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CHROMIUM REMOVAL BY PHYTOREMEDIATION AND BIOSORPTION

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การทดสอบความสามารถของการใช้วัชพืชที่พบในประเทศไทย เป็นการศึกษาการใช้เทคโนโลยีในการฟื้นฟูสภาพพื้นที่ที่ปนเปื้อนโครเมียมในดินและน้ำ การศึกษาเริ่มด้วยการสำรวจบริเวณพื้นที่โรงงานฟอกหนัง เพื่อคัดเลือกวัชพืชที่มีความสามารถบนพื้นฐานการสะสมโครเมียมสูงสุด วัชพืชที่คัดเลือกได้แก่ หญ้าแพรก ต้นขลู่ ต้นก้างปลา หญ้าข้าวนก หญ้าแฝก และต้นผักขม โดยนำวัชพืชดังกล่าวปลูกใส่กระถางไว้ในเรือนเพาะชำ และใส่เฮกซะวาเลนท์โครเมียมที่ระดับความเข้มข้น 100 มก.เฮกซะวาเลนท์โครเมียม/กก.ดินพบว่า วัชพืชมีการสะสมโครเมียมเท่ากับ 152.1, 151.8, 101, 77, 69 และ 0 มก./กก. ตามลำดับ ภายหลังจากใส่เฮกซะวาเลนท์โครเมียม 30 วัน พบว่า ต้นขลู่มีการสะสมเฮกซะวาเลนท์โครเมียมที่ราก ต้น และใบ เท่ากับ 29, 35 และ 73 มก./กก. ในขณะที่หญ้าแพรกมีการสะสมเฮกซะวาเลนท์โครเมียมที่ราก ต้น และใบ เท่ากับ 38, 18 และ 0 มก./กก.ของน้ำหนักพืชแห้ง ตามลำดับ หลังจากนั้นได้นำวัชพืชดังกล่าวในรูปของมวลชีวภาพมาทำการศึกษาบนแบตช์ไอโซเทอมและการศึกษาบนคอลัมน์ โดยใช้น้ำเสียสังเคราะห์ที่มีความเข้มข้นของเฮกซะวาเลนท์โครเมียมเท่ากับ 50 มก./ล.พบว่า ใบของต้นก้างปลา ต้นขลู่ และหญ้าแพรกมีความสามารถในการดูดซับเฮกซะวาเลนท์โครเมียมสูงสุดเท่ากับ 53, 45 และ 37 มก./ก.มวลชีวภาพ ที่พีเอช 2 ณ เวลาสมดุลที่ 24 ชม. นอกจากนี้ยังพบว่า ใบของมวลชีวภาพของวัชพืชทุกชนิดมีความสามารถในการดูดซับโครเมียมดีที่สุด โดยเฉพาะใบของต้นขลู่มีความสามารถในการดูดซับเฮกซะวาเลนท์โครเมียมสูงสุดเท่ากับ 51.3 มก./ก.มวลชีวภาพที่พีเอช 2 ณ เวลาสมดุลที่ 102 ชม. อัตราการไหล 1.3 มล./นาที อย่างไรก็ตาม เมื่อเปรียบเทียบความสามารถในการกำจัดเฮกซะวาเลนท์โครเมียมจากวัชพืชในดินและมวลชีวภาพในน้ำพบว่า ใบของต้นขลู่มีความสามารถในการสะสมและดูดซับได้ดีกว่าส่วนของรากและต้นของวัชพืชและมวลชีวภาพชนิดอื่นๆ ดังนั้น ผลการวิจัยครั้งนี้สามารถสรุปได้ว่า ใบของต้นขลู่มีศักยภาพและคุณสมบัติในการสะสมและการดูดซับเฮกซะวาเลนท์โครเมียมได้ดีที่สุดทั้งในดินและน้ำ

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต.....

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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PANTAWAT SAMPANPANISH : CHROMIUM REMOVAL BY PHYTOREMEDIATION AND BIOSORPTION. THESIS ADVISOR : ASSOC. PROF. WASANT PONGSAPICH, PH.D., THESIS CO-ADVISORS : ASST. PROF. EAKALAK KHAN, PH.D., AND ASST. PROF. SUTHA KHAODHIAR, PH.D., 112 PP. ISBN 974-17-6483-9.

The possibility of using phytoremediation and biosorption with weed plant species in Thailand to remove chromium (Cr) from soil and water was investigated. Six plant species, *Cynodon dactylon*, *Pluchea indica*, *Phyllanthus reticulatus*, *Echinochloa colonum*, *Vetiveria nemoralis* and *Amaranthus viridis*, were chosen for their abilities to accumulate total chromium (TCr). TCr accumulation capacities of these plants were 152.1, 151.8, 101, 77, 69 and 0 mg/kg, respectively, at a hexavalent Cr [Cr(VI)] concentration of 100 mg Cr(VI)/kg soil. Within 30 days of dosing, Cr(VI) accumulation by *Pluchea indica* occurred mainly in roots, stems and leaves at 29, 35 and 73 mg/kg biomass on a dry weight basis, respectively, whereas 38, 18 and 0 mg/kg accumulated in the roots, stems and leaves of *Cynodon dactylon*, respectively. Biosorption experiments were conducted in both batch and column reactors. A synthetic solution containing 50 ppm of Cr(VI) was used to represent Cr(IV) contaminated water. *Phyllanthus reticulates, Pluchea indica* and *Echinochloa colonum* showed the maximum Cr(VI) adsorption capacities of 53, 45 and 37 mg/g biomass, respectively, at a pH of 2 and an equilibrium time of 24 hours. Leaves were found to have the maximum adsorption capacity. In the column experiments, leaves of *Pluchea indica* had the maximum Cr(VI) adsorption capacity of 51.3 mg/g biomass at a pH of 2, a breakthrough time of 102 hours, and a flow rate of 1.3 ml/min. The relationship of Cr removal capacities of phytoremediation with living plants and biosorption using non living biomass are discussed. Leaves of *Pluchea indica* had greater Cr(VI) accumulation and adsorption than the other plants and are therefore the most effective for Cr phytoremediation and biosorption.

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ABBREVIATIONS

ANOVA	Analysis of Variance
ANRCP	Amarillo National Resource Center for Plutonium
CEC	Cation Exchange Capacity
Cr	Chromium
Cr(VI)	Hexavalent Chromium
Cr(III)	Trivalent Chromium
DIW	Department of Industrial Work
EBCT	Empty Bed Contact Time
GWRTAC	Ground Water Remediation Technologies Analysis Center
ITRC	Interstate Technology and Regulatory Cooperation
PZC	Point of Zero Charge
SAS	Statistic Analysis System
TCr	Total Chromium
US	United States
USEPA	United States Environmental Protection Agency

CHAPTER I

INTRODUCTION

1.1 Background

Environmental pollution problems are issues of great concern in many countries. There have been many conscious efforts to address industrial pollution problems through waste treatment, remediation and source reduction. In Thailand, the use of chromium (Cr) in the tannery industry has resulted in the contamination of soil and water. The contamination exists in surface water, localized vadose zones and in groundwater. Cr can exist in soils as a species of either hexavalent chromium [Cr(VI)] or trivalent chromium [Cr(III)]. Cr(VI) is of greater concern due to its toxic effect on biota and its higher mobility in aquatic environments and thus its consequent potential for groundwater contamination.

Phytoremediation and biosorption are two treatment techniques that may be used to remove Cr from soil and water, respectively. These methods are alternatives to other more expensive practices of cleaning up contaminated soil and water. In phytoremediation, living plants are specifically used to remove pollutants through their uptake of pollutants in both soil and water. Biosorption employs the biomass of plants to sorb pollutants in water.

The principal objective of the research is to investigate the uses of selected weed plant species for phytoremediation and biosorption of Cr contaminated soil and water, respectively. The weed plant species were studied because they have no value and are burdens to the environment as agriculture waste. There are four specific objectives.

1) To investigate the Cr removal capacities and to identify the removal mechanism(s) and the fate and transport of Cr in selected weed plants used for phytoremediation of Cr contaminated soil.

2) To determine the Cr adsorption capacities of selected weed plant biomass and the effect of pH on the capacities through batch biosorption isotherm experiment and to investigate the effects of empty bed contact time (EBCT) and initial Cr concentration on the Cr adsorption capacities of selected weed plant biomass through biosorption column experiment.

3) To determine whether those plants that are effective for use in phytoremediation would also provide effective biomass for biosorption.

The phytoremediation study began with soil and plant sampling at a site. Out of 34 weed plants found at the site, six plants were selected and studied in a nursery to identify the two plants that have the highest Cr accumulation ability. The two plant species were studied to determine the mechanisms of Cr removal and fate and transport of Cr in them. The biosorption research was performed using biomass of the six plants used in the phytoremedation study and consisted of 2 parts: 1) Batch isotherm experiments, to determine the Cr adsorption capacities of the biomass and the effect of pH on the capacities, 2) Column experiments, to study the effect of empty bed contact time and initial Cr concentration on the adsorption capacities. Statistical analyses were performed to determine whether plants that are effective for phytoremediation are also good biosorbents.

The research plan is shown in Figure 1-1.

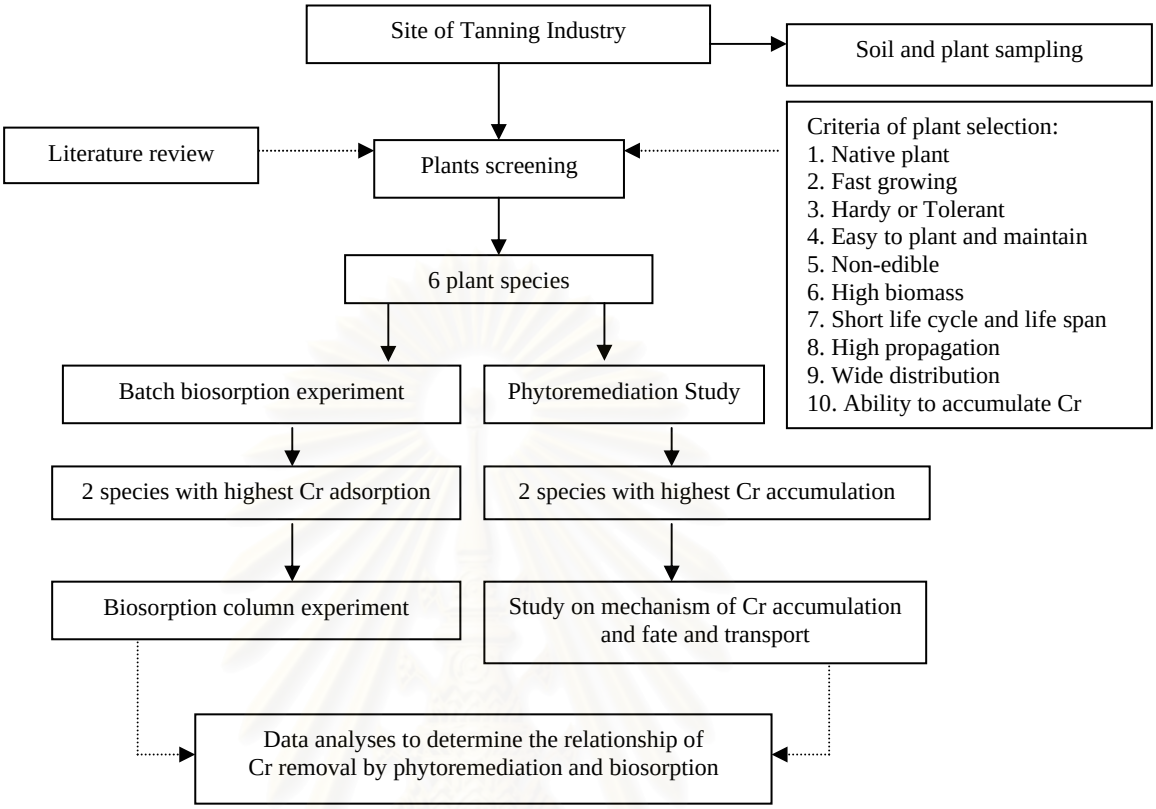


Figure 1-1. Diagram of research methodology for Cr removal from soil and water by phytoremediation and biosorption.

1.2 Dissertation Contents

This dissertation contains 5 chapters. The first chapter introduces the theoretical background and the merit of figure of this research. Chapter 2 presents the results of Cr removal from soil by phytoremediation with weed plant species in Thailand. The possibility of using weed plant species to remove Cr from soil was investigated. More over, the chapter illustrates the results of Cr accumulation and translocation in plant.

Chapter 3 presents the results of Cr removal from water by biosorption with non-living biomass of weed plant species. The selected biomass from weed plant species in Thailand was investigated for the possibility of the biosorption of Cr(VI) from contaminated wastewater.

Chapter 4 outlines the results of a alternative of Cr removal by phytoremediation and biosorption with weed plant species. The relationship of Cr removal by phytoremediation with living weed plant species and biosorption with non-living biomass species are also discussed in this chapter.

Finally, the conclusions of this research and recommendations for the further work are presented in Chapter 5.

CHAPTER II

CHROMIUM REMOVAL FROM SOIL BY PHYTOREMEDIATION WITH WEED PLANT SPECIES

2.1 Introduction

In Thailand, Cr use in the tannery industry has resulted in the contamination of soil. Altogether, it is improper solid waste, sludge, and wastewater disposal that causes the contamination. Cr(VI) of 0-252 mg/kg dry soil, Cr(III) of 7-34,292 mg/kg dry soil, and total chromium (TCr) of 7-34,300 mg/kg dry soil were found in soil at the Thai tannery sites (Department of Industrial Works of Thailand, 1999). The contamination has increased the human health risk around the sites. Currently, Thailand has no soil standard for Cr. Only the TCr standard of 1,000 mg/kg of dry weight basis in solid waste (sludge) from tanneries has been established by the Department of Industrial Works of Thailand (1997).

The United States Environmental Protection Agency (USEPA) and several state regulatory agencies have promulgated or proposed soil Cr standards based on potential health risks associated with inhalation or ingestion exposures. The New Jersey Department of Environmental Protection has proposed to regulate Cr(VI) in soil based on dermal contact by setting a Cr(VI) soil cleanup standard of 15 mg/kg. The cleanup level is designed to be protective of Cr(VI) sensitive individuals (Proctor et al., 1998). In Arizona, California, Hawaii, Nevada and the Pacific Islands (USEPA Region 9), preliminary remediation goals of 64 mg/kg and 450 mg/kg for Cr(VI) and TCr, respectively, have been established for industrial soil (USEPA, 2002).

Cr(VI) in soil tends to be reduced to Cr(III) by organic matter and Cr(III) is a more stable form (USEPA, 1998; Zayed and Terry, 2003). Cr(III) does not dissolve easily in water and attaches strongly to soil. The movement of Cr in soil depends on the type and condition of soil and environmental factors (Eco-USA, 2001). Cr contamination in soil can be remediated by several techniques: in situ physical and chemical processes (soil flushing, solidification and stabilization), in situ thermal processes (vitrification), ex situ physical and chemical processes (soil washing, chemical reduction and oxidation), and other processes such as excavation and off-site disposal (USEPA, 1993). These techniques are costly and inappropriate for Thailand because of its economic status.

Phytoremediation is an alternative for cleaning up contaminated soil. Its use for heavy metal removal from soil is environmentally sound and low in cost (Schnoor, 1997; USEPA, 2000). Salt et al. (1994) demonstrated that *Brassica juncea* (Indian mustard) could efficiently accumulate palladium (Pd), zinc (Zn), cadmium (Cd), nickel (Ni), copper (Cu) and Cr(VI) from soils or water in both roots and stems. Kumar et al. (1995) demonstrated the ability of six *Brassica* species, *B. nigra, B. oleracea, B. campestris, B. carinata, B. juncea* and *B. napus* to efficiently accumulate heavy metals. They found that Cr had the highest phytoextraction coefficient, followed by Cd, Ni, Zn and Cu. Lytle et al. (1998) found that *Eichhornia crassipes* concentration with Cr(VI), accumulated Cr(III) in root and stem tissues. After Cr(VI) was reduced to Cr(III) in the fine lateral roots, the Cr (III) was then translocated to leaf tissues. Zhu et al. (1999) reported that *Eichhornia crassipes* was a good accumulator of Cd and Cr, but a poor accumulator of arsenic (As) and Ni.

Pulford et al. (2001) investigated the concentrations of Cr and Zn in various tissues of different tree species, grown in both the field and a hydroponic system.

They found that Cr was taken up and accumulated mainly in the roots, whereas Zn was strongly translocated from the roots to the stems. Zavoda et al. (2001) found that *Helianthus annuus* (Dwarf sunflowers) accumulated Cd > Ni > Cr and *Brassica juncea* accumulated Cr > Ni > Cd. Chen and Cutright (2001) studied the chelating effect of ethylenediaminetriacetic acid and *N*-(2-hydroxyethyl)-ethylenediaminetriacetic acid on the uptake of Cd, Cr and Ni by *Helianthus annuus*. They reported that these chelators significantly enhanced the metal concentrations in plant tissues, but caused a severe loss of biomass of more than 50%. As a result, the total amount of metals removed by plants decreased. Aldrich et al. (2003) found that Mesquite (*Prosopis* spp.) can reduce Cr(VI) to Cr(III) in solid and hydroponic media. The X-ray absorption spectroscopy showed that Cr(VI) was taken up in the Mesquite roots and was fully reduced to Cr(III) in the leaf tissues.

Previous research on phytoremediation of Cr contaminated soil did not focus on high concentrations of Cr(VI) in soil (up to 400 ppm) and the use of weed plant species. This portion of the dissertation research investigated the possibility of using phytoremediation to remove Cr from contaminated soil with non-edible weed plant species found in the Thai tannery area. Since weed plant species are not edible, their use should prevent the accidental consumption by people and animals. They were tested in pots, which were maintained in a nursery. The Cr(VI) concentration level and the Cr(VI) accumulation in plants were determined based on a mass balance approach. The Cr removal capacity and mechanism, and the translocation of Cr in plants were investigated along with the effect of soil pH on Cr uptake.

2.2 Materials and Methods

2.2.1 Soil samples and plants

A total of 15 soil samples were collected at a depth of 0-30 cm from 10 locations within tannery sites, shown in Figures 1 and 2 of Appendix II, and from 5 locations outside the tanneries in Samutprakarn province, Thailand. They were analyzed for pH, moisture content, conductivity, soil texture, organic matter, cation exchange capacity (CEC), nitrogen (N), phosphorus (P), potassium (K) and TCr. Uncontaminated soil from outside the factory site, which exhibited similar properties as the contaminated soil found within the factory site, was excavated and used for the experiments.

Six out of 34 weed plant species found around the site were selected on the basis of their ability to accumulate TCr. These six species, three monocots and three dicots, are widely distributed, fast growing, hardy, easy to plant and maintain, and are non-edible. Moreover, they have a short life span, high rate of propagation, and a large biomass. The selected monocots were *Cynodon dactylon* (L.) Pers., *Vetiveria nemoralis* (A.) Camus. and *Echinochloa colonum* (L.) Link., while *Phyllanthus reticulatus* Poir., *Pluchea indica* Less., and *Amaranthus viridis* L were the selected dicots. They are shown in Figure 3 of Appendix II. These plants were obtained from uncontaminated areas in the Bangprahun district, Ayutthaya, Thailand.

2.2.2 Experimental design and procedure

2.2.2.1 TCr uptake experiment

This experiment was conducted in 12-in diameter pots, each with one 1-in diameter drainage hole. Saucers covered with plastic bags were placed under the pots to collect drainage water and this water was later poured back into the pots daily in order to prevent the loss of TCr through leaching. Five kilograms of soil was placed in each pot. Seedlings of the plant species were conducted and maintained in a nursery for 3-4 weeks in order to observe their hardiness before the Cr(VI) addition to the soil, shown in Figure 4 of Appendix II.

One hundred milliliters of three different concentrations of potassium dichromate ($K_2Cr_2O_7$) aqueous solutions, 14,100, 28,300, and 56,500 mg/L, were prepared using deionized water and applied to soil in the pots, yielding the concentrations of Cr(VI) of 100, 200, and 400 ppm (mg Cr(VI)/kg soil). A lower Cr(VI) concentration of 50 ppm or 100 mL of $K_2Cr_2O_7$ solution of 7,100 mg/L, was prepared for *Amaranthus viridis* L. because it died at Cr(VI) concentrations exceeding 50 ppm. For control pots, instead of applying potassium dichromate, 100 milliliters of water were applied. In addition, Cr(VI) was also applied to soil in pots without plants at Cr(VI) concentrations of 0, 100, 200 and 400 ppm (mg Cr(VI)/kg soil) to determine the extent of Cr adsorption and reduction by soil.

Five hundred milliliters of water were applied equally to each pot daily in the morning. One gram of 15% N, 15% P, and 15% K fertilizer was added to all pots, including the controls, every 30 days. The plants were harvested on days 30, 60, and 90. Based on 4 Cr(VI) concentrations (100, 200, 400 ppm and control), 3 harvesting times, and a triplication of experiments, 36 pots were required for each plant species.

As a result, a total of 216 pots for 6 plant species were used in this experiment. In addition, 9 pots were used for *Amaranthus viridis* L. at the Cr(VI) concentration of 50 ppm. A total of 40 pots for soils without plants were used. Only water was added to the soils of pots without plants. Soil in pots without plants and drainage water in the saucers were collected on days 1, 15, 30, 60 and 90 and analyzed for TCr, Cr(III), Cr(VI) and soil pH. This work on the pots with soil but without plant was conducted for quality assurance and material balance purposes.

Upon treatment with Cr(VI), the height of each plant was measured every 15 days. During harvesting, plant tissues (mixture of the whole plant), soil, and drainage water in the saucers were collected from each pot, and analyzed for TCr. To ensure that Cr from the roots was not contaminated with Cr from the soil for the TCr analysis, the roots were thoroughly washed with tap water. Soil pH was also measured during the harvesting. The main purpose of this TCr uptake experiment was to select two plant species, one dicot and one monocot, that showed the highest TCr accumulation for the investigation of the Cr removal mechanism in which the uptakes of different Cr species were also evaluated as described in the following section.

2.2.2.2 Cr removal mechanism experiment

The procedure of the removal mechanism experiment was similar to that of the TCr uptake experiment; however, only the Cr(VI) concentration of 100 ppm was used because it was below the mortality concentration determined in the TCr uptake experiment. The plants were harvested after 30, 60, 90, and 120 days. For each plant, this experiment used a total of 24 pots corresponding to two Cr(VI) concentrations (100 ppm and control), 4 harvesting times and a triplication of experiments. After

harvesting, the plants were cut into three parts, roots, stems, and leaves, and analyzed for Cr(VI), Cr(III), and TCr (see Figure 5 in Appendix II).

2.2.3 Analytical methods

The USEPA method 3052 (acid digestion/atomic absorption spectrometer) was used for the analysis of TCr (USEPA, 1996a). A Perkin Elmer atomic absorption spectrometer Model AAnalyst 800 (Perkin Elmer Instruments LLC, Unberlingen, Germany) was used. For the analysis of Cr(VI), alkaline digestion of soil and plant tissues were performed according to the USEPA method 3060A (USEPA, 1996b) and the 1,5-diphenylcarbohydrazide colorimetric method according to the USEPA method 7196A (USEPA, 1992) was used. The concentration of Cr(III) was determined from the difference between TCr and Cr(VI) concentrations. Moisture content, conductivity, soil texture, organic matter, CEC, N, P, K, and pH of soil samples were analyzed according to the methods of the Canadian Society of Soil Science (1993). The method for soil pH measurement was based on electrometry using a pH meter on a soil and deionized water mixture at 1:1 (w/w).

2.2.4 Statistical analyses

Chromium uptake data were analyzed using the analysis of variance (ANOVA) and the Duncan multiple range test with orthogonal contrast. The Duncan test was used to obtain the grouping of the mean values of chromium uptake that are not significantly different among themselves. The null hypothesis about the contrasts to be tested is the six plants provide mean TCr uptakes that are not significantly

different (H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$) and the alternative hypothesis (H_1) is at least one mean TCr uptake is not statistically equal to the others. These statistical analyses were conducted using the Statistic Analysis System version 8 (Statistic Analysis System Institute Inc., Cary, North Carolina, USA).

2.3 Results and Discussion

2.3.1 Soil properties and plant growth and survival

Soil samples were randomly collected from the pots before Cr(VI) was applied for an analysis of soil properties. The results of the soil analyses are shown in Table 2-1. The TCr background in the soil was 51 mg/kg on a dry weight basis. No Cr(VI) was found in the soil background, suggesting that TCr existed as Cr(III) due to the reduction of Cr(VI) to Cr(III). The properties of soil used in this study were within the ranges of properties of soil found within the factory site.

Table 2-1

Properties of soil used for the uptake experiment.

Parameters	Soil properties
pH	5.2±0.0
Conductivity (mS/cm)	4.0±0.4
Soil moisture (%)	0.5±0.0
Organic matter (%)	4.8±0.0
Nitrogen (%)	0.2±0.0
Phosphorus (mg/kg)	748±5
Potassium (mg/kg)	6170±28
CEC (meq/100g)	21.1±0.6
Total chromium (mg/kg soil dry weight)	51.2±1.2
Hexavalent chromium	Not detectable
Sand (%)	65
Silt (%)	8
Clay (%)	27
Soil texture	Sandy clay loam

Upon treatment with different Cr(VI) concentrations, all six plant species showed signs of stunted growth with dry and yellow leaves as well as loss of leaves in the first week. They are shown in Figure 6 of Appendix II. The heights of each plant, which were measured every 15 days, are shown in Table 1 of Appendix I. Similar observations of severe wilting and chlorosis as the initial symptoms of Cr toxicity on plants were also reported by ANRCP (1998). Fendorf (1995) reported that Cr(VI) as low as 0.5 ppm in solution or 5 ppm in soil can be toxic to plants. In the following weeks, all plants were found to be adapting and growing. Among the six species, *Cynodon dactylon* and *Phyllanthus reticulatus* grew best when considering all three Cr(VI) concentrations of 100, 200, and 400 ppm together.

When comparing the results at different Cr(VI) concentrations, all plants except *Amaranthus viridis* were tallest and survived best at the concentration of 100 ppm at all harvesting times (Table 2-2). *Amaranthus viridis* could not survive at concentrations above 50 ppm, possibly because the species is more sensitive to Cr toxicity. All *Vetiveria nemoralis, Echinochloa colonum,* and *Pluchea indica* plants died at the Cr(VI) concentration of 400 ppm. It is not clear why *Cynodon dactylon* and *Phyllanthus reticulatus*, the only two plants that survived at 400 ppm, had lower survival rates than the other three species at 100 ppm. Note that the survival rates of these two species at 100 ppm were not much less than that of the other three species. For example, the survival rate of 89% for *Cynodon dactylon* was a result of the plant death in 1 pot out of 9 pots tested.

Table 2-2

Survival percentage of six plant species during the uptake experiment.

Plant species	Percent survival of plants at different Cr(VI) concentrations (ppm)				
	Control	50	100	200	400
Cynodon dactylon (L.) Pers	100	-	89	78	67
Vetiveria nemoralis (A.) Camus	100	-	100	56	0
Echinochloa colonum (L.) Link	100	-	100	33	0
Phyllanthus reticulatus Poir	100	-	78	78	33
Pluchea indica Less.	100	-	100	33	0
Amaranthus viridis L.	100	100	0	0	0

2.3.2 TCr uptake experiment

2.3.2.1 TCr uptake capacities of plants

Figure 2-1 shows the mean TCr accumulation of the six plant species at different concentrations including the control and different harvesting times. The error bars represent the standard errors of the means. When only one plant out of the triplicate survived, the single TCr accumulation data point is reported with no error bar. At the Cr(VI) concentration of 200 ppm, the highest concentration that some of each of the plants tested except *Amaranthus viridis* survived, TCr accumulated in *Cynodon dactylon* much more than in *Pluchea indica, Echinochloa colonum, Phyllanthus reticulatus* and *Vetiveria nemoralis* on day 30. The TCr accumulations in *Cynodon dactylon, Pluchea indica, Echinochloa colonum, Phyllanthus reticulatus* and *Vetiveria nemoralis* were 451, 186, 141, 136, and 94 mg TCr/kg of plant on a dry weight basis, respectively. *Amaranthus viridis* at the Cr(VI) concentration of 50 ppm, accumulated only 8 mg/kg on day 60 while no TCr was detected in its tissues on day 30 (Figure 1f). It should be noted that at the Cr(VI) concentration of 400 ppm, *Cynodon dactylon* that survived (67% of the total number tested) behaved as a TCr hyperaccumulator (TCr accumulation > 1000 mg/kg, Lasat, 2002). The mass balances of TCr at the end of the experiments were performed and TCr in plant, soil, and drainage water combined was > 94% of the total chromium input for all cases, shown in Table 2 of Appendix I.

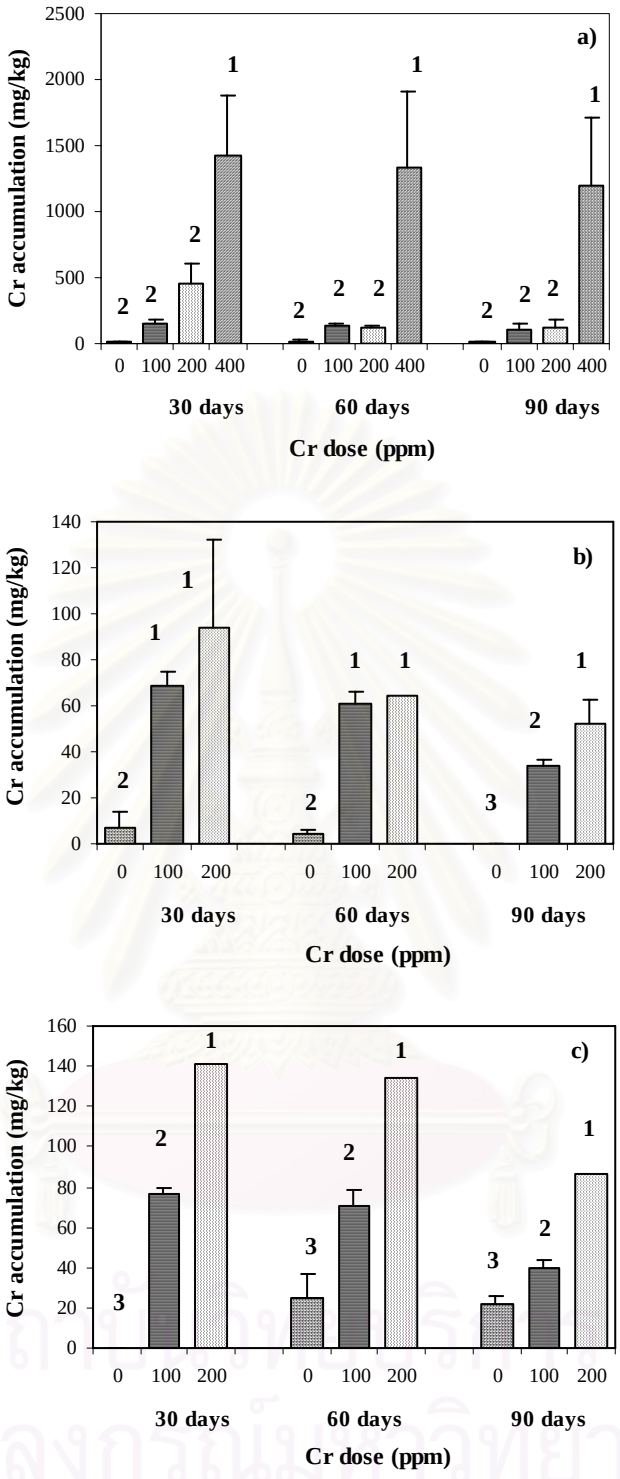


Figure 2-1. TCr accumulation in six plant species: a) *Cynodon dactylon* (L.) Pers., b) *Vetiveria nemoralis* (A.) Camus., c) *Echinochloa colonum* (L.) Link, d) *Phyllanthus reticulatus* Poir., e) *Pluchea indica* Less., and f) *Amaranthus viridis* L.

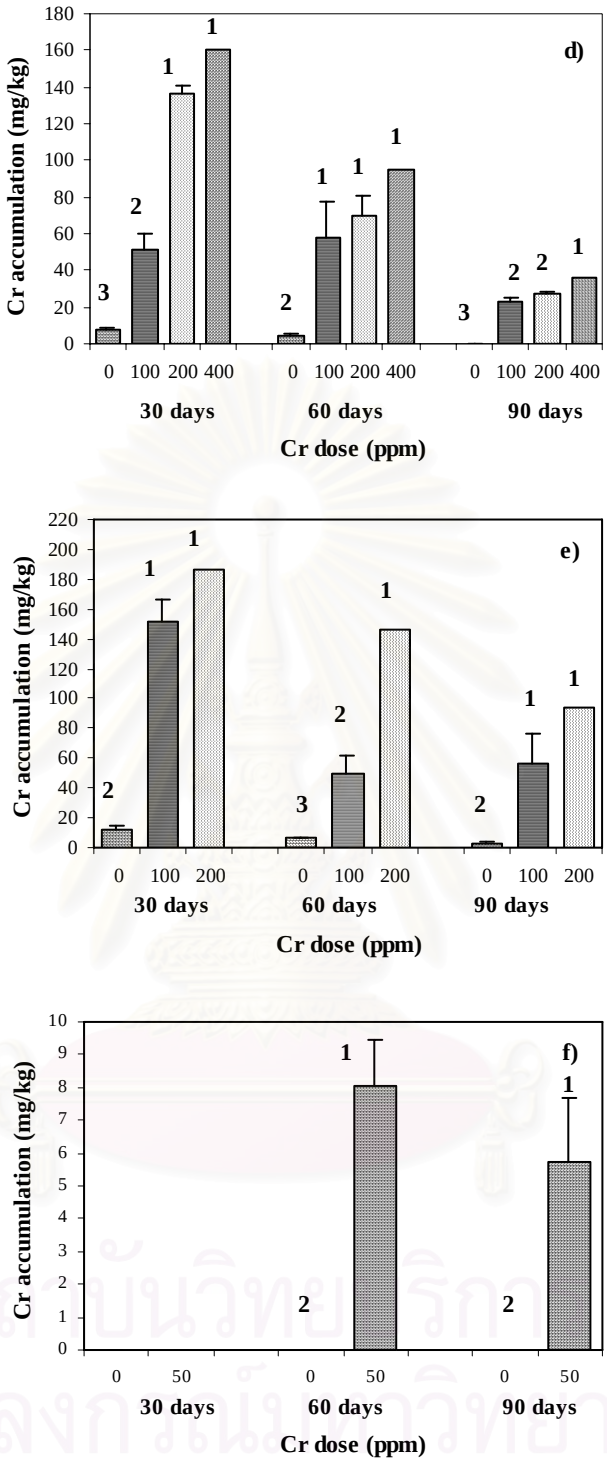


Figure 2-1. (Cont.)

Significant differences ($p < 0.05$, one-way ANOVA) in TCr accumulation were observed among different concentrations and the control at all harvesting times for all six species. The effect of Cr(VI) concentration was further analyzed using the Duncan multiple range test ($p < 0.05$) and the results were not the same for all six species. The numbers (1, 2, and 3) above the bars in Figure 2-1 are the groups of the means that were not statistically different according to the Duncan test. For example, *Cynodon dactylon* accumulated significantly higher TCr at the concentration of 400 ppm than at other concentrations and the control whose means were in the same group (not statistically different) although their mean TCr accumulations were quite different quantitatively. These statistical results suggest that Cr(VI) concentration tended to affect the TCr uptake capacities of the plants and that the effect was species dependent.

The TCr uptake capacities of all plant species except *Amaranthus viridis* peaked on day 30, and decreased on days 60 and 90. The reason for the decrease could have been due to the fact that the plants had grown well and the resulting high biomass on days 60 and 90 lessened the concentrations of TCr in the plants. Table 2-3 shows the dry biomass data of the six plant species on days 30, 60, and 90. The data indicate that the Cr uptake occurred mostly or ceased on day 30 or earlier. Except for *Echinochloa colonum* at a Cr(VI) concentration of 100 ppm and *Amaranthus viridis* , the plant dry weights on day 90 were 1.5 to 3 times of those on day 30. The dry biomass data agree with the TCr accumulation in Figure 2-1 in most cases. The discrepancies between the ratio of dry biomass data and the ratio of chromium accumulation on days 30 and 90 of *Phyllanthus reticulatus* (2 fold increases versus 3-4 fold decreases) cannot be clearly explained and may be due to experimental variation and error.

Table 2-3

The dry weight of six plant species on days 30, 60 and 90.

Harvesting time (day)	Cr(VI) concentration (ppm)	Mean dry weight ± standard error (g)					
		Cynodon dactylon	*Vetiveria nemoralis*	*Echinochloa colonum*	*Phyllanthus reticulatus*	*Pluchea indica*	*Amaranthus viridis*
30	0 (control)	30.1 ± 2.7	58.6 ± 7.2	25.7 ± 0.5	34.9 ± 2.8	36.2 ± 4.9	6.3 ± 0.2
	50	-	-	-	-	-	1.1 ± 0.4
	100	24.6 ± 1.2	36.7 ± 1.3	22.3 ± 9.1	28.4 ± 9.9	23.8 ± 5.6	-
	200	21.8 ± 0.0	26.0 ± 11.9	15.7	22.2 ± 0.2	18.1	-
	400	16.8 ± 3.2	-	-	18.0	-	-
60	0 (control)	35.2 ± 4.9	70.4 ± 6.7	29.3 ± 1.7	61.7 ± 0.9	45.7 ± 2.5	7.8 ± 0.5
	50	-	-	-	-	-	5.6 ± 0.5
	100	29.9 ± 1.2	59.8 ± 8.0	25.7 ± 2.4	45.7 ± 5.3	39.8 ± 1.2	-
	200	24.7 ± 3.7	45.1	20.0	42.4 ± 7.5	21.0	-
	400	21.0 ± 2.0	-	-	19.7	-	-
90	0 (control)	39.7 ± 0.5	104.4 ± 9.6	29.8 ± 3.1	64.9 ± 4.2	64.1 ± 4.4	8.8 ± 2.0
	50	-	-	-	-	-	8.2 ± 3.1
	100	35.4 ± 2.6	99.4 ± 11.6	26.5 ± 3.6	54.8 ± 4.2	59.0 ± 25.6	-
	200	30.4 ± 1.1	52.5 ± 4.2	24.3	46.4 ± 2.7	36.2	-
	400	28.2 ± 4.0	-	-	40.8	-	-

The one-way ANOVA showed that, for the monocots, significant differences ($p < 0.05$) in the TCr uptake were found between *Cynodon dactylon* and the other two species, *Vetiveria nemoralis* and *Echinochloa colonum* at all harvesting times and Cr(VI) concentrations. No statistical difference was found between the TCr uptakes by *Vetiveria nemoralis* and *Echinochloa colonum*. For the dicots, *Phyllanthus reticulatus* and *Pluchea indica* had uptakes that were not significantly different but were significantly higher than TCr uptakes by *Amaranthus viridis* at all harvesting times and Cr(VI) concentrations.

Figure 2-2 shows that the harvesting time was found to affect the TCr uptake in *Vetiveria nemoralis, Echinochloa colonum*, and *Pluchea indica* at the Cr(VI) concentration of 100 ppm, and in *Amaranthus viridis* at the 50 ppm concentration ($p < 0.05$, one-way ANOVA). The effect of the harvesting time at Cr(VI) concentrations above 100 ppm was not statistically examined because the data at some harvesting times were unavailable (due to high mortality). For *Cynodon dactylon* and *Phyllanthus reticulatus*, the harvesting time had no significant effect on the TCr uptake ($p > 0.05$, one-way ANOVA).

Table 2-4 shows the ranking of plant species according to TCr accumulation. When the TCr accumulation of the six plant species at the Cr(VI) concentrations of 50 ppm for *Amaranthus viridis* and 100 ppm for the other five species was ranked, the monocot, *Cynodon dactylon*, and the dicot, *Pluchea indica*, had the highest TCr accumulation capacities of 152.1 and 151.8 mg/kg, respectively, on day 30. The Duncan multiple range test on the data confirmed that these capacities are statistically in the same group and are significantly different to the other accumulation capacities shown in Table 2-4. The two plant species were therefore selected for the study of the Cr removal mechanism.

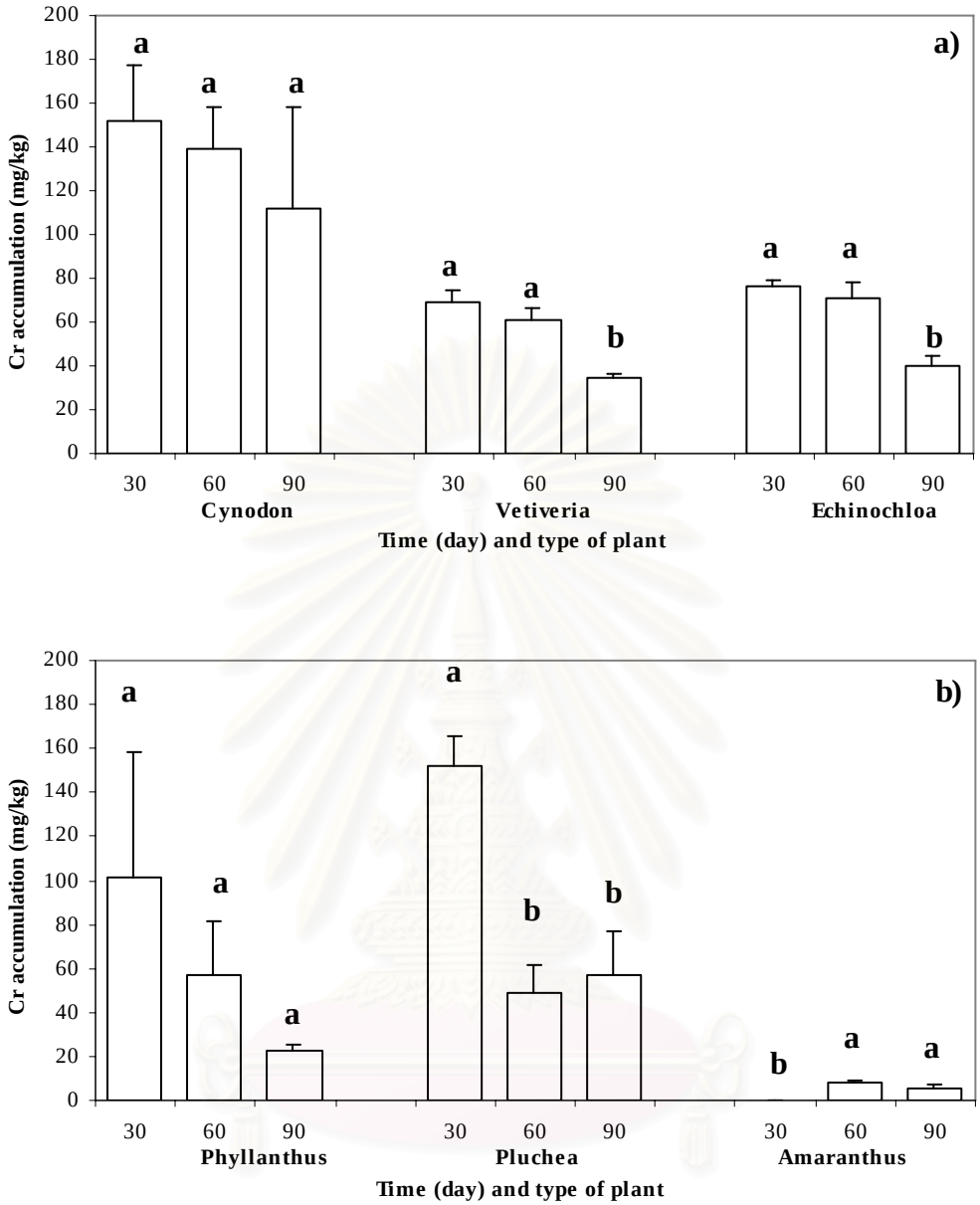


Figure 2-2. Effect of harvesting time on Cr accumulation in six plant species at Cr(VI) concentration level of 100 ppm: a) Monocot plants and b) Dicot plants.

Table 2-4

The TCr accumulation rankings of the six plant species at Cr(VI) concentration of 50 ppm for *Amaranthus viridis* and 100 ppm for the other five species.

Rank	Plant species and harvesting time	Mean TCr accumulation ±standard error (mg/kg)	Group[1]
1	*Cynodon*-day 30	152.1±24.9	1
2	*Pluchea*-day 30	151.8±14.0	1
3	*Cynodon*-day 60	138.9±19.3	2
4	*Cynodon*-day 90	111.6±46.3	3
5	*Phyllanthus*-day 30	101.2±57.3	4
6	*Echinochloa*-day 30	76.5±2.8	5
7	*Echinochloa*-day 60	71.0±7.1	6
8	*Vetivaria*-day 30	68.7±5.8	6
9	*Vetivaria*-day 60	60.9±5.1	7
10	*Phyllanthus*-day 60	57.4±24.2	7
11	*Pluchea*-day 90	56.7±19.9	7
12	*Pluchea*-day 60	49.1±12.6	7
13	*Echinochloa*-day 90	40.1±4.2	8
14	*Vetivaria*-day 90	34.3±2.0	9
15	*Phyllanthus*-day 90	22.7±3.1	10
16	*Amaranthus*-day 60	8.1±1.4	11
17	*Amaranthus*-day 90	5.7±2.0	11
18	*Amaranthus*-day 30	0.0±0.0	12

[1] Differences among specific means were evaluated using the Duncan multiple range test. The means with different group numbers differ significantly (*F*-test, $p < 0.05$).

2.3.2.2 Effect of soil pH at the harvesting time on TCr uptake

Table 2-5 presents soil pH at harvesting times of the six of plant species. Decreases in pH from 5.2 (for soil as collected before the addition of $K_2Cr_2O_7$) to about 4 were observed in most cases. The pH decreases were probably because of the addition of the $K_2Cr_2O_7$ solutions. Table 2-6 shows the effect of soil pH (at the harvesting time) range on the TCr accumulation in six plant species regardless of the harvesting time. Based on the one-way ANOVA ($p < 0.05$) and Duncan multiple range ($p < 0.05$) tests, pH affected the TCr uptakes of *Cynodon dactylon* and *Phyllanthus reticulatus*; pH > 4.5 was more favorable for the TCr accumulation in these two plants. The higher the pH, the more chance Cr will be hexavalent (depending on redox potential). Cr(VI) is more mobile and more bioavailable than Cr(III). This explains the greater Cr uptake of the two plant species at pH > 4.5. TCr accumulation of the other four species was not pH dependent; the mean TCr uptakes of these plants at the three pH ranges were not significantly different.

Table 2-5

Soil pH at the harvesting time of the six plant species.

Plant species	Harvesting time (days)	Mean soil pH ± standard error
Cynodon dactylon	30	4.4 ± 0.1
	60	4.1±0.1
	90	4.1± 0.2
Vetiveria nemoralis	30	4.4±0.1
	60	4.3± 0.2
	90	4.4± 0.2
Echinichloa colonum	30	4.0±0.1
	60	3.8±0.1
	90	4.1± 0.1
Phyllanthus reticulatus	30	4.1± 0.2
	60	3.9±0.1
	90	4.0±0.2
Pluchea indica	30	3.8±0.1
	60	3.8±0.1
	90	3.6±0.1
Amaranthus viridis	30	4.1±0.1
	60	4.2±0.2
	90	4.0±0.1

Table 2-6

Effect of soil pH at the harvesting time on TCr accumulation in six plant species.

Plant species	pH range	Mean TCr accumulation ±standard error (mg/kg)	*p* value[1]	Group[2]
Cynodon dactylon	< 4.0	82.6±22.2	0.0001	1
	4.0-4.5	388.5±106.0		2
	> 4.5	1074.1±350.5		2
Vetiveria nemoralis	< 4.0	32.9±9.6	0.1594	1
	4.0-4.5	34.2±8.2		1
	> 4.5	45.2±16.0		1
Echinochloa colonum	< 4.0	53.5±11.1	0.1909	1
	4.0-4.5	40.1±11.6		1
	> 4.5	-[3]		1
Phyllanthus reticulatus	< 4.0	19.1±4.3	0.0001	1
	4.0-4.5	56.0±16.2		2
	> 4.5	125.6±39.4		3
Pluchea indica	< 4.0	60.2±15.4	0.1075	1
	4.0-4.5	132.3±103.7		1
	> 4.5	-[3]		1
Amaranthus viridis	< 4.0	2.8±0.8	0.1485	1
	4.0-4.5	4.9±0.2		1
	> 4.5	4.1±0.1		1

[1]One-way ANOVA test

[2]Duncan multiple range test ($p < 0.05$)

[3]Soil pH was ≤ 4.5 for all pots planted with *Echinochloa colonum* and *Pluchea indica*

2.3.2.3 Characteristics of soil and drainage water from pot without plants

Figure 2-3a shows that the highest amount of Cr(VI) accumulation in soil from the pots without plants was at day 1 and decreased through days 15, 30, 60 and 90, respectively, for Cr(VI) concentration levels of 100, 200 and 400 ppm. Cr(VI) solution was applied to soil samples in the pots without a plant in order to determine the extent of Cr adsorption and reduction by the soil. However, both Cr(III) and TCr levels increased over time to day 90 for all Cr(VI) concentration levels. Table 2-7 shows that the soil pH in the pots without plants was 5.2-5.5 for pots with no Cr amendment and were 5.2-5.7, 5.3-5.7 and 5.2-5.8, for the pots amended with Cr(VI) at 100, 200, and 400 ppm respectively. Moreover, soil pH in pots without plants at Cr(VI) concentrations of 100, 200 and 400 ppm not significant differently to the soil pH in pots without Cr(VI) and without plants. Figure 2-3b shows that the highest level of Cr accumulation in soil for Cr(VI), Cr(III) and TCr occurred when the pH was >5.5.

Figure 2-4a shows that the highest amount of Cr(VI), Cr(III) and TCr accumulation in drainage water from saucers of pots without plants occurred at day 1 and reduced with time on days 15, 30, 60 and 90, respectively, for Cr concentration levels of 100, 200, and 400 ppm. Table 2-8 shows that the drainage water in the saucers of the pots without the Cr addition were pH 6.6-7.7 while the pH of drainage water from the saucers of the pots dosed with Cr(VI) at 100, 200, and 400 ppm were 6.4-7.8, 6.1-7.5, and 6.0-7.4, respectively. Figure 2-4b shows that the highest level of Cr accumulation in drainage water from saucers for Cr(VI), Cr(III) and TCr occurred at pH of <6.5. The pH of drainage water from saucers had a higher pH than that of soil in pots.

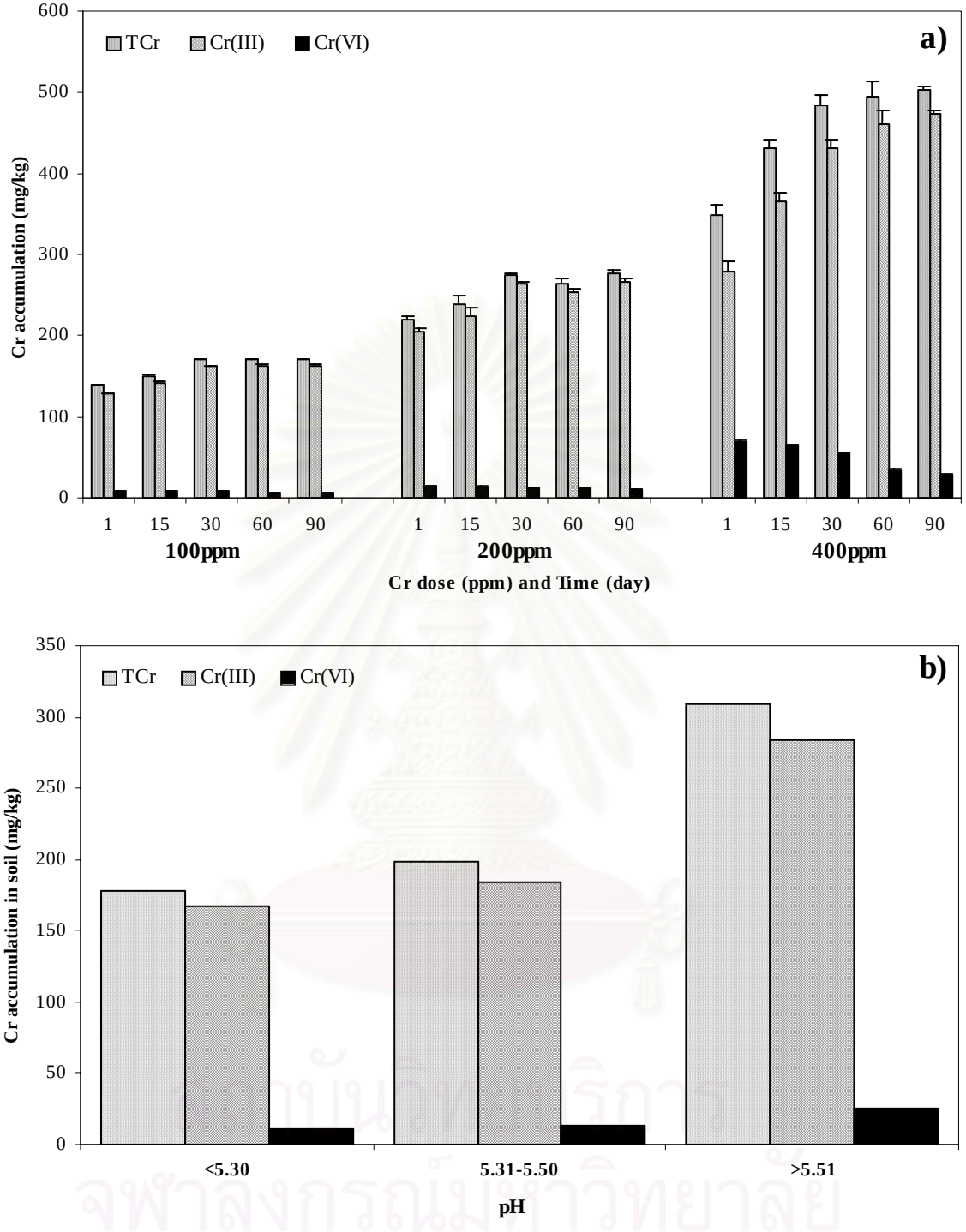


Figure 2-3. Soil pH and Cr accumulation in soil in the pots without plants: a) Cr accumulation and b) Soil pH.

Table 2-7

TCr, Cr(III) and Cr(VI) in soils and soil pH in the pots without plants at days 1, 15, 30, 60 and 90.

Cr(VI) concentration (ppm)	Time (day)	pH	Mean TCr accumulation ±standard error (mg/kg)	Mean Cr(III) accumulation ±standard error (mg/kg)	Mean Cr(VI) accumulation ±standard error (mg/kg)
0	1	5.3	54.4±0.4	54.4±0.4	0.0±0.0
	15	5.2	55.9±1.6	55.9±1.6	0.0±0.0
	30	5.2	55.5±0.5	55.5±0.5	0.0±0.0
	60	5.5	54.0±1.5	54.0±1.5	0.0±0.0
	90	5.5	54.4±1.5	54.4±1.5	0.0±0.0
100	1	5.2	138.6±3.7	129.4±3.8	9.2±0.6
	15	5.3	150.5±10.3	141.5±10.2	8.9±0.3
	30	5.4	171.1±1.9	162.3±2.7	8.7±1.1
	60	5.5	170.2±4.5	162.9±4.3	7.2±0.4
	90	5.7	170.3±3.3	163.6±3.2	6.7±0.5
200	1	5.3	219.3±12.5	204.8±12.1	14.5±0.8
	15	5.5	238.4±10.5	224.8±10.4	13.6±0.6
	30	5.4	275.7±12.4	263.5±12.4	12.2±0.6
	60	5.5	265.1±17.8	253.3±16.9	11.7±0.9
	90	5.7	276.9±3.8	267.1±4.1	9.9±0.6
400	1	5.4	349.1±36.5	278.7±35.9	70.4±1.1
	15	5.6	431.8±9.4	366.1±11.2	65.7±8.7
	30	5.2	484.6±6.8	430.1±23.8	54.5±20.5
	60	5.7	495.3±9.7	461.0±7.6	34.3±4.0
	90	5.8	502.3±4.7	474.1±7.5	28.2±4.2

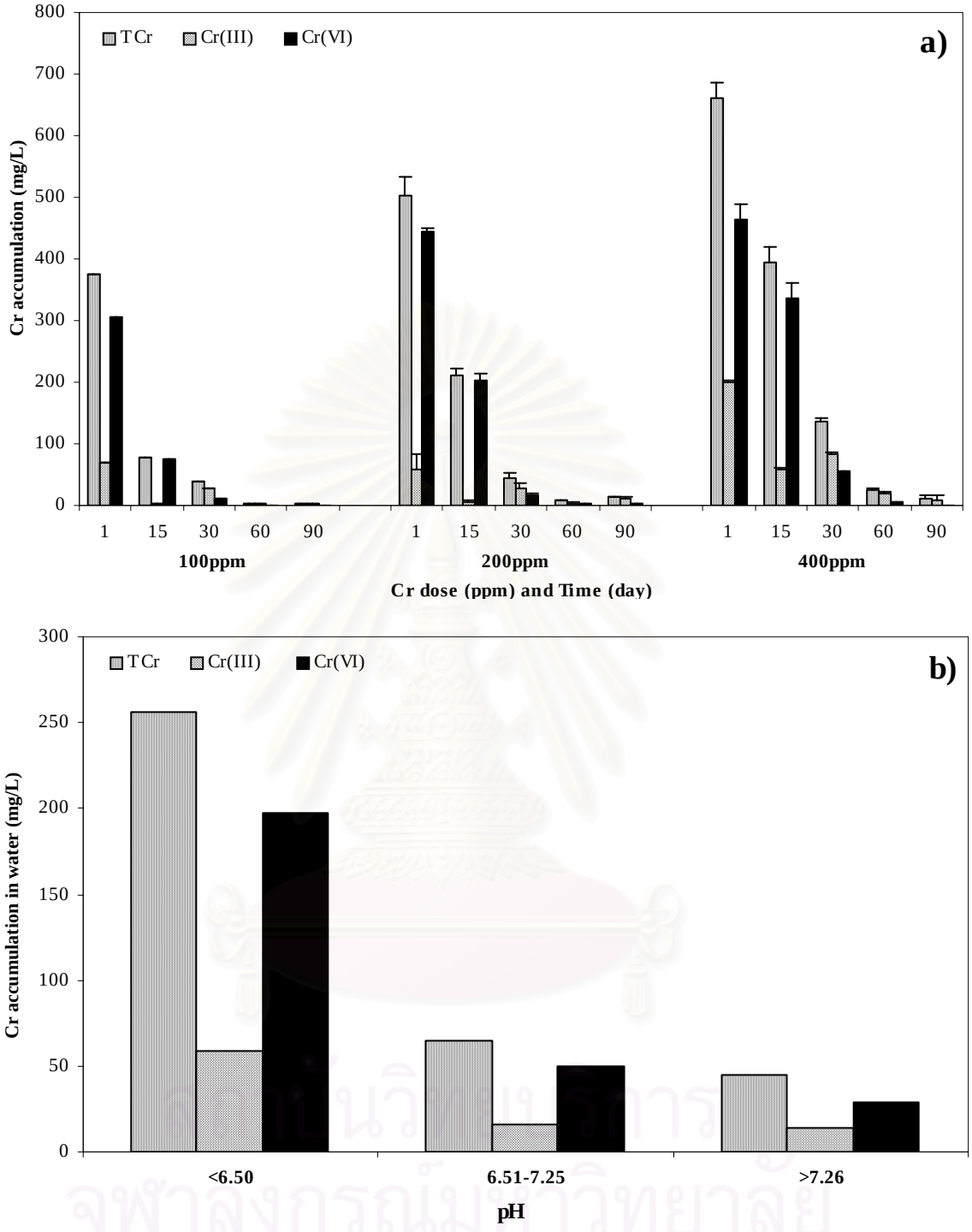


Figure 2-4. Cr accumulation and pH in drainage water from saucers below the pots containing soil without plants: a) Cr accumulation and b) Drainage water pH.

Table 2-8

TCr, Cr(III) and Cr(VI) in drainage water from saucers below the pots containing soil without plants at days 1, 15, 30, 60 and 90.

Cr(VI) concentration (ppm)	Time (day)	pH	Mean TCr accumulation ±standard error (mg/L)	Mean Cr(III) accumulation ±standard error (mg/L)	Mean Cr(VI) accumulation ±standard error (mg/L)
0	1	7.1	0.0±0.0	0.0±0.0	0.0±0.0
	15	7.7	0.0±0.0	0.0±0.0	0.0±0.0
	30	7.6	0.0±0.0	0.0±0.0	0.0±0.0
	60	6.6	0.0±0.0	0.0±0.0	0.0±0.0
	90	7.2	0.0±0.0	0.0±0.0	0.0±0.0
100	1	6.5	374.0±31.6	69.6±25.7	304.5±5.8
	15	7.7	78.9±12.0	3.0±2.0	75.9±9.9
	30	7.8	38.8±6.6	28.2±8.9	10.7±2.3
	60	6.4	3.1±0.8	1.9±0.7	1.1±0.04
	90	7.4	3.6±0.8	3.1±0.7	0.5±0.1
200	1	6.1	502.0±22.6	57.6±3.9	444.4±26.5
	15	7.5	210.6±24.6	6.7±0.7	203.8±23.8
	30	7.3	45.4±5.4	28.6±3.5	16.8±1.9
	60	6.3	6.9±3.3	3.9±1.6	3.1±1.7
	90	7.2	13.9±6.8	12.3±6.5	1.6±0.3
400	1	6.0	662.2±0.6	198.9±38.6	463.3±38.1
	15	7.4	395.5±1.6	59.4±29.3	336.1±30.9
	30	6.5	137.0±19.2	82.8±8.0	54.2±11.2
	60	6.7	25.0±10.7	20.7±10.7	4.40.0
	90	7.2	10.2±0.4	9.5±0.3	0.7±0.1

2.3.3 Cr removal mechanisms experiment

Figure 2-5 shows the Cr(VI), Cr(III), and TCr accumulations in roots, stems, and leaves of *Cynodon dactylon* and *Pluchea indica* on days 30, 60, 90, and 120 at the Cr(VI) concentration of 100 ppm. Similar to Figure 2-1, the data with no error bar resulted from the cases where only one plant out of the triplicate survived. *Cynodon dactylon* and *Pluchea indica* accumulated Cr(VI) and Cr(III) in roots throughout the experimental period. The accumulations of both Cr forms reached the highest values in roots on day 30, and gradually decreased on days 60, 90, and 120.

Cynodon dactylon showed the accumulations in stems until day 60, but no accumulations in the leaves. *Pluchea indica* accumulated Cr in stems and leaves until day 60. On days 90 and 120, Cr did not disappear from the stems of *Cynodon dactylon* and the stems and leaves of *Pluchea indica* but were diluted by plant growth (with minimal or no Cr uptake) to the levels that were lower than the detection limit (about 30 mg/kg for TCr). It should be noted that the TCr levels in the stems of *Cynodon dactylon* and *Pluchea indica* were close to 30 mg/kg on day 60. The TCr level of about 50 to 75 mg/kg in the leaves of *Pluchea indica* on day 60 was diluted by a factor of 2.1 between days 60 and 90. Table 2-9 presents the dry biomass data that support this explanation. Table 3 in Appendix I shows the mass balances of TCr, Cr(III) and Cr(VI) at the end of the experiments in parts of plant, soil and drainage water. The translocation of both Cr forms from the roots to the above ground parts of the plants suggests that phytoaccumulation is the main removal mechanism.

The accumulations of TCr on day 30 in roots, stems and leaves of *Cynodon dactylon* were 62, 40, and 0 mg/kg corresponding to 51%, 49% and 0% of the TCr mass

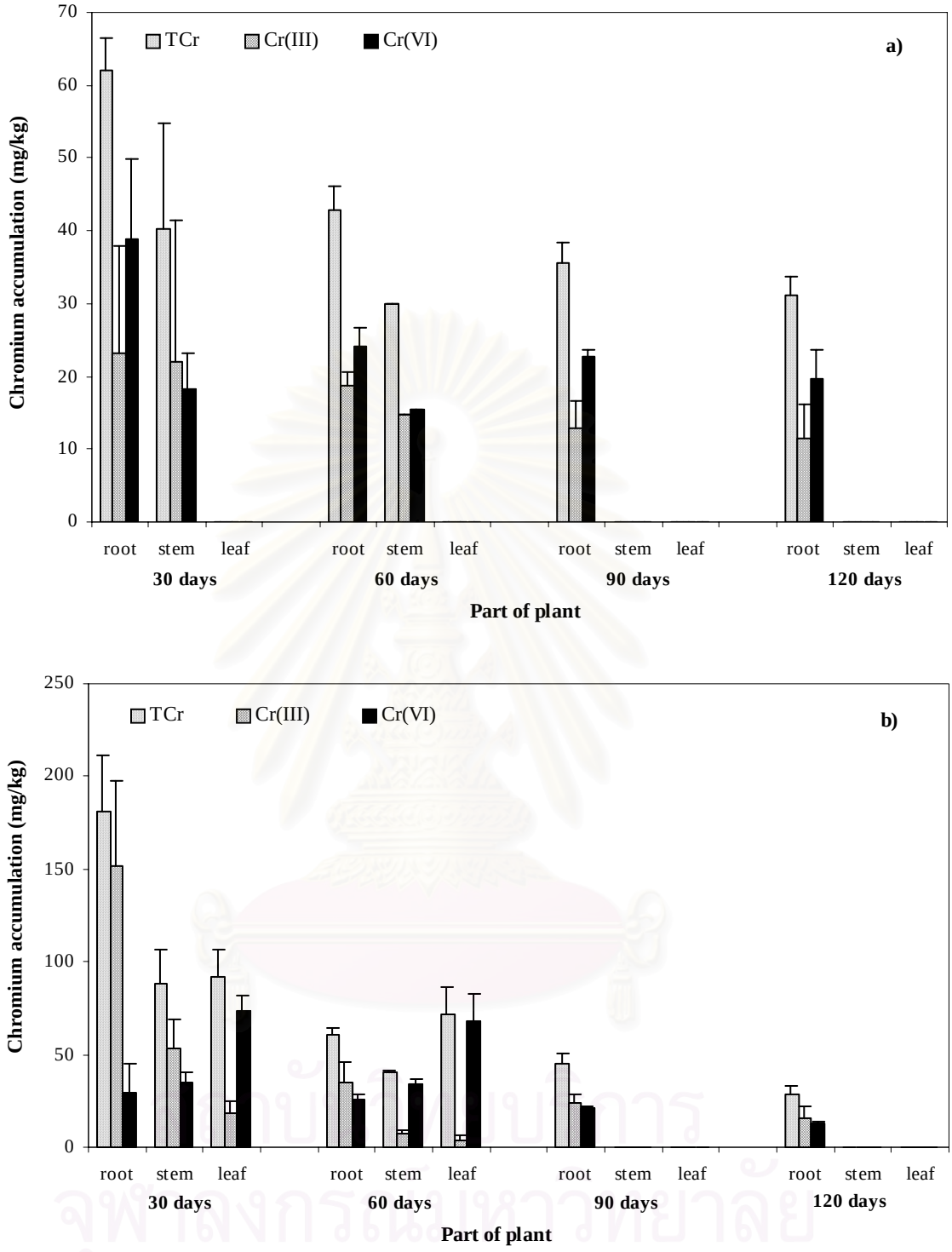


Figure 2-5. Cr(VI), Cr(III) and TCr accumulation in roots, stems and leaves of a) *Cynodon dactylon* (L.) Pers. and b) *Pluchea indica* Less.

Table 2-9

The dry weight of different parts of *Cynodon dactylon* and *Pluchea indica* on days 30, 60, 90 and 120 at Cr(VI) concentration of 100 ppm and control (0 ppm).

Plant species	Harvesting time (days)	Mean dry weight of plant parts ± standard error (g)		
		Root	Stem	Leaf
Cynodon dactylon	30	3.0 ± 0.3	3.8 ± 0.9	2.3 ± 0.3
	60	2.6 ± 0.4	3.7 ± 0.1	2.4 ± 0.4
	90	3.2 ± 0.7	5.0 ± 0.6	3.6 ± 0.8
	120	3.5 ± 0.9	5.3 ± 0.6	4.4 ± 0.8
Control	30	2.7 ± 0.1	3.8 ± 0.6	2.1 ± 0.2
	60	2.8 ± 0.6	3.6 ± 0.4	2.8 ± 0.6
	90	2.9 ± 0.3	5.0 ± 0.7	3.7 ± 0.6
	120	2.6 ± 0.2	4.1 ± 0.6	4.1 ± 0.7
Pluchea indica	30	0.4 ± 0.0	1.2 ± 0.2	1.1 ± 0.3
	60	1.6 ± 0.4	3.5 ± 1.3	1.3 ± 0.9
	90	1.7 ± 0.5	4.5 ± 0.7	2.7 ± 0.7
	120	1.9 ± 0.5	6.1 ± 1.4	3.5 ± 0.8
Control	30	1.4 ± 0.2	2.8 ± 0.5	2.7 ± 0.3
	60	1.6 ± 0.3	4.1 ± 1.8	3.0 ± 0.7
	90	1.9 ± 0.6	5.5 ± 1.3	3.7 ± 0.0
	120	3.3 ± 0.4	6.7 ± 0.7	4.8 ± 0.7

uptake, respectively. For *Pluchea indica*, TCr accumulated in roots, stems, and leaves on day 30 at 180, 86, and 90 mg/kg or 27%, 38%, and 35% of the TCr mass uptake, respectively. Accumulations of Cr(III) in *Cynodon dactylon* were observed along with accumulations of Cr(VI) in all cases at comparable levels or slightly to moderately less than the levels of Cr(VI) accumulation. While Cr(VI) accumulation increased from roots to leaves for *Pluchea indica*, the results were the opposite for Cr(III). Less and least accumulations of Cr(III) in stems and leaves of *Pluchea indica* suggests that the plant has less ability to translocate Cr(III) compared to Cr(VI).

The finding, that *Cynodon dactylon* accumulated the highest Cr(III) and Cr(VI) in roots (24 and 38 mg/kg) on day 30, agrees with the result of a previous study by Arteaga et al. (2000). They reported that *Larrea tridentata* (Creosote bush) could accumulate TCr well in roots. The difference in the TCr accumulation in plant tissues of *Larrea tridentata* was suggested to be because of the difference in the molecular components of the tissues. Roots and stems contain more cellulose and hemicellulose than leaves, which consist of mostly proteins; therefore, roots and stems have more hydroxyl groups which can coordinate with Cr and assist its uptake inside their tissues better than in leaves (Gardea-Torresdey et al., 1998).

The more favorable accumulation of Cr(VI) in the leaves of *Pluchea indica* at 73 mg/kg on day 30 was expected. ANRCP (1998) summarized that dicots such as buckwheat and rutabaga absorbed more Cr through their roots and transported more Cr to the above ground parts than did monocots such as corn and barley. The difference in the TCr accumulation in different tissues was reportedly due to the differences in the rooting patterns, transpiration rates and metabolisms between monocot and dicot species.

2.4 Conclusions

This portion of dissertation research studied the possibility of using weed plant species in Thailand to remove Cr from soil. Cr accumulation capacities of *Cynodon dactylon*, *Pluchea indica*, *Echinochloa colonum*, *Vetiveria nemoralis*, *Phyllanthus reticulatus*, and *Amaranthus viridis* were investigated. Results indicate that the Cr(VI) concentration affected the Cr uptake capacities of all six plants while the effect of harvesting time on the Cr accumulation was significant for *Vetiveria nemoralis*, *Echinochloa colonum*, *Pluchea indica*, and *Amaranthus viridis*. Only the TCr uptakes of *Cynodon dactylon* and *Phyllanthus reticulatus* were affected by soil pH and pH > 4.5 provided higher Cr accumulations. *Cynodon dactylon* and *Pluchea indica* had the highest Cr accumulation capacities when grown in Cr(VI) contaminated soil and were further studied for the removal mechanism. Cr was translocated from the roots all the way to the leaves of *Pluchea indica* while the translocation stopped at the stems for *Cynodon dactylon*. The translocations of Cr to the above ground parts suggest phytoaccumulation as a key removal mechanism. It may be possible to use *Cynodon dactylon* and *Pluchea indica* to remediate Cr contaminated soil. Although *Cynodon dactylon* is more tolerant and is considered a Cr hyperaccumulator at the Cr(VI) concentration of 400 ppm, for the remediation of Cr, it has to be harvested and replanted since it does not translocate Cr to the leaves. If *Pluchea indica* is used for the remediation of Cr in soil, its leaves should be stripped on day 30 and discarded in secured landfills. After the leaves are stripped, this plant can grow its leaves back again and can uptake more Cr. *Pluchea indica* is thus better than *Cynodon dactylon* for the remediation of Cr contaminated soil.

CHAPTER III

CHROMIUM REMOVAL FROM WATER BY BIOSORPTION WITH NON LIVING BIOMASS OF WEED PLANT SPECIES

3.1 Introduction

Chromium (Cr) is used widely in many industries and particularly in tanning processes of the Thai tannery industry. The improper management of waste is the principle cause of Cr contamination of soil and groundwater. Cr is found in the natural environment in two oxidation states; trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)] (Palmer et al., 1994). Cr(VI) is more toxic than Cr(III) (McGrath, 1982). Normally, tanning industries discharge both Cr(III) and Cr(VI) into waterways. Cr(VI) is of particular concern because it will dissolve in water and is then able to move deeper into the soil via groundwater systems (Eco-USA, 2001).

The Department of Industrial Works of Thailand (1997) has set the Cr effluent standard content in wastewater at 0.75 mg/l for Cr(III) and 0.25 mg/l for Cr(VI). In comparison, the effluent standard for wastewater in Japan is 0.5 mg/l for Cr(VI) and the United States have set the standard for total chromium (TCr) at 0.3 mg/l (Kura et al., 1996). Cr contamination in wastewater can be remediated by many techniques: biological treatment (aerobic, anaerobic, bioreactor, biofilter, surfactant-mediated bioremediation, in situ bioremediation processes); chemical and physical treatment (advanced oxidation and reduction processes) (USEPA, 1993). However, the operational costs for all of these techniques are high.

Biosorption is an alternative to other more expensive methods of cleaning up contaminated water. Its use for heavy metal removal from water has a low

environmental impact and is cost effective (Schnoor, 1997; USEPA, 2000). Kratochvil et al. (1998) found that the maximum uptake of Cr(VI) by protonated *Sargassum* biomass was achieved at pH 2. At pH < 2, the reduction of Cr(VI) to Cr(III) shows the dominance of equilibrium in batch systems. Cabatingan et al. (2001) has shown that chromate (CrO_4^{2-}) can be sorbed by brown seaweed *Sargassum siliquosum* at an optimum pH level of 2. Sudha et al. (2001) investigated the sorption ability of Cr(VI) with a powdered biomass of *Rhizopus nigricans*. They found that the optimum pH for biosorption of Cr(VI) was also 2. More than 75% of the Cr(VI) was removed within 30 minutes of contact. Maximum removal was obtained after 8 hours. Gupta et al. (2001) studied the sorption of Cr(VI) from solution by a biomass of filamentous *Spirogyra* algae. The adsorption capacity of the biomass strongly depends on equilibrium pH levels. Maximum removal of Cr(VI) was 14,700 mg Cr(VI)/kg of dry weight biomass at a pH of 2 in 120 minutes with an initial concentration of 5 mg/l. Iqbal et al. (2002) examined the biosorption of Pb^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+}, Cr^{3+} and Zn^{2+} contaminated water by petiolar felt-sheath of palm. They found that the biomass efficiently removes all the toxic metal ions with the following selectivity order: $Pb^{2+} > Cd^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cr^{3+}$.

The previous research on biosorption did not focus on high Cr(VI) concentration levels in water, nor on the use of weed plant species (biomass). The objective of this research was to investigate the possibility of using biosorption to remove Cr(VI) from contaminated water found in the Thai tannery area using biomass. The Cr(VI) adsorption capacities of the biomass were also determined, where the roots, stems and leaves were measured for their adsorption efficiency. The research determined the effect of pH and empty bed contact time (EBCT) on Cr(VI) removal capacities of each biomass in batch and continuous column modes.

3.2 Materials and Methods

3.2.1 Plants for Biomass

Six out of thirty-four weed plant species occurring around the Thai tannery sites were used in the biosorption study. They were selected on the basis of their ability to accumulate TCr. The six plant species were classified into two groups; the dicot species and monocot species. The dicot species included *Phyllanthus reticulatus* Poir., *Pluchea indica* Less. and *Amaranthus viridis* L. The monocot species included *Cynodon dactylon* (L.) Pers., *Vetiveria nemoralis* (A.) Camus. and *Echinochloa colonum* (L.) Link. However, the six plant species selected for this study had been collected from uncontaminated areas at Bangprahun, Ayutthaya province, Thailand. They were washed with tap water and distilled water to remove dirt and then air dried at room temperature. Roots, stems and leaves were cut and wrapped with foil and then oven-dried again at 70 ^{o}C for 2-3 days. Each part was then milled with mortar and pestle and separately screened through a 2-3 mm mesh. They are shown in Figure 7 of the appendix. No background TCr was found in these plant biomasses and they were used as the biosorption biomass for all experiments.

3.2.2 Experimental Design and Procedure

3.2.2.1 Batch Experiment

Two hundred milliliters of potassium dichromate ($K_2Cr_2O_7$) solution, at 50 ppm of Cr(VI) and at pH levels of 2, 4, 6 and 8 were prepared. Each biomass (root, stem and leaf) was added separately to the solutions in dosages of 0.1, 0.25, 0.5, 1, 1.5

and 2 grams. Two hundred milliliters of these solutions were put into a 500 milliliter volumetric flask, which was then continuously shaken at 120 rpm. The aqueous phase was sampled and analyzed for Cr(VI) concentrations at 15, 30, 60, 120 and 180 minute intervals and then at three hour intervals until the equilibrium time was reached where there was no further change in Cr(VI) concentration. The Freundlich isotherm and the Langmuir isotherm were used to model the results.

3.2.2.2 Column Experiment

The parts of the biomass of one dicot species and one monocot species which provided the maximum Cr(VI) removal capacity were selected from the batch experiments. Acrylic columns with a diameter of 1.85 cm and a length of 20 cm were used. The biomass was prepared in the same way as in the batch experiment, weighed at 8.0 grams, and packed into each column. Synthetic influent water containing 50 ppm of Cr(VI) was used for this experiment. Initial pH of the influent was adjusted to 2, 4, 6 or 8 for each column. The influent was continually upflow-fed into a column using perlistatic pumps (Cole Parmer Instrument Co. Model 7553-60) at EBCTs of 10, 20 and 30 minutes, yielding flow rates of 4, 2 and 1.3 milliliters/minute, respectively. The aqueous effluent phase was analyzed for Cr(VI) concentration. The experiment was done in duplicated to ensure the reproducibility of the results.

3.2.3 Analyses

The USEPA method 3052 (acid digestion/atomic absorption spectrometer) was used for the analysis of TCr (USEPA, 1996). Cr(VI) was analyzed following the USEPA method 7196A (1,5 diphenylcarbohydrazide colorimetric method) (USEPA, 1992). This experiment was also conducted in order to analyze using the analysis of variance (ANOVA). These statistical analyses were conducted through the Statistic Analysis System version 8.

3.3 Results and Discussion

3.3.1 Batch experiment

3.3.1.1 Type of biomass

Figure 3-1 shows the Cr(VI) adsorption capacity of the six plant species at a pH of 2, biomass mass of 0.1, 0.25, 0.5, 0.75, 1 and 2 grams and an equilibrium time of 24 hours. The leaves of the dicot species of *Phyllanthus reticulatus*, *Pluchea indica* and *Amaranthus viridis* showed maximum Cr(VI) adsorption capacities of 53, 45 and 36.4 mg/g, respectively, at biomass mass of 0.1 gram. These were greater than the adsorption capacities of the leaves of the monocot species of *Echinochloa colonum, Cynodon dactylon* and *Vetiveria nemoralis,* which yielded maximum Cr(VI) adsorption capacities of 36.6, 33.9 and 27.5 mg/g, respectively, at biomass mass of 0.1 gram.

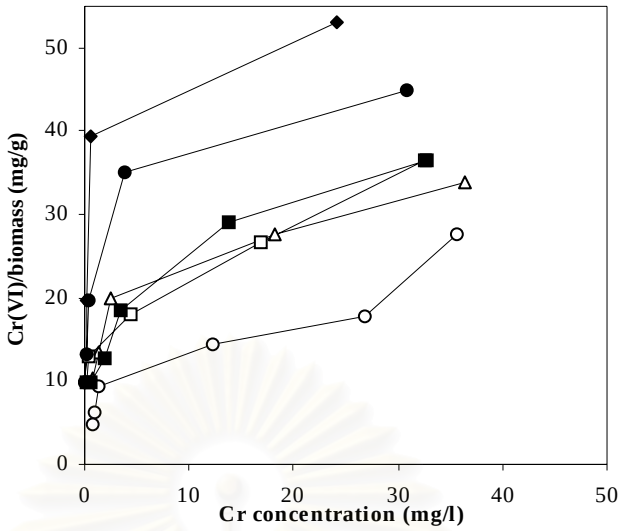


Figure 3-1. Isotherm for leaves of six plant species at the maximum Cr(VI) adsorption capacity at pH of 2.

Symbol:

△ *Cynodon dactylon*

○ *Vetiveria nemoralis*

□ *Echinochloa colonum*

◆ *Phyllanthus reticulatus*

● *Pluchea indica*

■ *Amaranthus viridis*

3.3.1.2 Effect of pH

Figure 3-2 shows the isotherm of dicot and monocot species which recorded the maximum Cr(VI) adsorption capacities, at biomass mass of 0.1, 0.25, 0.5, 0.75, 1 and 2 grams. Figure 3-2a shows that the leaf of *Phyllanthus reticulatus* had maximum Cr(VI) adsorption capacities of 53, 25.1, 21.4 and 12 mg/g, respectively, at biomass mass of 0.1 gram and pH levels of 2, 4, 6 and 8. Figure 3-2b shows that the leaf biomass of *Echinochloa colonum* had maximum Cr(VI) adsorption capacities of 36.6, 7.7, 7.3 and 11.6 mg/g, respectively, at biomass mass of 0.1 gram and pH levels of 2, 4, 6 and 8. However, these results show that the Cr(VI) removal efficiency decreased as pH increased.

The adsorption of metal ions depends on the pH level of the contaminated solution because acidity strongly influences the electrostatic binding of these ions to corresponding functional groups of OH^-, COO^-, –COOH or $–NH_2$, etc. Sudha et al. (2001) found that the *Rhizopus* biomass serves as a matrix of –COOH and $–NH_2$ groups, which in turn take part in the binding of metal ions. At low pH levels, Cr(VI) is mostly found in $HCrO_4^-$, $Cr_2O_7^{2-}$, $Cr_4O_{13}^{2-}$ and $Cr_3O_{10}^{2-}$. Thus, the increased binding of Cr(VI) ions can be explained as being due to electrostatic binding to positively charged groups, such as amino and carboxyl groups in plant cell walls (Gardea-Torresdey et al., 1998; Amino acid, 2001). Baig et al. (1999) explained that the biomass binds more Cr(VI) at a pH of 2 than at a pH of 5. They had attempted to determine the mechanism of Cr(VI) binding by the biomass and settled the hypotheses of research on the following two processes. Firstly, where Cr(VI) occurs as an oxo-anion, such as CrO_4^{2-}, $HCrO_4^-$, and $Cr_2O_7^{2-}$, reducing at higher pHs is highly unlikely where negatively charged carboxylate ions prevail. Secondly, it had been previously

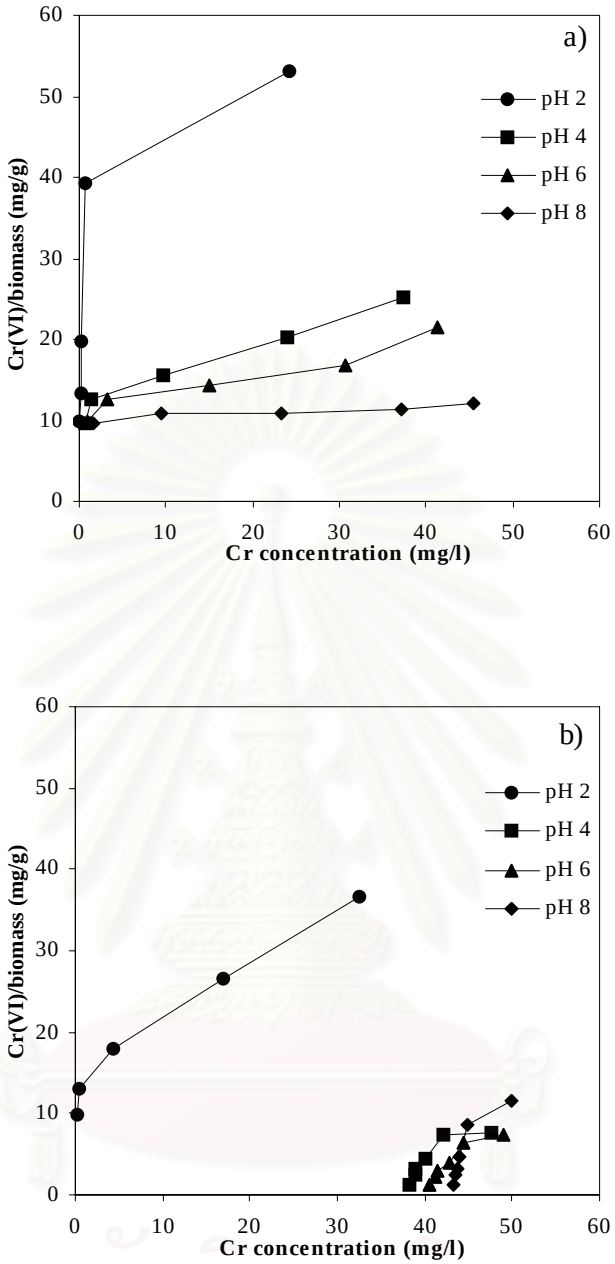


Figure 3-2. Isotherm of plant species at the maximum Cr(VI) adsorption capacity:

a) Leaves of *Phyllanthus reticulatus* and

b) Leaves of *Echinochloa colonum*

reported that Cr(VI) is reduced to Cr(III) by some biomasses at lower pHs. In addition, optimum Cr removal by biosorption at a pH of 2 has been reported for protonated *Sargassum* biomass (Kratochvil et al., 1998), brown seaweed *Sargassum siliquosum* (Cabatingan et al., 2001), *Rhizopus nigricans* (Sudha et al., 2001), and biomass of filamentous algae *Spirogyra* species (Gupta et al., 2001).

Figure 3-3 shows the proportion of Cr(VI) adsorption to the leaves of six biomasses, *Phyllanthus reticulatus*, *Pluchea indica*, *Echinochloa colonum*, *Amaranthus viridis*, *Cynodon dactylon* and *Vetiveria nemoralis*, at a pH of 2, 4, 6 and 8. These biomasses have been found to have a maximum Cr(VI) removal capacity of 99.8%, 99.8%, 99.7%, 99%, 98.4% and 97.2%, respectively, at a pH of 2, biomass mass of 1 gram and an equilibrium time of 24 hours. The adsorption capacities of *Phyllanthus reticulatus* were 98.4%, 98.1% and 96.7%, at pH levels of 4, 6 and 8, respectively. The results for this plant showed that the percentage Cr(VI) adsorption capacity decreased by 2% with each 2 point increase in Cr(VI) solution pH. pH was shown to have little effect on Cr(VI) adsorption by this plant because after experiment seems to be finely powdered biomass. Moreover, in many cases colloids are least stable, that is, they tend to aggregate, at pH_{pzc} (pH of point of zero charge is defined as pH at which surface charge is zero) for colloids such as metal oxides and can have a significant impact on the adsorption of metals, ions, and other molecules (Sawyer, 1994). However, Cr(VI) adsorption capacity of *Pluchea indica* decreased from 99.8% to 31.7% and that of *Echinochloa colonum* decreased from 99.7% to 22.3% as pH levels were altered from 2 to 4. As pH levels were increased from 4 to 8 for *Pluchea indica* and *Echinochloa colonum*, results showed a 5% reduction of Cr(VI) adsorption capacity with each 2 point increase in Cr(VI) solution pH. In comparison, leaves from *Amaranthus viridis*, *Cynodon dactylon* and *Vetiveria nemoralis* had even lower

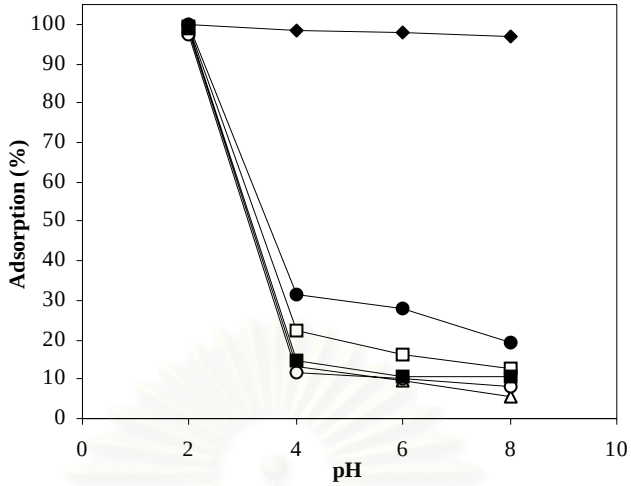


Figure 3-3. The percentage removal of Cr(VI) by leaves of six plant species at a pH of 2, 4, 6 and 8, at an equilibrium time of 24 hours.

Symbol:

△ *Cynodon dactylon*

○ *Vetiveria nemoralis*

□ *Echinochloa colonum*

◆ *Phyllanthus reticulatus*

● *Pluchea indica*

■ *Amaranthus viridis*

Cr(VI) adsorption capacities. It was observed that the maximum Cr(VI) adsorption capacity occurred at a pH level of 2 for each of the biomasses. More importantly, Sharma et al. (1993) found that for sphagnum moss peat as the dose increased from 1-10g, the Cr(VI) removal efficiency increased from 56-83%, but the adsorption densities dropped from 28 mg/g to as low as 4 mg/g, at pH 2. Sudha et al. (2001) show that for the biomass dosage of *Rhizopus nigricans* at 0.5%(w/v) and pH of 2, more than 75% of the Cr(VI) ions were removed within 30 minutes of contact and maximum removal was obtained after 8 hours. This plant showed 99-100% adsorption efficiencies at Cr concentration of 50 mg/l. Gupta et al. (2001) found that the biomass of filamentous algae *Spirogyra* species had a maximum Cr(VI) removal capacity of 14.7 mg/g biomass at a pH of 2, in 120 rpm and 5 mg/l of Cr initial concentration. Thus Cr(VI) is considerably more soluble than Cr(III). Chromate, CrO_4^{2-}, which is the predominant form at pH of more than 6, exists in a pH dependent equilibrium with other forms of Cr(VI) such $HCrO_4^-$ and dichromate ($Cr_2O_7^{2-}$). Cr(VI) can be adsorbed by positively charged surfaces of biomasses. Adsorption of Cr(VI) was considerably less at neutral alkaline pH than at more acidic pH values (USEPA, 2000).

3.3.1.3 Effect of various parts of plants

These results showed that the type of plant part used (root, stem or leaf) for six plant species (eighteen types of biomass) had affected Cr(VI) adsorption capacities. Of the three parts, leaves were found to adsorb most effectively. Gardea-Torresdey et al. (1998) summarized that the three different biomass types (roots, stems and leaves) all had different Cr(VI) adsorption capacities. Both roots and stems are composed of woody material necessary to physically support the plant. Leaves are not composed of woody material and so may have other types of metal binding

compounds which differ from those found in stems or roots. The main components of woody material in roots and stems are cellulose and hemicellulose. Both of these contain hydroxyl groups that are able to bind with metal ions, but to a lesser extent than carboxyl groups. Therefore, leaves may contain higher protein levels that will supply sulhydryl, amino and carboxyl groups. These groups may cause the reduction of Cr(VI) when using leaves of weed plants.

3.3.1.4 Adsorption isotherm of Cr(VI)

Figure 3-4 shows equilibrium data on Cr(VI) adsorption capacity at the optimum condition and were found to have a better fit to the Freundlich isotherm than to the Langmuir isotherm. Table 3-1 shows the linear correlation coefficient (R^2) values on the roots, stems and leaves of these biomasses at a pH of 2.

3.3.1.5 Cr(VI) adsorption capacities of biomass

Table 3-2 shows the ranking of biomass species according to Cr(VI) adsorption at the Cr(VI) concentration of 50 ppm in solute for different parts of plants: root, stem and leaf. The leaves of the dicots *Phyllanthus reticulatus, Pluchea indica* and the monocot, *Echinochloa colonum*, had the maximum Cr(VI) adsorption capacities of 53, 45 and 36.4 mg/g biomass, respectively, at biomass dosage of 0.1 gram, pH of 2 and equilibrium time of 24 hours. The Duncan multiple range test on the data confirmed that these capacities are statistically in the same group and are significantly different to the other accumulation capacities shown in Table 3-2. Leaf biomass of *Phyllanthus reticulatus, Pluchea indica* and *Echinochloa colonum* were therefore selected for the study of the column modes.

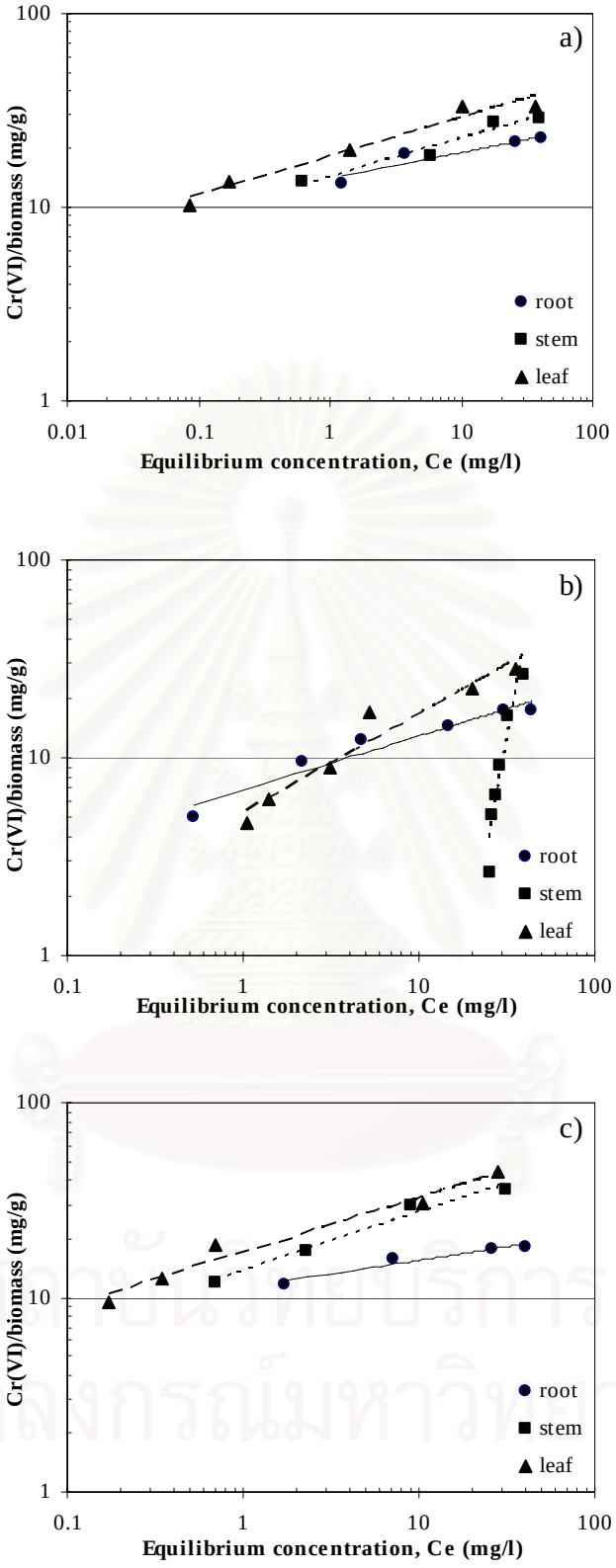
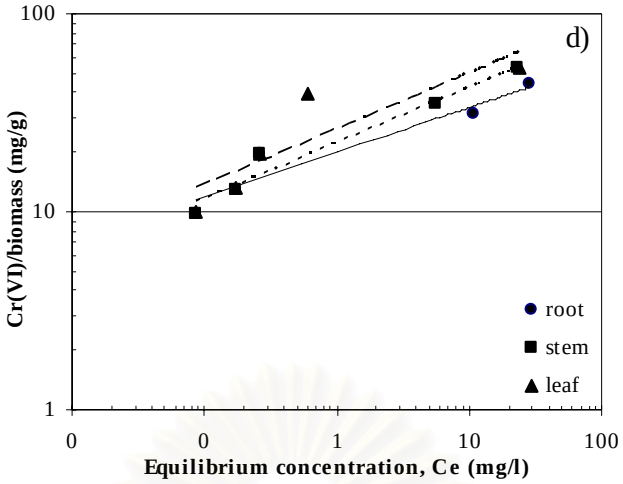
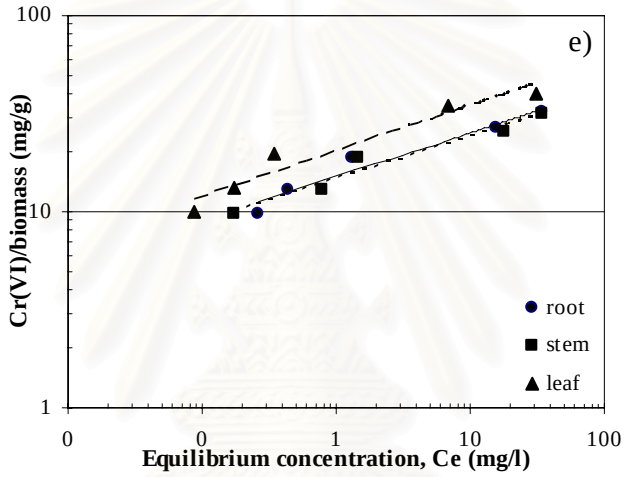


Figure 3-4. The determining Freunlich isotherm for six plant species at pH 2: a) *Cynodon dactylon*, b) *Vetiveria nemoralis*, c) *Echinochloa colonum*, d) *Phyllanthus reticulatus*, e) *Pluchea indica* and f) *Amaranthus viridis*.





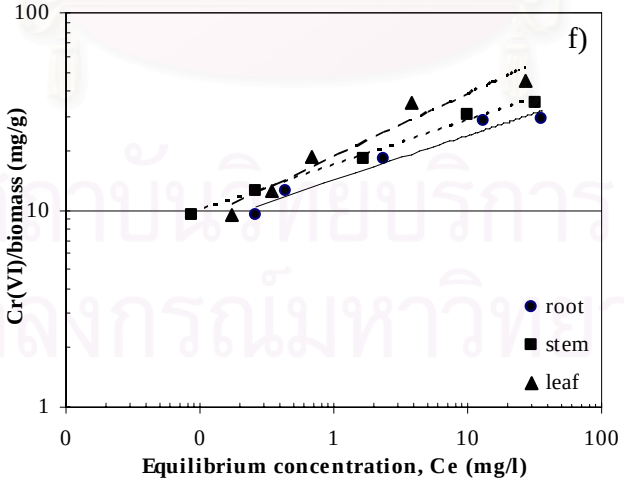


Figure 3-4. (Cont.)

Table 3-1

The Freundlich isotherm of six plant species or biomass on the linear correlation coefficient value.

Species of biomass	Y equation	R^2	N
Cynodon dactylon			
-leaves	$18.228x^{0.2011}$	0.96	5
-stems	$14.367x^{0.1957}$	0.95	4
-roots	$14.019x^{0.1358}$	0.88	4
Vetiveria nemoralis			
-leaves	$5.228x^{0.5007}$	0.94	6
-stems	$8E\text{-}07x^{4.7986}$	0.90	6
-roots	$6.914x^{0.2713}$	0.94	6
Echinochloa colonum			
-leaves	$17.047x^{0.2783}$	0.97	6
-stems	$13.741x^{0.2976}$	0.97	6
-roots	$11.231x^{0.1404}$	0.95	6
Phyllanthus reticulatus			
-leaves	$26.077x^{0.2816}$	0.77	6
-stems	$22.269x^{0.2783}$	0.96	6
-roots	$20.058x^{0.2243}$	0.92	5
Pluchea indica			
-leaves	$20.384x^{0.2302}$	0.94	6
-stems	$14.705x^{0.2150}$	0.96	6
-roots	$15.100x^{0.2234}$	0.95	6
Amaranthus viridis			
-leaves	$18.490x^{0.3167}$	0.94	6
-stems	$16.718x^{0.2283}$	0.99	6
-roots	$14.131x^{0.2269}$	0.96	6

Table 3-2

The Cr(VI) adsorption rankings of the six biomass species at Cr(VI) concentration of 50 ppm.

Rank	Biomass species and part of plants	Mean Cr(VI) adsorption ±standard error (mg/g)	Group[1]
1	*Phyllanthus*-leaf	53.0±0.2	1
2	*Phyllanthus*-stem	52.0±0.2	2
3	*Pluchea*-leaf	45.0±0.4	3
4	*Phyllanthus*-root	43.6±0.3	4
5	*Echinochloa*-leaf	36.4±0.2	5
6	*Amaranthus*-leaf	36.0±0.0	5
7	*Cynodon*-leaf	33.7±0.2	6
8	*Pluchea*-root	33.4±0.3	6
9	*Pluchea*-stem	32.9±0.0	7
10	*Vetivaria*-leaf	27.7±0.1	8
11	*Echinochloa*-stem	27.3±0.2	8
12	*Vetivaria*-stem	26.4±0.1	9
13	*Cynodon*-stem	25.4±0.3	10
14	*Cynodon*-root	24.6±0.2	11
15	*Amaranthus*-stem	23.3±0.1	12
16	*Amaranthus*-root	21.3±0.0	13
17	*Vetivaria*-root	20.7±0.2	13
18	*Echinochloa*-root	18.4±0.0	14

[1] Differences among specific means were evaluated using Duncan multiple range test. Numbers with different superscript differ significantly (*F*-test, $P < 0.05$).

3.3.2 Column experiment

3.3.2.1 Biomass for column modes

From the batch experiment, the maximum capacity for Cr(VI) adsorption in dicot leaves was shown by *Phyllanthus reticulatus* and in monocot leaves by *Echinochloa colonum*. These species were thus selected for the column study on the basis of their ability to adsorb Cr(VI). However, dicots are more interesting than monocots in terms of Cr(VI) adsorption. The leaf of *Pluchea indica* had the second highest adsorption capacity of the dicots and was thus also chosen for the column study, which was conducted on three plant species. The results of the column experiment show that leaves of *Pluchea indica* have a maximum Cr(VI) adsorption capacity which is greater than that of the leaves of *Echinochloa colonum* and *Phyllanthus reticulatus*.

From part of the batch isotherm experiment, *Phyllanthus reticulatus* showed the maximum Cr(VI) adsorption capability. During that experiment the solution containing biomass was shaken constantly which caused a reduction in biomass size, a fracturing of biomass structure and which resulted in an increase in surface area and charge for surface adsorption. An explanation of this result can be gained from observing the leaf characteristics of this species. The leaves are thin and easily broken and after shaking were in powderous form. However, in the column experiment, the columns were not shaken. Thus, leaves of *Phyllanthus reticulates* adsorbed Cr(VI) at lower capacities than *Pluchea indica*.

3.3.2.2 Effect of pH

Figure 3-5 shows the breakthrough curves of *Pluchea indica*, *Echinochloa colonum* and *Phyllanthus reticulates* leaves at pH differences of 2, 4, 6 and 8 and at a flow rate of 1.3 ml/min. The results show that the *Pluchea indica* leaves had the maximum Cr(VI) adsorption capacity of 51.3, 0.5, 0.3 and 0.1 mg/g biomass at breakthrough times of 102 hours, 60 minutes, 30 minutes and 15 minutes, respectively. However, leaves from *Echinochloa colonum* had Cr(VI) adsorption capacities lower than that at all the pH levels, at 12.1, 0.1, 0.1 and 0.1 mg/g biomass, and with breakthrough times of 24 hours, 15 minutes, 15 minutes and 15 minutes, respectively. Leaves from *Phyllanthus reticulatus* had even lower Cr(VI) adsorption capacities at all pH levels of 6.1, 0.3, 0.1 and 0.1 mg/g biomass in breakthrough times of 12 hours, 30 minutes, 15 minutes and 10 minutes, respectively. Altogether, this experiment had procedure and sample correction until the equilibrium time was reached and no further change in Cr(VI) concentration occurred.

3.3.2.3 Effect of flow rate (EBCT)

Figure 3-6 shows the breakthrough curves of *Pluchea indica*, *Echinochloa colonum* and *Phyllanthus reticulatus* leaves at flow rate differences of 1.3, 2 and 4 ml/min, at a pH of 2. The results show that the *Pluchea indica* leaves had the maximum Cr(VI) adsorption capacities for all flow rates at 51.3, 18.1 and 9.4 mg/g biomass with breakthrough times of 102, 24 and 6 hours, respectively. Leaves from *Echinochloa colonum* had Cr(VI) adsorption capacities which were lower than these at all flow rates. The adsorption capacities were 12.1, 6.9 and 1.5 mg/g biomass with breakthrough times of 24, 9 and 1 hour, respectively. Leaves from *Phyllanthus reticulates* had even lower adsorption capacities at these flow rates. This plant had

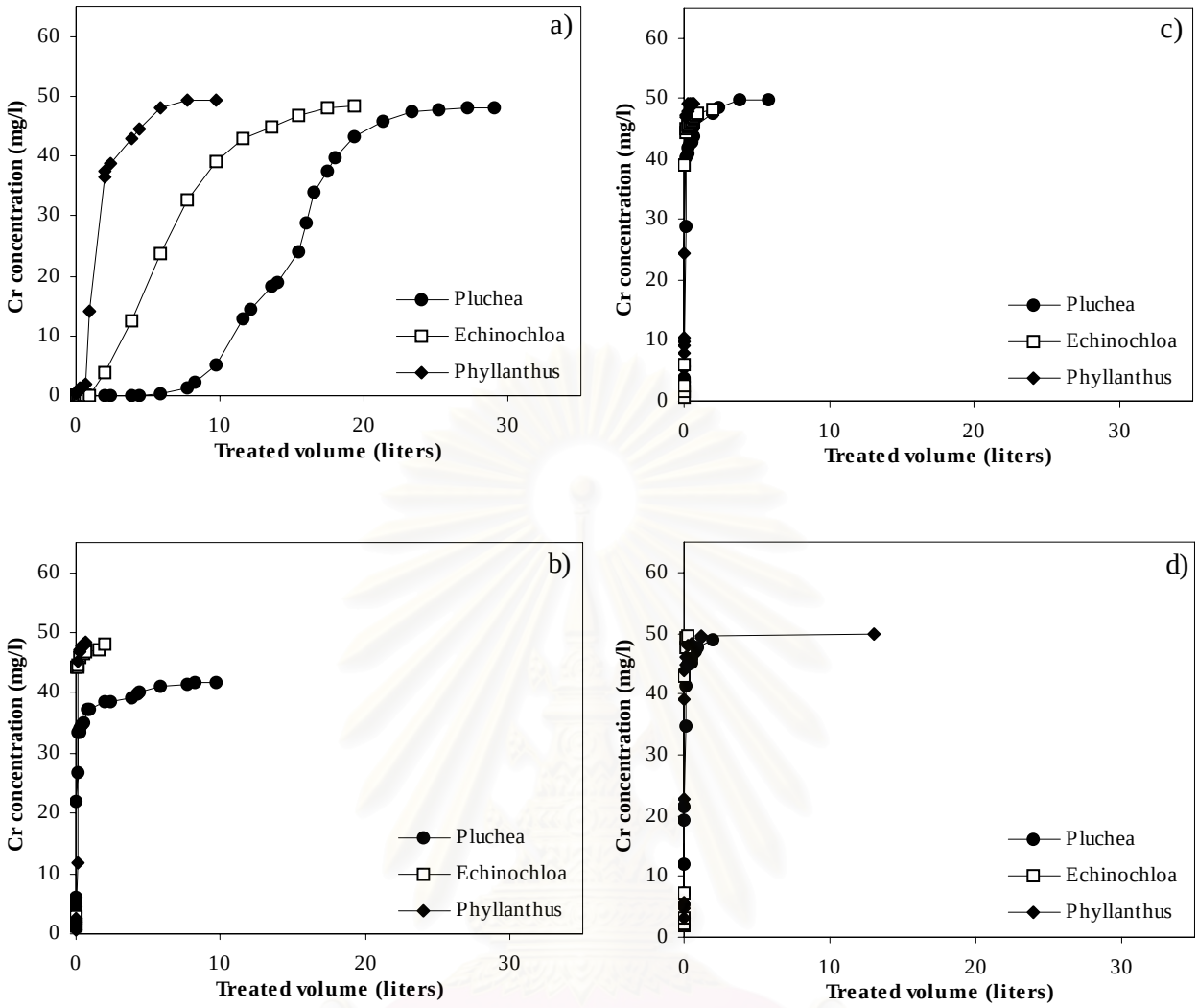


Figure 3-5. Breakthrough curve of *Pluchea indica*, *Echinochloa colonum* and *Phyllanthus reticulatus*, at EBCT of 30 minutes: a) pH 2, b) pH 4, c) pH 6 and d) pH 8.

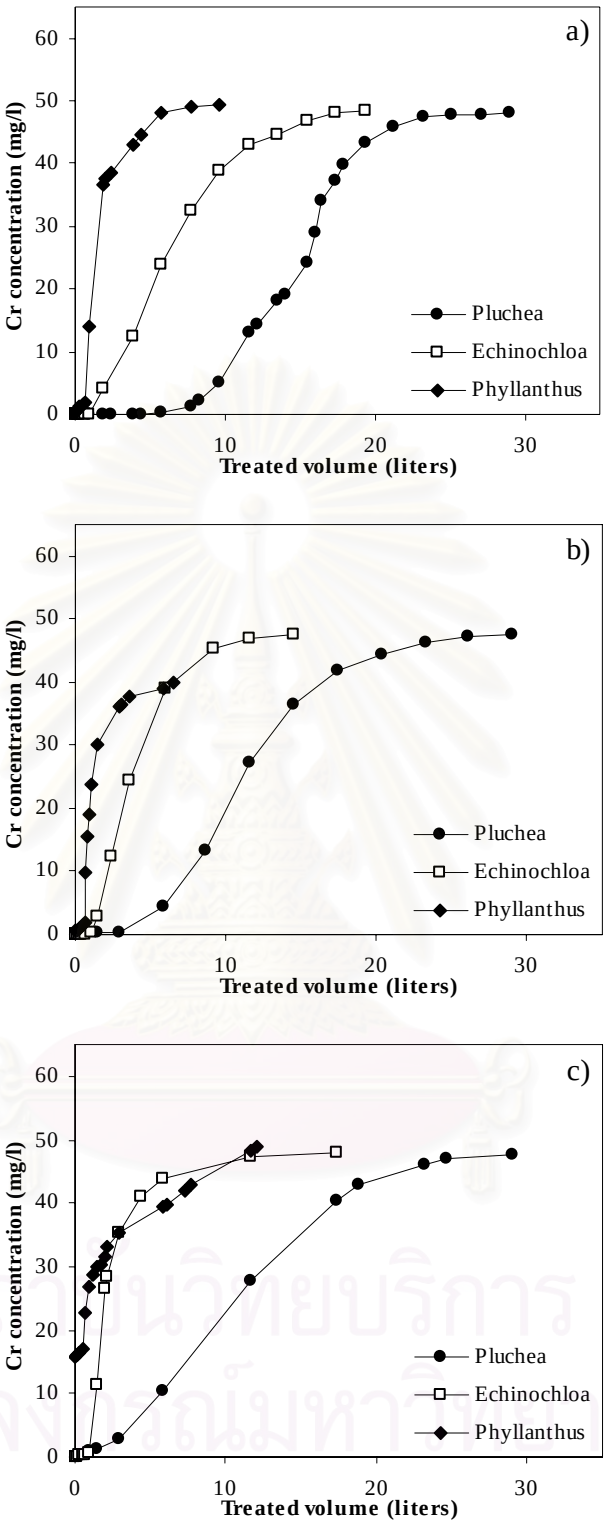


Figure 3-6. Breakthrough curve of *Pluchea indica*, *Echinochloa colonum* and *Phyllanthus reticulatus*, at pH of 2: a) EBCT 30 minutes, b) EBCT 20 minutes and c) EBCT 10 minutes.

adsorption capacities of 6.1, 4.6 and 1.5 mg/g biomass with breakthrough times of 12, 6 and 1 hour, respectively. Therefore, the effect of pH and the effect of flow rate can be summarized as in Figure 3-7. Figure 3-7 shows the breakthrough curve of *Pluchea indica*, *Echinochloa colonum* and *Phyllanthus reticulatus* leaves, at the maximum Cr(VI) adsorption capacities, pH of 2 and at a flow rate of 1.3 ml/min. However, the Cr(VI) adsorption capacity decreased as a pH increased from 2 to 8, and also decreased as flow rate increased from 1.3 to 4 ml/min.

3.4 Conclusions

The maximum Cr(VI) adsorption capacity of the leaves of six plant species can be used to remediate Cr(VI) contaminated wastewater. From batch experiments, biomass of the dicot species showed higher Cr(VI) adsorption capacities than the monocot species. However, increasing the monocot species biomass dosage may result in greater Cr(VI) adsorption. The pH level of 2 was found to be the optimum pH for Cr(VI) adsorption for all parts of the plants; root, stem and leaves, and particularly for the leaves of *Phyllanthus reticulatus* and *Pluchea indica*. These plants showed maximum Cr(VI) adsorption capacities of 99-100%, at a biomass mass of 1 gram and an equilibrium time of 24 hours. Results from the column experiments found that the leaves of *Pluchea indica* had the maximum Cr(VI) adsorption capacity of 51.3 mg/g biomass, at a pH of 2, a flow rate of 1.3 ml/min and a breakthrough time of 102 hours. Therefore, further research in this field will be oriented towards the development of a suitable biofiltation particle for repeated applications of these results. Weed plant species in Thailand have the potential to be used as a biomaterial

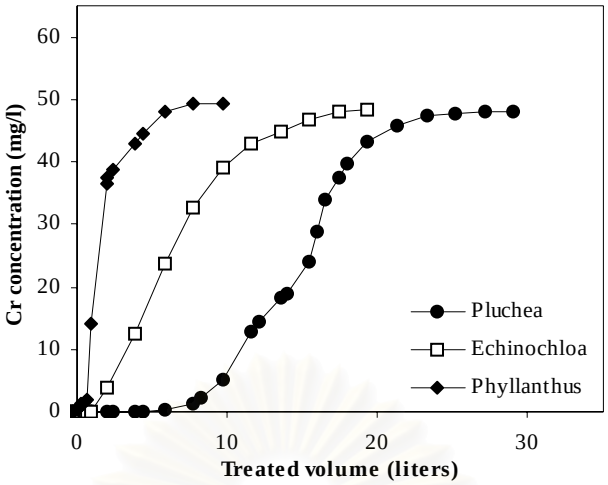


Figure 3-7. Breakthrough curve of *Pluchea indica*, *Echinochloa colonum* and *Phyllanthus reticulatus*, at pH of 2 and an EBCT of 30 minutes.

for Cr(VI) removal from contaminated water. Thus, the ability to effectively remove Cr(VI) from wastewater indicates the potential use of biomass derived from leaves of *Pluchea indica* to treat industrial wastewater effluents and cleanse the environment. More importantly, these results indicate that biomass can reduce Cr(VI) on the maximum uptake, which took place at a pH of 2. Thus, leaves of *Pluchea indica* could potentially be used to remediate Cr(VI) contaminated wastewater effluents more effectively than leaves of other plant species. For potential remedial applications, the leaves of weed plant species in Thailand could be used as a biofilter for treating Cr contaminated wastewater because these plants have no economic value and their use is cost effective. In addition, further studies are needed in understanding the interaction behavior between the activated biomass and Cr(VI) ions in batch and column study.

CHAPTER IV

ALTERNATIVE OF CHROMIUM REMOVAL BY PHYTOREMEDIATION AND BIOSORPTION WITH WEED PLANT SPECIES

4.1 Introduction

Chromium (Cr) is found in two oxidation states in the natural environment: trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. Of these two forms, essentially immobile Cr(III) compounds are the predominant species in most environmental settings (Eco-USA, 2001). It is known to be less toxic than Cr(VI). Industry can and does discharge both Cr(III) and Cr(VI) into the environment. Cr is used in many industries and in particular in Thai tanning factories which has resulted in Cr contamination of soil and water. Phytoremediation and biosorption are two treatment techniques that may be used to solve or mitigate this problem.

Phytoremediation is the direct use of living plants for in-situ, or on-site remediation of contaminated soils, sludges, sediments and ground water, through contaminant removal, degradation or containment (USEPA, 1998; 1999). Phytoremediation can be used to remediate various contaminants including metals, pesticides, solvents, explosives, petroleum hydrocarbons, polycyclic aromatic hydrocarbons and landfill leachates. Phytoremediation has been studied extensively in research and small-scale demonstrations, but full-scale applications are currently limited to only a small number of projects (ITRC, 1999).

Biosorption is the use of dead biomass to bind and concentrate heavy metals from dilute aqueous solutions. Biomass exhibits this property by acting just as a chemical substance, as an ion exchanger of biological origin. The cell wall structure of

certain algae, fungi and bacteria was found to be particularly responsible for this phenomenon. The opposite of biosorption is the metabolically driven active bioaccumulation by living cells. That is an altogether different phenomenon requiring a different approach for its exploration (Volesky, 1990).

The objective of this chapter is to summarize the alternative between Cr removal by phytoremediation with weed plant species and biosorption with biomass. It's been discussed that the plants that are effective for use in phytoremediation would also provide effective biomass for biosorption. This research focuses on weed plant species from phytoremediation that can be used for biosorption. Both methods can be effectively utilized for remediating Cr(VI) contaminated soil and wastewater.

Moreover, the potential for alternative mitigating processes based on relationship between phytoremediation and biosorption is one objective of the research. The salient point is that these two areas have never been related even though they both rely on plants one on a live plant and the other on a dead plant. There might or might not be a relationship between these two areas.

4.2 Materials and Methods

The experiment used plants from a tannery site. Six out of thirty four weed plant species found around the tannery site were selected on the basis of their ability to accumulate TCr. The selected monocots were *Cynodon dactylon* (L.) Pers., *Vetiveria nemoralis* (A.) Camus. and *Echinochloa colonum* (L.) Link., while *Phyllanthus reticulatus* Poir., *Pluchea indica* Less., and *Amaranthus viridis* L were the selected dicots. These plant species were studied in phytoremediation and biosorption experiments.

4.2.1 Phytoremediation experiment

This experiment, soil samples were collected from 10 locations within the tannery and from 5 locations outside. Six plant species were studied in pots, with plastic bag enclosed saucers, at a nursery. One hundred milliliters of potassium dichromate ($K_2Cr_2O_7$) solution of various concentrations was applied to soil in the pots. This yielded soil Cr(VI) concentrations at 100, 200 and 400 ppm (mg Cr(VI)/kg soil). For the control pots, only water was applied. Water was applied to each pot daily in the morning. 15% of N, P, and K fertilizer were added to all pots, including the controls, every 30 days. The plants were harvested on days 30, 60 and 90. During harvesting, plant tissues were collected from each pot and analyzed for TCr concentrations. Altogether, one dicot and one monocot species, that showed the highest TCr accumulation were chosen for the Cr(VI) removal mechanism process. However, the procedure of the removal mechanism experiment was similar to that of the previous section except that only a Cr(VI) concentration of 100 ppm was used. The plants were harvested after 30, 60, 90, and 120 days. After harvesting, the plants were cut into three parts, roots, stems, and leaves, and analyzed for Cr(VI), Cr(III), and TCr concentrations.

4.2.2 Biosorption experiment

This experiment, six plant species were collected from uncontaminated areas and then washed and air dried at room temperature. Roots, stems and leaves were cut and wrapped with foil and then oven dried at 70^{o}C for 2-3 days and milled with mortar and pestle and then separately screened through a 2-3 mm mesh. In addition,

potassium dichromate ($K_2Cr_2O_7$) solutions, at 50 ppm (Cr(VI)/L) and at pH levels of 2, 4, 6 and 8 were also prepared for batch and column studies. Biomass (root, stem and leaf) was used in dosages of 0.1, 0.25, 0.5, 1.0, 1.5 and 2 grams on batch isotherm and 8 gram on column modes. The results from the batch isotherm showed the maximum Cr(VI) adsorption capacities of the biomass of one dicot and one monocot species. The column study used EBCTs of 10, 20 and 30 minutes, yielding flow rates of 4, 2 and 1.3 milliliters/minute, respectively. The aqueous phase was sampled and analyzed for Cr(VI) concentrations.

4.2.3 Phytoremediation and Biosorption relationship

Data analyses were carried out to determine whether plants that are effective for phytoremediation are also effective biosorbents.

4.2.4 Analyses and Statistics

The USEPA method 3052 (acid digestion/atomic absorption spectrometer) was used for the analysis of TCr (USEPA, 1996a). For the analysis of Cr(VI), alkaline digestion of plant tissues was performed according to the USEPA method 3060A (USEPA, 1996b) and the 1,5 diphenylcarbohydrazide colorimetric method according to the USEPA method 7196A (USEPA, 1992). The concentration of Cr(III) was determined from the difference between measured TCr and Cr(VI) concentrations. Phytoremediation and biosorption data were also analyzed using the analysis of variance (ANOVA). These statistical analyses were conducted using the Statistic Analysis System (SAS) version 8 programs.

4.3 Results and Discussion

4.3.1 Phytoremediation experiment

The results showed that the TCr capacities of *Cynodon dactylon, Pluchea indica, Phyllanthus reticulatus, Echinochloa colonum* and *Vetiveria nemoralis* were 152.1, 151.8, 101, 77 and 69 mg TCr/kg of plant on a dry weight basis on day 30 at a Cr(VI) concentration of 100 ppm, respectively. While, for *Amaranthus viridis* at the Cr(VI) concentration of 50 ppm, no TCr was detected in its tissues on day 30. Thus, Cr(VI) concentration had a significant effect on TCr uptake capacities of plants. By testing with one-way ANOVA, significant differences ($p < 0.05$) in TCr accumulation were observed among the 6 plant species. This research shows that the monocot, *Cynodon dactylon,* and dicot, *Pluchea indica,* had the highest Cr accumulation capacities. These plants accumulated Cr(VI), Cr(III) and TCr in roots throughout the experimental period. *Cynodon dactylon* showed TCr accumulation in stems until day 60, but no TCr accumulation in the leaves. *Pluchea indica* accumulated TCr in stems and leaves until day 60. The corresponding Cr(VI) uptake results of *Cynodon dactylon* in root, stem and leaf were 38, 18 and 0 mg/kg of plant on a dry weight basis on day 30, respectively. For *Pluchea indica*, the corresponding Cr(VI) uptake results in root, stem and leaf were 29, 35 and 73 mg/kg, respectively.

4.3.2 Biosorption experiment

The results show that of the dicots, the leaves of *Phyllanthus reticulatus* had Cr(VI) adsorption capacities greater than those for *Pluchea indica* and *Amaranthus viridis*. These were 53,000, 45,000 and 36,400 mg/kg, at a pH of 2 and a Cr(VI) concentration of 50 ppm. Of the monocots, the leaves of *Echinochloa colonum* had Cr(VI) adsorption capacities which were higher than for *Cynodon dactylon* and *Vetiveria nemoralis.* These were 36,600, 33,900 and 27,500 mg/kg, respectively. Among the three parts of the plant, leaves were found to adsorb most effectively, while stems and roots had lower Cr(VI) adsorption capacities. This research shows that leaves of *Pluchea indica* were found to have maximum Cr(VI) adsorption capacity, at a pH of 2 and an EBCT of 30 minutes, at a Cr(VI) concentration of 50 ppm, which was higher than that for other biomass. This biomass had a breakthrough volume of 51.3 mg/g biomass, at 102 hours. Moreover, this research found that the Cr(VI) adsorption capacity increased as pH decreased and that the flow rate decreased over time.

4.3.3 Relationship of Phytoremediation and Biosorption

4.3.3.1 Cr(VI) contamination medias

The results of the phytoremediation study show that *Cynodon dactylon* had the highest TCr uptake capacities at a Cr(VI) concentration of 400 ppm and acted as a TCr hyperaccumulator. Phytoremediation using weed plant species can clean up large areas of Cr(VI) contamination as an in-situ procedure. Another salient point is that, high Cr(VI) concentrations may inhibit plant growth and thus Cr(VI) uptake capacity. The success of phytoremediation may be seasonal, depending on geographical location. Other climatic factors will also influence the effectiveness with which plants can remove Cr(VI) by direct uptake. In contrast, biosorption with biomass can operate in areas of higher Cr(VI) concentrations and as an ex-situ procedure. Whilst biosorption cannot process large areas of Cr(VI) contamination this method does prevent contaminants moving in soils or groundwater. Biomass can remove Cr(VI) by direct adsorption.

4.3.3.2 Possibility of business management

In phytoremediation, plant species must be used for a long time in order to clean up a site. Their maintenance and successful growth relies on effective operational measures such as irrigation and fertilizer application. This is costly and time consuming, where plant species used for phytoremediation may take several growing seasons to clean a site. Moreover, Cr(VI) accumulation in plants may pose a risk to animals that eat these plants. Phytoremediation was also slower than biosorption treatment. However, phytoremediation has higher public acceptance than biosorption technology. The biosorption process requires significant amounts of

biomass material and there is difficulty in finding and collecting this material. Biosorption with biomass has shown short times in the consumption of toxins to clean wastewater and has no negative effect on animals.

4.3.3.3 Type of plant

The research using phytoremediation of weed plant species for the clean up Cr contaminants from soil found that *Pluchea indica* and *Cynodon dactylon* had TCr uptake capacities which were more than those for *Echinochloa colonum, Vetiveria nemoralis, Phyllanthus reticulatus* and *Amaranthus viridis*. The biosorption of biomass experiment found that of the leaves, *Pluchea indica, Echinochloa colonum* and *Phyllanthus reticulatus* showed the maximum Cr(VI) adsorption capacities. Both of these experiments tested different plant material for Cr removal from soil and water. Thus, both of them showed that the leaves of *Pluchea indica* had greater accumulation than that for other weed plants. This plant had greater Cr(VI) adsorption capacities over other species and other parts of plants. In addition, plants can absorb both Cr(VI) and Cr(III), although the more toxic Cr(VI) could be more easily transported through an active mechanism inside the plant than the less toxic Cr(III) which was transported through a passive mechanism (Skeffington et al, 1976). This indicates that the two forms do not share common uptake mechanisms.

4.3.3.4 Efficiency of Cr accumulation in plants and Cr adsorption in biomass

Results from the phytoremediation research show that the plant species *Cynodon dactylon, Pluchea indica, Phyllanthus reticulatus, Echinochloa colonum, Vetiveria nemoralis and Amaranthus viridis* had TCr accumulation values of 0.152, 0.151, 0.101, 0.077, 0.069 and 0 mg/g of plant on a dry weight basis on day 30 at a Cr(VI) concentration of 100 ppm, respectively. Altogether, roots, stems and leaves of *Cynodon dactylon* had Cr(VI) uptake results of 0.038, 0.018 and 0 mg/g, respectively. For *Pluchea indica,* the Cr(VI) uptake results for roots, stems and leaves were 0.029, 0.035 and 0.073 mg/g, respectively. In contrast, the results from the biosorption experiment found that of the leaves of *Phyllanthus reticulatus, Pluchea indica, Echinochloa colonum, Amaranthus viridis, Cynodon dactylon* and *Vetiveria nemoralis* had Cr(VI) adsorption capacities of 53, 45, 36.6, 36.4, 33.9 and 27.5 mg/g, respectively, at a pH of 2 and at a Cr(VI) concentration of 50 ppm. Altogether, this research found that *Pluchea indica, Echinochloa colonum* and *Phyllanthus reticulatus* had Cr(VI) adsorption capacities of 51.3, 12.1 and 6.1 mg/g, respectively, at a flow rate of 1.3 ml/min and pH of 2.

Table 4 in the appendix I shows the Cr uptake and adsorption ranking for functional parameters of the six plant and biomass species in the phytoremediation and biosorption study. This research found that *Phyllanthus reticulatus* leaf, had the maximum Cr(VI) adsorption capacity of 53000 mg/kg biomass on a dry weight basis, at a pH of 2, an equilibrium time of 24 hr., a biomass mass of 0.1 gram and a Cr(VI) concentration of 50 ppm on batch experiment. While *Pluchea indica* leaf, had the maximum Cr(VI) adsorption capacity of 51300 mg/kg biomass, at a pH of 2, a flow rate of 1.3 ml/min. and a Cr(VI) concentration of 50 ppm on column experiment. The

ranking shows higher Cr(VI) adsorption in biosorption than from Cr(VI) accumulation in phytoremediation. The Duncan multiple range test on the data confirmed that these capacities are statistically in the same group and are significantly different to the other accumulation capacities. They are shown in Table 4 of the appendix I.

In addition, Table 4-1 shows the ranking of plant species used in phytoremediation and biomass species used in biosorption according to Cr(VI) accumulation and adsorption at Cr(VI) concentrations of 100 ppm and 50 ppm, respectively. In the phytoremediation and biosorption study, *Pluchea indica* leaves, had the highest Cr(VI) accumulation and/or adsorption capacities of 73 mg/kg of plant on dry weight basis and 51.3 mg/g of biomass on dry weight basis, respectively.

While, of monocot species, the root of *Cynodon dactylon* in the phytoremediation study and the leaf of *Echinochloa colonum* in the biosorption study had the highest Cr(VI) accumulation and/or adsorption capacities of 38 mg/kg of plant and 12.1 mg/g of biomass, at a biomass dosage of 0.1 gram, pH of 2 and equilibrium time of 24 hours, respectively. Thus, leaves of both the plant and the biomass of *Pluchea indica* had the highest uptake capacities and thus were selected to solve the problem of Cr contamination in both soil and water. The Duncan multiple range test on the data confirmed that these capacities are statistically in the same group and are significantly different to the other accumulation capacities.

Table 4-1

The Cr(VI) accumulation and adsorption rankings of plant species for the phytoremediation and biomass species for biosorption study.

Rank	Phytoremediation[1]		Biosorption[2]	
	Plant species and part of plants	Mean Cr(VI) accumulation ±standard error (mg/kg)	Biomass species and part of plants	Mean Cr(VI) adsorption ±standard error (mg/g)
1	*Pluchea*-leaf	73±8.3	*Pluchea*-leaf	51.3±0.2
2	*Cynodon*-root	38±11.1	*Echinochloa*-leaf	12.1±0.1
3	*Pluchea*-stem	35±5.6	*Phyllanthus*-leaf	6.1±0.2
4	*Pluchea*-root	29±15.5		
5	*Cynodon*-stem	18±4.9		
6	*Cynodon*-leaf	0±0.0		

[1] Cr(VI) removal mechanism part of phytoremediation study, at the Cr(VI) concentration of 100 ppm on day 30.

[2] Cr(VI) adsorption capacities in column modes part of biosorption study, at the Cr(VI) concentration of 50 ppm, pH of 2 and a flow rate of 1.3 ml/min.

Moreover, the salient point is that, leaves of *Pluchea indica* in both the phytoremediation and biosorption sections of the experiment, had effected the highest Cr(VI) removal capacities. The dominance of this species shows the relationship between phytoremediation and biosorption in two areas of the research. Thus, this relationship needs further research in regards to chemical relationships and interaction behavior between living plants and non-living plants.

4.3.3.5 Cr(VI) removal after plants treatments

The management of plants after they have accumulated Cr(VI) in phytoremediation needs further research. Biosorption biomasses after Cr(VI) adsorption can be used in solidification and stabilization processes and can then be securely stored as landfill. However, harvested plants used in phytoremediation and biomass on biosorption may require disposal as hazardous waste.

4.4 Conclusions

The alternative between Cr removal capacities of phytoremediation with weed plant species and biosorption with biomass were discussed. Plant species used in phytoremediation can also be used as biomass for biosorption. The weed plant species used for phytoremediation in this research were *Cynodon dactylon*, *Vetiveria nemoralis*, *Echinochloa colonum*, *Phyllanthus reticulatus*, *Pluchea indica* and *Amaranthus viridis*. These plants can also be used as biomass for biosorption and both methods can be effectively utilized for remediating Cr(VI) contaminated soil and wastewater. The results of phytoremediation found that *Pluchea indica* and *Cynodon dactylon* had the highest TCr uptake capacities. The biosorption of biomass research

found that the leaves of *Pluchea indica, Phyllanthus reticulatus* and *Echinochloa colonum* had maximum Cr(VI) adsorption. The Cr removal mechanisms experiment showed that the leaves of *Pluchea indica* had the highest Cr(VI) accumulation capacities and were higher than those for *Cynodon dactylon*. The biosorption on column experiment found that the leaves of *Pluchea indica* had the maximum adsorption capacities. Both the phytoremediation and biosorption experiments incorporated different media for Cr remediation of both soil and water. However, both of these showed that the leaves of *Pluchea indica* had higher Cr accumulation and adsorption than the other weed species.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

This research investigated the possibility of using weed plant species in Thailand to remove Cr from contaminated soil and water. Cr accumulation capacities of *Cynodon dactylon, Pluchea indica, Echinochloa colonum, Vetiveria nemoralis, Phyllanthus reticulatus* and *Amaranthus viridis* were investigated. Results indicate that the Cr(VI) input dosage affected Cr uptake capacities of all six plants, while the effect of harvesting time on Cr accumulation was significant for *Vetiveria nemoralis, Echinochloa colonum, Pluchea indica* and *Amaranthus viridis*. *Cynodon dactylon* and *Pluchea indica* recorded the highest Cr accumulation capacities when grown in Cr(VI) contaminated soil. They were further studied to identify removal mechanisms. Cr was translocated from the roots to the leaves of *Pluchea indica* but did not move further than the stems for *Cynodon dactylon*. *Pluchea indica* is thus more effective than *Cynodon dactylon* for the remediation of Cr contaminated soil. The translocation of Cr to the above ground plant parts suggests that phytoaccumulation is a key removal mechanism. Altogether, the Cr(VI) adsorption capacity of the leaves of *Pluchea indica*, *Phyllanthus reticulatus* and *Echinochloa colonum* can be used to remediate Cr(VI) contaminated wastewater. In contrast, biomass of the monocot species showed lower Cr(VI) adsorption capacities than those of the dicot species. Additionally, leaves of *Pluchea indica* showed the maximum Cr(VI) adsorption capacities. This ability to effectively remove Cr(VI) from wastewater indicates the potential use of biomass derived from leaves of *Pluchea indica* to also remove other toxic metals from industrial wastewater effluent. Thus, leaves of *Pluchea indica* could be used to remediate Cr(VI) contaminated wastewater effluents more effectively than

leaves of other plants. The relationship between Cr removal capacities of phytoremediation with weed plant species and biosorption with biomass were discussed. It was found that plant species used in phytoremediation can also be used as biomass for biosorption. The results from phytoremediation report that *Cynodon dactylon* and *Pluchea indica* had the highest TCr uptake capacities. The results from biosorption of biomass studies found that the leaves of *Pluchea indica, Phyllanthus reticulatus* and *Echinochloa colonum* had maximum Cr(VI) adsorption. The Cr removal mechanisms experiment showed that the leaves of *Pluchea indica* had the highest Cr(VI) accumulation capacities and were more than those for *Cynodon dactylon*. The biosorption on column experiment found that the leaves of *Pluchea indica* had the maximum adsorption capacities. Thus, both of these experiments showed that the leaves of *Pluchea indica* had Cr(VI) accumulation which was greater than that of other weed species.

Additionally, this research found that weed plant species can uptake both Cr(VI) and Cr(III) and transport these within the plant. Some Cr(VI) had also been reduced by soil in pots without plants.

The research found TCr to accumulate more extensively in soil than in drainage water collected from the saucers, suggesting that most TCr was absorbed in soil. TCr accumulation in both soil and drainage water was highest on day 30 and decreased on days 60 and 90. In part of the Cr mechanisms section, the discussion focused almost exclusively on Cr(VI) because Cr(VI) is more toxic to plants than any other Cr species form. Due to this, this research was interested only in Cr(VI).

This experiment was conducted on particular criterion including the following:

1. The plants had performed in individual soil pots. Whole parts of plants (root, stem and leaf) were harvested on time study to analyze Cr accumulation.

2. The sample weight taken from the whole plant was only 0.5 grams for TCr analysis and 1-2.5 grams for Cr(VI) analysis.

A widely held assumption exists that at days 90 and 120 soil in pots may be adsorbing more Cr than that adsorbed by the root. However, from observation, Cr may be accumulating in or transported to the rhizome and/or shoots of plants. More importantly, the reasons for the decrease of Cr should be that the plants had grown well and that the high dry weight of biomass at times, often increased. The results show the difference and non-equality between Cr uptake rates by plants and growth rates of plants. Cr uptake rates were lower than the growth rate of plants. Thus, Cr may be accumulating in plants in their lowest physiological levels and could not be detected in samples taken of stems and leaves. Thus, Cr accumulation in plants is likely to depend on both plant growth rate and Cr uptake rate.

Finally, the recommendations derived from this study are as follows:

1. Plants should perform in plot scale available containers (plots) and/or large areas as this will decrease the difference between soil and plant separation.

2. The size of the biomass on biosorption experiment should be consistent or have a homogeneous make up to determine the factors affecting of Cr accumulation of biomass.

3. The biomass on batch was shaken constantly, which reduced the size and also fractured the biomass. This resulted in an increase in surface area and charge for surface adsorption. Constant shaking should thus be part of biosorption applications.

4. The leaves of *Pluchea indica* had the highest Cr from soil accumulation and they also had the maximum Cr from water adsorption on biomass. Further studies to determine the factors or reasons for this result are recommended, with particular focus on chemical relationships and interaction behavior between the living plant as phytoremediation and non-living biomass as biosorption.

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APPENDICES

APPENDIX I

TABLES

Table 1

Plant growth in height (cm) for six plant species on days 15, 30, 45, 60, 75, and 90.

Cr(VI) concentration	Time and Height of Plants (cm.)						
(ppm)	0 days	15 days	30 days	45 days	60 days	75 days	90 days
Cynodon dactylon							
0	53.4	56.0	58.2	59.3	62.2	65.3	65.7
100	60.6	52.9	39.3	40.5	43.3	45.3	48.3
200	58.1	47.2	37.9	29.8	32.5	33.3	39.3
400	66.7	36.7	28.2	28.5	31.2	28.7	32.3
Vetiveria nemoralis							
0	60.8	89.3	128.4	145.8	152.2	158.3	157.3
100	74.4	83.8	127.2	136.2	137.8	135.7	134.3
200	69.0	43.4	72.1	76.8	77.8	73.7	72.7
400	68.7	10.3	16.4	20.5	20.7	42.3	42.0
Echinochloa monodum							
0	38.9	56.1	74.6	83.5	85.3	97.0	101.0
100	40.8	62.2	67.3	75.8	78.5	78.0	81.7
200	36.6	9.2	17.6	25.0	25.5	31.0	31.3
400	36.7	8.8	13.4	20.7	21.3	23.3	20.7
Phyllanthus reticulatus							
0	50.7	77.9	87.9	106.2	107.7	116.7	120.3
100	43.4	74.6	94.1	106.8	108.5	104.3	107.7
200	36.7	36.0	58.6	94.0	96.2	93.0	95.3
400	45.2	13.9	32.3	50.6	52.0	62.8	66.5

Table 1 (Cont.)

Cr(VI) concentration (ppm)	Time and Height of Plants (cm.)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
Pluchea indica							
0	48.1	56.0	65.2	72.7	75.5	86.3	90.0
100	46.6	52.7	48.2	58.5	60.8	69.0	75.0
150	36.3	16.6	22.1	22.0	23.5	15.0	15.0
200	48.1	15.1	11.7	11.2	11.7	14.8	15.3
400	49.2	13.9	0.0	0.0	0.0	0.0	0.0
Amaranthus viridis							
0	32.7	35.3	37.8	40.0	42.0	44.3	48.3
50	38.0	39.0	42.0	43.0	44.0	45.0	45.0
100	51.2	0.0	0.0	0.0	0.0	0.0	0.0
200	41.9	0.0	0.0	0.0	0.0	0.0	0.0
400	46.9	0.0	0.0	0.0	0.0	0.0	0.0

Table 2

The mass balance of TCr accumulation in plants, soil and drainage water on six plant species, Cr(VI) concentrations of 0, 100, 200 and 400 ppm, at days 30, 60 and 90.

Time	Cr(VI) concentration (ppm)	Total Cr accumulation in plant (mg)	in soil (mg)	in water (mg)	Sum of Total Cr (mg)	Total Cr accumulation (%)
Cynodon dactylon						
30	0	0.480	240.000	0.117	240.597	94.352
	100	2.754	534.565	0.873	538.192	71.284
	200	18.183	757.130	1.834	777.147	61.924
	400	22.453	1828.130	1.930	1852.513	82.151
60	0	0.716	251.000	0.208	251.925	98.794
	100	7.186	446.130	1.224	454.540	60.204
	200	2.361	821.463	1.784	825.608	65.786
	400	13.004	1763.797	3.386	1780.187	78.944
90	0	0.339	213.750	0.057	214.146	83.979
	100	3.985	615.630	0.156	619.771	82.089
	200	1.057	840.130	1.190	842.377	67.122
	400	11.810	1699.832	3.326	1714.967	76.052
Vetiveria nemoralis						
30	0	0.291	251.000	0.006	251.297	98.548
	100	1.824	541.280	0.478	543.583	71.998
	200	1.967	829.130	0.783	831.880	66.285
60	0	0.259	223.000	0.017	223.276	87.559
	100	3.705	488.797	0.284	492.786	65.270
	200	2.898	921.130	0.912	924.940	73.700
90	0	0.000	217.667	0.000	217.667	85.359
	100	3.396	572.130	0.136	575.662	76.247
	200	2.679	1027.380	0.299	1030.358	82.100

Table 2 (Cont.)

Time	Cr(VI) concentration (ppm)	Total Cr accumulation in plant (mg)	in soil (mg)	in water (mg)	Sum of Total Cr (mg)	Total Cr accumulation (%)
Echinochloa colonum						
30	0	0.000	247.167	0.029	247.195	96.939
	100	1.663	587.130	0.469	589.263	78.048
	200	3.351	960.630	0.782	964.762	76.873
60	0	0.525	253.333	0.045	253.903	99.570
	100	2.002	528.630	0.251	530.883	70.316
	200	2.008	885.630	0.344	887.982	70.756
90	0	0.428	243.333	0.000	243.761	95.592
	100	0.663	515.297	0.627	516.587	68.422
	200	1.841	935.130	1.169	938.140	74.752
Phyllanthus reticulatus						
30	0	0.258	251.833	0.006	252.097	98.861
	100	1.775	595.463	0.284	597.522	79.142
	200	0.773	1042.380	5.559	1048.713	83.563
	400	2.893	2188.630	3.186	2194.709	97.326
60	0	0.286	253.333	0.069	253.688	99.485
	100	1.939	436.364	0.448	438.752	70.369
	200	2.719	1157.130	0.641	1160.490	92.469
	400	1.863	1974.630	0.872	1977.365	87.688
90	0	0.000	237.833	0.151	237.985	93.327
	100	1.275	515.380	0.965	517.621	68.559
	200	1.170	975.030	1.035	977.236	86.972
	400	1.460	1801.630	0.998	1804.088	80.004

Table 2 (Cont.)

Time	Cr(VI) concentration (ppm)	Total Cr accumulation in plant (mg)	in soil (mg)	in water (mg)	Sum of Total Cr (mg)	Total Cr accumulation (%)
Pluchea indica						
30	0	0.457	253.500	0.084	254.040	99.624
	100	3.764	571.630	3.758	579.152	76.709
	150	4.918	673.130	0.584	678.632	67.526
	200	3.361	769.130	0.620	773.111	61.602
60	0	0.279	253.330	0.085	253.698	99.489
	100	2.759	486.963	0.873	490.594	64.979
	150	1.692	806.630	0.700	809.022	80.500
	200	1.176	1158.130	2.064	1161.370	92.539
90	0	0.125	238.667	0.193	238.985	93.719
	100	2.345	511.797	1.284	515.425	68.268
	150	1.307	743.630	0.402	745.339	74.163
	200	4.348	966.130	1.017	971.494	77.410
Amaranthus viridis						
30	0	0.000	254.667	0.016	254.683	99.876
	50	0.000	499.687	1.352	501.039	99.216
60	0	0.000	217.500	0.142	217.642	85.350
	50	0.044	352.297	3.494	355.835	70.462
90	0	0.000	254.333	0.007	254.340	99.741
	50	0.035	424.630	0.174	424.839	84.126

Table 3

The mass balance of TCr, Cr(III), Cr(VI) from varies part of plant species at Cr(VI) concentration of 100 ppm, at days 30, 60, 90 and 120.

Type of plant	root						stem						leaf					
and times	Cr(III) (mg)	Cr(III) (%)	Cr(VI) (mg)	Cr(VI) (%)	TCr (mg)	TCr (%)	Cr(III) (mg)	Cr(III) (%)	Cr(VI) (mg)	Cr(VI) (%)	TCr (mg)	TCr (%)	Cr(III) (mg)	Cr(III) (%)	Cr(VI) (mg)	Cr(VI) (%)	TCr (mg)	TCr (%)
Pluchea indica																		
day30	0.06	0.01	0.01	0.00	0.07	0.01	0.06	0.01	0.04	0.01	0.10	0.01	0.02	0.00	0.08	0.01	0.09	0.01
day60	0.07	0.01	0.05	0.01	0.12	0.02	0.03	0.00	0.12	0.02	0.14	0.02	0.01	0.00	0.18	0.03	0.19	0.03
day90	0.02	0.00	0.05	0.01	0.27	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
day120	0.03	0.00	0.06	0.01	0.09	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cynodon dactylon																		
day30	0.08	0.01	0.11	0.02	0.19	0.03	0.12	0.02	0.06	0.01	0.18	0.02	0.00	0.00	0.00	0.00	0.00	0.00
day60	0.15	0.02	0.06	0.01	0.21	0.03	0.05	0.01	0.06	0.01	0.11	0.02	0.00	0.00	0.00	0.00	0.00	0.00
day90	0.04	0.01	0.13	0.02	0.18	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
day120	0.04	0.01	0.07	0.01	0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3 (Cont.)

Type of plant and times	Soil					
	Cr(III) (mg)	Cr(III) (%)	Cr(VI) (mg)	Cr(VI) (%)	TCr (mg)	TCr (%)
Pluchea indica						
day30	518.32	72.91	191.84	26.98	710.17	99.89
day60	474.76	66.69	236.24	33.19	711.00	99.88
day90	499.55	70.05	262.79	36.85	712.17	99.87
day120	490.79	67.80	268.21	37.05	723.50	99.94
Cynodon dactylon						
day30	506.87	69.57	220.46	30.26	727.33	99.83
day60	468.68	63.66	293.48	39.86	735.33	99.87
day90	423.77	56.79	368.23	49.34	745.33	99.87
day120	469.38	62.28	323.28	42.89	750.00	99.51

Table 3 (Cont.)

Type of plant and times	Water					
	Cr(III) (mg)	Cr(III) (%)	Cr(VI) (mg)	Cr(VI) (%)	TCr (mg)	TCr (%)
Pluchea indica						
day30	0.38	0.05	0.14	0.02	0.52	0.07
day60	0.36	0.05	0.04	0.00	0.40	0.06
day90	0.60	0.08	0.05	0.01	0.64	0.09
day120	0.28	0.04	0.04	0.01	0.31	0.04
Cynodon dactylon						
day30	0.78	0.11	0.05	0.01	0.84	0.11
day60	0.38	0.05	0.25	0.03	0.63	0.09
day90	0.75	0.10	0.01	0.00	0.76	0.10
day120	3.53	0.47	0.02	0.00	3.56	0.47

Table 3 (Cont.)

Type of plant and times	TCr root (mg)	TCr stem (mg)	TCr leaf (mg)	Sum of Cr in plant (mg)	Sum of Cr in soil (mg)	Sum of Cr in water (mg)	Sum TCr (mg)	TCr (%)
Pluchea indica								
day30	0.07	0.10	0.09	0.26	710.17	0.52	711	94.2
day60	0.12	0.14	0.19	0.44	711.00	0.40	712	94.3
day90	0.27	0.00	0.00	0.27	712.17	0.64	713	94.4
day120	0.09	0.00	0.00	0.09	723.50	0.31	724	95.9
Cynodon dactylon								
day30	0.19	0.18	0.00	0.37	727.33	0.84	729	96.5
day60	0.21	0.11	0.00	0.32	735.33	0.63	736	97.5
day90	0.18	0.00	0.00	0.18	745.33	0.76	746	98.8
day120	0.10	0.00	0.00	0.10	750.00	3.56	754	99.8

Table 4

The Cr uptake and adsorption rankings of the six plant and biomass species in the phytoremedaition and biosorption study.

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
1	Phyllanthus-leaf-pH2-24hr-0.1g-50ppm	53000.0	1
2	Phyllanthus-stem-pH2-24hr-0.1g-50ppm	52800.5	2
3	Pluchea-leaf-1.3ml/min-pH2-50ppm	51300.0	3
4	Pluchea-leaf-pH2-24hr-0.1g-50ppm	45000.0	4
5	Phyllanthus-root-pH2-24hr-0.1g-50ppm	43900.5	5
6	Echinochloa-leaf-pH2-24hr-0.1g-50ppm	36600.0	6
7	Amaranthus-leaf-pH2-24hr-0.1g-50ppm	36400.0	7
8	Cynodon-leaf-pH2-24hr-0.1g-50ppm	33900.0	8
9	Pluchea-root-pH2-24hr-0.1g-50ppm	33100.5	9
10	Pluchea-stem-pH2-24hr-0.1g-50ppm	32900.5	10
11	Vetiveria-leaf-pH2-24hr-0.1g-50ppm	27500.0	11
12	Echinochloa-leaf-pH2-24hr-0.1g-50ppm	27100.5	12
13	Vetiveria-stem-pH2-24hr-0.1g-50ppm	26300.5	13
14	Cynodon-stem-pH2-24hr-0.1g-50ppm	25700.5	14
15	Phyllanthus-leaf-pH4-24hr-0.1g-50ppm	25100.5	15
16	Cynodon-root-pH2-24hr-0.1g-50ppm	24400.0	16
17	Amaranthus-stem-pH2-24hr-0.1g-50ppm	23500.5	17
18	Phyllanthus-leaf-pH6-24hr-0.1g-50ppm	21400.5	18
19	Amaranthus-root-pH2-24hr-0.1g-50ppm	21300.5	19
20	Vetiveria-root-pH2-24hr-0.1g-50ppm	20900.5	20

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
21	Echinochloa-root-pH2-24hr-0.1g-50ppm	18400.5	21
22	Pluchea-leaf-2ml/min-pH2-50ppm	18100.5	22
23	Echinochloa-leaf-1.3ml/min-pH2-50ppm	12100.0	23
24	Phyllanthus-leaf-pH8-24hr-0.1g-50ppm	12000.5	24
25	Echinochloa-leaf-pH8-24hr-0.1g-50ppm	11600.5	25
26	Amaranthus-leaf-pH8-24hr-0.1g-50ppm	11100.5	26
27	Amaranthus-stem-pH4-24hr-0.1g-50ppm	9200.5	27
28	Pluchea-leaf-4ml/mim-pH2-50ppm	9100.5	28
29	Vetiveria-stem-pH6-24hr-0.1g-50ppm	8900.5	29
30	Cynodon-stem-pH6-24hr-0.1g-50ppm	8900.5	29
31	Amaranthus-root-pH4-24hr-0.1g-50ppm	8600.5	30
32	Vetiveria-root-pH6-24hr-0.1g-50ppm	8600.5	30
33	Phyllanthus-stem-pH2-24hr-0.1g-50ppm	8400.5	31
34	Cynodon-root-pH8-24hr-0.1g-50ppm	8200.5	32
35	Cynodon-root-pH6-24hr-0.1g-50ppm	7800.5	33
36	Echinochloa-leaf-pH4-24hr-0.1g-50ppm	7700.5	34
37	Echinochloa-stem-pH4-24hr-0.1g-50ppm	7600.5	35
38	Echinochloa-stem-pH8-24hr-0.1g-50ppm	7400.5	36
39	Cynodon-leaf-pH4-24hr-0.1g-50ppm	7400.5	36
40	Phyllanthus-root-pH6-24hr-0.1g-50ppm	7400.5	36
41	Echinochloa-stem-pH6-24hr-0.1g-50ppm	7300.5	37
42	Echinochloa-root-pH6-24hr-0.1g-50ppm	7100.5	38

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
43	Pluvhea-stem-pH6-24hr-0.1g-50ppm	7100.5	38
44	Vetiveria-stem-pH4-24hr-0.1g-50ppm	6900.5	39
45	Cynodon-stem-pH8-24hr-0.1g-50ppm	6800.5	40
46	Phyllanthus-leaf-2ml/min-pH2-50ppm	6800.5	40
47	Cynodon-stem-pH4-24hr-0.1g-50ppm	6300.5	41
48	Amaranthus-leaf-pH4-24hr-0.1g-50ppm	6200.5	42
49	Echinochloa-root-pH8-24hr-0.1g-50ppm	6100.5	43
50	Vetiveria-root-pH4-24hr-0.1g-50ppm	6100.5	43
51	Phyllanthus-leaf-1.3ml/min-pH2-50ppm	6100.0	43
52	Phyllanthus-stem-pH6-24hr-0.1g-50ppm	5900.5	44
53	Pluchea-leaf-pH6-24hr-0.1g-50ppm	5600.5	45
54	Echinochloa-root-pH4-24hr-0.1g-50ppm	5500.5	46
55	Amaranthus-leaf-pH8-24hr-0.1g-50ppm	5400.5	47
56	Phyllanthus-root-pH4-24hr-0.1g-50ppm	5300.5	48
57	Amaranthus-root-pH6-24hr-0.1g-50ppm	5100.5	49
58	Pluchea-root-pH6-24hr-0.1g-50ppm	4900.5	50
59	Amaranthus-leaf-pH6-24hr-0.1g-50ppm	4800.5	51
60	Cynodon-leaf-pH6-24hr-0.1g-50ppm	4700.5	52
61	Pluchea-stem-pH8-24hr-0.1g-50ppm	4600.5	53
62	Pluchea-leaf-pH4-24hr-0.1g-50ppm	4500.5	54
63	Phyllanthus-leaf-2ml/min-pH2-50ppm	4500.5	54
64	Amaranthus-stem-pH6-24hr-0.1g-50ppm	4400.5	55

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
65	Cynodon-root-pH4-24hr-0.1g-50ppm	3800.5	56
66	Amaranthus-root-pH8-24hr-0.1g-50ppm	3700.5	57
67	Echinochloa-leaf-pH6-24hr-0.1g-50ppm	3500.5	58
68	Pluchea-root-pH8-24hr-0.1g-50ppm	3400.5	59
69	Cynodon-leaf-pH8-24hr-0.1g-50ppm	3100.5	60
70	Pluchea-root-pH4-24hr-0.1g-50ppm	3100.5	60
71	Phyllanthus-stem-pH8-24hr-0.1g-50ppm	2900.5	61
72	Vetiveria-leaf-pH2-24hr-0.1g-50ppm	2800.5	62
73	Vetiveria-root-pH8-24hr-0.1g-50ppm	2700.5	63
74	Vetiveria-stem-pH8-24hr-0.1g-50ppm	2700.5	63
75	Phyllanthus-root-pH8-24hr-0.1g-50ppm	2500.5	64
76	Pluchea-leaf-pH8-24hr-0.1g-50ppm	2300.5	65
77	Pluchea-stem-pH4-24hr-0.1g-50ppm	2100.5	66
78	Vetiveria-leaf-pH4-24hr-0.1g-50ppm	1900.5	67
79	Vetiveria-leaf-pH6-24hr-0.1g-50ppm	1500.5	68
80	Phyllanthus-leaf-4ml/min-pH2-50ppm	1500.5	68
81	Echinochloa-leaf-4ml/min-pH2-50ppm	1500.5	68
82	Cynodon-30day-400ppm-TCr	1428.6	69
83	Cynodon-60day-400ppm-TCr	1337	70
84	Cynodon-90day-400ppm-TCr	1199.2	71
85	Pluchea-leaf-1.3ml/min-pH2-50ppm	500.5	72
86	Cynodon-30day-200ppm-TCr	452.3	73

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
87	Phyllanthus-leaf-1.3ml/min-pH4-50ppm	300.5	74
88	Pluchea-leaf-4ml/min-pH4-50ppm	300.5	74
89	Pluchea-leaf-4ml/min-pH8-50ppm	300.5	74
90	Echinochloa-leaf-4ml/min-pH4-50ppm	300.5	74
91	Pluchea-leaf-1.3ml/min-pH6-50ppm	300.5	74
92	Pluchea-leaf-4ml/min-pH6-50ppm	300.5	74
93	Pluchea-leaf-2ml/min-pH8-50ppm	200.5	75
94	Pluchea-leaf-2ml/min-pH4-50ppm	200.5	75
95	Phyllanthus-leaf-2ml/min-pH4-50ppm	200.5	75
96	Pluchea-leaf-2ml/min-pH6-50ppm	200.5	75
97	Phyllanthus-leaf-2ml/min-pH6-50ppm	200.5	75
98	Pluchea-30day-200ppm-TCr	186.5	76
99	Phyllanthus-30day-400ppm-TCr	161.4	77
100	Pluchea-30day-100ppm-TCr	152.3	78
101	Cynodon-30day-100ppm-TCr	152.1	78
102	Pluchea-60day-200ppm-TCr	147.3	79
103	Echinochloa-30day-200ppm-TCr	142.0	80
104	Cynodon-60day-100ppm-TCr	138.9	81
105	Phyllanthus-30day-200ppm-TCr	136.5	82
106	Echinochloa-60day-200ppm-TCr	134.2	83
107	Cynodon-60day-200ppm-TCr	121.1	84
108	Cynodon-90day-200ppm-TCr	114.4	85

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
109	Cynodon-90day-100ppm-TCr	112.1	86
110	Echinochloa-leaf-2ml/min-pH8-50ppm	100.5	87
111	Echinochloa-leaf-4ml/min-pH8-50ppm	100.5	87
112	Phyllanthus-leaf-1.3ml/min-pH8-50ppm	100.5	87
113	Echinochloa-leaf-2ml/min-pH4-50ppm	100.5	87
114	Pluchea-leaf-1.3ml/min-pH8-50ppm	100.5	87
115	Phyllanthus-leaf-4ml/min-pH4-50ppm	100.5	87
116	Phyllanthus-leaf-4ml/min-pH8-50ppm	100.5	87
117	Phyllanthus-leaf-1.3ml/min-pH6-50ppm	100.5	87
118	Echinochloa-leaf-1.3ml/min-pH4-50ppm	100.5	87
119	Echinochloa-leaf-2ml/min-pH6-50ppm	100.5	87
120	Echinochloa-leaf-1.3ml/min-pH8-50ppm	100.5	87
121	Echinochloa-leaf-1.3ml/min-pH6-50ppm	100.5	87
122	Echinochloa-leaf-2ml/min-pH8-50ppm	100.5	87
123	Phyllanthus-leaf-4ml/min-pH6-50ppm	100.5	87
124	Echinochloa-leaf-4ml/min-pH6-50ppm	100.5	87
125	Phyllanthus-60day-400ppm-TCr	95.2	88
126	Pluchea-90day-200ppm-TCr	94.6	88
127	Vetiveria-30day-200ppm-TCr	94.1	88
128	Echinochloa-90day-200ppm-TCr	87.0	89
129	Echinochloa-30day-100ppm-TCr	76.5	90
130	Pluchea-leaf-30day-100ppm-Cr(VI)	73.5	91

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
131	Echinochloa-60day-100ppm-TCr	71.5	92
132	Phyllanthus-60day-200ppm-TCr	70.1	93
133	Vetiveria-30day-100ppm-TCr	69.2	93
134	Pluchea-leaf-60day-100ppm-Cr(VI)	68.4	93
135	Vetiveria-60day-200ppm-TCr	64.8	94
136	Vetiveria-60day-100ppm-TCr	61.4	95
137	Phyllanthus-60day-100ppm-TCr	57.9	96
138	Pluchea-90day-100ppm-TCr	57.2	96
139	Vetiveria-90day-200ppm-TCr	52.4	97
140	Phyllanthus-30day-100ppm-TCr	50.9	98
141	Pluchea-60day-100ppm-TCr	49.6	98
142	Echinochloa-90day-100ppm-TCr	40.6	99
143	Cynodon-root-30day-100ppm-Cr(VI)	38.8	100
144	Phyllanthus-90day-400ppm-TCr	36.3	101
145	Pluchea-stem-30day-100ppm-Cr(VI)	35.6	101
146	Vetiveria-90day-100ppm-TCr	34.8	101
147	Pluchea-stem-60day-100ppm-Cr(VI)	34.3	102
148	Pluchea-root-30day-100ppm-Cr(VI)	29.3	103
149	Phyllanthus-90day-200ppm-TCr	27.6	104
150	Pluchea-root-60day-100ppm-Cr(VI)	26.0	105
151	Cynodon-root-60day-100ppm-Cr(VI)	24.5	106
152	Phyllanthus-90day-100ppm-TCr	23.2	106

Table 4 (Cont.)

Rank	Plant and biomass species	Mean Cr accumulation and adsorption (mg/kg)	Group[1]
153	Cynodon-root-90day-100ppm-Cr(VI)	23.2	106
154	Pluchea-root-90day-100ppm-Cr(VI)	21.5	107
155	Cynodon-root-120day-100ppm-Cr(VI)	20.3	107
156	Cynodon-stem-30day-100ppm-Cr(VI)	18.8	108
157	Cynodon-stem-60day-100ppm-Cr(VI)	15.9	109
158	Pluchea-root-120day-100ppm-Cr(VI)	13.3	110
159	Amaranthus-60day-50ppm-TCr	8.1	111
160	Amaranthus-90day-50ppm-TCr	6.2	112

[1] Differences among specific means were evaluated using Duncan multiple range test. Numbers with different superscript differ significantly (*F*-test, $P < 0.05$).

APPENDIX II

FIGURES

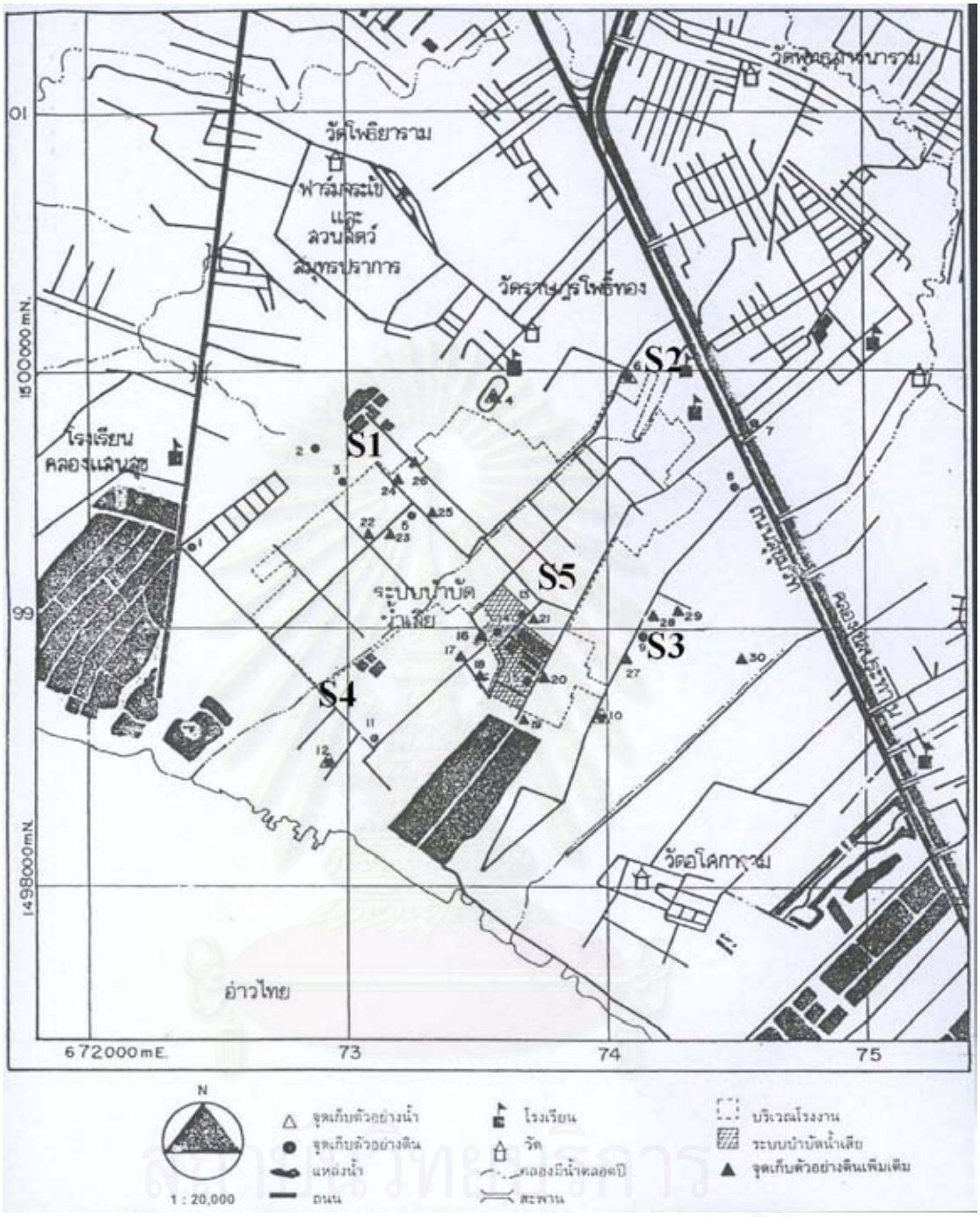

Figure 1. Soil sampling at 5 points within the Thai tannery industry site at Km. 30, Samutprakarn province.

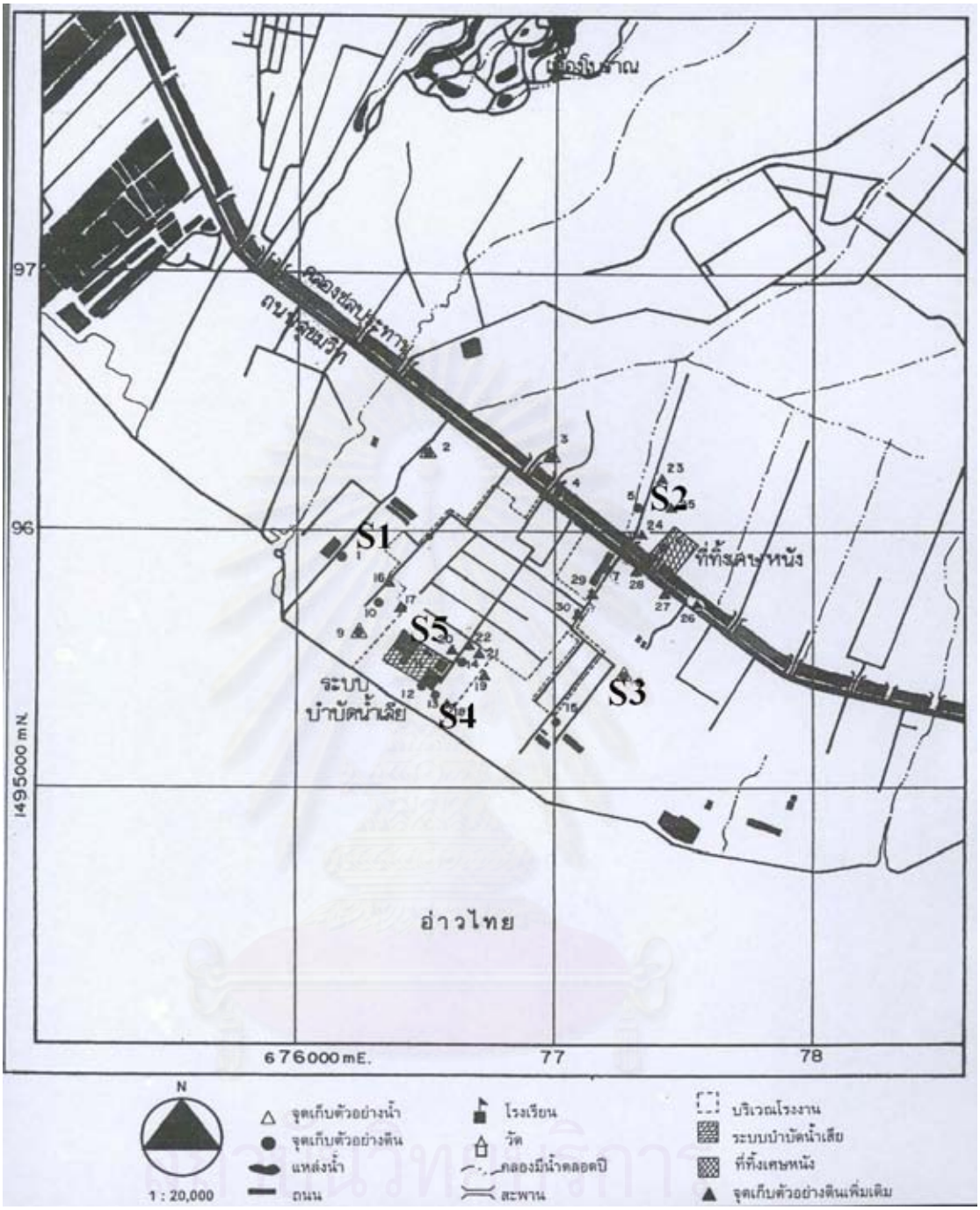

Figure 2. Soil sampling at 5 points within the Thai tannery industry site at Km. 34, Samutprakarn province.

Cynodon dactylon

Vetiveria nemoralis

Echinochloa colonum

Figure 3. Six plant species consisting of three monocot and three dicot species were used in this research.

Phyllanthus reticulatus

Pluchea indica

Amaranthus viridis

Figure 3. (Cont.)

Cynodon dactylon *Vetiveria nemoralis* *Echinochloa colonum*

Phyllanthus reticulatus *Pluchea indica* *Amaranthus viridis*

Figure 4. Cr uptake studies of six plant species in soil pots. Each pot was placed on a covered saucer and plastic bags were placed under the pots. A triplication of the experiment was conducted.

Roots Stems Leaves

Cynodon dactylon

Roots Stems Leaves

Pluchea indica

Figure 5. Preparation of plant species which were cut into the three parts of roots, stems and leaves for analysis of, Cr(VI), Cr(III) and TCr.

Monocot plant species

Dicot plant species

Figure 6. Symptoms of six plant species in the first week after Cr(VI) concentration were applied to soils.

Figure 7. Plants for biomass were separated into roots, stems and leaves and then air dried at room temperature before being wrapped in foil and oven dried.

BIOGRAPHY

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1. Sampanpanish, P., Pongsapich, W., Khaodhiar, S., and Khan, E. Chromium Removal from Soil by Phytoremediation with Weed Plant Species in Thailand. *Accepted with Pending Revision for Publication in Water, Air, and Soil Pollution.*
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