinach after 30 days of cultivation

Initial concentrations of cadmium content in root, stem and leaf were 0.07, 0.11, 0.06 mg/kg wet weight, respectively. In high cadmium concentrated water (7.6 mg/L) cadmium accumulation in root, stem and leaf were 171.69, 36.52 and 58.82 mg/kg wet weight, respectively. After 30 days of cultivation, the cadmium concentrations in water spinach were highest in the roots, leaves and finally the stems. Suppradid (2005) investigated cadmium transport in kale cultivation when irrigated with cadmium contaminated water at different concentrations and found cadmium contamination in kale in the similar descending order of roots, leaves and stems.

Landberg et al. (1996) reported that a larger proportion of the metals was retained in the roots and thereby prevented them from interfering with sensitive metabolic reactions in the shoots. This is probably an internal mechanism to avoid toxic metal concentrations in the shoot, which has also been observed elsewhere. The higher accumulation of metals in the roots than in the shoots is possible because of a greater tolerance to toxic metals in the roots than in the shoots. The roots of water spinach accumulated much higher concentrations of the metals before toxic effects occurred. The mechanism behind the higher tolerance of the roots may include the binding of the positively charged toxic metal ions to negative charges in the cell walls (Beauford et al., 1977; Wierzbicka, 1998). Part of the metal that has been taken up into the root may be transported further through the plasma membrane and bind to macromolecules, organic acids, or sulfur-rich polypeptides, like phytochelatins, and accumulate in the cytoplasm or the vacuole and thereby be detoxified (Mathys, 1977; Grill et al., 1985; Steffens, 1990; Harmens et al., 1994).

At the low cadmium concentrations in water (0.0 mg/L), cadmium accumulation in each part of the water spinach was 0.033 mg/kg wet weight in the stem, 0.012 mg/kg wet weight in the root and 0.037 mg/kg wet weight in the leaf. The cadmium concentrations were within the range of concentrations in aquatic plants from unpolluted sites and were insignificant different in each part of the water spinach.

The Cd accumulation in edible parts (stem and leave) of the water spinach at high cadmium concentrations was more than 1000 times higher than that of the control group (0.0 mg/L). It can be concluded that the water spinach grown in cadmium concentrations equal to or exceeding 7.6 mg/L are not safe for consumption according to the EU standard of0.2 mg/kg wet weight.

The cadmium accumulation in water spinach increased as the cadmium concentration in the water increased, which is in agreement with how the cadmium content in roots of garlic increased as the solution concentration of Cd^{+2} increased. (Jiang et al., 2004)

The cadmium contents in the plants were analyzed by flame atomic absorption at high cadmium concentrations and graphite furnace atomic absorption at low cadmium concentrations, according to the US EPA standard.

4.2. Cadmium uptake under long-term cultivation.

4.2.1 Characteristics of water.

The cadmium concentrations in the water used in these experiments are presented in Table 4.4

Table 4.4: Actual cadmium concentrations of the water in the cadmium uptake under long-term cultivation

	Actual cadmium concentration								
Target / cultivation	0 mg/L	0.005 mg/L	0.01 mg/L	0.03 mg/L	0.05 mg/L	0.1 mg/L	0.15 mg/L	0.2 mg/L	0.5 mg/L
1	0.00052	0.00300	0.01780	0.04060	0.05050	0.10400	0.13300	0.21800	0.42500
2	0.00081	0.00872	0.03520	0.03760	0.04670	0.19200	0.15400	0.22000	0.46700
3	0.00040	0.00535	0.01140	0.04340	0.05870	0.16200	0.13600	0.18200	ND
4	0.00061	0.00164	0.01140	0.01990	0.05440	0.12400	ND	0.18300	ND
5	0.00000	0.00457	0.01010	0.03280	0.05620	0.09460	ND	ND	ND
Average	0.00047	0.00466	0.01720	0.03490	0.05330	0.13500	0.14100	0.20100	0.44600
SD.	0.00030	0.00268	0.01051	0.00924	0.00475	0.04090	0.01136	0.02109	0.02969

Remark : ND = No data

The cadmium concentrations in the water were expected to be 0, 0.005, 0.01, 0.03, 0.05, 0.1, 0.2 and 0.5 mg/L, respectively. However, a discrepancy in the preparation of the cadmium resulted in some variations and actual concentrations were different from the targeted concentrations. Figure 4.8 showes the actual cadmium concentrations of water in each cultivation which were close to target concentrations.

Additional investigations at a cadmium concentration of 0.141 mg/L were conducted during the later part of the study because water spinach in plots with a cadmium concentration of 0.5 mg/L died within 40 days. There were empty plots available for additional study which began on the 40th day of long-term cultivation. The cultivation period in these plots started after the stems were planted.

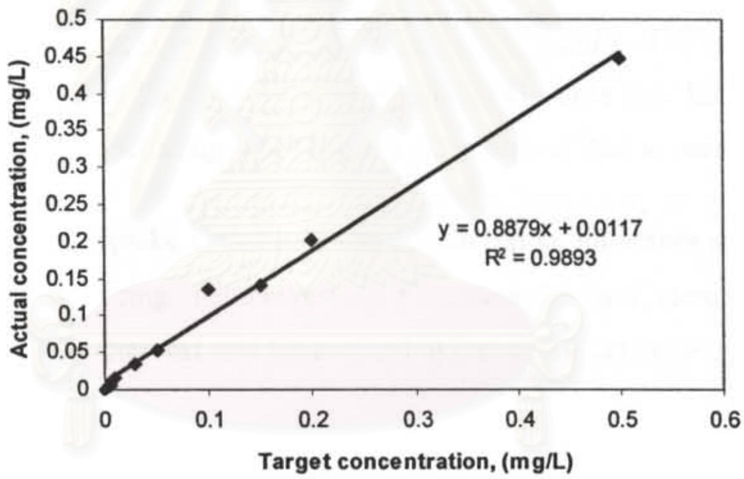


Figure 4.9: Relationship between target and actual cadmium concentrations in the water

pH of water

The pH of the water was in the alkaline range with pH values ranging from 6.06-10.55, as shown in Appendix D Table D.1 The tolerance limit of pH for water spinach cultivation ranges from 6.5 to 9.0 (Rattan et al., 2005 and Patel et al., 2004) and for the most part, the water in this study had pH values within the permissible limit.

According to the rather high pH, some parts of the cadmium was in its oxidized form and not bioavailable to the water spinach. It should be reminded that the cadmium in the water was measured in its filterable (0.45μm) form.

To control pH variations to remain within the alkaline range, 10% HNO_3 and 10 N Sodium bicarbonate were added every 3 day interval to control the pH value to be within 6.5-7.5.

4.2.2 Water spinach

4.2.2.1 Yield of water spinach

The water spinach cultivation period in this experiment, 4 replications and 5 cultivations, had been conducted over the period of 130 days during July 2005 to October 2005. This period could be classified as the rainy season in Thailand. Meteorology reports from this periods are as follows: the average temperature range was 26.9-28.6°C, the humidity ranged from 83-89 % and rain intensity ranged from 155.2-436.3 mm. (http://www.tmd.go.th/program/ngr_n.php). The yields of water spinach are presented in Figure 4.9 and in Appendix D Table D.8. Edible part weight covered leaves, stems including shoot in designated area of plot as mention in 3.4.

For cadmium uptake under long- term cultivation, all leaves and stems above water were removed during each harvesting. Plant samples (leaf, stem) were collected from part 1 in the first harvest and the remaining harvests were done according to part number. The leaves and stems were removed from plots according to their location. The plots were divided into 6 parts, separate by plastic strips. This methods ensured that roots, leaves and stems in each part be exposed to cadmium for the required cultivation periods.

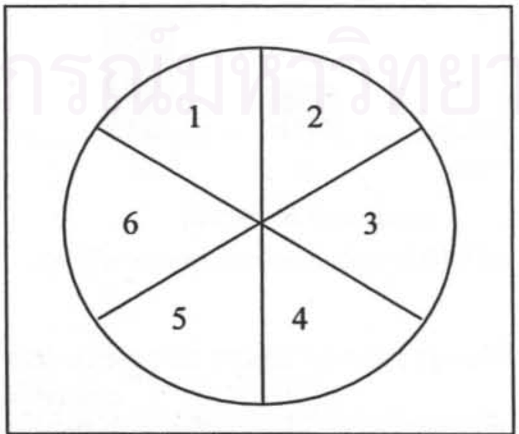


Figure 3.4: Plant sampling points

The cadmium concentrations in the water were expected to be 0, 0.005, 0.01, 0.03, 0.05, 0.1, 0.2 and 0.5 mg/L, respectively. However, a discrepancy in the preparation of the cadmium resulted in some variations and actual concentrations were different from the targeted concentrations. Figure 4.8 showes the actual cadmium concentrations of water in each cultivation which were close to target concentrations.

Additional investigations at a cadmium concentration of 0.141 mg/L were conducted during the later part of the study because water spinach in plots with a cadmium concentration of 0.5 mg/L died within 40 days. There were empty plots available for additional study which began on the 40th day of long-term cultivation. The cultivation period in these plots started after the stems were planted.

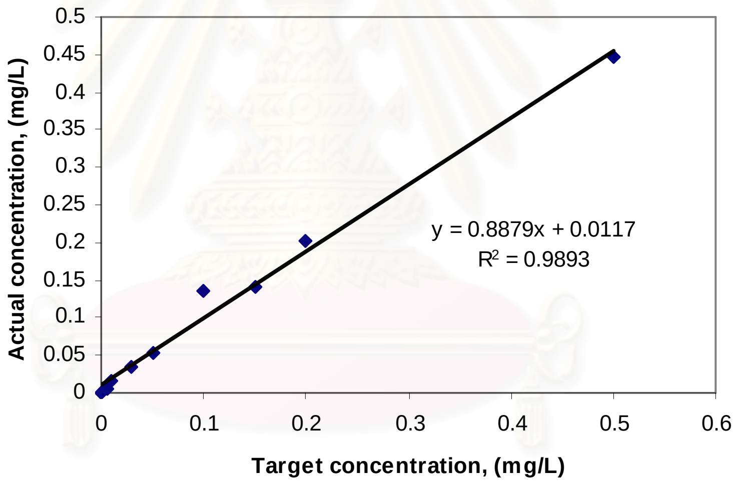


Figure 4.9: Relationship between target and actual cadmium concentrations in the water

pH of water

The pH of the water was in the alkaline range with pH values ranging from 6.06-10.55, as shown in Appendix D Table D.1 The tolerance limit of pH for water spinach cultivation ranges from 6.5 to 9.0 (Rattan et al., 2005 and Patel et al., 2004) and for the most part, the water in this study had pH values within the permissible limit.

According to the rather high pH, some parts of the cadmium was in its oxidized form and not bioavailable to the water spinach. It should be reminded that the cadmium in the water was measured in its filterable (0.45μm) form.

To control pH variations to remain within the alkaline range, 10% HNO_3 and 10 N Sodium bicarbonate were added every 3 day interval to control the pH value to be within 6.5-7.5.

4.2.2 Water spinach

4.2.2.1 Yield of water spinach

The water spinach cultivation period in this experiment, 4 replications and 5 cultivations, had been conducted over the period of 130 days during July 2005 to October 2005. This period could be classified as the rainy season in Thailand. Meteorology reports from this periods are as follows: the average temperature range was 26.9-28.6^{o}C, the humidity ranged from 83-89 % and rain intensity ranged from 155.2-436.3 mm. (http://www.tmd.go.th/program/ngr_n.php). The yields of water spinach are presented in Figure 4.9 and in Appendix D Table D.8. Edible part weight covered leaves, stems including shoot in designated area of plot as mention in 3.4.

For cadmium uptake under long- term cultivation, all leaves and stems above water were removed during each harvesting. Plant samples (leaf, stem) were collected from part 1 in the first harvest and the remaining harvests were done according to part number. The leaves and stems were removed from plots according to their location. The plots were divided into 6 parts, separate by plastic strips. This methods ensured that roots, leaves and stems in each part be exposed to cadmium for the required cultivation periods.

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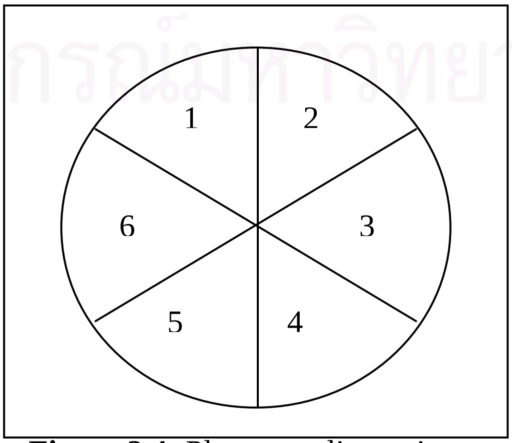
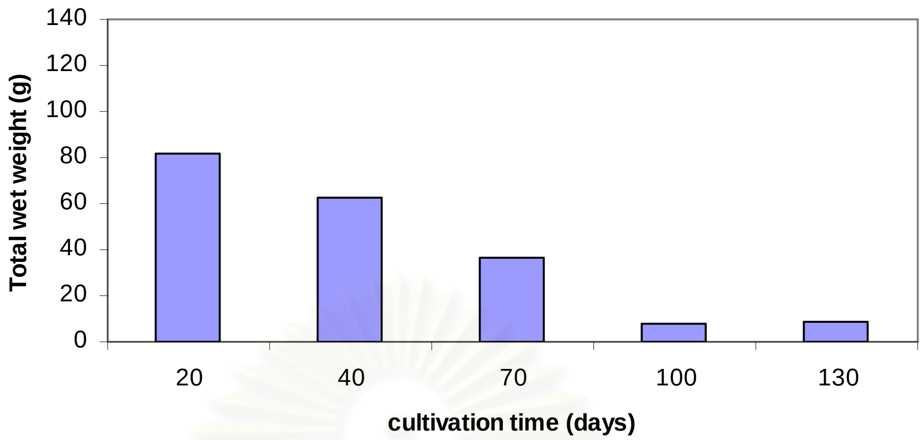
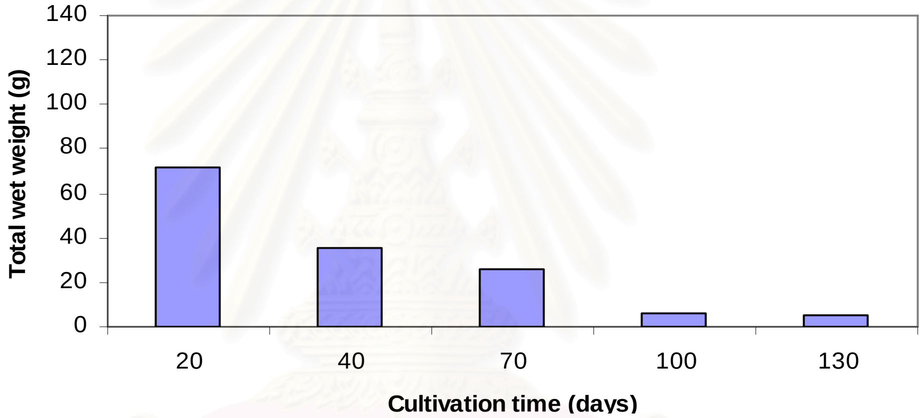


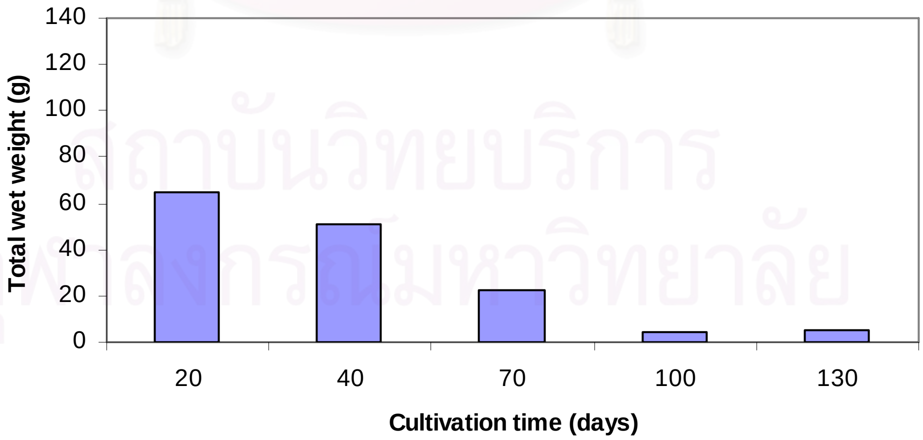
Figure 3.4: Plant sampling points



a) Cadmium concentration 0.00 mg/L

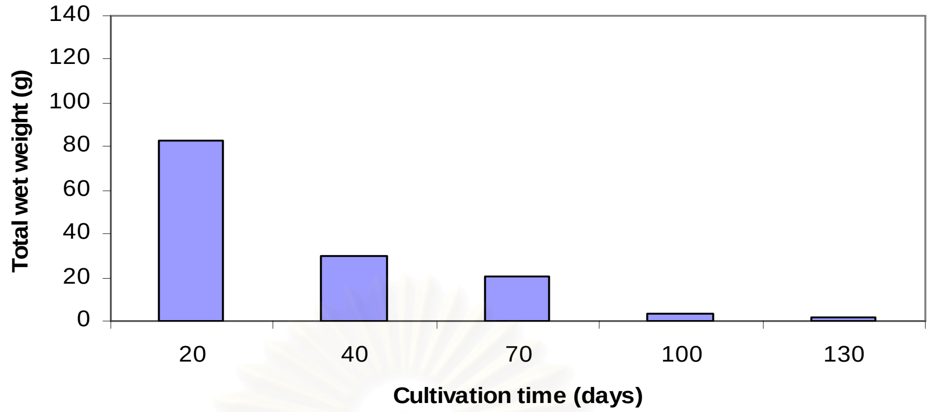


b) Cadmium concentration 0.005 mg/L

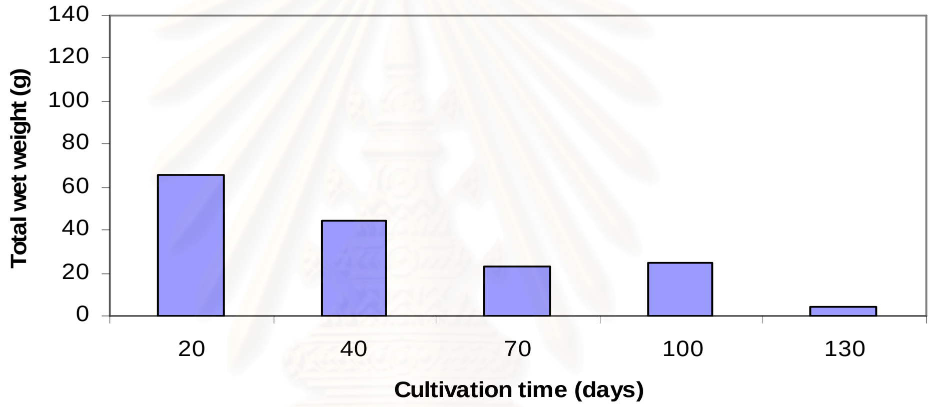


c) Cadmium concentration 0.017 mg/L

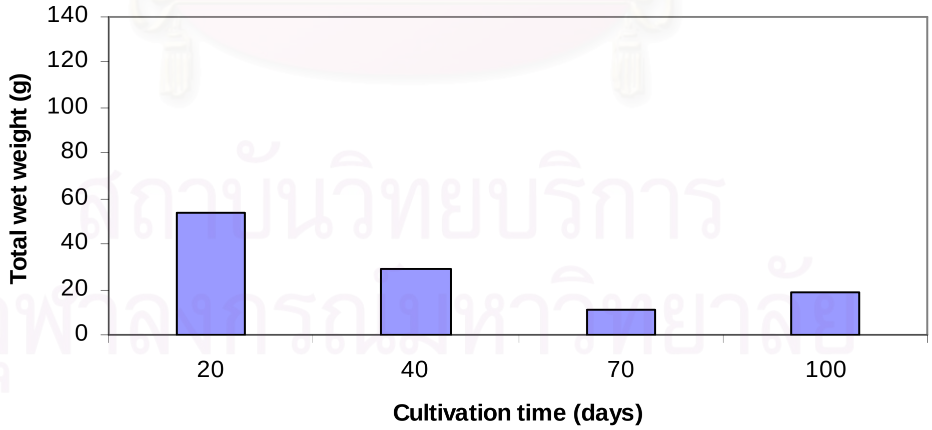
Figure 4.10: Total edible part weight of water spinach in long-term cultivation



d) Cadmium concentration 0.035 mg/L

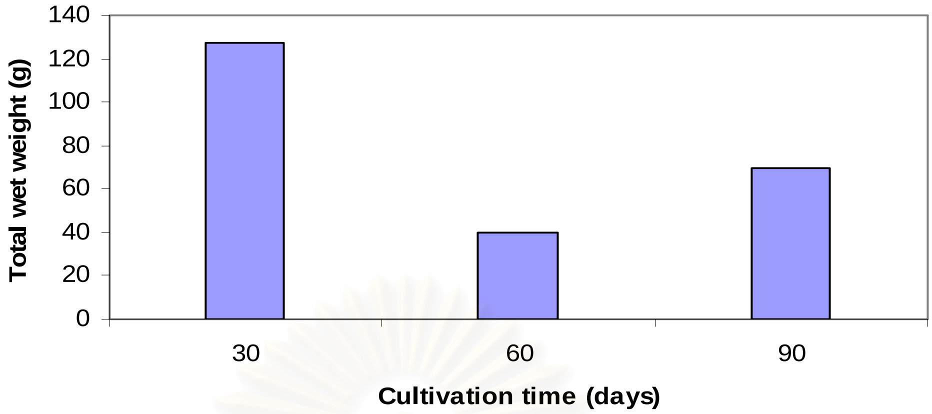


e) Cadmium concentration 0.053 mg/L

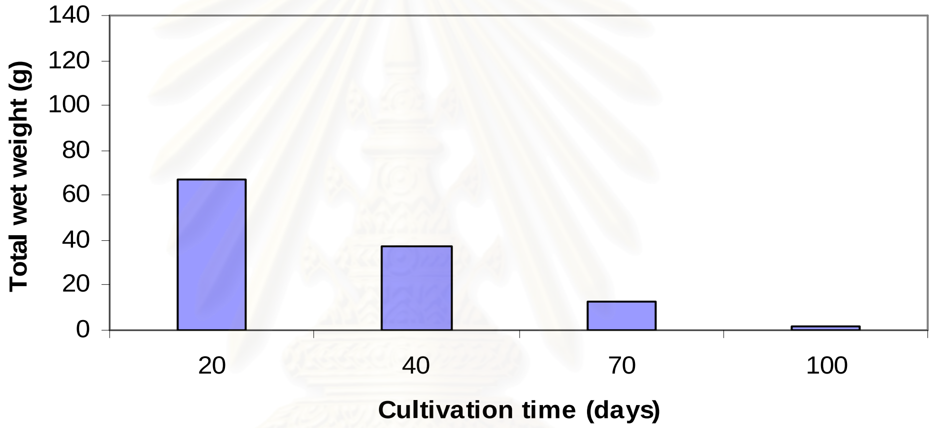


f) Cadmium concentration 0.135 mg/L

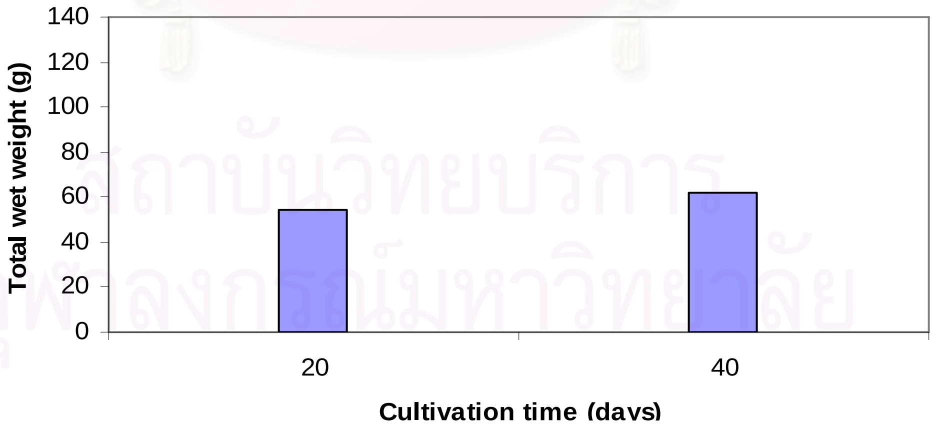
Figure 4.10: Total edible part weight of water spinach in long- term cultivation (continue)



g) Cadmium concentration 0.141 mg/L *



h) Cadmium concentration 0.201 mg/L



i) Cadmium concentration 0.446 mg/L

Remark * Additional investigations at cadmium concentration of 0.141 mg/L were conducted during the later part of studies at the harvesting time 30, 60 and 90 days

Figure 4.10: Total edible part weight of water spinach in long term cultivation (continue)

After starting long-term cultivation, water spinach in some plots showed obvious toxicity effects and finally died in the following manner: on the 40th day cultivation in 0.446 mg/L cadmium concentration, on the 100 th day cultivation in the cadmium concentrations of 0.135 and 0.201 mg/L (Figure 4.9 –g,h,i)

The water spinach used in these experiments was Thai water convolvulus. It is an annual aquatic plant that grows on the water surface or the ground with a life cycle of the species normally not exceeding 1 season (160 days) based on unpolluted sites. This experiment was conducted under stress conditions (cadmium contaminated) so the life cycle of water spinach could be shorter (Pancho, J. et al., 1978) and the toxicity of cadmium could have occurred in the other plants same as Jiang et al.(2004) investigated the effect of Cd^{+2}concentrations on the root, bulk and root growth of garlic and the uptake and accumulation of Cd^{+2} by garlic roots, bulbs and shoots. The range of the cadmium chloride concentration was 10^{-6}-10^{-2} M. The results their study indicated that Cd^{+2} could significantly inhibit the root growth of garlic only above 10^{-3} M, and it had a stimulatory effect on root length at lower concentrations of 10^{-6}-10^{-5} M. According to Figure 4.9-a, the harvesting weights of control plots (0 mg Cd/L) in this study gradually decreased and finally died off in replications 2, 3 and 4. It is obvious that the growing season (rainy) and long cultivation period have effects on water spinach harvesting weights.

A comparison between the growth of water spinach in 0.053 mg Cd/L in 130 days cultivation and 7.6 mg Cd/L in 30 days of toxicity test (section 4.1.2.2) showed that long-term cultivation had lower growth. It was expected due to the different seasons during the different cultivation times.

The toxicity of cadmium in water may increase the rate in which plants died-off as the cultivation time increased. Figure 4.10 (a-f) shows water spinach growth in the cadmium concentration of 0.053 mg/L at various cultivation times. It was found that water spinach had rapid growth in the initial cultivation periods (30-60 days) before slowly declining, as shown in Figure 4.10 (d-f). The similar low patterns were observed in plots with low Cd concentrations, i.e. 0.005-0.053 mg/L.

a) The 0 th day

b) The 20 th day

c) The 40 th day

d) The 70 th day

e) The 100 th day

f) The 130 th day

Figure 4.11: Photograph of water spinach growth at the cadmium concentration of 0.053

There were little variations of growth among the replications for each cadmium concentration during the initial period (0-40 days). After long-term cultivation, variations occurred between the replications, they are shown in Figure 4.9a) - 4.9i). This was probably due to damaging from pest in some plots as shown in Figure 4.11 and Figure 4.12. The pests in these experiments were cotton aphid and cut worm (Appendix F). The Cotton aphid damaged replications 1, 3 and 4 of cadmium concentration 0.053 mg/L at 40^{th} day. When this occurred, leaves began to curl and pucker; seedling plants become stunted and died as shown in Figure 4.11. The cut worm, as shown in Figure 4.12, ate whole leaves and even flowers of the water spinach. They damaged the replication 4 of cadmium concentration 0.201 mg/L at the 20^{th} day.

Figure 4.12: Photograph of water spinach that was infected with cotton aphid.

Figure 4.13: Photograph of water spinach that damaged by the cut worm

The symptoms caused by cadmium toxicity in this experiment were leaf yellowing or chlorosis that unusually found in the natural and control groups. Figure 4.13 a) shows the symptoms which also appeared on the water spinach treated with nickel, zinc, chromium, cadmium, and manganese (En, 1998) and occurred in the toxicity test of water spinach growth. The other symptom were the dwarf and stem crisp symptom as shown in Figure 4.13 b). There was little information on this symptom, which requires further study.

a) Leaf yellowing b) Dwarf and stem crisp symptom

Figure 4.14: Photographs of cadmium toxicity symptoms in water spinach

The yields of the edible parts (stems and leaves) are presented in Table 4.5. They gradually decreased with cultivation time.

Table 4.5: Yields of the edible parts in water spinach grown in long-term cultivation.

Cd concentration (mg/L)	**Yield of edible part (kg/ha)**				
	Cultivation 1	**Cultivation 2**	**Cultivation 3**	**Cultivation 4**	**Cultivation 5**
0.00	2893	2220	1292	266	321
0.005	2528	1260	919	214	185
0.017	2295	1806	806	145	173
0.035	2931	1070	710	131	53
0.053	2319	1583	824	890	139
0.135	1907	1026	398	657	ND
0.141*	4498	1399	2455	ND	ND
0.201	2381	1331	457	55	ND
0.446	1914	2199	ND	ND	ND

Remark ND = No data, plant dies

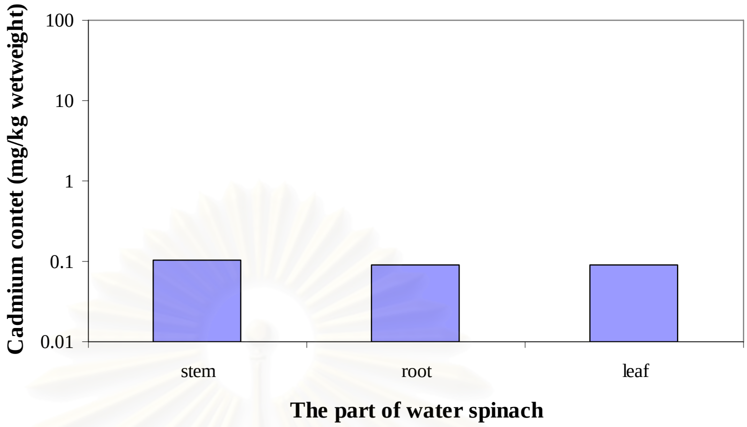
* Cultivation days were 30^{th}, 60^{th} and 90^{th} respectively

It was found that the yields of water spinach in each cultivation with different concentration of cadmium were not statistically different (P>95%).

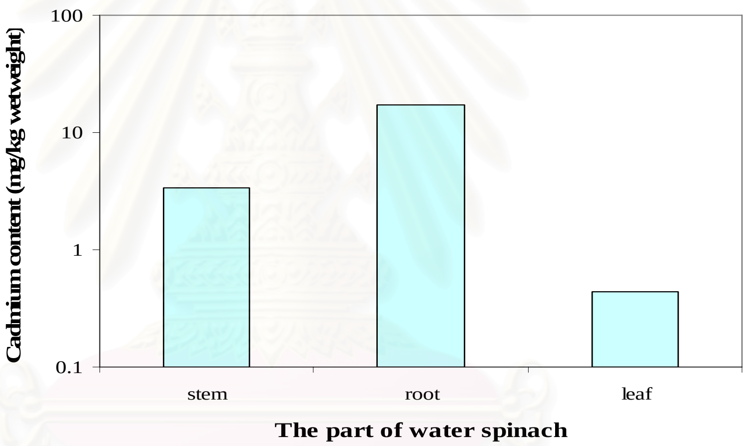
4.2.2.2 Cadmium accumulation in water spinach

The cadmium distribution in the parts of the water spinach can be divided into three parts which are its roots, stems including shoots, and leaves. The variations of cadmium contamination in each part of water spinach is shown in Figure 4.14

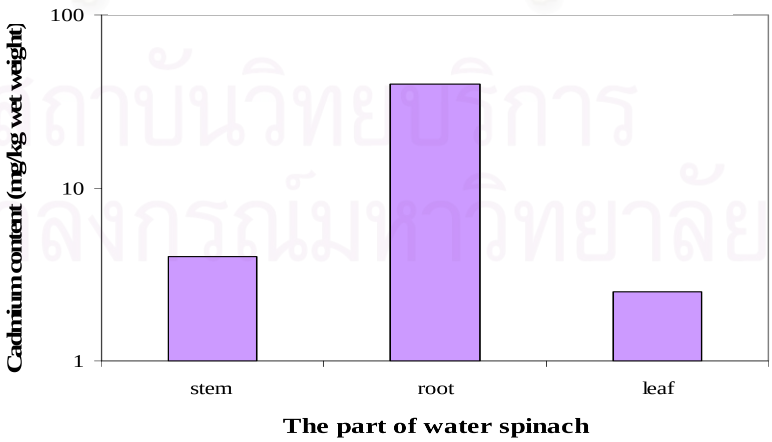
The variations among replications at each cadmium concentration depended on the amount of cadmium in the water and plant's uptake. Although cultivation conditions in the replications were similar, there were some variations of cadmium in the roots while leaves and stems had lesser degrees of variation. The mechanisms of cadmium uptake in water spinach are through both absorption and adsorption so many factors could cause variations such as the pH of the water, light, the growing season, etc.



a) Cadmium concentration 0.00 mg/L



b) Cadmium concentration 0.0035 mg/L



c) Cadmium concentration 0.446 mg/L

Figure 4.15 : Variations of cadmium contamination in each part of the water spinach in the 1st cultivation.

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The cadmium contents in plants were analyzed by flame atomic absorption for high cadmium concentrations and graphite furnace atomic absorption for low cadmium concentrations according to the US EPA standard. In this experiment, cadmium concentrations over 0.2 mg Cd/L were analyzed by flame atomic absorption.

The cadmium accumulation in each part of water spinach under long–term cultivation could be described as the control group (0.00 mg /L), low cadmium concentration group and high cadmium concentration group.

Although the control group was without cadmium addition, it had an actual cadmium concentration equal to 0.00047 mg /L. The cadmium might have come from laboratories or other domestic sources in Chiang Mai University which entered wastewater treatment plant. The cadmium would practically adsorb in an activated sludge system before being releasing in effluent. It could be considered that the cadmium contamination at 0.00047 mg /L was relatively low and therefore, it was used as the baseline concentration. The contamination was less than Thailand industrial effluent standard of 0.03 mg/L and class-3 water quality standard of 0.005 mg/L. The cadmium accumulation in water spinach of the control group are shown in Figure 4.15 and cadmium accumulation as dry weight basis are in Appendix D.

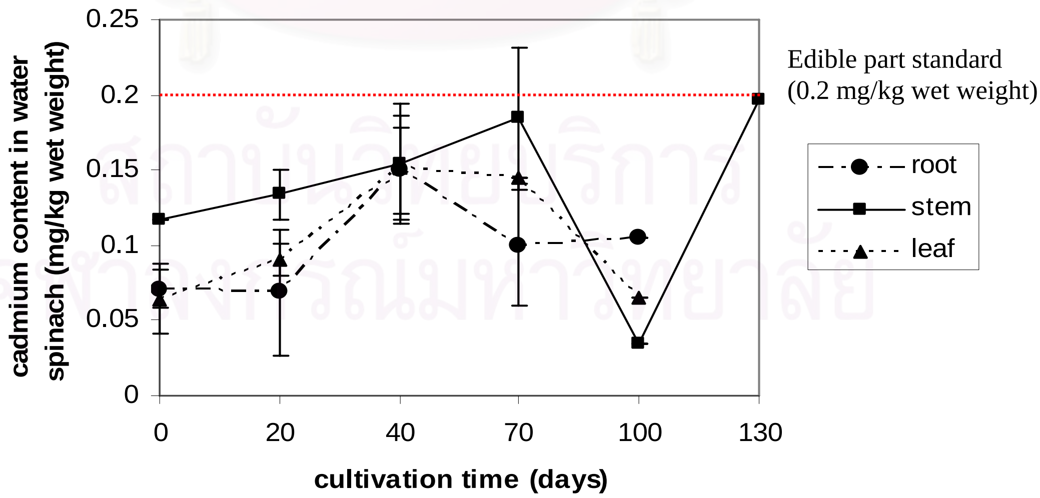
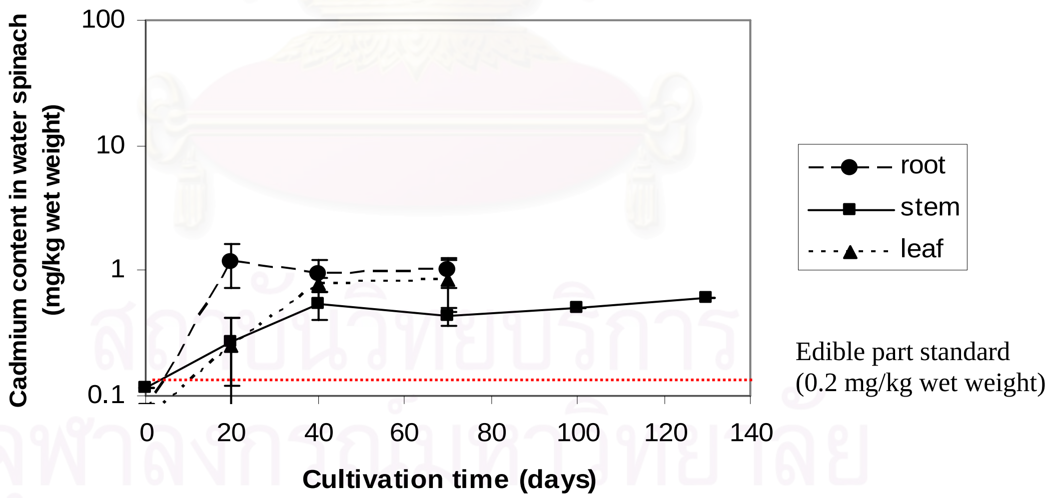


Figure 4.16: Cadmium content in root stem and leaf of water spinach at cadmium concentration 0.00047 mg/L

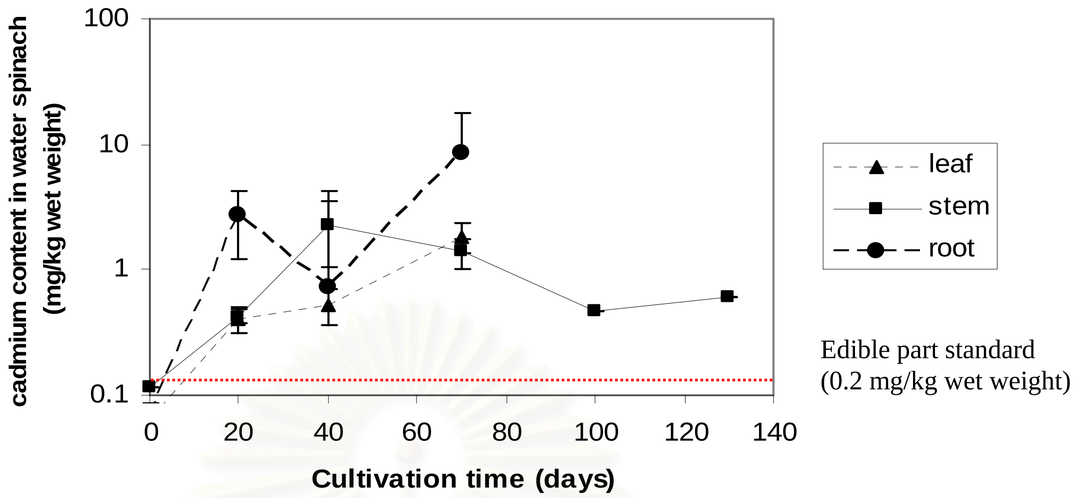
The cadmium accumulations in the roots, stems and leaves were not significatly different and no distinct pattern was found over long-term cultivation. It is hypothesized to be due to the low concentration of cadmium. Therefore, the plant uptake did not show any differences in the various parts under long-term cultivation. Although cadmium in water was very lower, high cadmium still absorb in plant affectively especially in stems. This indicates the high bioavailability of cadmium in water spinach. The amounts of cadmium that accumulated in all parts of water spinach grown in this cultivation did not exceed the cadmium level on crop safety (leaf and stem) of the European Commission (EU) standard, 0.2 mg/kg wet weight, and it can be concluded that the water spinach grown under this condition is safe for consumption.

The cadmium accumulations in plants of the low cadmium concentration group, which consisted of cadmium concentrations of 0.005, 0.017, 0.035 and 0.053 mg/L, are shown in Figure 4.16 and 4.17.

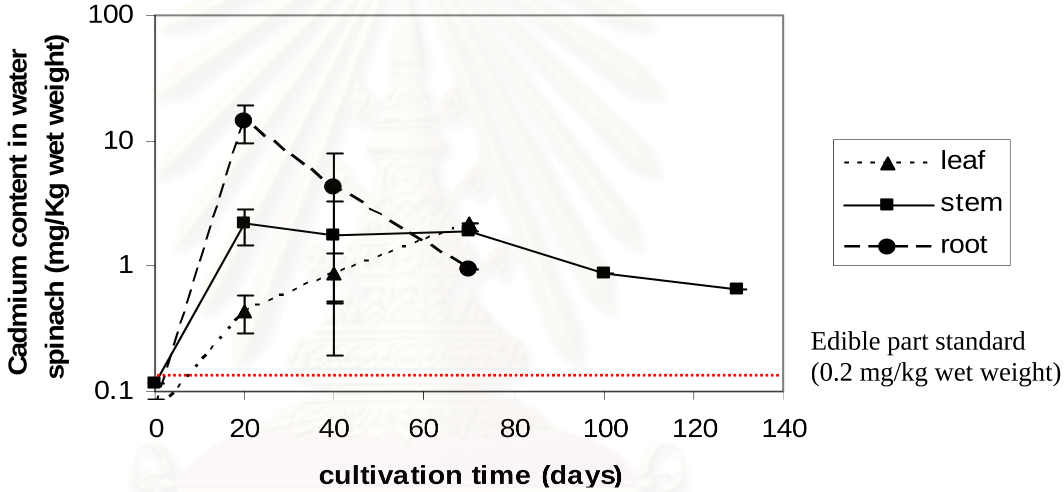


a) Cadmium concentration 0.005 mg/L

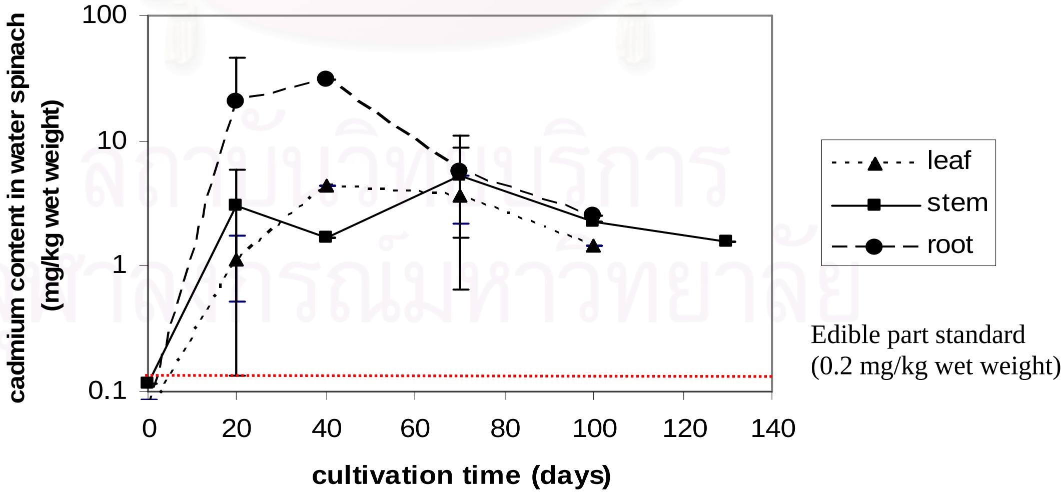
Figure 4.17: Cadmium content in root, stem and leaf of water spinach as a function of cultivation time at low cadmium concentration group including 0.005, 0.017, 0.035, and 0.053 mg/L respectively.



b) Cadmium concentration 0.017 mg/L



c) Cadmium concentration 0.035 mg/L



d) Cadmium concentration 0.053 mg/L

Figure 4.17: Cadmium content in root, stem and leaf of water spinach as a function of cultivation time at low cadmium concentration group including 0.005, 0.017, 0.035, and 0.053 mg/L respectively (cont).

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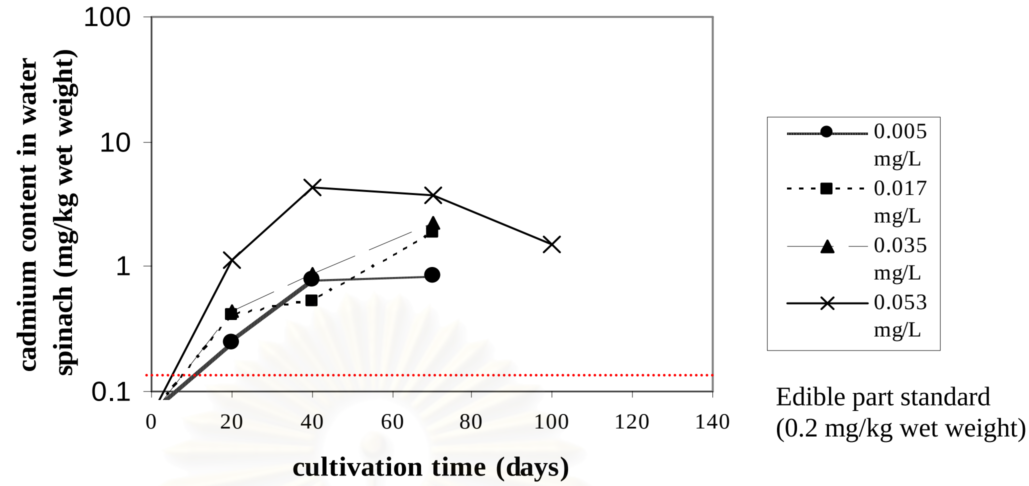
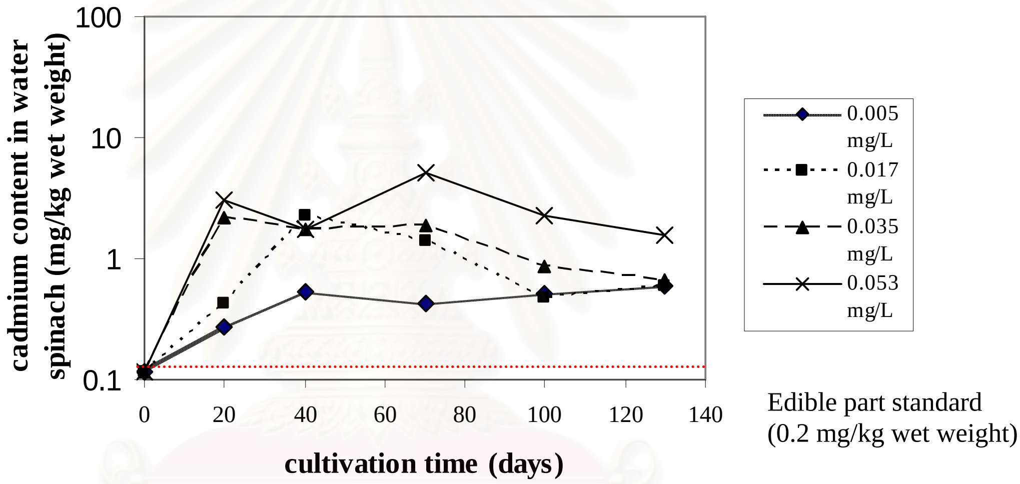
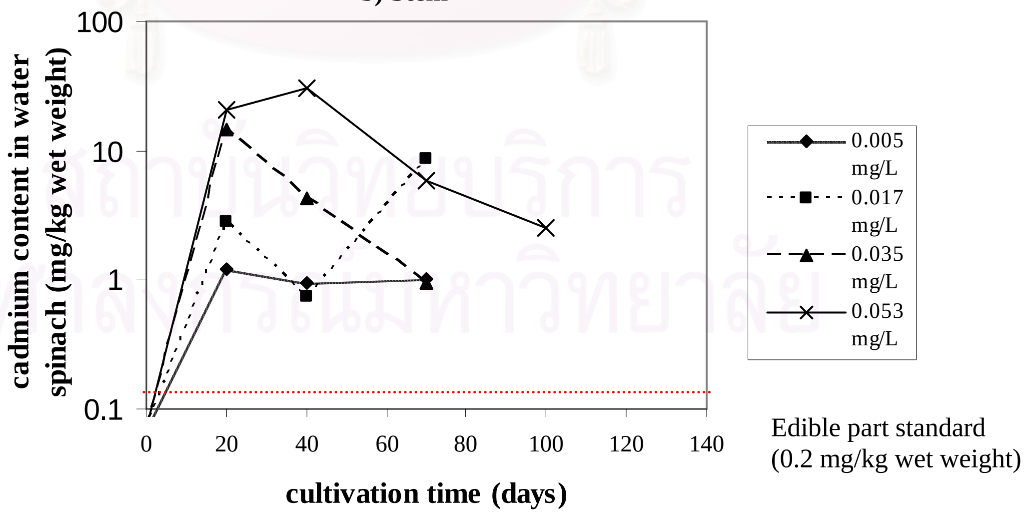


a) Leaf



b) Stem



c) Root

Figure 4.18 Cadmium content in water spinach as a function of cultivation time at low cadmium concentration group including 0.005, 0.017, 0.035, and 0.053 mg/L respectively in each part of water spinach.

The tendency towards cadmium accumulation in stems increased with increasing cultivation time until the cadmium content approximately equaled to 2 mg/kg wet weight (20 mg/kg dry weight)which occurred in the 20 th day (cultivation 1) and the 40 th day (cultivation 2). Cadmium in stems grown under low concentrations probably reached its maximum level around this value.

The tendency for cadmium to accumulate in roots increased with increasing cultivation time but without a distinct pattern. For cadmium in water at 0.005 and 0.017 mg/L, the concentration showed an increasing trend but for cadmium at 0.035 and 0.053 mg/L, the concentration fluctuated and decreased during the last period of cultivation. This was probably due to the life cycle and the cadmium toxicity as mentioned in topic 4.2.2.1 the yield of water spinach, water spinach is an annual aquatic plant that grows on the water surface or the ground with the life cycle of the species normally not exceeding 1 seasons (160 days) based on unpolluted sites. This experiment was conducted under stress conditions (cadmium contaminated) so the life cycle of water spinach could be shorter (Pancho, J. et al., 1978). At the last period of cultivation time the water spinach declined.

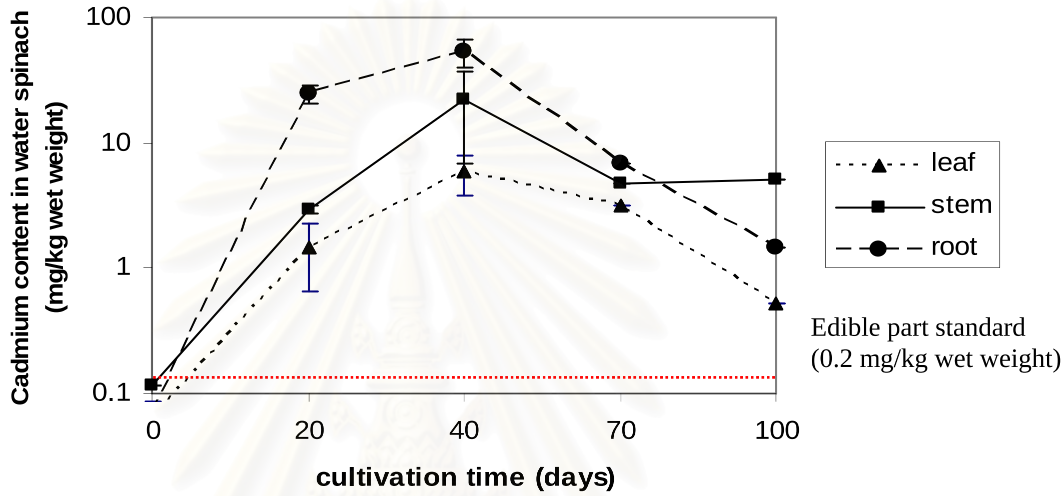
The tendency of cadmium accumulation in leaves increased with increasing cultivation time until the cadmium content approximately equaled to 2-4 mg/kg wet weight (12-15 mg/kg dry weight) that commonly occurred in the 40 th day (cultivation 2) and the 70 th (cultivation 3). In most cultivation, plants declined and did not have sufficient leaves for analysis. The data of cadmium in leaves were not available. However, plots grown in cadmium at 0.053 mg/L, showed that cadmium in leaves decreased during the last period (Figure 4.16-d). The value of around 4 mg/kg wet weight was probably the maximum level accumulated in the leaf of water spinach grown in this condition. In the leaf, the cadmium transfer mechanism was absorption. The principal of the absorption mechanism was passing through phloem and xylem in stem before existed in leaf.

Under low cadmium conditions can be concluded that maximum amount of cadmium is in roots, stems and leaves were in the same range. The higher cadmium concentration in the roots may be the result of contamination of the root surface which had direct contact with the cadmium contaminated water. Secondly it may be dye to the reason mention earlier in topic 4.1.2.3 regarding the cadmium accumulation in water spinach in which Landberg et al. (1996) reported a larger proportion of the metals were retained in the roots and thereby prevented them from interfering with the sensitive metabolic reactions in the shoots. This is probably an internal mechanism to avoid toxic metal concentrations in the shoot, which has also been observed elsewhere. The higher accumulation of metals in the roots rather than in the shoots is possible because of a greater tolerance to toxic metals in the roots than in the shoots. The roots of water spinach accumulated much higher concentrations of the metals before toxic effects occurred. The mechanism behind the higher tolerance of the roots may include the binding of positively charged toxic metal ions to negative charges in the cell walls (Beauford et al., 1977; Wierzbicka, 1998). Part of the metal that has been taken up into the root may be transported further through the plasma membrane and binded to macromolecules, organic acids, or sulfur-rich polypeptides, like phytochelatins, and accumulated in the cytoplasm or the vacuole and thereby be detoxified (Mathys, 1977; Grill et al., 1985; Steffens, 1990; Harmens et al., 1994).

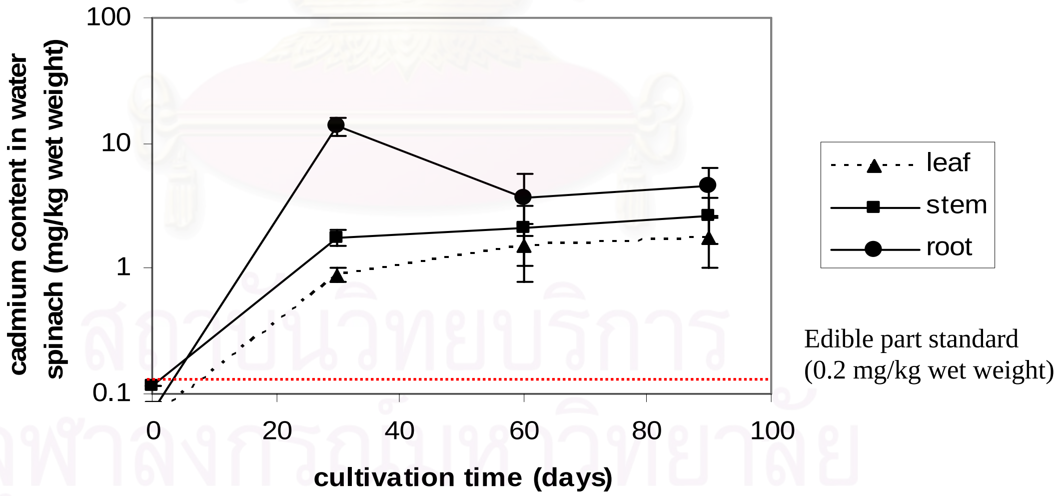
Another possible reason for this may be the cadmium transfer mechanism. The cadmium transfer mechanisms in roots were adsorption on roots' surface and absorption, in stems absorption and some adsorption but in leaves was only with absorption.

For cadmium contamination in this group every part of the water spinach exceeded the EU standard (0.2 mg/kg wet weight). The dietary intake from the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended Provisional Tolerable Weekly Intake (PTWI) of cadmium of 7 micrograms per kilogram of body weight (µg/kg) (http://www.fao.org/english/newsroom/news/2003/19783-en.html). In one day the maximum intake of a human (assume average weight is 70 kg) was 490 micrograms it means that in one week a human can consume approximately 1700 g of water spinach grown in a cadmium concentration of 0.005 mg/L and can consume approximately 400 g of water spinach grown in cadmium concentrations of 0.017, 0.03 and 0.05 mg/L. It could be therefore possible to consume water spinach grown in a cadmium concentration of 0.005 mg/L.

The cadmium accumulations in water spinach in the high cadmium concentration group, which consisted of cadmium concentrations of 0.135, 0.141, 0.201 and 0.446 mg/L, are shown in Figure 4.18 and 4.19.



a) Cadmium concentration 0.135 mg/L



b) Cadmium concentration 0.141 mg/L

Figure 4.19: Cadmium content in leaf, stem and root of water spinach as a function of cultivation time at high cadmium concentration group including 0.135, 0.141, 0.201, and 0.446 mg/L respectively.

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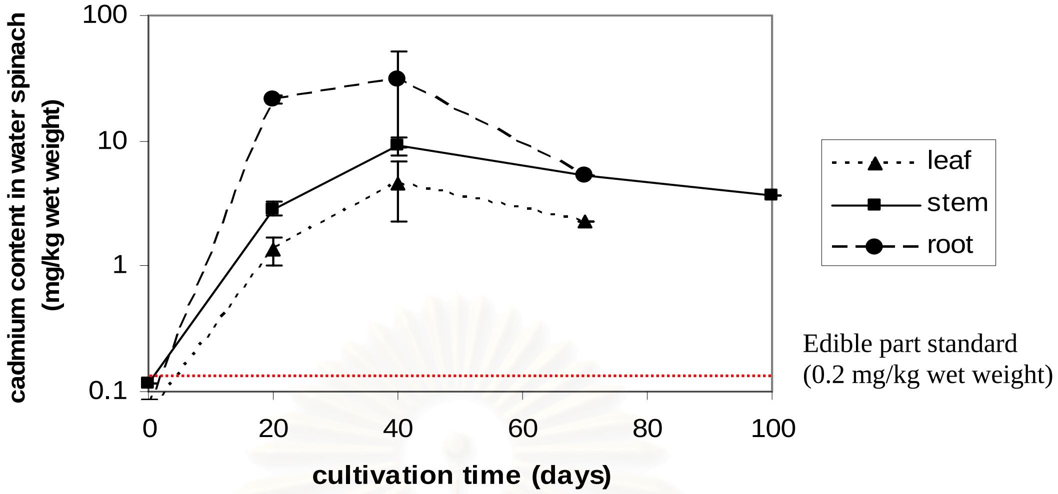
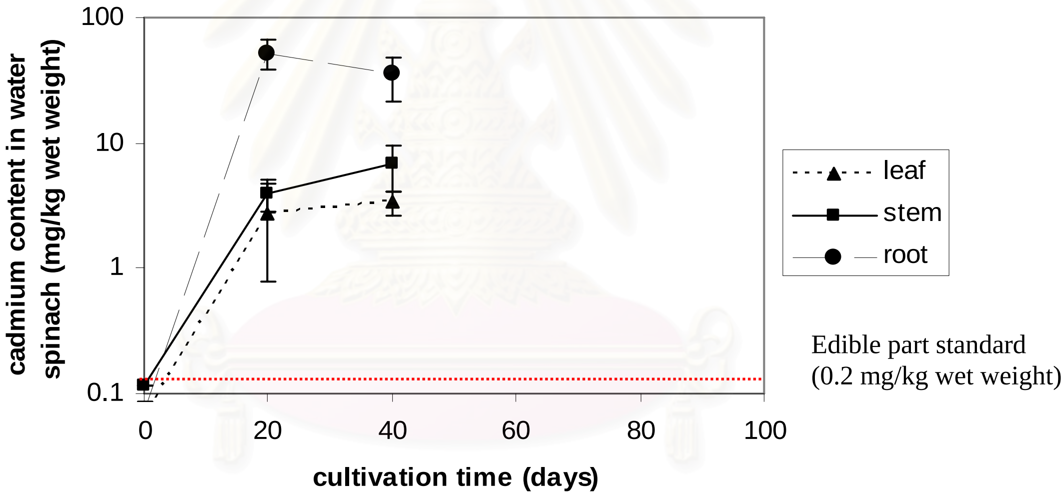
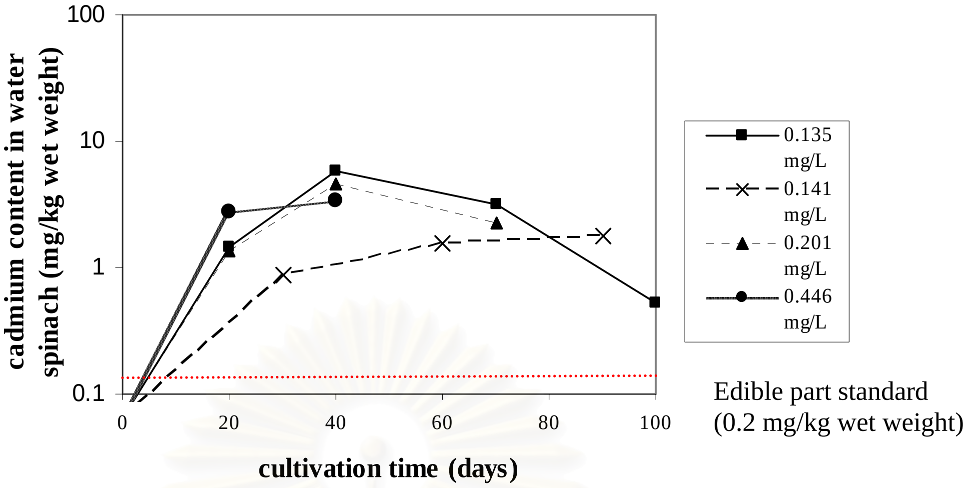


c) Cadmium concentration 0.201 mg/L

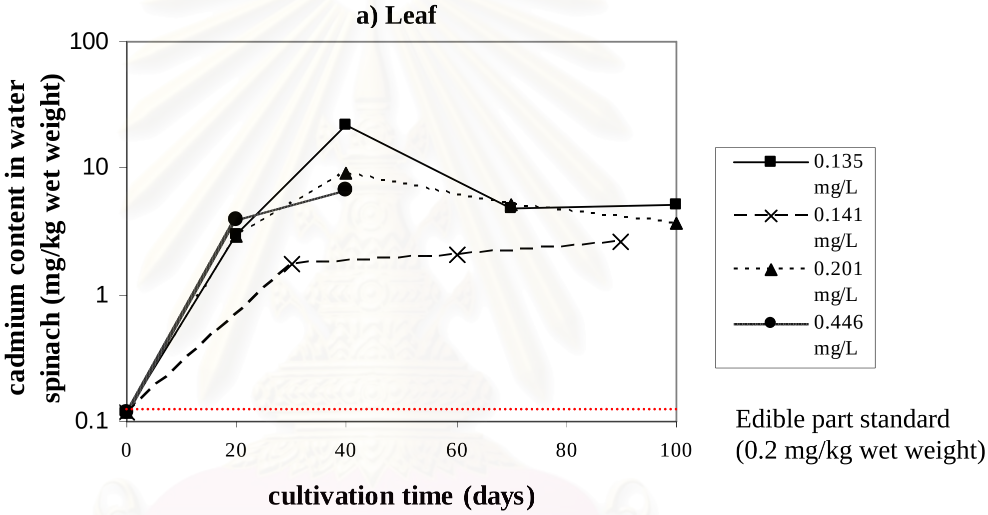


d) Cadmium concentration 0.446 mg/L

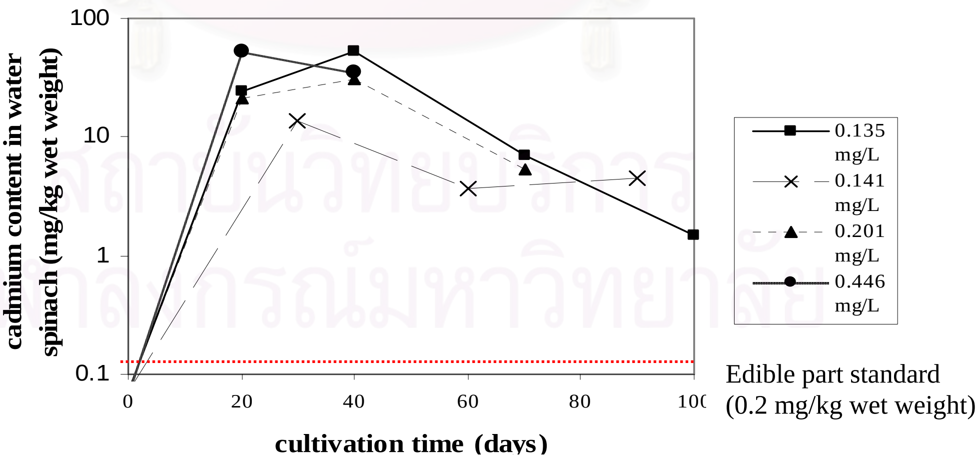
Figure 4.19: Cadmium content in leaf, stem and root of water spinach as a function of cultivation time at high cadmium concentration group including 0.135, 0.141, 0.201, and 0.446 mg/L respectively. (cont.)



a) Leaf



b) Stem



c) Root

Figure 4.20: Cadmium content in water spinach as a function of cultivation time at high cadmium concentration group including 0.135, 0.141, 0.201, and 0.446 mg/L respectively in each part of water spinach.

The cadmium accumulations in stems increased with increasing cultivation time until the cadmium content approximately equaled to 5-20 mg/kg wet weight(50-70 mg/kg dry weight) that commonly occurred on the 20 th day (cultivation 1) to the 40 th day (cultivation 2) then it decreased as the cultivation time increased. This value was probably the maximum level accumulated in stems of water spinach grown under this condition.

The cadmium accumulations in roots increased with increasing cultivation time until the cultivation time equaled to 20 and 40 days (cultivation 1 and 2) then it decreased with increasing cultivation time. This was probably because of the life cycle and the effects of cadmium toxicity as mentioned in topic 4.2.2.1 the yield of water spinach, water spinach is an annual aquatic plant that grows on the water surface or the ground with the life cycle of the species normally not exceeding 1 season (160 days) based on unpolluted sites. [confusing and long run-on sentence] This experiment was conducted under the stress condition (cadmium contaminated) so the life cycle of the water spinach could have been shorter (Pancho, J. et al., 1978). Therefore, at these concentrations, the water spinach had declining state.

The cadmium accumulated in leaves increased with increasing cultivation time until the cadmium content approximately equaled to 3-5 mg/kg wet weight (10-15 mg/kg dry weight) that commonly occurred on the 20 th day (cultivation 1) to the 40 th day (cultivation 2) then it decreased as cultivation time increased. This value was probably the maximum level accumulated in the leaves of the water spinach grown in this condition and this value and was close to value in the stems.

The amounts of cadmium accumulated in parts of water spinach were found to be present from highest to lowest in roots, stems and leaves, respectively. The highest cadmium concentration observed in the water spinach's roots may be the result as mention in topic 4.1.2.3 Landberg et al. (1996) reported a larger proportion of the metals was retained in the roots and thereby prevented them from interfering with sensitive metabolic reactions in the shoots. This is probably an internal mechanism to avoid toxic metal concentrations in the shoots, which has also been observed elsewhere. The higher accumulation of metals in the roots than in the shoots is possible because of a greater tolerance to toxic metals in the roots than in the shoots. The roots of water spinach accumulated much higher concentrations of the metals before the toxic effects occurred. The mechanism behind the higher tolerance of the roots may include the binding of the positively charged toxic metal ions to negative charges in the cell walls (Beauford et al., 1977; Wierzbicka, 1998). Part of the metal that has been taken up into the root may be transported further through the plasma membrane and bind to macromolecules, organic acids, or sulfur-rich polypeptides, like phytochelatins, and accumulate in the cytoplasm or the vacuole and thereby be detoxified (Mathys, 1977; Grill et al., 1985; Steffens, 1990; Harmens et al., 1994).

Another possible reason for this may be the cadmium transfer mechanism. The cadmium transfer mechanisms in the roots were adsorption on the roots' surface and absorption in stems absorption and some adsorption but in leaves was only with absorption.

This result agreed with Göthberg (2002) who reported that water spinach exposed to metals retained a major proportion of the metals in the roots, which had a higher tolerance than the shoots for high internal metal concentrations. It could be concluded that water spinach may be a hyper accumulated plant for cadmium.

The cadmium accumulations in every part of the water spinach exceeded the EU standard. The dietary intake from the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended Provisional Tolerable Weekly Intake (PTWI) of cadmium of 7 micrograms per kilogram of body weight (μg/kg) (http://www.fao.org/english/newsroom/news/2003/19783-en.html). In one day, the maximum intake for a human (assume average weight is 70 kg) was 490 micrograms. According to results obtained, a human can consume leaves of water spinach grown in a high cadmium concentration of approximately 80-300 g per week and can consume approximately 22-185 g of stems per week. This is an unlikely consumption rate, so the water spinach grown in this condition should be avoided.

4.3 Cadmium uptake under shock load condition

4.3.1 Characteristic of water

Cadmium concentration in water

The water initially used in this experiment was treated effluent from Chiang Mai University's wastewater treatment plant. Water spinach was initially grown in control water prior to being transferred to the shock load condition. The separate cultivation plots with initial cadmium concentrations of 0.2 mg/L were used as growing units for periods of 1, 3, 5, 7 and 9 days, respectively.

After the shock period was over, water spinach was rinsed with tap water before being put back to the initial cultivation plots until the end of experiment, i.e. 20 days. The actual cadmium concentration in the water is shown in Table 4.6. In this experiment the cadmium concentrations in the water were expected at concentration 0.2 mg/L.

Table 4.6: Actual cadmium concentration of water in cadmium uptake under the shock load condition

Sampling Time (days)	Shock loading period (days)					
	Actual cadmium concentration (mg/L)					
	0	**1**	**3**	**5**	**7**	**9**
0	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.144	0.136	0.145	0.149	0.164
8	0.000	0.000	0.033	ND	ND	ND
10	0.000	0.000	0.000	ND	ND	0.034
11	0.000	0.000	0.000	0.039	ND	ND
12	0.000	0.000	0.000	0.000	0.022	ND
14	0.000	0.000	0.000	0.000	0.000	0.029
18	0.000	0.000	0.000	0.000	0.000	0.000
20	0.000	0.000	0.000	0.000	0.000	0.000

Remark ND not determined

The actual average cadmium concentration was 0.148 mg/L. This experiment was based on a scenario of a river receiving industrial effluent containing cadmium while water spinach cultivation along river banks was common. There were possible fluctuations in the cadmium concentration of the river water if the wastewater treatment plant were not well operated temporarily. The target concentration was assumed to represent the concentration of the river for a short period of time.

The pH of the water was in the alkaline range with pH values ranging from 6.06-8.65, as shown in Appendix E Table E.1. The tolerance limit of pH for water spinach cultivation ranges from 6.5 to 9.0 (Rattan et al., 2005 and Patel et al., 2004) and the water had pH levels within the permissible limit.

4.3.2 Water spinach

4.3.2.1 Yield of water spinach

The yields of water spinach are presented in Appendix E Figure E.1 and Table E.2. It was found that the yields of water spinach which were grown in this experiment was not statistically different (Appendix H). The time that the water spinach had been grown in cadmium contaminated water was too short so the toxicity of cadmium did not affect water spinach growth and yield.

4.3.2.2 Cadmium accumulation in water spinach

The cadmium accumulations in parts of the water spinach were separated into three parts, i.e. the roots, stems including shoot, and leaves. The variations of cadmium contamination in each part of water spinach are shown in Figure 4.18, and Table 4.7 shows how the concentrations of cadmium accumulation by the root, stem and leaf increased as the shock loading period increased.

Table 4.7: Cadmium accumulation in parts of water spinach grown in cadmium uptake under the shock load condition

Shock loading periods (days)	Cadmium concentration in leaves (mg/kg wet weight)	Cadmium concentration in stems (mg/kg wet weight)	Cadmium concentration in roots (mg/kg wet weight)	EU* (mg/kg)
0 (Before)	0.019	0.089	0.020	
0	0.043	0.032	0.076	0.1 (root)
1	0.103	0.405	0.860	0.2 (leafy)
3	0.244	0.495	2.348	
5	0.440	0.825	3.939	
7	0.729	1.181	4.260	
9	0.832	0.848	6.913	

Remark * Based on plant's wet weight

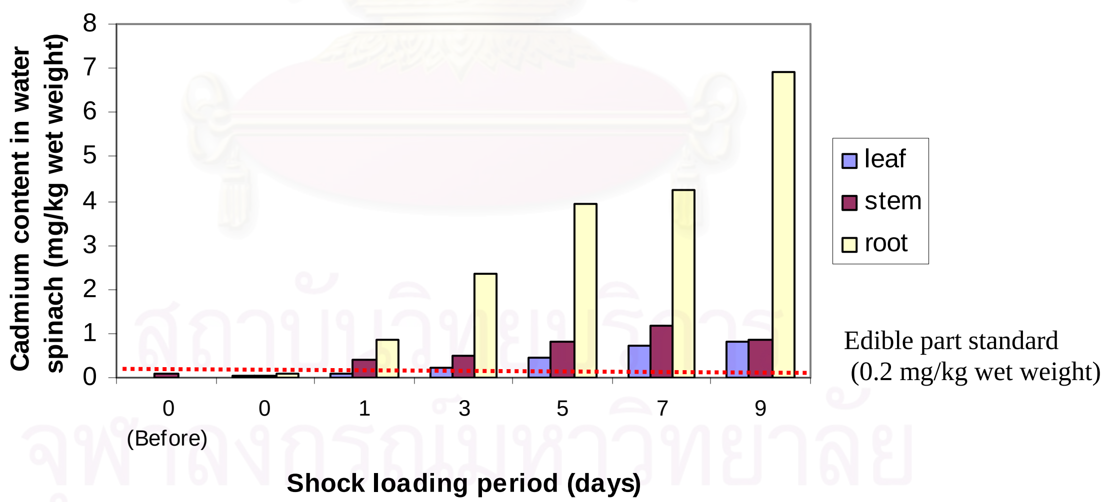


Figure 4.21: Cadmium accumulation in parts of water spinach grown in cadmium

The cadmium accumulated mainly in the roots and some portion of cadmium translocated to the leaves and the stem as shown in Figure 4.18 .The highest cadmium concentration was observed in water spinach's root, a the result of the contamination of the root surface which had direct contact with the cadmium contaminated water.

The cadmium uptake under long-term cultivation in water with a cadmium concentration equal to 0.141 mg/L, had higher cadmium content than this study. A possible reason was the shorter exposure period of the water spinach with the cadmium in the water.

In the control plots without the shock load condition cadmium concentrations, all part of the water spinach were within the EU standard. The cadmium accumulation in the leaves of the water spinach with a 1-day shock load condition was within the EU standard; however, accumulations in the roots and stems exceeded the standard. The roots had the highest amounts of cadmium, about 2 times that of stems, while the cadmium in the stems was about 4 times higher than in the leaves. This indicated that cadmium had transfered from the roots to the stems and then the leaves. For water spinach grown under a longer shock loading condition, the cadmium content in the plant exceeded the EU standard. Figure 4.18 shows a similar pattern of metal transfer where roots absorbed and adsorbed the highest concentration of cadmium. Based on dietary intake standards of the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO), the maximum weekly intakes for human (assuming the average weight is 70 kg) of the edible part of water spinach grown in 1, 3, 5, 7 and 9 day shock loading conditions are approximately 1929 g, 1326 g, 774 g, 513 g and 583 g, respectively. It is possible to consume the first 2 groups at these rates (1 and 3 day shock load) for a longer period. It can be concluded that water spinach grown in a shock loading period of 0.148 mg Cd/L for 1 day receive no adverse effects and is safe for consumption at weekly rate of 1929 g.

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CHAPTER V

CONCLUSIONS

The major objective of this research was to investigate cadmium transport from cadmium contaminated water, at various concentrations, to water spinach. Based on the state condition, the following conclusions can be drawn.

1. Toxicity level in the water spinach was found during its cultivation in water containing 59.0 mg Cd/L and plant died within 6 days. Cadmium contamination in the water spinach was highest in roots followed by the stems, and lowest in the leaves. Nevertheless, the leaves exceeded the European Commission (EU) standard for vegetables.

2. In long-term cultivation, 130 days, cadmium accumulation increases with increasing cadmium concentrations in the water. There is natural pattern of plant die-off under long-term cultivation, even in the control plots (0 mg Cd/L). The maximum amounts of cadmium accumulation in stems and leaves are about 5-20 and 2-4 mg/kg wet weight, respectively. The cadmium contamination in water spinach from highest in roots to lowest in the following order: stems, and then leaves respectively. The edible part (stems and leaves) in most cultivation had cadmium contamination levels that exceeded the European Commission (EU) standard, with the exception of the control plots. Based on the WHO dietary intake data, water spinach grown in cadmium water at 0.005 mg/L at this condition should not be consumed more than 1,700 g/week. Water spinach was found to be a hyper accumulated plant for cadmium.

3. The water spinach grown under a shock load condition of 0.148 mg Cd/L for 1 day was determined safe for consumption, based on the dietary intake rate of 1,929 g/week.

4. In the case of the water spinach was grown in water resources locating near the factories which have the cadmium in their effluents. The knowledge from this study could be applied for monitoring the contamination and accumulation of cadmium in water spinach when the leakage of wastewater containing high cadmium

concentration was observed because water spinach will die and have the symptom (leaf yellowing) when contact with water containing high cadmium concentration. And the results from this study can be used as the database for estimation and comparison of cadmium concentration in the other plants when they are grown in cadmium contaminated water. The cadmium content in the edible part of water spinach can be used for set the standard of cadmium in aquatic vegetable for consumption and standard of cadmium content in water which is used for agriculture purposes.

Another application of this study is phytoremediation. Because of water spinach was found to be a hyper accumulated plant for cadmium so it possible to use this plant to remediate cadmium contaminated in the polluted water resources.

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CHAPTER VI

RECOMMENDATIONS FOR FUTURE WORK

Based on the results of this study, some recommendations for further studies are proposed.

1. In this study, cadmium was taken into water spinach from water without identifying the form of cadmium, and the mechanism of cadmium distribution. Therefore, the form of cadmium should be investigated in a further study.

2. The high accumulation of cadmium in water spinach was high possible due to the direct contact of cadmium on the surface of the roots. A similar study should be conducted using other aquatic plants such as water mimosa, water hyacinth and lotus to compare their accumulation of cadmium.

3. The distribution of cadmium from water to plant was investigated but an investigation on the distribution of cadmium from contaminated plants to animals was not done. Therefore, the distribution of cadmium further down the food chain should be investigated.

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REFERENCES

Alloway, B. J., Andrew P. J, and Morgan, H. (1990). The accumulation of cadmium by vegetables grown on soil contaminated from a variety of sources. The science of the total Environmental 91: 223-236.

All information on cadmium. [online]. Available from. http://www.cadmium.org/ [2005, March 6].

Alia-Saradhi, P.P(1991). Proline accumulation under heavy metal stress. Journal of Plant Physiology138: 554–558.

Antil, R. S, Gupta, A.P. and Narwal, R.P. (2001). Nitrogen transformation and microbial biomass content in soil contaminated with nickel and cadmium from industrial wastewater irrigation. Urban Water 3: 299-302.

ANZFA (Australia New Zealand Food Authority). (2006). Australia New Zealand Food Standards [online], Available from: http://www.foodstandards.gov.au/foodstandardscode/index.cfm#_FSCchapter2 [2006, March 24].

Armstrong. (2004). Water spinach (*Ipomoea aquatica*) [online] Available from :http:// waynesword.palomar.edu/ ww0804.htm [2006, March 20].

APHA, AWWA and WPCF. (1998). Standard method for the examination of water and wastewater. 20 th edition. American Public Health Association, Washinton DC.

ATSDR (Agency for Toxic Substances and Disease Registry). (1990). Toxicological Profiles for cadmium. [online] Available from http://www.atsdr.cdc.gov/toxprofiles/tp5-c3.pdf [2006, March 6].

Beauford, W., Barber, J., and Barringer ,A.R (1977). Uptake and distribution of mercury within higher plants. Physiol. Plant. 39:261–265.

Bingham FT, Page AL, Strong JE. (1980). Yield and cadmium content of rice grain in relation to addition reates of cadmium, copper, nickel and zinc with sewage sludge and liming. Soil Science. 130: 32-38.

Broyer, T.C., Johnson, C.N. and Paull. R.E. (1972). Plant soil.36.301.

Cadmium elements. [online] Available from: http://www.lenntech.com-Periodic-chart-elements-Cd-en_15_gif [2005, Desember 2].

Chen, Y.X., He, Y.F., Luo, Y.M., Yu, Y.L., Lin, Q. and Wong M.H. (2003). Physiological mechanism of plant roots exposed to cadmium. Journal of Chemosphere: 50, 789–793

Costa, G. and Morel I.L. (1993) Cadmium uptake by Lupinus albus (L). Cadmium excretion, a possible mechanism of cadmium tolerance. Journal of Plant Nutrient:16.

Cox sam. (2000). Mechanisms and Strategies for Phytoremediation of Cadmium [online],Available from http://lamar.colostate.edu /INTRODUCTION.html., [2005, November 21].

George, K. (2003). UN Committee recommends new dietary intake limits for mercury. UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO). [online], Available from http://www.fao.org/english/ newsroomnews/2003/19783-en., [2006, January 20]

Gothberg, A. (2004). Influence of Nutrient Levels on Uptake and Effects of Mercury, Cadmium, and Lead in Water Spinach. Journal of Environmental Quality. 33: 1247-1255.

Jackson, A. P. and Alloway, B. J. (1992). The transfer of cadmium from agricultural soils to the human food chain. Advances in Trace Substance Research. Boca Raton, USA. Lewis Publishers: 109-150.

Jain, S. K., Gujral, G. S., and Vasudevan, P. (1987). Potential Utilization of Water Spinach (Ipomoea aquatica). Journal of Sciencetific & Industrial Research. 46: 77-78.

Jaipakdee, R. (2001). Illustrated Book for Teaching Aid (Thai/English). First publication.156: 41-44.

Jiang, W., Liv, D. and Hou, W. (2001). Hyperaccumulation of cadmium by roots, blubs and shoots of garlic (*Allium sativum L.*). Bioresource Technology. 76: 9-13.

Karnchanawong, S., Silprasert, A., Keawvichit, R. and Prapamontol, T. (2002). Contamination levels of crops irrigated by effluent from domestic wastewater treatment plant. Proceedings of the international symposium on lowland technology, Saga University, Saga, 18-20 September.

Kijjanapanich, V. and Karnchanawong, S. (2004). Water quality of infiltrated from laboratory-scale plots irrigated by effluent from domestic wastewater treatment plants. Proceeding IWA International Conference on Wastewater Treatment for Nutrient Removal and Reuse, Asian Institute of Technology, Bangkok 26-29 January.

Landberg, T., and Greger, M. (1996). Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl. Geochem. 11:175–180.

Casarett, L.J. And Doull, J.M.D.(2003) Toxicology the basic science of poisons : Macmillan Publishing Co., Inc.

Mathys, W. (1977). The role of malate, oxalate and mustard oil glucosides in the evolution of zinc-resistance in herbage plants. Physiol. Plant. 40:130–136.

Meteorological Department (2006). [online], Available from http://www.tmd.go.th/monthly_report/cur_temp.php., [2006, March 27].

McLaughlin, M.J. and Singh, B.R. (1999). Cadmium in soils and Plants. Dordrecht ; Boston : Kluwer Academic Publishers.

U.S.Occupational Safety & Health Administration.(2006). Law and regulation.[online], Available from http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=PREAMBLES&p_id=818., [2005, November 21].

Pancho, J. and Soerjanj, M. (1978). Aquatic weeds of southeast Asia. First published. Quezon city : National Publishing Cooperative Incorporated,

Prayut, S. (1999). Distribution of Cadmium and Zinc in Soil from Zinc Mining Activity: A Case Study of Zinc Mine, Mae Sot District, Tak Province. Thesis of Master Degree, Major Field of Technology of Environmental Planning For Rural Development, Mahidol University, Nakhon Pathom 73170, Thailand. From website: http://www.eric.chula.ac.th/gcrc/abstract/PAGES8/741-45.html.

Rai, UN. and Sinha, S. (2001). Distribution of metals in aquatic edible plants: Trapa natans (Roxb.) Makino and Ipomoea aquatica Forsk**.** Environmental Monitoring and Assessment, 70(3):241-252.

Rattan, R.K., Datta, S.P. Chhonkar, P.K., Suribabu, K., and Singh, A.K. (2005). Long-term impact of irrigated with sewage effluents on heavy metal content in soil, crops and groundwater-a case study. Agriculture, Ecosystems and Environment. Available online at www.sciencedirect.com.

Sharma, S.S., Schat, H., and Vooijs, R. (1998). In vitro alleviation of heavy metal-induced enzyme inhibition by praline. Phytochemistry49 (6): 1531–1535.

Stanley, E. (1992). Toxicological chemistry. Second edition: Manahan Lewis publishers, 449: 154-162.

Sun, E. J. and Wu, F.Y. (1998). Along-vein necrosis as indicator symptom on water spinach caused by nickel in water culture. Botanical Bulletin of Academia Sinica. 39: 255-259.

Suppradid, J. (2005). Distribution of cadmium in laboratory-scale kale cultivation irrigated with cadmium contaminated water. Master of Science, Graduate School, Chulalongkorn University.

Taylor, M.D. (1997). Accumulation of cadmium derived from fertilizer in New Zealand soils. The science of the total Environment, 208: 123-126.

Tech Tree Dream Team Members. (2002). Physical Solidification/Stabilization. [online], Available from http://www.cpeo.org/techtree/ttdescript/solidsta.htm., [2006, January 21].

Theerapunsatien, S. (1995). Cadmium and zinc in water, sediment and mussel Hyriopsis myersiana of the Ping River. Master of Science, Graduate School, Chulalongkorn University, p 2.

Vig, K., Megharaj, M., Sethunathan, N. and Naidu, R. (2003). Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. Advances in Environmental Research., 8, 121-135.

Vassilev A, Tsonev T, Yordanov I. (1998). Physiological response of barley plants (Hordeum vulgare) to cadmium contamination in soil during ontogenisis. Environmental Pollution 103: 287-293.

Wanleelag, N. (1987). Insect pests of vegetables in Thailand. Department of Entomology. Kasetsart University: 6-10, 16-19, 59-61 and 83-85.

Youn-Joo An (2004). Soil ecotoxicity assessment using cadmium sensitive plants. Environmental Pollution.127: 21-26.

APPENDICES

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APPENDIX A

Acid digestion by block digester

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Digestion Blocks

Digestion Blocks are designed to be used when wet acid ashing or digestion is required as a sample preparation technique. Most common is the sulfuric acid digestion required for determination of Kjeldahl nitrogen/protein or of total phosphorus. Digestion Blocks can also be used where acid digestion is required prior to trace metal analysis. (http://www.rosesci.com/Products/Chemical%20Analysis/Digestion%20BlocksOverview.htm)

In this experiment used digestion blocks for digest plant sample and the model that used is Description the *Digi*PREP Jr. 24 position as shown in Figure A.1

Figure A.1: The picture of digestion block

(Adapt from http//www.scpscience.com/.../ DigiPREP%20Jr_3.gif)

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A. Acid digestion

A.1 METHOD 3050B

This method has been written to provide two separate digestion procedures, one for the preparation of sediments, sludges, and soil samples for analysis by flame atomic absorption spectrometry (FLAA) or inductively coupled plasma atomic emission spectrometry (ICP-AES) and another one for the preparation of sediments, sludges, and soil samples for analysis of samples by Graphite Furnace AA (GFAA) or inductively coupled plasma mass spectrometry (ICP-MS).

Sample 0.5 g

Reagents

Nitric acid (concentrated), HNO_3. Acid should be analyzed to determine levels of impurities. If method blank is < MDL, the acid can be used.

Hydrogen peroxide. (30%), H_2O_2 Oxidant should be analyzed to determine level of impurities. If method blank is < MDL, the peroxide can be used.

Hydrochloric acid (concentrated), HCl. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

Procedure

- Weigh to the nearest 0.01 g and transfer a 1-2 g sample (wet weight) or 0.5 -1 g sample (dry weight) to a digestion vessel. may be used as long as digestion is completed.

NOTE: All steps requiring the use of acids should be conducted under a fume hood by properly trained personnel using appropriate laboratory safety equipment. The use of an acid vapor scrubber system for waste minimization is encouraged.

- For the digestion of samples for analysis by GFAA or ICP-MS, add 10 mL of 1:1 HNO_3 , mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to 95^{o}C ± 5^{o}C and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO_3 , replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3 , repeat this step (addition of 5 mL of conc. HNO_3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO_3 . Using a ribbed watch glass or vapor recovery system, either allow the solution to evaporate to approximately 5 mL without boiling or heat at $95^oC \pm 5^oC$ without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

NOTE: Alternatively, for direct energy coupling devices, such as a microwave, digest samples for analysis by GFAA or ICP-MS by adding 10 mL of 1:1 HNO_3 , mixing the slurry and then covering with a vapor recovery device. Heat the sample to $95^oC \pm 5^oC$ and reflux for 5 minutes at $95^oC \pm 5^oC$ without boiling. Allow the sample to cool for 5 minutes, add 5 mL of concentrated HNO_3 , heat the sample to $95^oC \pm 5^oC$ and reflux for 5 minutes at $95^oC \pm 3\ 5^oC$. If brown fumes are generated, indicating oxidation of the sample by HNO_3 , repeat this step (addition of 5 mL concentrated HNO_3) until no brown fumes are given off by the sample 3 indicating the complete reaction with HNO_3 . Using a vapor recovery system, heat the sample to $95^oC \pm 5^oC$ and reflux for 10 minutes at $95^oC \pm 5^oC$ without boiling

- After the previous step has been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H_2O_2 . Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel.

- Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged but do not add more than a total of 10 mL 30% H_2O_2 .

- Cover the sample with a ribbed watch glass or vapor recovery device and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at $95^oC \pm 5^oC$ without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

- After cooling, dilute to 100 mL with water. Particulates in the digestate should then be removed by filtration, Filter through Whatman No. 41 filter paper (or equivalent).
- For the analysis of samples for FLAA or ICP-AES, add 10 mL conc. HCl to the sample digest from H_2O_2 and cover with a watch glass or vapor recovery device. Place the sample on/in the heating source and reflux at 95 $^oC \pm 5^oC$ for 15 minutes.
- Filter the digestate through Whatman No. 41 filter paper (or equivalent) and collect filtrate in a 100-mL volumetric flask. Make to volume and analyze by FLAA or ICP-AES.

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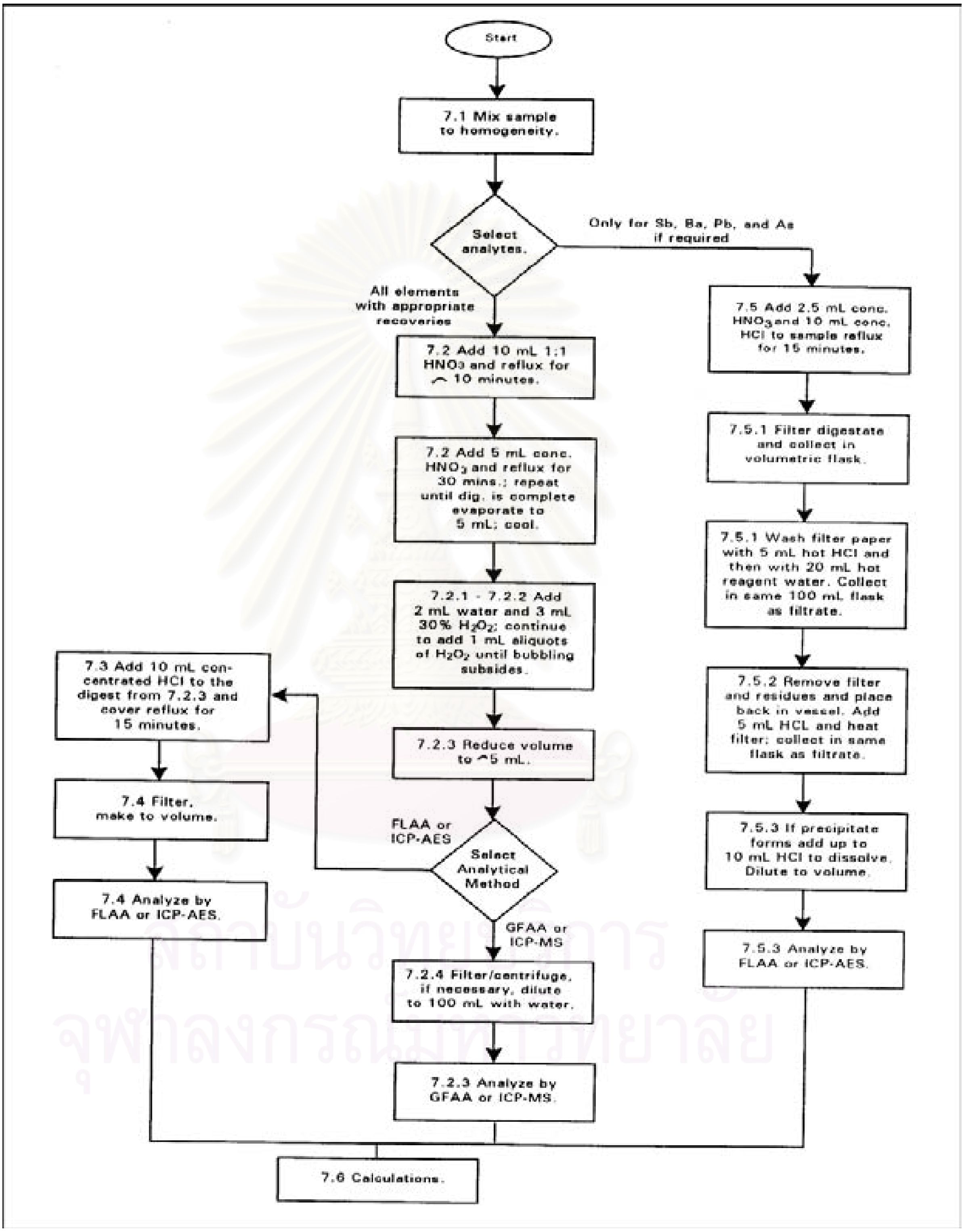

Figure A.2: The flow chart of acid digestion method 3050B
(Adapt from http://www.epa.gov/sw-846/pdfs/3050b.pdf)

APPENDIX B

Analytical Methods

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B Analytical Methods

B.1 pH.

The pH was directly measured by Horiba pH-meter, Model F-21 with an accuracy of ± 0.01. The pH meter was calibrated daily with buffer solution at pH 4.00, 7.00 and 9.00, respectively.

B.2 Atomic Absorption Spectroscopy

Cadmium concentration was directly measured by flame and graphite furnace atomic absorption spectroscopy.

B.2.1 Flame atomic absorption

Lamp current: 3.0 mA.

Flame type: Air- Acetylene (oxidizing)

Flame emission:

Wavelength: 228.8 nm.

Slit width: 0.2 nm.

B.2.2 Graphite Furnace atomic absorption

Wavelength: 228.8 nm

Slit: 0.5 nm.

Lamp current: 3 mA.

Table B.1: Atomic absorption spectrophotometer condition for cadmium analysis

Element	Max Ash in HNO_3 ˚C	Atomize Temp ˚C	Characteristic concentration Ar ng/mL	Characteristic concentration N_2 ng/mL	Characteristic Mass Ar pg	Typical respond (conc. for 20 µL to give approx. 0.3 abs) Ar ng/mL
Cadmium	300	1800	0.013	0.013	0.25	1.0

Remark: Final volume was diluted to be 50 mL.

B. 2.3 Data interpretation

Standard solution at desire concentration was measured absorbance for create standard calibration curve. The absorbance of the sample was determined the concentration by using the standard calibration curve. The example of data interpretation is presented as follows.

Concentration of heavy metal from standard calibration curve is x µg/ml
The total volume of sample is 50 ml equal to heavy metal 50 x µg.
The samples 50 ml prepare from plant dry weight 0.5 g.
Thus, plant dry weight 0.5 g has heavy metal 50x µg.
Plant dry weight 1.0 g has heavy metal 50x/0.5 µg/g.

APPENDIX C

Toxicity test on water spinach growth

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Table C.1 pH of water in toxicity test on water spinach growth cultivation.

Sampling time (days)	pH					
	Cadmium concentration (mg/L)					
	0	**7.6**	**59**	**118.9**	**242.6**	**553.7**
0	7.37	7.3	7.33	7.33	8.1	7.8
7	8.03	8.08	7.84	7.88	7.43	8.4
15	8.835	8.41	6.08	9.3	7.29	8.45
21	8.12	7.69	7.56	7.52	7.16	8.18
30	7.52	8.975	8.645	7.985	8.36	8.69
Average	7.975	8.091	7.491	8.003	7.668	8.304
SD.	0.57808	0.64611	0.93208	0.77199	0.52988	0.33501

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Table C.2 Cadmium content in part of water spinach (wet weight) of toxicity test on water spinach growth cultivation (Harvesting date June, 30 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg wetweight)								
		stem			root			leaf		
				Avarage			Avarage			Avarage
0	0	rep 1	3.21E-02	3.34E-02	rep 1	7.08E-02	1.20E-02	rep 1	ND	3.68E-02
		rep 2	3.02E-02		rep 2	1.58E-02		rep 2	2.97E-02	
		rep 3	4.29E-02		rep 3	2.98E-03		rep 3	4.40E-02	
		rep 4	2.86E-02		rep 4	1.73E-02		rep 4	ND	
1	7.6	rep 1	36.52	36.52	rep 1	171.70	171.70	rep 1	58.82	58.82
2		rep 2	ND		rep 2	ND		rep 2	ND	
3		rep 3	ND		rep 3	ND		rep 3	ND	
4		rep 4	ND		rep 4	ND		rep 4	ND	

Table C.3 Cadmium content in part of water spinach (dry weight) of toxicity test on water spinach growth cultivation (Harvesting date June, 30 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg dryweight)								
		stem			root			leaf		
				Avarage			Avarage			Avarage
0	0	rep 1	3.37E-01	3.01E-01	rep 1	1.99E-01	1.45E-01	rep 1	ND	2.33E-01
		rep 2	2.48E-01		rep 2	3.28E-02		rep 2	2.33E-01	
		rep 3	3.81E-01		rep 3	2.04E-01		rep 3	ND	
		rep 4	2.39E-01		rep 4	ND		rep 4	ND	
1	7.6	rep 1	349.15	349.15	rep 1	2489.58	2489.58	rep 1	526.70	526.70
2		rep 2	ND		rep 2	ND		rep 2	ND	
3		rep 3	ND		rep 3	ND		rep 3	ND	
4		rep 4	ND		rep 4	ND		rep 4	ND	

APPENDIX D

Cadmium uptake under long term cultivation

Table D.1 pH of water in cadmium uptake under long term cultivation

Sampling time (days)	pH								
	Cadmium concentration (mg/L)								
	0	**0.0047**	**0.0172**	**0.0349**	**0.0533**	**0.1352**	**0.1408**	**0.201**	**0.446**
0	7.37	7.30	7.33	7.33	8.10	7.80	ND	8.06	8.23
3	9.03	8.08	7.84	7.88	7.43	9.40	ND	9.23	9.32
6	9.84	9.41	6.08	9.30	7.29	8.45	ND	7.15	5.92
9	8.12	7.69	7.56	7.52	7.16	8.18	ND	8.53	9.09
12	8.52	8.98	8.65	7.99	8.36	8.69	ND	9.21	6.42
15	8.56	9.00	8.51	8.49	7.22	7.91	ND	9.12	6.31
18	7.91	7.57	7.37	7.39	7.26	7.30	ND	7.78	8.16
20	7.95	7.63	7.48	7.39	7.65	7.49	ND	7.87	8.16
21	9.32	9.83	8.96	9.22	9.11	8.78	ND	9.49	10.04
24	10.13	9.70	9.46	9.39	9.95	9.92	ND	9.61	9.69
27	10.07	10.13	9.45	9.47	9.67	9.09	ND	9.45	10.52
30	7.71	9.60	9.36	9.35	9.24	9.67	ND	9.44	9.54
33	7.88	10.45	9.78	9.22	9.64	9.88	ND	9.84	10.55
36	7.81	7.99	8.66	8.11	7.88	8.13	ND	7.74	8.96
39	8.45	7.34	7.38	7.45	7.65	7.20	ND	6.65	7.69
42	7.70	7.07	8.96	7.53	7.72	7.40	ND	7.22	7.26
43	6.06	6.23	6.65	7.03	6.51	7.36	ND	6.72	ND
46	9.86	6.15	8.60	8.60	9.26	9.06	ND	9.48	ND
49	6.28	6.61	7.19	7.18	7.51	7.65	ND	7.95	ND
52	9.31	6.66	7.20	7.46	7.52	7.34	ND	7.08	ND
55	6.71	6.69	7.59	8.43	7.59	7.40	7.53	7.28	ND
58	7.28	7.19	8.34	7.50	7.56	7.39	7.59	7.21	ND
61	6.79	7.34	8.12	7.31	7.48	6.59	8.12	7.42	ND
64	6.77	6.31	6.81	6.23	6.02	6.44	7.69	5.75	ND
67	7.00	7.11	7.13	7.12	7.90	7.40	7.39	7.35	ND
70	7.35	7.40	7.72	7.56	7.41	7.12	6.95	7.16	ND
73	7.12	7.08	7.03	6.97	7.74	7.76	7.77	7.71	ND
74	8.53	8.66	8.59	8.50	8.43	8.54	7.34	7.46	ND
77	6.99	6.45	6.56	6.65	6.32	6.66	7.44	7.84	ND
80	7.59	7.86	7.52	7.25	7.46	7.23	7.63	7.65	ND
83	6.98	8.72	7.78	7.86	7.69	7.56	7.82	7.89	ND
86	7.56	9.12	8.65	8.64	8.54	8.24	8.03	8.15	ND
89	6.77	6.31	6.81	6.23	6.02	6.44	5.75	6.73	ND
92	6.89	6.97	7.23	7.06	7.55	7.25	6.13	6.99	ND
95	7.56	7.46	7.65	7.45	7.79	7.46	7.02	7.05	ND
98	7.82	7.85	7.56	8.12	7.95	7.75	7.78	7.45	ND
101	6.95	8.56	7.02	6.98	8.25	8.02	7.99	7.59	ND
104	7.04	6.82	7.71	7.19	6.72	6.75	6.64	6.23	ND
105	6.15	7.60	6.56	7.81	7.75	ND	7.59	ND	ND
108	7.84	7.61	7.73	7.73	8.59	ND	7.69	ND	ND
111	8.01	7.73	7.77	8.01	7.04	ND	8.52	ND	ND
114	7.95	7.73	7.91	8.24	7.77	ND	7.88	ND	ND
117	7.89	7.37	7.67	7.83	7.82	ND	8.11	ND	ND

Table D.1 pH of water in cadmium uptake under long term cultivation (cont.)

Sampling time (days)	pH								
	Cadmium concentration (mg/L)								
	0	**0.0047**	**0.0172**	**0.0349**	**0.0533**	**0.1352**	**0.1408**	**0.201**	**0.446**
120	8.03	7.36	8.09	7.01	8.12	ND	7.84	ND	ND
123	8.12	8.47	7.97	6.59	6.98	ND	8.56	ND	ND
126	7.95	8.18	8.08	7.25	7.12	ND	7.89	ND	ND
129	7.91	7.95	7.99	7.48	7.54	ND	7.52	ND	ND
132	7.66	7.56	7.77	7.78	7.84	ND	6.25	ND	ND
135	7.82	7.24	7.51	7.98	8.04	ND	7.15	ND	ND
Average	7.66	7.64	7.67	7.60	7.62	7.66	7.26	7.66	8.49
SD.	1.46	1.52	1.37	1.35	1.38	1.54	1.49	1.59	1.48

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Table D.2 Actual cadmium concentration of water in the first cultivation of the cadmium uptake under long-term cultivation

Sampling Time (days)	Target concentration in water (ug/L)							
	0	5	10	30	50	100	200	500
0	3.00	2.68	3.17	8.49	14.88	218.55	87.20	238.75
3	0.00	4.07	21.41	215.40	344.75	128.30	136.60	117.50
6	0.00	2.28	25.62	35.85	36.98	24.35	63.20	78.00
9	0.00	0.83	0.90	3.21	57.48	4.20	180.09	283.50
12	0.00	1.11	5.47	3.65	10.95	39.75	33.70	125.75
15	0.17	0.00	24.94	14.03	287.25	143.65	199.30	243.13
18	0.00	6.01	16.09	8.05	60.75	143.95	156.50	216.25
20	1.01	7.04	45.14	35.83	71.25	128.75	168.80	698.88
Average	0.52	3.00	17.84	40.56	110.53	103.94	128.17	250.22
SD.	1.06	2.07	10.61	71.87	129.51	73.61	59.89	195.12

Table D.3 Actual cadmium concentration of water in the second cultivation of the cadmium uptake under long-term cultivation

Sampling Time (days)	Target concentration in water (ug/L)							
	0	5	10	30	50	100	200	500
21	0.00	7.73	50.22	18.39	31.70	215.60	264.40	959.50
23	0.03	30.06	165.10	86.65	56.73	268.50	139.60	884.50
26	0.00	10.66	5.79	5.35	70.10	87.60	323.00	221.75
29	0.00	1.36	12.47	14.11	51.23	236.60	127.70	231.00
32	0.08	1.59	12.67	77.90	21.20	73.40	182.10	268.50
35	0.31	0.56	3.42	8.06	15.90	155.75	212.20	127.75
38	2.09	8.78	15.80	48.91	66.20	243.60	290.50	523.00
40	3.96	9.06	15.81	41.44	60.25	256.60	224.30	517.75
Average	0.81	8.72	35.16	37.60	46.66	192.21	220.48	466.72
SD.	1.46	10.26	58.20	31.64	20.90	77.01	69.74	314.62

Table D.4 Actual cadmium concentration of water in the third cultivation of the cadmium uptake under long-term cultivation.

Sampling Time (days)	Target concentration in water (ug/L)							
	0	5	10	30	50	100	150	200
41	0.14	4.06	13.12	90.60	166.00	156.00	ND	208.80
43	0.32	7.50	18.58	44.25	119.20	238.40	ND	224.90
46	0.92	7.73	11.93	46.66	93.05	191.45	ND	202.60
49	0.14	0.00	12.59	30.67	89.50	151.30	130.50	154.10
52	0.09	2.86	4.75	23.87	86.43	127.65	ND	175.40
55	0.44	0.00	2.71	22.46	87.88	127.90	ND	154.40
58	0.38	29.80	34.14	207.60	55.75	535.00	141.70	322.60
60	0.74	1.19	12.60	23.55	27.25	166.25	159.00	135.20
64	0.85	0.73	0.93	0.40	1.48	7.15	112.20	181.10
67	0.07	0.10	0.68	1.00	1.75	5.30	111.60	114.10
70	0.00	4.92	13.15	33.07	24.23	67.65	140.56	154.80
Average	0.39	5.35	11.38	43.35	58.65	161.81	132.59	181.92
SD.	0.34	10.56	10.39	59.72	42.31	143.45	18.46	58.84

Table D.5 Actual cadmium concentration of water in the forth cultivation of the cadmium uptake under long-term cultivation.

Sampling Time (days)	Target concentration in water (ug/L)							
	0	5	10	30	50	100	150	200
71	0.00	1.39	0.00	11.86	34.88	67.65	111.50	192.40
76	0.19	1.62	3.18	8.18	49.78	43.35	49.00	152.30
80	1.26	0.86	10.50	25.36	45.87	86.59	156.35	203.69
87	0.61	0.74	41.19	34.03	65.35	217.85	307.10	172.00
97	0.39	3.19	6.24	12.20	56.48	146.60	104.00	205.90
100	0.34	2.07	7.04	18.58	40.25	118.45	95.70	230.20
Average	0.46	1.65	11.36	18.37	48.77	123.60	137.28	192.75
SD.	0.47	1.13	17.53	11.92	8.53	62.86	111.01	25.92

Table D.6 Actual cadmium concentration of water in the fifth cultivation of the cadmium uptake under long-term cultivation.

Sampling Time (days)	Target concentration in water (ug/L)						
	0	**5**	**10**	**30**	**50**	**100**	**150**
101	0.00	0.00	0.00	0.00	80.40	123.56	0.00
103	0.00	2.37	10.23	32.65	69.65	56.98	150.65
106	0.00	5.69	15.26	25.48	76.59	98.75	145.85
109	0.00	3.69	24.90	75.89	56.39	125.89	200.56
112	0.00	4.87	29.58	45.68	84.69	0.00	149.85
115	0.00	0.00	1.53	9.80	0.00	250.65	0.00
118	0.00	5.98	3.25	36.59	48.95	95.87	158.65
121	0.00	9.36	8.57	25.64	56.32	87.45	178.95
124	0.00	5.47	5.69	16.58	56.48	95.95	185.63
127	0.00	6.95	5.98	65.48	23.55	105.36	123.54
130	0.00	5.87	6.58	26.98	65.48	94.59	198.75
Average	0.00	4.57	10.14	32.80	56.23	67.58	135.68
SD.	0.00	2.86	9.48	22.61	25.21	59.93	71.12

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Table D.7 Cadmium content in part of water spinach (dry weight) in cultivation 1 of cadmium uptake under long-term cultivation (Havesting date July 30, 2005

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg wetweight)								
		stem Avarage			root Avarage			leaf Avarage		
0	0	rep 1	1.770	1.770	rep 1	0.475	0.541	rep 1	0.735	1.013
		rep 2	ND		rep 2	0.490		rep 2	1.328	
		rep 3	ND		rep 3	0.663		rep 3	0.713	
		rep 4	ND		rep 4	0.538		rep 4	1.275	
1	0	rep 1	1.578	1.658	rep 1	0.547	1.576	rep 1	1.605	1.480
2		rep 2	1.660		rep 2	2.080		rep 2	1.178	
3		rep 3	1.735		rep 3	1.493		rep 3	1.593	
4		rep 4	ND		rep 4	2.185		rep 4	1.545	
5	0.0046	rep 1	0.295	1.621	rep 1	15.965	13.136	rep 1	3.018	2.001
6		rep 2	3.753		rep 2	6.035		rep 2	0.985	
7		rep 3	0.763		rep 3	17.408		rep 3	ND	
8		rep 4	1.675		rep 4	ND		rep 4	ND	
9	0.017	rep 1	ND	4.702	rep 1	ND	34.974	rep 1	10.943	5.757
10		rep 2	5.635		rep 2	40.655		rep 2	3.890	
11		rep 3	4.050		rep 3	12.025		rep 3	3.433	
12		rep 4	4.420		rep 4	52.243		rep 4	4.763	
13	0.035	rep 1	19.300	24.100	rep 1	96.900	183.400	rep 1	2.763	3.630
14		rep 2	25.300		rep 2	131.700		rep 2	3.435	
15		rep 3	27.700		rep 3	199.000		rep 3	2.993	
16		rep 4	ND		rep 4	306.000		rep 4	5.330	
17	0.053	rep 1	5.300	33.750	rep 1	28.500	336.050	rep 1	0.185	2.396
18		rep 2	17.400		rep 2	26.700		rep 2	2.168	
19		rep 3	65.400		rep 3	838.000		rep 3	3.446	
20		rep 4	46.900		rep 4	451.000		rep 4	3.784	
21	0.135	rep 1	26.100	30.225	rep 1	85.500	422.625	rep 1	1.209	12.277
22		rep 2	27.300		rep 2	400.000		rep 2	13.500	
23		rep 3	29.800		rep 3	701.000		rep 3	16.100	
24		rep 4	37.700		rep 4	504.000		rep 4	18.300	
25	0.201	rep 1	23.100	26.033	rep 1	409.000	389.000	rep 1	14.800	11.300
26		rep 2	24.900		rep 2	364.000		rep 2	9.500	
27		rep 3	30.100		rep 3	394.000		rep 3	9.600	
28		rep 4	ND		rep 4	ND		rep 4	ND	
29	0.446	rep 1	45.400	48.700	rep 1	672.000	852.500	rep 1	19.900	24.850
30		rep 2	53.400		rep 2	637.000		rep 2	16.700	
31		rep 3	67.900		rep 3	1729.000		rep 3	55.300	
32		rep 4	28.100		rep 4	372.000		rep 4	7.500	

Table D.7 Cadmium content in part of water spinach (dry weight) in cultivation 2 of cadmium uptake under long-term cultivation
(Havesting date August 30, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg wetweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
0	0	rep 1	1.770	1.770	rep 1	0.475	0.541	rep 1	0.735	1.013
		rep 2	ND		rep 2	0.490		rep 2	1.328	
		rep 3	ND		rep 3	0.663		rep 3	0.713	
		rep 4	ND		rep 4	0.538		rep 4	1.275	
1	0	rep 1	1.170	1.323	rep 1	3.055	2.414	rep 1	1.155	1.152
2		rep 2	1.573		rep 2	3.565		rep 2	1.110	
3		rep 3	1.448		rep 3	1.668		rep 3	1.353	
4		rep 4	1.100		rep 4	1.370		rep 4	0.990	
5	0.0046	rep 1	5.368	5.223	rep 1	10.283	9.161	rep 1	4.885	4.906
6		rep 2	4.623		rep 2	8.693		rep 2	4.703	
7		rep 3	5.283		rep 3	10.505		rep 3	5.745	
8		rep 4	5.620		rep 4	7.165		rep 4	4.290	
9	0.017	rep 1	4.555	4.064	rep 1	12.800	8.913	rep 1	4.170	3.769
10		rep 2	3.573		rep 2	7.158		rep 2	4.330	
11		rep 3	ND		rep 3	6.783		rep 3	2.808	
12		rep 4	ND		rep 4	ND		rep 4	ND	
13	0.035	rep 1	12.000	18.450	rep 1	21.200	41.214	rep 1	1.852	2.447
14		rep 2	26.300		rep 2	30.300		rep 2	1.213	
15		rep 3	11.300		rep 3	77.800		rep 3	3.096	
16		rep 4	24.200		rep 4	35.556		rep 4	3.627	
17	0.053	rep 1	6.885	6.885	rep 1	341.000	341.000	rep 1	27.500	27.500
18		rep 2	ND		rep 2	ND		rep 2	ND	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
21	0.135	rep 1	104.600	157.475	rep 1	504.000	688.250	rep 1	27.300	35.333
22		rep 2	238.000		rep 2	786.000		rep 2	27.900	
23		rep 3	51.300		rep 3	838.000		rep 3	50.800	
24		rep 4	236.000		rep 4	625.000		rep 4	ND	
25	0.201	rep 1	54.100	78.600	rep 1	407.000	308.450	rep 1	2.964	4.619
26		rep 2	103.400		rep 2	487.000		rep 2	6.274	
27		rep 3	93.600		rep 3	312.000		rep 3	ND	
28		rep 4	63.300		rep 4	27.800		rep 4	ND	
29	0.446	rep 1	47.400	75.300	rep 1	508.000	399.324	rep 1	25.200	31.468
30		rep 2	69.800		rep 2	322.973		rep 2	29.405	
31		rep 3	108.700		rep 3	367.000		rep 3	39.800	
32		rep 4	ND		rep 4	ND		rep 4	ND	

Table D.7 Cadmium content in part of water spinach (dry weight) in cultivation 3 of cadmium uptake under long-term cultivation
(Havesting date September 20, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg wetweight)								
		stem Avarage			root Avarage			leaf Avarage		
0	0	rep 1	1.770	1.770	rep 1	0.475	0.541	rep 1	0.735	1.013
		rep 2	ND		rep 2	0.490		rep 2	1.328	
		rep 3	ND		rep 3	0.663		rep 3	0.713	
		rep 4	ND		rep 4	0.538		rep 4	1.275	
1	0	rep 1	1.451	1.503	rep 1	1.541	2.049	rep 1	1.394	1.394
2		rep 2	1.343		rep 2	1.862		rep 2	ND	
3		rep 3	1.562		rep 3	1.598		rep 3	ND	
4		rep 4	1.656		rep 4	3.197		rep 4	ND	
5	0.0046	rep 1	5.655	4.990	rep 1	16.310	9.983	rep 1	6.125	5.860
6		rep 2	4.185		rep 2	3.655		rep 2	5.160	
7		rep 3	5.180		rep 3	ND		rep 3	5.225	
8		rep 4	4.940		rep 4	ND		rep 4	6.930	
9	0.017	rep 1	22.080	30.450	rep 1	25.710	25.710	rep 1	13.520	15.610
10		rep 2	9.300		rep 2	ND		rep 2	17.700	
11		rep 3	16.280		rep 3	ND		rep 3	ND	
12		rep 4	74.140		rep 4	ND		rep 4	ND	
13	0.035	rep 1	17.800	17.800	rep 1	23.800	23.800	rep 1	12.100	12.100
14		rep 2	ND		rep 2	ND		rep 2	ND	
15		rep 3	ND		rep 3	ND		rep 3	ND	
16		rep 4	ND		rep 4	ND		rep 4	ND	
17	0.053	rep 1	7.700	15.900	rep 1	147.900	90.710	rep 1	16.700	13.900
18		rep 2	24.100		rep 2	33.520		rep 2	11.100	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
21	0.135	rep 1	44.900	44.900	rep 1	140.800	140.800	rep 1	29.700	29.700
22		rep 2	ND		rep 2	ND		rep 2	ND	
23		rep 3	ND		rep 3	ND		rep 3	ND	
24		rep 4	ND		rep 4	ND		rep 4	ND	
25	0.141	rep 1	17.200	20.000	rep 1	170.800	198.267	rep 1	5.700	6.600
26		rep 2	17.000		rep 2	235.000		rep 2	7.600	
27		rep 3	25.800		rep 3	189.000		rep 3	6.500	
28		rep 4	ND		rep 4	ND		rep 4	ND	
29	0.201	rep 1	57.400	57.400	rep 1	107.400	107.400	rep 1	21.600	21.600
30		rep 2	ND		rep 2	ND		rep 2	ND	
31		rep 3	ND		rep 3	ND		rep 3	ND	
32		rep 4	ND		rep 4	ND		rep 4	ND	

Table D.7 Cadmium content in part of water spinach (dry weight) in cultivation 4 of cadmium uptake under long-term cultivation
(Havesting date October 10, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg wetweight)								
		stem	Avarage		root	Avarage		leaf	Avarage	
0	0	rep 1	0.390	0.390	rep 1	0.277	0.277	rep 1	0.504	0.504
		rep 2	ND		rep 2	ND		rep 2	ND	
		rep 3	ND		rep 3	ND		rep 3	ND	
		rep 4	ND		rep 4	ND		rep 4	ND	
1	0	rep 1	0.035	0.035	rep 1	0.105	0.105	rep 1	0.066	0.066
2		rep 2	ND		rep 2	ND		rep 2	ND	
3		rep 3	ND		rep 3	ND		rep 3	ND	
4		rep 4	ND		rep 4	ND		rep 4	ND	
5	0.0046	rep 1	5.117	5.117	rep 1	ND	ND	rep 1	ND	ND
6		rep 2	ND		rep 2	ND		rep 2	ND	
7		rep 3	ND		rep 3	ND		rep 3	ND	
8		rep 4	ND		rep 4	ND		rep 4	ND	
9	0.017	rep 1	3.904	3.904	rep 1	ND	ND	rep 1	ND	ND
10		rep 2	ND		rep 2	ND		rep 2	ND	
11		rep 3	ND		rep 3	ND		rep 3	ND	
12		rep 4	ND		rep 4	ND		rep 4	ND	
13	0.035	rep 1	10.482	10.482	rep 1	ND	ND	rep 1	ND	ND
14		rep 2	ND		rep 2	ND		rep 2	ND	
15		rep 3	ND		rep 3	ND		rep 3	ND	
16		rep 4	ND		rep 4	ND		rep 4	ND	
17	0.053	rep 1	19.600	19.600	rep 1	15.668	15.668	rep 1	10.304	10.304
18		rep 2	ND		rep 2	ND		rep 2	ND	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
21	0.135	rep 1	47.200	47.200	rep 1	11.348	11.348	rep 1	6.331	6.331
22		rep 2	ND		rep 2	ND		rep 2	ND	
23		rep 3	ND		rep 3	ND		rep 3	ND	
24		rep 4	ND		rep 4	ND		rep 4	ND	
25	0.141	rep 1	22.200	25.775	rep 1	53.000	46.450	rep 1	9.293	9.772
26		rep 2	24.000		rep 2	32.500		rep 2	12.230	
27		rep 3	17.100		rep 3	74.500		rep 3	4.367	
28		rep 4	39.800		rep 4	25.800		rep 4	13.198	
29	0.201	rep 1	30.600	30.600	rep 1	ND	ND	rep 1	ND	ND
30		rep 2	ND		rep 2	ND		rep 2	ND	
31		rep 3	ND		rep 3	ND		rep 3	ND	
32		rep 4	ND		rep 4	ND		rep 4	ND	

Table D.7 Cadmium content in part of water spinach (dry weight) in cultivation 5 of cadmium uptake under long-term cultivation
(Havesting date October 30, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg wetweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
0	0	rep 1	0.390	0.390	rep 1	0.277	0.277	rep 1	0.504	0.504
		rep 2	ND		rep 2	ND		rep 2	ND	
		rep 3	ND		rep 3	ND		rep 3	ND	
		rep 4	ND		rep 4	ND		rep 4	ND	
1	0	rep 1	2.025	2.025	rep 1	ND	ND	rep 1	ND	ND
2		rep 2	ND		rep 2	ND		rep 2	ND	
3		rep 3	ND		rep 3	ND		rep 3	ND	
4		rep 4	ND		rep 4	ND		rep 4	ND	
5	0.0046	rep 1	4.955	4.955	rep 1	ND	ND	rep 1	ND	ND
6		rep 2	ND		rep 2	ND		rep 2	ND	
7		rep 3	ND		rep 3	ND		rep 3	ND	
8		rep 4	ND		rep 4	ND		rep 4	ND	
9	0.017	rep 1	4.233	4.233	rep 1	ND	ND	rep 1	ND	ND
10		rep 2	ND		rep 2	ND		rep 2	ND	
11		rep 3	ND		rep 3	ND		rep 3	ND	
12		rep 4	ND		rep 4	ND		rep 4	ND	
13	0.035	rep 1	7.615	7.615	rep 1	ND	ND	rep 1	ND	ND
14		rep 2	ND		rep 2	ND		rep 2	ND	
15		rep 3	ND		rep 3	ND		rep 3	ND	
16		rep 4	ND		rep 4	ND		rep 4	ND	
17	0.053	rep 1	16.855	16.855	rep 1	ND	ND	rep 1	ND	ND
18		rep 2	ND		rep 2	ND		rep 2	ND	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
25	0.141	rep 1	57.900	29.500	rep 1	82.200	46.174	rep 1	29.715	13.935
26		rep 2	23.700		rep 2	10.148		rep 2	6.591	
27		rep 3	35.500		rep 3	ND		rep 3	5.499	
28		rep 4	0.900		rep 4	ND		rep 4	ND	

Table D.8 Cadmium content in part of water spinach (wet weight) in cultivation 1 of cadmium uptake under long-term cultivation
(Havesting date July 30, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg dryweight)								
		stem	Avarage		root	Avarage		leaf	Avarage	
0	0	rep 1	0.11704	0.11704	rep 1	0.08254	0.07059	rep 1	0.06629	0.06429
		rep 2	ND		rep 2	0.08007		rep 2	0.09687	
		rep 3	ND		rep 3	0.05686		rep 3	0.04528	
		rep 4	ND		rep 4	0.06288		rep 4	0.04873	
1	0	rep 1	0.08903	0.10738	rep 1	0.02138	0.06886	rep 1	0.08946	0.09018
2		rep 2	0.12184		rep 2	0.11911		rep 2	0.07646	
3		rep 3	0.11128		rep 3	0.05117		rep 3	0.09407	
4		rep 4	ND		rep 4	0.08375		rep 4	0.10072	
5	0.0046	rep 1	0.14741	0.27328	rep 1	1.35153	1.18608	rep 1	0.49017	0.2511
6		rep 2	0.48968		rep 2	0.65714		rep 2	0.27156	
7		rep 3	0.18669		rep 3	1.54958		rep 3	0.1396	
8		rep 4	0.26933		rep 4	ND		rep 4	0.10306	
9	0.017	rep 1	3.57962	0.42359	rep 1	7.42617	2.7489	rep 1	1.46888	0.40952
10		rep 2	0.43651		rep 2	6.72709		rep 2	0.37487	
11		rep 3	0.36488		rep 3	1.24083		rep 3	0.33912	
12		rep 4	0.46939		rep 4	4.54929		rep 4	0.51456	
13	0.035	rep 1	6.96705	2.15057	rep 1	8.66227	14.4076	rep 1	0.38638	0.43937
14		rep 2	1.62904		rep 2	13.0396		rep 2	0.34391	
15		rep 3	2.95263		rep 3	15.5788		rep 3	0.36385	
16		rep 4	1.87004		rep 4	20.3498		rep 4	0.66334	
17	0.053	rep 1	0.53833	2.99827	rep 1	2.25685	20.4041	rep 1	0.26262	1.14661
18		rep 2	0.8842		rep 2	2.15506		rep 2	1.13783	
19		rep 3	6.63109		rep 3	58.589		rep 3	1.68261	
20		rep 4	3.93946		rep 4	18.6154		rep 4	1.50339	
21	0.135	rep 1	2.86742	2.9908	rep 1	8.16667	24.3943	rep 1	0.26444	1.44246
22		rep 2	2.73358		rep 2	20.4966		rep 2	1.72424	
23		rep 3	3.17178		rep 3	41.8764		rep 3	1.85817	
24		rep 4	3.19044		rep 4	27.0375		rep 4	1.923	
25	0.201	rep 1	2.49396	2.88331	rep 1	19.562	21.2205	rep 1	1.77502	1.37308
26		rep 2	3.13688		rep 2	21.6183		rep 2	1.17961	
27		rep 3	3.01909		rep 3	22.4812		rep 3	1.16461	
28		rep 4	ND		rep 4	ND		rep 4	ND	
29	0.446	rep 1	3.57147	3.99528	rep 1	41.3674	52.032	rep 1	2.51368	2.74779
30		rep 2	4.42508		rep 2	47.3338		rep 2	2.09574	
31		rep 3	5.29049		rep 3	95.3493		rep 3	5.49503	
32		rep 4	2.69407		rep 4	24.0777		rep 4	0.8867	

Table D.8 Cadmium content in part of water spinach (wet weight) in cultivation 2 of cadmium uptake under long-term cultivation
(Havesting date August 30, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg dryweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
0	0	rep 1	0.11704	0.11704	rep 1	0.08254	0.07059	rep 1	0.06629	0.06429
		rep 2	ND		rep 2	0.08007		rep 2	0.09687	
		rep 3	ND		rep 3	0.05686		rep 3	0.04528	
		rep 4	ND		rep 4	0.06288		rep 4	0.04873	
1	0	rep 1	0.15119	0.15478	rep 1	0.15709	0.14964	rep 1	0.15471	0.15162
2		rep 2	0.16867		rep 2	0.16483		rep 2	0.1266	
3		rep 3	0.19716		rep 3	0.10772		rep 3	0.19947	
4		rep 4	0.10209		rep 4	0.1689		rep 4	0.12572	
5	0.0046	rep 1	0.69163	0.54183	rep 1	1.32495	0.94358	rep 1	0.76742	0.78105
6		rep 2	0.47403		rep 2	0.89139		rep 2	0.73133	
7		rep 3	0.39364		rep 3	0.78281		rep 3	0.91641	
8		rep 4	0.60803		rep 4	0.77518		rep 4	0.70904	
9	0.017	rep 1	3.36667	2.26654	rep 1	7.77143	3.66371	rep 1	0.5924	0.5292
10		rep 2	2.49419		rep 2	1.27911		rep 2	0.60556	
11		rep 3	0.93876		rep 3	1.9406		rep 3	0.70502	
12		rep 4	ND		rep 4	ND		rep 4	ND	
13	0.035	rep 1	0.65071	1.73293	rep 1	1.02815	4.23289	rep 1	0.73573	0.87707
14		rep 2	1.62904		rep 2	2.90171		rep 2	0.45129	
15		rep 3	0.50733		rep 3	9.80672		rep 3	1.29	
16		rep 4	1.95033		rep 4	2.04342		rep 4	1.03126	
17	0.053	rep 1	1.72059	1.72059	rep 1	30.5599	30.5599	rep 1	4.33559	4.33559
18		rep 2	ND		rep 2	ND		rep 2	ND	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
21	0.135	rep 1	12.8877	22.0971	rep 1	37.6641	52.7592	rep 1	4.41764	5.84062
22		rep 2	40.5929		rep 2	64.8064		rep 2	4.9953	
23		rep 3	6.54591		rep 3	62.9458		rep 3	8.10892	
24		rep 4	28.362		rep 4	45.6204		rep 4	ND	
25	0.201	rep 1	9.36081	9.06584	rep 1	50.4248	30.465	rep 1	2.96352	4.61863
26		rep 2	9.35122		rep 2	43.3715		rep 2	6.27375	
27		rep 3	10.4734		rep 3	26.1122		rep 3	ND	
28		rep 4	7.07793		rep 4	1.95171		rep 4	ND	
29	0.446	rep 1	4.9146	6.81418	rep 1	34.3696	52.032	rep 1	2.91517	3.37897
30		rep 2	5.67358		rep 2	22.0074		rep 2	2.94048	
31		rep 3	9.85436		rep 3	48.9333		rep 3	4.28127	
32		rep 4	ND		rep 4	ND		rep 4	ND	

Table D.8 Cadmium content in part of water spinach (wet weight) in cultivation 3 of cadmium uptake under long-term cultivation
(Havesting date September 20, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg dryweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
0	0	rep 1	0.11704	0.11704	rep 1	0.08254	0.07059	rep 1	0.06629	0.06429
		rep 2	ND		rep 2	0.08007		rep 2	0.09687	
		rep 3	ND		rep 3	0.05686		rep 3	0.04528	
		rep 4	ND		rep 4	0.06288		rep 4	0.04873	
1	0	rep 1	0.15889	0.17702	rep 1	0.07934	0.10543	rep 1	0.14465	0.14465
2		rep 2	0.15072		rep 2	0.08378		rep 2	ND	
3		rep 3	0.24744		rep 3	0.09361		rep 3	ND	
4		rep 4	0.15104		rep 4	0.16501		rep 4	ND	
5	0.0046	rep 1	0.40393	0.4323	rep 1	1.18884	0.99663	rep 1	1.36111	0.84986
6		rep 2	0.36273		rep 2	0.80442		rep 2	0.60917	
7		rep 3	0.4219		rep 3	ND		rep 3	0.51733	
8		rep 4	0.54063		rep 4	ND		rep 4	0.91184	
9	0.017	rep 1	1.60145	1.3842	rep 1	2.04168	8.64281	rep 1	1.51056	1.84668
10		rep 2	0.87945		rep 2	ND		rep 2	2.18279	
11		rep 3	1.31477		rep 3	ND		rep 3	ND	
12		rep 4	6.83003		rep 4	ND		rep 4	ND	
13	0.035	rep 1	1.91428	1.91428	rep 1	5.28159	5.28159	rep 1	2.20468	2.20468
14		rep 2	ND		rep 2	ND		rep 2	ND	
15		rep 3	ND		rep 3	ND		rep 3	ND	
16		rep 4	ND		rep 4	ND		rep 4	ND	
17	0.053	rep 1	7.69486	5.21273	rep 1	9.38534	5.77106	rep 1	2.63462	3.705
18		rep 2	2.73059		rep 2	2.15678		rep 2	4.77538	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
21	0.135	rep 1	4.77763	4.77763	rep 1	6.92023	6.92023	rep 1	3.21126	3.21126
22		rep 2	ND		rep 2	ND		rep 2	ND	
23		rep 3	ND		rep 3	ND		rep 3	ND	
24		rep 4	ND		rep 4	ND		rep 4	ND	
25	0.141	rep 1	1.61217	1.7763	rep 1	11.626	13.6938	rep 1	0.8241	0.88735
26		rep 2	1.64883		rep 2	15.899		rep 2	1.01851	
27		rep 3	2.06791		rep 3	13.5563		rep 3	0.81944	
28		rep 4	ND		rep 4	ND		rep 4	ND	
29	0.201	rep 1	5.22214	5.22214	rep 1	5.35661	5.35661	rep 1	2.28014	2.28014
30		rep 2	ND		rep 2	ND		rep 2	ND	
31		rep 3	ND		rep 3	ND		rep 3	ND	
32		rep 4	ND		rep 4	ND		rep 4	ND	

Table D.8 Cadmium content in part of water spinach (wet weight) in cultivation 4 of cadmium uptake under long-term cultivation
(Havesting date October 10, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg dryweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
0	0	rep 1	0.11704	0.11704	rep 1	0.08254	0.07059	rep 1	0.06629	0.06429
		rep 2	ND		rep 2	0.08007		rep 2	0.09687	
		rep 3	ND		rep 3	0.05686		rep 3	0.04528	
		rep 4	ND		rep 4	0.06288		rep 4	0.04873	
1	0	rep 1	0.03463	0.03463	rep 1	0.10526	0.10526	rep 1	0.06564	0.06564
2		rep 2	ND		rep 2	ND		rep 2	ND	
3		rep 3	ND		rep 3	ND		rep 3	ND	
4		rep 4	ND		rep 4	ND		rep 4	ND	
5	0.0046	rep 1	0.50747	0.50747	rep 1	ND	ND	rep 1	ND	ND
6		rep 2	ND		rep 2	ND		rep 2	ND	
7		rep 3	ND		rep 3	ND		rep 3	ND	
8		rep 4	ND		rep 4	ND		rep 4	ND	
9	0.017	rep 1	0.47379	0.47379	rep 1	ND	ND	rep 1	ND	ND
10		rep 2	ND		rep 2	ND		rep 2	ND	
11		rep 3	ND		rep 3	ND		rep 3	ND	
12		rep 4	ND		rep 4	ND		rep 4	ND	
13	0.035	rep 1	0.8735	0.8735	rep 1	ND	ND	rep 1	ND	ND
14		rep 2	ND		rep 2	ND		rep 2	ND	
15		rep 3	ND		rep 3	ND		rep 3	ND	
16		rep 4	ND		rep 4	ND		rep 4	ND	
17	0.053	rep 1	2.26625	2.26625	rep 1	2.53664	2.53664	rep 1	1.48162	1.48162
18		rep 2	ND		rep 2	ND		rep 2	ND	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
21	0.135	rep 1	5.07107	5.07107	rep 1	1.45896	1.45896	rep 1	0.52214	0.52214
22		rep 2	ND		rep 2	ND		rep 2	ND	
23		rep 3	ND		rep 3	ND		rep 3	ND	
24		rep 4	ND		rep 4	ND		rep 4	ND	
25	0.141	rep 1	1.83276	2.10367	rep 1	2.81659	3.71901	rep 1	2.38591	1.54428
26		rep 2	2.18978		rep 2	1.89404		rep 2	1.03404	
27		rep 3	3.48433		rep 3	6.27557		rep 3	0.38771	
28		rep 4	0.9078		rep 4	3.88985		rep 4	1.48077	
29	0.201	rep 1	3.67975	3.67975	rep 1	ND	ND	rep 1	ND	ND
30		rep 2	ND		rep 2	ND		rep 2	ND	
31		rep 3	ND		rep 3	ND		rep 3	ND	
32		rep 4	ND		rep 4	ND		rep 4	ND	

Table D.8 Cadmium content in part of water spinach (wet weight) in cultivation 5 of cadmium uptake under long-term cultivation
(Havesting date October 30, 2005)

plot number	Actual con of water (mg/L)	Cadmium content (mg/kg dryweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
0	0	rep 1	0.11704	0.11704	rep 1	ND	ND	rep 1	ND	ND
		rep 2	ND		rep 2	ND		rep 2	ND	
		rep 3	ND		rep 3	ND		rep 3	ND	
		rep 4	ND		rep 4	ND		rep 4	ND	
1	0	rep 1	0.19626	0.19626	rep 1	ND	ND	rep 1	ND	ND
2		rep 2	ND		rep 2	ND		rep 2	ND	
3		rep 3	ND		rep 3	ND		rep 3	ND	
4		rep 4	ND		rep 4	ND		rep 4	ND	
5	0.0046	rep 1	0.59687	0.59687	rep 1	ND	ND	rep 1	ND	ND
6		rep 2	ND		rep 2	ND		rep 2	ND	
7		rep 3	ND		rep 3	ND		rep 3	ND	
8		rep 4	ND		rep 4	ND		rep 4	ND	
9	0.017	rep 1	0.59729	0.59729	rep 1	ND	ND	rep 1	ND	ND
10		rep 2	ND		rep 2	ND		rep 2	ND	
11		rep 3	ND		rep 3	ND		rep 3	ND	
12		rep 4	ND		rep 4	ND		rep 4	ND	
13	0.035	rep 1	0.65997	0.65997	rep 1	ND	ND	rep 1	ND	ND
14		rep 2	ND		rep 2	ND		rep 2	ND	
15		rep 3	ND		rep 3	ND		rep 3	ND	
16		rep 4	ND		rep 4	ND		rep 4	ND	
17	0.053	rep 1	1.58686	1.58686	rep 1	ND	ND	rep 1	ND	ND
18		rep 2	ND		rep 2	ND		rep 2	ND	
19		rep 3	ND		rep 3	ND		rep 3	ND	
20		rep 4	ND		rep 4	ND		rep 4	ND	
25	0.141	rep 1	4.2995	2.64204	rep 1	7.36358	4.55389	rep 1	3.77003	1.7855
26		rep 2	2.41602		rep 2	1.375		rep 2	0.79671	
27		rep 3	3.85013		rep 3	ND		rep 3	0.78975	
28		rep 4	0.0942		rep 4	ND		rep 4	ND	

Table D.9 Yield of water spinach grown in the cadmium uptake under long term cultivation

Plot group	Actual con in water (mg/L)	Replication	Total weight before cultivation (gram)	Total weight after cultivation* (gram)				
				cultivation 1	cultivation 2	cultivation 3	cultivation 4	cultivation 5
1	0	1	233.95	104.54	54.75	38.29	7.53	9.08
	0	2	201.77	56.86	72.99	33.13	ND	ND
	0	3	268.74	103.34	37.71	27.29	ND	ND
	0	4	205.78	62.37	85.58	47.37	ND	ND
2	0.00466	1	205.05	64.62	34.48	14.11	6.05	5.23
	0.00466	2	206.73	61.66	35.84	25.63	ND	ND
	0.00466	3	200.24	74.52	31.03	23.60	ND	ND
	0.00466	4	203.60	85.05	41.12	40.57	ND	ND
3	0.01718	1	222.56	53.46	34.20	15.28	4.12	4.89
	0.01718	2	243.05	53.94	56.93	31.30	ND	ND
	0.01718	3	240.89	81.46	54.89	19.98	ND	ND
	0.01718	4	207.38	70.61	58.24	24.63	ND	ND
4	0.03485	1	202.46	96.98	38.23	35.51	3.72	1.50
	0.03485	2	212.85	96.08	38.48	36.62	ND	ND
	0.03485	3	201.71	69.34	20.96	6.59	ND	ND
	0.03485	4	212.08	68.93	23.38	1.59	ND	ND
5	0.05329	1	203.35	71.33	ND	7.93	25.17	3.93
	0.05329	2	201.80	95.71	44.74	61.82	ND	ND
	0.05329	3	201.85	55.18	ND	4.29	ND	ND
	0.05329	4	204.29	39.95	ND	19.20	ND	ND
6	0.13523	1	203.30	43.49	26.20	5.79	18.57	death
	0.13523	2	201.94	44.71	33.11	36.19	ND	death
	0.13523	3	204.56	65.64	35.46	1.93	ND	death
	0.13523	4	203.27	61.82	21.26	1.17	ND	death
7	0.14079	1	505.46	ND	ND	135.08	60.63	223.62
	0.14079	2	506.10	ND	ND	107.06	24.25	6.82

Remark * stems, leaves and roots in designated area

Table D.9 Yield of water spinach grown in the cadmium uptake under long term cultivation (cont.)

Plot group	Actual con in water (mg/L)	Replication	Total weight before cultivation (gram)	Total weight after cultivation* (gram)				
				cultivation 1	cultivation 2	cultivation 3	cultivation 4	cultivation 5
7	0.14079	3	517.47	ND	ND	158.80	31.19	34.73
	0.14079	4	529.21	ND	ND	107.52	42.13	12.39
8	0.20095	1	213.27	67.41	32.85	40.35	1.58	death
	0.20095	2	205.65	66.50	31.09	1.41	ND	death
	0.20095	3	211.99	67.97	45.53	2.79	ND	death
	0.20095	4	209.70	ND	41.04	7.19	ND	death
9	0.44597	1	213.65	57.37	110.97	death	death	death
	0.44597	2	221.31	47.09	17.28	death	death	death
	0.44597	3	200.74	58.91	90.25	death	death	death
	0.44597	4	202.39	53.10	30.12	death	death	death

Remark * stems, leaves and roots in designated area

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APPENDIX E

Cadmium uptake under shock condition

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Table E.1 pH of water in cadmium uptake under shock load condition cultivation.

Sampling time (days)	Cadmium concentration					
	G1 0 day	G2 1 day	G3 3 day	G4 5 day	G5 7 day	G6 9 day
0	7.23	7.25	7.63	7.46	7.12	7.05
6	8.35	8.25	8.64	8.57	7.79	7.80
8	7.22	7.36	7.45	7.16	7.14	7.23
10	8.32	7.22	7.26	6.69	6.98	7.05
11	7.21	7.25	7.32	7.01	7.23	7.15
12	7.50	7.42	7.64	7.42	7.30	7.25
14	6.89	6.78	7.62	8.65	7.46	7.32
18	8.11	7.90	7.97	7.87	8.64	7.78
20	6.98	8.52	6.06	6.15	6.32	6.45
Average	7.53	7.55	7.51	7.44	7.33	7.23
SD.	0.57	0.56	0.68	0.82	0.63	0.41

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Table E.2 Yield of water spinach grown in the cadmium uptake under shock load condition

Group study	Day in shock con.	Total weight before cultivation weight (gram)				Total weight after cultivation weight (gram)			
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
1	0	275.26	259.30	263.25	276.60	280.40	360.15	240.65	320.24
2	1	286.13	270.31	250.01	286.30	280.23	280.46	100.45	120.97
3	3	262.72	286.97	284.94	264.47	120.98	240.32	180.63	240.32
4	5	288.00	252.70	250.03	270.30	220.22	200.01	220.03	200.96
5	7	268.77	286.74	291.80	269.08	320.41	300.23	240.67	280.95
6	9	282.92	269.52	263.23	298.24	260.35	300.43	340.54	280.56

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Table E.3 Cadmium content in part of water spinach (wet weight) of cadmium uptake under shock load condition (harvesting date November, 20 2005)

plot number	group (day)	Cadmium content (mg/kg wetweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
1	1	rep 1	0.027	0.049	rep 1	0.113	0.099	rep 1	0.081	0.047
2	(0 day)	rep 2	0.063		rep 2	0.008		rep 2	0.033	
3		rep 3	0.051		rep 3	0.037		rep 3	0.026	
4		rep 4	0.054		rep 4	0.239		rep 4	0.049	
5	2	rep 1	0.395	0.635	rep 1	0.458	0.301	rep 1	0.674	0.476
6	(1 day)	rep 2	0.502		rep 2	0.326		rep 2	0.663	
7		rep 3	0.923		rep 3	0.037		rep 3	0.305	
8		rep 4	0.717		rep 4	0.382		rep 4	0.261	
9	3	rep 1	0.366	0.338	rep 1	2.748	2.119	rep 1	0.454	0.555
10	(3 day)	rep 2	0.014		rep 2	2.460		rep 2	0.380	
11		rep 3	0.422		rep 3	2.492		rep 3	0.559	
12		rep 4	0.551		rep 4	1.692		rep 4	0.826	
13	4	rep 1	0.807	0.635	rep 1	5.547	3.940	rep 1	0.393	0.382
14	(5 day)	rep 2	1.014		rep 2	5.193		rep 2	0.557	
15		rep 3	0.069		rep 3	2.376		rep 3	0.197	
16		rep 4	0.650		rep 4	2.643		rep 4	0.381	
17	5	rep 1	1.104	1.181	rep 1	4.294	4.260	rep 1	0.806	0.787
18	(7day)	rep 2	1.055		rep 2	4.245		rep 2	0.078	
19		rep 3	1.179		rep 3	4.243		rep 3	1.611	
20		rep 4	1.388		rep 4	0.476		rep 4	0.652	
21	6	rep 1	0.037	0.848	rep 1	5.961	6.913	rep 1	1.153	0.833
22	(9 day)	rep 2	0.444		rep 2	8.201		rep 2	1.362	
23		rep 3	0.671		rep 3	7.227		rep 3	1.051	
24		rep 4	0.657		rep 4	6.263		rep 4	1.000	

Table E.4 Cadmium content in part of water spinach (dry weight) of cadmium uptake under shock load condition (harvesting date November, 20 2005)

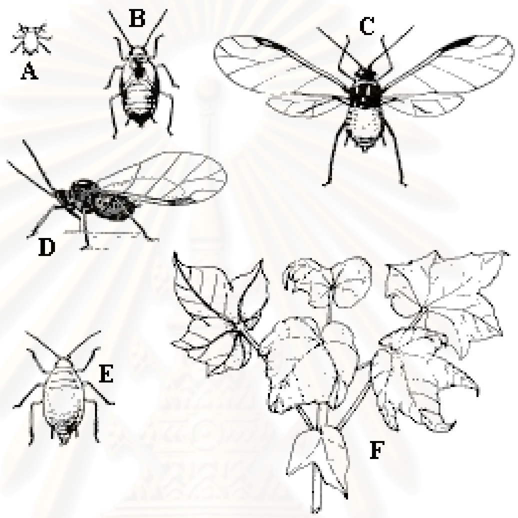
plot number	group (day)	Cadmium content (mg/kg dryweight)								
		stem		Avarage	root		Avarage	leaf		Avarage
1	1	rep 1	0.150	0.279	rep 1	0.686	0.578	rep 1	0.298	0.183
2	(0 day)	rep 2	0.362		rep 2	0.053		rep 2	0.143	
3		rep 3	0.307		rep 3	0.230		rep 3	0.102	
4		rep 4	0.298		rep 4	1.343		rep 4	0.188	
5	2	rep 1	2.601	3.467	rep 1	2.591	2.591	rep 1	2.846	1.964
6	(1 day)	rep 2	3.068		rep 2	2.489		rep 2	2.858	
7		rep 3	4.667		rep 3	2.694		rep 3	1.184	
8		rep 4	3.533		rep 4	ND		rep 4	0.970	
9	3	rep 1	2.452	2.744	rep 1	27.600	24.475	rep 1	1.846	2.180
10	(3 day)	rep 2	2.569		rep 2	28.600		rep 2	1.563	
11		rep 3	3.211		rep 3	25.000		rep 3	2.483	
12		rep 4	ND		rep.4	16.700		rep 4	2.827	
13	4	rep 1	6.385	4.137	rep 1	60.500	44.675	rep 1	1.511	1.561
14	(5 day)	rep 2	6.009		rep 2	49.300		rep 2	2.080	
15		rep 3	0.262		rep 3	32.900		rep 3	1.240	
16		rep 4	3.894		rep 4	36.000		rep 4	1.412	
17	5	rep 1	17.900	11.765	rep 1	60.000	48.350	rep 1	7.500	6.075
18	(7day)	rep 2	3.643		rep 2	61.000		rep 2	6.600	
19		rep 3	6.916		rep 3	65.700		rep 3	4.000	
20		rep 4	18.600		rep 4	6.700		rep 4	6.200	
21	6	rep 1	0.568	6.188	rep 1	81.200	102.975	rep 1	4.239	4.589
22	(9 day)	rep 2	5.685		rep 2	106.200		rep 2	5.401	
23		rep 3	10.238		rep 3	123.800		rep 3	4.904	
24		rep 4	8.263		rep 4	100.700		rep 4	3.811	

APPENDIX F

Pests

Cotton aphis

Common name : Cotton aphis
Scientific name : *Aphis gossypii Glov*.
Family : Aphididae
Order : Homoptera



Cotton aphid. A-B, Nymphs. C-E, Adults. F, Damage.

Figure D1: The picture of Cotton aphid
(Adapted from: http://ipm.ncsu.edu/AG271/cotton/aphids.html)

DESCRIPTION

Adult - Several species of aphids attack cotton but the cotton aphid and the cowpea aphid are most common. Adult aphids are about 2 mm long and may be winged or wingless. The cotton aphid is pale to dark green in cool seasons and yellow in hot, dry summers. The adult cowpea aphid is shiny black with white appendages.

Nymph - Cotton aphid nymphs are smaller than but similar in shape and color to the wingless adults. Cowpea aphid nymphs are pale green to gray with a powdery coating.

BIOLOGY

Distribution - Generally distributed throughout temperate, subtropic, and tropic zones.

Host plants - The cotton aphid infests cotton, okra, hibiscus, cowpea, citrus, cucurbits, strawberry, bean, spinach, tomato, clover, asparagus, catalpa, violet, hydrangea, begonia, ground ivy, gardenia, and several weeds. Host plants of the cowpea aphid include alfalfa, apple, carrot, cotton, cowpea, dandelion, dock, goldenrod, kidney bean, lambsquarters, lettuce, lima bean, pinto bean, peanut, pepperweed, pigweed, red clover, shepherds purse, vetch, wheat, white sweet clover, and yellow sweet clover.

Damage - In spite of the fact that many aphids infest cotton, these insects are only secondary pests. Congregating on lower leaf surfaces and on terminal buds, aphids extract plant sap. If weather is cool during the spring, populations of natural enemies will be slow in building up and heavy infestations of aphids may result. When this occurs, leaves begin to curl and pucker; seedling plants become stunted and may die. Most aphid damage is of this type. If honeydew resulting from late season aphid infestations falls onto open cotton, it can act as a growing medium for sooty mold. Cotton stained by this black fungus is reduced in quality and brings a low price for the grower.

Life history - Little information is available on the biology of the cowpea aphid although the life history of the cotton aphid is well known. Cotton aphids over winter as adults in the cotton belt, and as eggs in cooler climates. In the cotton belt, winged adults hibernate in soil or field debris. During warm periods, they fly to weedy hosts, and continue their life cycle until cool weather forces them back into hibernation. In spring, winged females fly to suitable host plants and give birth to living young. In the cotton belt, all progeny generally develop into wingless females. Whenever crowding occurs or food becomes scarce, winged adults develop and fly to new host plants. Male and female adults occur in northern states.

Females produce an average of 84 nymphs. Under favorable conditions, a nymph will mature within 4 or 5 days and begin producing its own progeny. In the South, reproduction may continue all winter so that as many as 57 generations may be produced each year.

Cut worm

Common name : Tobacco cutworm, cotton worm, cotton leaf worm.

Scientific name : *Spodotera litura Fabricius*.

Family : Noctuidae

Order : Lepidoptera

Figure D3 : The picture of Cut worm
(Adapted from http://www.usyd.edu.au/macleay/larvae/acro/litura.html)

They eat whole leaves, and even flowers and fruit. They become brown with three thin yellow lines down the back: one in the middle and one each side. A row of black dots run along each side, and a conspicuous row of dark triangles decorate each side of the back.

Host plants are from over 40 families, such as : Allium (Alliaceae); Mangifera (Anacardiaceae) and Ipomoae (Convolvulaceae).

APPENDIX G

Solidification/ Stabilization

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Solidification/stabilization

Solidification/stabilization (S/S) techniques are akin to locking the contaminants in the soil. It is a process that physically encapsulates the contaminant. This technique can be used alone or combined with other treatment and disposal methods.

The most common form of S/S is a cement process. It simply involves the addition of cement or a cement-based mixture, which thereby limits the solubility or mobility of the waste constituents. These techniques are accomplished either in-situ, by injecting a cement based agent into the contaminated materials or ex situ, by excavating the materials, machine-mixing them with a cement-based agent, and depositing the solidified mass in a designated area. The goal of the S/S process is to limit the spread, via leaching, of contaminated material. The end product resulting from the solidification process is a monolithic block of waste with high structural integrity. Types of solidifying/stabilizing agents include the following: Portland; gypsum; modified sulfur cement, consisting of elemental sulfur and hydrocarbon polymers; and grout, consisting of cement and other dry materials, such as acceptable fly ash or blast furnace slag. Processes utilizing modified sulfur cement are typically performed ex situ. (http://www.cpeo.org/techtree/ttdescript/solidsta.htm)

Cadmium can be a potential threat to wide range of biota because it is non-essential and non-metabolic, it is toxic to humans at concentration lover than those toxic to plants, it more mobile and bioavailability than other metals, and its effect on humans are accumulative.

After this experiments, cadmium contaminated water was mixed with lime to pH 12 precipitated as cadmium hydroxide in sludge forms. The method selected for hazardous waste treatment was solidification by using Portland cement.

Materials

1. Portland cement
2. Sand
3. Sediment
4. Trowel
5. Plastic bowl 45 L

Procedure

1. Mix the sludge to be homogeneous.
2. Mix the Portland cement and sand together in 45 L plastic bowls.
3. Slowly pour the homogeneous sediment into mix ingredient of sand and Portland.
4. Slowly mix all ingredients to be homogeneous.
5. Let it setting.
6. String solidification waste in laboratory for future disposed.

Figure E1: The procedure of solidification

APPENDIX H

Statistical Data

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H1 Statistical analysis for the yield of water spinach grown in cadmium uptake under long term condition

One way

Descriptives

V3

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Maximum	Minimum
					Lower Bound	Upper Bound		
1	3	-49.8833	45.98475	26.54931	-164.1158	64.3491	-100.40	-10.46
2	4	-68.0275	21.45039	10.72520	-102.1599	-33.8951	-83.60	-36.86
3	4	-87.4875	24.83747	12.41873	-127.0095	-47.9655	-110.61	-53.90
4	4	-72.7975	45.44425	22.72213	-145.1094	-.4856	-118.18	-26.52
5	4	-95.5100	68.00089	34.00045	-203.7146	12.6946	-145.14	.47
6	4	-104.4325	13.12454	6.56227	-125.3166	-83.5484	-119.02	-87.93
7	4	-278.5050	133.01194	66.50597	-490.1567	-66.8533	-367.97	-86.13
8	4	-108.7250	38.18000	19.09000	-169.4779	-47.9721	-161.47	-71.08
9	4	-93.2500	54.04595	27.02298	-179.2492	-7.2508	-156.94	-45.31
Total	35	-108.1311	83.18224	14.06037	-136.7052	-79.5570	-367.97	.47

ANOVA

V3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	140998.238	8	17624.780	4.862	.001
Within Groups	94257.479	26	3625.288		
Total	235255.717	34			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: V3
Scheffe

(I) V2	(J) V2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
1	2	18.1442	45.98642	1.000	-179.9939	216.2823
	3	37.6042	45.98642	.999	-160.5339	235.7423
	4	22.9142	45.98642	1.000	-175.2239	221.0523
	5	45.6267	45.98642	.998	-152.5114	243.7648
	6	54.5492	45.98642	.992	-143.5889	252.6873
	7	228.6217	45.98642	.014	30.4836	426.7598
	8	58.8417	45.98642	.987	-139.2964	256.9798
	9	43.3667	45.98642	.998	-154.7714	241.5048
2	1	-18.1442	45.98642	1.000	-216.2823	179.9939
	3	19.4600	42.57516	1.000	-163.9802	202.9002
	4	4.7700	42.57516	1.000	-178.6702	188.2102
	5	27.4825	42.57516	1.000	-155.9577	210.9227
	6	36.4050	42.57516	.999	-147.0352	219.8452
	7	210.4775	42.57516	.015	27.0373	393.9177
	8	40.6975	42.57516	.998	-142.7427	224.1377
	9	25.2225	42.57516	1.000	-158.2177	208.6627
3	1	-37.6042	45.98642	.999	-235.7423	160.5339
	2	-19.4600	42.57516	1.000	-202.9002	163.9802
	4	-14.6900	42.57516	1.000	-198.1302	168.7502
	5	8.0225	42.57516	1.000	-175.4177	191.4627
	6	16.9450	42.57516	1.000	-166.4952	200.3852
	7	191.0175	42.57516	.036	7.5773	374.4577
	8	21.2375	42.57516	1.000	-162.2027	204.6777
	9	5.7625	42.57516	1.000	-177.6777	189.2027
4	1	-22.9142	45.98642	1.000	-221.0523	175.2239
	2	-4.7700	42.57516	1.000	-188.2102	178.6702
	3	14.6900	42.57516	1.000	-168.7502	198.1302
	5	22.7125	42.57516	1.000	-160.7277	206.1527
	6	31.6350	42.57516	1.000	-151.8052	215.0752
	7	205.7075	42.57516	.018	22.2673	389.1477
	8	35.9275	42.57516	.999	-147.5127	219.3677

	9	20.4525	42.57516	1.000	-162.9877	203.8927
5	1	-45.6267	45.98642	.998	-243.7648	152.5114
	2	-27.4825	42.57516	1.000	-210.9227	155.9577
	3	-8.0225	42.57516	1.000	-191.4627	175.4177
	4	-22.7125	42.57516	1.000	-206.1527	160.7277
	6	8.9225	42.57516	1.000	-174.5177	192.3627
	7	182.9950	42.57516	.051	-.4452	366.4352
	8	13.2150	42.57516	1.000	-170.2252	196.6552
	9	-2.2600	42.57516	1.000	-185.7002	181.1802
6	1	-54.5492	45.98642	.992	-252.6873	143.5889
	2	-36.4050	42.57516	.999	-219.8452	147.0352
	3	-16.9450	42.57516	1.000	-200.3852	166.4952
	4	-31.6350	42.57516	1.000	-215.0752	151.8052
	5	-8.9225	42.57516	1.000	-192.3627	174.5177
	7	174.0725	42.57516	.074	-9.3677	357.5127
	8	4.2925	42.57516	1.000	-179.1477	187.7327
	9	-11.1825	42.57516	1.000	-194.6227	172.2577
7	1	-228.6217	45.98642	.014	-426.7598	-30.4836
	2	-210.4775	42.57516	.015	-393.9177	-27.0373
	3	-191.0175	42.57516	.036	-374.4577	-7.5773
	4	-205.7075	42.57516	.018	-389.1477	-22.2673
	5	-182.9950	42.57516	.051	-366.4352	.4452
	6	-174.0725	42.57516	.074	-357.5127	9.3677
	8	-169.7800	42.57516	.089	-353.2202	13.6602
	9	-185.2550	42.57516	.046	-368.6952	-1.8148
8	1	-58.8417	45.98642	.987	-256.9798	139.2964
	2	-40.6975	42.57516	.998	-224.1377	142.7427
	3	-21.2375	42.57516	1.000	-204.6777	162.2027
	4	-35.9275	42.57516	.999	-219.3677	147.5127
	5	-13.2150	42.57516	1.000	-196.6552	170.2252
	6	-4.2925	42.57516	1.000	-187.7327	179.1477
	7	169.7800	42.57516	.089	-13.6602	353.2202
	9	-15.4750	42.57516	1.000	-198.9152	167.9652
9	1	-43.3667	45.98642	.998	-241.5048	154.7714
	2	-25.2225	42.57516	1.000	-208.6627	158.2177
	3	-5.7625	42.57516	1.000	-189.2027	177.6777
	4	-20.4525	42.57516	1.000	-203.8927	162.9877
	5	2.2600	42.57516	1.000	-181.1802	185.7002
	6	11.1825	42.57516	1.000	-172.2577	194.6227
	7	185.2550	42.57516	.046	1.8148	368.6952
	8	15.4750	42.57516	1.000	-167.9652	198.9152

* The mean difference is significant at the .05 level.

Homogeneous Subsets

V3

Scheffe

V2	N	Subset for alpha = 0.05	
		1	2
7	4	-278.5050	
8	4	-108.7250	-108.7250
6	4	-104.4325	-104.4325
5	4	-95.5100	-95.5100
9	4	-93.2500	-93.2500
3	4		-87.4875
4	4		-72.7975
2	4		-68.0275
1	3		-49.8833
Sig.		0.053	0.982

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.857.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

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H2 Statistical analysis for the yield of water spinach grown in cadmium uptake under shock load condition

Oneway

Descriptives

V2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	40.283	62.0337	35.8152	-113.817	194.384	-23.3	100.7
2	4	-78.188	92.8027	46.4013	-225.857	69.482	-166.3	9.7
3	4	-79.775	53.9449	26.9725	-165.613	6.063	-142.7	-24.5
4	4	-55.258	18.5438	9.2719	-84.765	-25.750	-70.3	-30.0
5	4	5.903	42.6750	21.3375	-62.003	73.808	-51.8	51.2
6	4	16.523	46.8640	23.4320	-58.049	91.094	-22.9	76.8
Total	23	-27.927	69.2923	14.4484	-57.892	2.037	-166.3	100.7

ANOVA

V2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50283.878	5	10056.776	3.089	.037
Within Groups	55347.355	17	3255.727		
Total	105631.233	22			

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Post Hoc Tests

Multiple Comparisons

Dependent Variable: V2
Scheffe

(I) V1	(J) V1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	118.471	43.5795	.248	-44.880	281.821
	3	120.058	43.5795	.236	-43.292	283.409
	4	95.541	43.5795	.468	-67.810	258.891
	5	34.381	43.5795	.985	-128.970	197.731
	6	23.761	43.5795	.997	-139.590	187.111
2	1	-118.471	43.5795	.248	-281.821	44.880
	3	1.588	40.3468	1.000	-149.646	152.821
	4	-22.930	40.3468	.997	-174.163	128.303
	5	-84.090	40.3468	.522	-235.323	67.143
	6	-94.710	40.3468	.395	-245.943	56.523
3	1	-120.058	43.5795	.236	-283.409	43.292
	2	-1.588	40.3468	1.000	-152.821	149.646
	4	-24.518	40.3468	.995	-175.751	126.716
	5	-85.678	40.3468	.502	-236.911	65.556
	6	-96.298	40.3468	.378	-247.531	54.936
4	1	-95.541	43.5795	.468	-258.891	67.810
	2	22.930	40.3468	.997	-128.303	174.163
	3	24.518	40.3468	.995	-126.716	175.751
	5	-61.160	40.3468	.801	-212.393	90.073
	6	-71.780	40.3468	.677	-223.013	79.453
5	1	-34.381	43.5795	.985	-197.731	128.970
	2	84.090	40.3468	.522	-67.143	235.323
	3	85.678	40.3468	.502	-65.556	236.911
	4	61.160	40.3468	.801	-90.073	212.393
	6	-10.620	40.3468	1.000	-161.853	140.613
6	1	-23.761	43.5795	.997	-187.111	139.590
	2	94.710	40.3468	.395	-56.523	245.943
	3	96.298	40.3468	.378	-54.936	247.531
	4	71.780	40.3468	.677	-79.453	223.013
	5	10.620	40.3468	1.000	-140.613	161.853

Homogeneous Subsets

V2

Scheffe

V1	N	Subset for alpha = 0.05
		1
3	4	-79.775
2	4	-78.188
4	4	-55.258
5	4	5.903
6	4	16.523
1	3	40.283
Sig.		.194

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.789.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

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