ACCUMULATION AND TOXICITY OF LEAD AND CHROMIUM IN *HYDROCOTYLE UMBELLATA* L.

JIRAPORN YONGPISANPHOP

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Thesis
Entitled

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ACCUMULATION AND TOXICITY OF LEAD AND CHROMIUM IN *HYDROCOTYLE UMBELLATA* L.

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ABSTRACT

The aquatic plant, *Hydrocotyle umbellata,* was studied for its accumulation and toxicity of lead and chromium in a synthetic solution. Plants were cultured in modified Hoagland's nutrient solutions supplemented with 20, 40, 60, 80 and 100 mg Pb/L as lead nitrate $Pb(NO_3)_2$ and other with 2, 4, 6, 8, and 10 mg Cr/L as potassium dichromate $K_2Cr_2O_7$. They were separately harvested after 3, 6, 9, and 12 days. Lead and chromium led to a significant decrease ($P \leq 0.05$) in the relative growth, biomass productivity, and chlorophyll contents of *H. umbellata* when the exposure time and heavy metal concentration were increased. However, they did not significantly decrease on day 3. The EC_{50} of chromium was lower than that of lead during the same exposure time, suggesting that chromium was comparatively more toxic than lead. The accumulation of the above heavy metals in the plants significantly increased ($P \leq 0.05$), but it was not linear with exposure time and heavy metal concentration. Both lead and chromium were accumulated higher in the roots than in the shoots. The average accumulation of lead and chromium in the roots decreased sharply during the first 6 days of the experiment. The bioconcentration factor (BCF) of lead was higher than that of chromium at the same exposure time, indicative of a higher accumulation potential of lead than of chromium in *H. umbellata.*

The toxicity symptoms of *H. umbellata* exposed to heavy metals at different concentrations and exposure times showed that there had been a reduction in the production of new plantlets, a withering of petioles, and the color of the roots had changed from light green to dark brown. Lead caused leaf chlorosis, whereas chromium caused leaf necrosis. The toxicity symptoms increased when exposure time and heavy metals concentration were increased.

KEY WORDS : LEAD / CHROMIUM / ACCUMULATION / TOXICITY / *HYDROCOTYLE UMBELLATA* L.

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การสะสมและความเป็นพิษของตะกั่วและโครเมียมในต้นแว่นแก้ว *HYDROCOTYLE UMBELLATA* L. ACCUMULATION AND TOXICITY OF LEAD AND CHROMIUM IN *HYDROCOTYLE UMBELLATA* L.

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บทคัดย่อ

การทดสอบการสะสมและความเป็นพิษของตะกั่วและโครเมียม โดยใช้พืชน้ำ (ต้นแว่นแก้ว) *Hydrocotyle umbellata* L. ทำการทดลองโดยใช้สารละลายอาหาร Hoagland ที่เติมโลหะหนักที่ความเข้มข้นต่าง ๆดังนี้ ตะกั่ว 20, 40, 60, 80 และ 100 มิลลิกรัมต่อลิตร และโครเมียม 2, 4, 6, 8 และ 10 มิลลิกรัมต่อลิตร และเก็บต้นแว่นแก้วมาวิเคราะห์ในวันที่ 3, 6, 9 และ 12 ผลการทดลองพบว่า อัตราการเจริญเติบโต ผลผลิตมวลชีวภาพ และ ปริมาณคลอโรฟิลล์ของแว่นแก้วลดลงอย่างมีนัยสำคัญทางสถิติ ($P\leq0.05$) เมื่อความเข้มข้นของโลหะหนัก และระยะเวลาในการทดลองเพิ่มขึ้น อย่างไรก็ตามพบว่าวันที่ 3 การลดลงไม่มีนัยสำคัญทางสถิติ ($P>0.05$) ค่า EC_{50} ของโครเมียมมีค่าต่ำกว่าของตะกั่วในช่วงเวลาเดียวกัน แสดงว่าต้นแว่นแก้วสามารถทนต่อพิษของตะกั่วได้ดีกว่าของโครเมียม ปริมาณการสะสมของตะกั่วและโครเมียมในพืชเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P\leq0.05$) เมื่อความเข้มข้นของโลหะหนัก และระยะเวลาในการทดลองเพิ่มขึ้น การสะสมของตะกั่วและโครเมียมในรากสูงกว่าในต้นและใบ ค่าเฉลี่ยการสะสมของโลหะหนักในรากพืชลดลงอย่างรวดเร็วใน 6 วันแรกของการทดลอง ค่าBCF ของโครเมียมต่ำกว่าของตะกั่ว แสดงว่าต้นแว่นแก้วมีประสิทธิภาพในการสะสมตะกั่วได้ดีกว่าโครเมียม

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CHAPTER I
INTRODUCTION

During the first three years of Thailand's Golden Era, between 1987 and 1997, Thailand enjoyed a rapid economic growth and it was hailed as one of the world's fastest growing economies. As a result of rapid growth in the industrial sector, without suitable environmental management, Thailand is now encountering several environmental problems especially contamination of heavy metals in water. Other problems are air pollution, solid waste and toxic and hazardous substance disposal problem.

Heavy metals – a group of metal with density higher than 5 g/cm^3-such as Cd, Cr, Hg, Pb, Sn, etc., are important environmental pollutants (Toppi and Gabbrielli, 1999). Heavy metal pollution of water is a major environmental problem facing the modern world (Dushenkov *et al*., 1995). The global heavy metal pollution is increasing in the environment due to increasing of human activities. Moreover, it is gaining in importance day by day due to its obvious impact on human health through the food chain (Prassad, 1997). The danger of heavy metals is aggravated by their almost indefinite persistence in the environment because they cannot be destroyed biologically (no degradation, change in the nuclear structure of the elements, occurs) but are only transformed from oxidation state or organic complex to another. In addition, they are highly toxic for both higher organisms and microorganisms (Garbisu and Alkorta, 2001). So, we must reduce heavy metal level in the environment to an acceptable level. Lead and chromium were selected as toxicants for the present study because they are used in several industries in Thailand and they are highly toxic to human, animals and plants.

There are several methods of removing heavy metals from water based on ion exchange or chemical and microbiological precipitation, which have been developed and used with some success. These technologies have different efficiencies for different metals and may be very costly if large volume, low metal concentration and high clean up

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standards are involved (Dushenkov *et al*., 1995).

Phytoremediation is being developed as an alternative technology for the clean up of contaminated environment. It has been estimated to be four to 1,000 times cheaper, on a per volume basis, than current non-biological technologies (Sadowsky, 1999). It takes advantage of the fact that a living plant can be considered a solar-driven pump, which can extract and concentrate particular elements from the environment (Raskin *et al*., 1997).

The use of aquatic plants to remove heavy metal is simple, economic and environmentally friendly technology. Aquatic plants have been shown to have a tremendous capacity to reduce the level of toxic metals (Cr, Pb, Cd, Hg etc), nutrients (N and P), biological oxygen demand (BOD) and total suspended solids from polluted water (Vajpayee *et al*., 2001). The selection of a suitable aquatic plant species for metal removal will depend on the ease of growth of the plant, yield of biomass and ease of harvest under the conditions of application (Rahmani and Sternberg, 1999). So, in the present study *Hydrocotyle umbellata* L. commonly known as water pennywort, aquatic plant of the Umbelliferae family, was used. The accumulation of lead and chromium by *H. umbellata* and their toxicities were determined.

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CHAPTER II

OBJECTIVES

The objectives of this study were as follows:

1. To study the accumulation of lead and chromium by *Hydrocotyle umbellata*.
2. To study the toxicities of lead and chromium to *H. umbellata*.

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CHAPTER III
LITERATURE REVIEW

3.1 Lead (Pb)

Lead is the 36th most abundant element in the earth's crust, with an average concentration of 15 mg/kg (Moore, 1991). In nature lead is a ubiquitous element. Moreover, in the last fifty years great amounts of lead have been extracted, concentrated, and used by man, and re-emitted into the environment. Further reduction of lead emissions is necessary because lead is not decomposed and accumulates locally in biological organisms (Ewers and Schlipkoter, 1991).

3.1.1 Physical and chemical properties

Lead has an atomic number of 82, an atomic mass of 207.19, and is located in group IVa of the periodic table. It is a bluish-white, soft metal with a density of 11.34 g/cm^3, a melting point is 327.5°C and a boiling point of 1,740°C. Natural lead consists of 52%^{208}Pb, 24%^{206}Pb, 23%^{207}Pb, and 1%^{204}Pb. Radioactive ^{210}Pb from radon decay plays also a role, for instance, in waters and sediments (Ewers and Schlipkoter, 1991). Electronic structure, covalent radius and Pauling's electronegativity value of lead is $4f^{14}\ 5d^{10}\ 6s^2\ 6p^2$, 1.46 °A and 1.8, respectively (Lee, 1996). Moreover, lead is high opacity to gamma and x-ray energy, exceptional malleability, high corrosion resistance and stability, which made it extremely useful in industries (WHO, 1996).

In most inorganic compounds lead is in the +2 oxidation state. The salts of Pb (II), lead oxides and lead sulfide, are not readily soluble in water, with the exception of lead acetate, lead chlorate, and, to some extent, lead chloride. Some of the salt formed with organic acid such as oxalate, are also insoluble (WHO, 1989). Pb (IV) compounds are unstable and strong oxidizing agents. Teramethyllead and tetraethyllead are important organolead compounds. Both are colorless liquids with boiling points of 220°C and 200°C, respectively (Ewers and Schlipkoter, 1991).

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3.1.2 Occurrence

Lead is a naturally occurring element, which represents as almost ever-present constituent of the earth's crust. It can be found in all environmental media and in all compounds of the biosphere. The most important of lead minerals, which found in over 200 minerals, are galena or lead sulfide (PbS), cerrusite or lead carbonate ($PbCO_3$), and anglesite or lead sulfate ($PbSO_4$). Galena occurs mostly in deposits, which contain zinc minerals and small amount of copper, iron, cadmium, and other metals (Ewers and Schlipkoter, 1991).

3.1.3 Production

Galena is the most stable solid form within the soil matrix and form under reducing conditions when increased concentrations of sulfide are present. It is the most important source of primary lead. It is black, shiny, very dense and easy to extract. There is two method of extracting the element.

1. Roast in air to give PbO, and reduce with coke or Co in a blast furnace.

$$2PbS + 3O_2 \longrightarrow 2PbO \longrightarrow 2Pb\ (liquid) + CO_2\ (gas)$$
$$+SO_2$$

2. PbS is oxidized by heating and blowing air through it. After some time the air is turned off and heating is continued, and the mixture undergoes self-reduction

$$3PbS \longrightarrow PbS + 2PbO \longrightarrow 3Pb(liquid) + SO_2\ (gas)$$

World production of lead was 5.3 million tones in 1992. Of this 3.1 million tones was primary production from lead mineral which is galena (PbS). The recycling of scrap lead yielded 2.2 million tones (Lee, 1996).

3.1.4 Consumption

About 55% of lead production is used to make lead/acid storage batteries. Over 80% of battery lead is recovered from worn-out batteries and recycled. About 15 % of production is used for lead sheets, lead pipers and solder. The manufacture of $PbEt_4$ as an additive to petrol at one time used for 10-20%. Ten percent is used in paints and pigment. Red lead (Pb_3O_4) is used for rustproofing metal and white lead $(PbCO_3)_2.Pb(OH)_2$ was at some time widely used as an opacifier in paint. Calcium

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plumbate (Ca_2PbO_4) is used for rustpoofing corrugated steel sheets, and $PbCrO_4$ is used as a strong yellow pigment for road signs and markings. Lead compounds are also included in crown glass and cut glass, and in ceramic glazes (Lee, 1996).

3.1.5 Source of lead in the environment

Lead and its compounds may enter the environment by natural and antropogenic sources. Estimates of the dispersal of lead emissions into the environmental media include the atmosphere, which is the major initial recipient. Mobile and stationary sources of lead emissions tend to be concentrated in areas of high population density, and near smelter (Ewers and Schlipkoter, 1991). The data of worldwide lead and chromium emissions into the environmental media is adapted from Nriagu and Pacyna (1988).

The global emission of lead into the atmosphere from antropogenic and natural sources in unit of 10^6 kg/yr. For antropogenic sources, the total value is 332 as follows: automobile (253); nonferrous metal production (49); coal combustion (8.2); steel and iron manufacturing (7.6); cement production (7.1); oil combustion equal to refuse incineration (2.4); wood combustion (2.1); and phosphate fertilizers (0.76). Whereas, the total value of lead emission from natural sources is 12.24, which is from different sources: windblown (3.9); volcano (3.3); wild forest fires (1.9); sea salt (1.4); continental biosphere-particulate (1.3); marine biosphere-volatile (0.24); and continental biosphere-volatile (0.2) (Salbu and Steinner, 1995).

Worldwide emissions of total lead input into soil (10^6 kg/yr) are between 479 to 1113. The values is 5-49 from agriculture and animal waste fertilizers; 6.6-8.2 from logging and wood waste; 20.8-7.3 from urban refuse and sewage/sludge; 45-242 from fly ash; 202-263 from atmospheric fallout; and 199-478 from the other and wastage of commercial product (Ross, 1994).

Worldwide emissions of lead input to freshwater from different sources in unit of thousand metric ton/yr are as follows: atmospheric fallout (87-113); manufacturing process includes metal (2.5-22), chemical (0.4-3), pulp and paper (0.01-0.9), and petroleum products (0.02-0.1); dumping of sewage sludge (2.9-10); domestic wastewater includes central (0.9-7.2), and non central (0.6-4.8); smelting and

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refining includes nonferrous metal (1.0-6.0), and iron and steel (1.4-2.8); and stream electrical production (0.2-1.2) (Moore, 1991).

3.1.6 Concentration of lead in the environment

3.1.6.1 Rock

Lead in rock is often found at concentrations of 10-20 mg/kg (Ewers and Schlipkoter, 1991). In the igneous rock, which is serpentine, basalt, and granite, lead level is 0.1-14 μg/g, 3-5 μg/g, and 20-24 μg/g, respectively. Lead concentrations in the sedimentary rocks is 5.7-7 μg/g of limestone, 8-10 μg/g of sandstone, and 20-23 μg/g of shale (Ross, 1994). Generally, lead content in continental crust is 15 mg/kg, and in oceanic crust is 0.89 mg/kg. For lead concentrations in mica schists, greywackes, and granulites rocks are 16, 14, and 9.8 mg/kg in order of abundance (Wedepohl, 1991).

3.1.6.2 Soil

Average concentration of lead in normal soil range from 10-40 mg/kg. Most values are below 20 mg/kg. Background of lead levels in upper limit of non -polluted soil is 50 μg/g in sandy soil, which is equal to that in loam soil (Greger, 1999). Significantly increased lead concentrations in soils have been found particularly in inner city areas, near busy highway and near lead worker (Ewers and Schlipkoter, 1991). Deposition of atmospheric lead into the top layers of soil usually contains higher lead level than deeper layers. It was reported that lead is mainly existing in the upper 20 cm (Baumhardt and Welch, 1972). The lower layer of soil remote from man activities lead concentration is about 5-40 μg/g DW, which is equal to in rural soil but less than in urban soil 10-50 μg/g DW, and in man smelters soil 20-2000 μg/g DW. Whereas lead concentration in upper layer of soil is 5-40 μg/g DW in remote area, 5-60 μg/g DW in rural area, 50-300 μg/g DW in urban area, and 300-20000 μg/g DW in near smelters. Under the oxic condition, the principal chemical species of lead in acid soil solution are Pb^{2+}, $PbSO_4$, and $PbHCO_3^+$; for alkaline soil solutions are $PbCO_3$, $PbHCO_3^+$, $Pb(CO_3)_2^{2-}$, and $PbOH^+$. The retention time of lead in soil is between 150 to 5,000 years (Kumar, 1995).

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3.1.6.3 Water

Natural lead concentration is 0.001-0.015 μg/L in seawater and 0.2 μg/L in freshwater (Greger, 1999). The lead content of lake and river waters usually is in the range of 0.1-10 μg/L. The average lead concentration in surface ocean water is about 0.01-0.03 μg/L, and in deep ocean water it is 0.001-0.004 μg/L. The global range of lead concentration in ground water varied from 0.001 to 0.06 mg/L (Singh *et al.,* 1997). Lead level normally found in drinking water is of the range 2-25 μg/L. The maximum admissible lead concentration in drinking water is 50 μg/L. If water level is above 20 μg/L after corrosion control treatment, public must be warned (Ewers and Schlipkoter, 1991).

Total lead in surface water is highly variable, but is typically less than 0.05 mg/L. Although maximum residues are often associated with poorly treated industrial or mining effluents, the input of lead into remote water is due primary to atmospheric deposition. Particulate lead generally accounts for more than 75% of residues in flowing waters and more than 50 % in standing water (Moore, 1991).

In freshwater, lead forms a number of complexes of low solubility with many of the major anions, including hydroxides, carbonates, sulfides, and (less commonly) sulfates. Lead also partitions favorably with humic and fulvic acids, forming moderately strong chelates. At pH 10, $Pb(OH)^+$ dominates all other species. Approximately 75% of lead in rivers is in suspension and 25% in solution, but in saltwater, the corresponding ratio is about 50:50 (Moore, 1991).

3.1.6.4 Atmosphere

Atmospheric lead levels in remote continental areas fall in the range of 0.1-10 ng/m^3. Marine atmospheres generally contain less lead than continental area. The lowest atmospheric lead concentration measured anywhere was 0.046 ng/m^3 found in the west winds of the North Atlantic. Urban area has significantly higher air lead levels than rural areas. In the 1960s and 1970s the annual mean air lead levels in towns and cities in the USA, Canada, and Europe were found to be in the range of 0.4-4 μ g/m^3. In central urban areas with high traffic density, lead levels of up to 10 μg/m^3 were recorded. The air lead level presently found in Europe and North American cities

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was in the range of 0.2-0.8 μ g/m^3. In rural area the air lead levels usually are in the range of 0.05-0.3 μg/m^3 (Ewers and Schlipkoter, 1991).

Lead particles present in the ambient air are reported to have a mass median equivalent diameter of between 0.25 μm and 0.69 μm but they are rarely, if ever, spherical. The resident time of lead in the air varies with particle size, wind and rainfall, but in the lower troposphere it ranges from about 6days to 14days, whilst in the upper troposphere it ranges from 14days to 28days (Waldron, 1980).

3.1.7 Fate and transport of lead in environment

The transport and distribution of lead from stationary and mobile sources is mainly through the atmosphere. Leads are released into the atmosphere, both as particles and vapors. Chemical species of lead particle in the exhaust gases, which emitted by motor car using petrol containing lead alkyls is initially in the form of halides (i.e., $PbBr_2$, PbBrCl and $PbCl_2$) which are then converted to oxides (i.e., Pb (OH)Br, $(PbO)_2Pb{\cdot}Br_2$, and $(PbO)_2{\cdot}PbBrCl$), and sulphates [$(PbO)_2{\cdot}PbSO_4$] and carbonates ($PbCO_3$) (Waldron, 1980).

Depending on the particle size, airborne suspended particles may have a long residence time. In remote areas, lead-containing particles are transported over substantial distance, up to thousands of kilometers, by generally weather system. According to the distance of atmospheric transport, the transport pattern has been classified as near fall out was defined as the deposition in the immediate surroundings of the emission sources. Far fall out was defined as fall out away from the emission sources, but within the geographic area (20-200 km). Airborne designated small particles, which were subject to long-range air transport and ultimately deposited elsewhere (Ewers and Schlipkoter, 1991).

Lead can be removed from the atmosphere and transferred to environmental surfaces and component by wet deposition about 40-70%, which is more important than dry deposition. The most efficient clearing mechanism is rain (WHO, 1989). Rainwater contained between 18 μg/L and 34 μg/L of lead (Waldron, 1980). In most cases, it is poorly soluble and either precipitates out in soils and sediments or is bound to organic matter in these components. So, lead is not readily removed and tends to accumulate in these ecosystems where it is deposited (WHO, 1996).

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Large discharges of lead may also occur directly into natural water and onto the land, but in such case lead tends to be localized near the point of discharge because of the low solubility of the lead compounds that are formed upon contact with soil and water (WHO, 1989). Ionic lead (Pb^{2+}), lead oxides, hydroxides, and lead-metal oxyanion complexes are the generals from of lead that are released into the soil, groundwater and surface waters. Atmospheric lead deposited on soil is retained in the upper soil layers, especially in soils with at least 5% organic matter and pH of 5 or above (Ewers and Schlipkoter, 1991). Transport within soil and the bioaviailability of lead from soil are dependent upon pH, mineral composition of the soil and amount and type of organic material. If pH is range 4 to 6, organic lead complexes become more soluble and the lead more available for plant uptake and reaching into water (WHO, 1989).

There is still much to be learnt about the transport of lead in the environment and about the chemical reactions which may take place along the way. Recently, it has been shown that microorganisms found in lake sediments can transform inorganic and organic leads compound into volatile tetramethyllead (Waldron, 1980).

3.2 Chromium (Cr)

Chromium is the 21st most abundant element in the earth crust at about 100 mg/ kg (Barnhart, 1997). Over the past several decades, increased quantities of chromium compounds have been used by men and introduced into the environment. The danger of it depends on the oxidation state. Chromium (VI) is 100 to 1,000 times more toxic than the trivalent compounds (Gauglhofer and Bianchi, 1991).

3.2.1 Physical and chemical properties

Chromium with the atomic number 24 and atomic mass 51.996 exists in all oxidation states from -2 to +6, but only the trivalent and hexavalent compounds and the metallic chromium are of practical importance. The naturally occurring isotopic mixture consists mainly of ^{52}Cr (83-76%) together with three other isotopes. ^{51}Cr has a half-life of 27.8 days and is the most stable among the radioactive isotopes. Chromium is a silvery, shiny, malleable metal with a density of 7.2 g/cm^3. It melts at about 1,860 °C and boils at about 2,670°C. The surface of pure chromium and its alloy can be

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passivated by treatment with a strong oxidative agent, which makes it largely resistant to corrosion (Gauglhofer and Bianchi, 1991). Electronic structure of chromium is $3d^2$ $4s^1$. It has covalent radius 1.17 °A and Pauling's electronegativity value is 1.6 (Lee, 1996).

Cr (III) is the most stable oxidation state. It is classified as a hard acid and forms relatively strong complex with oxygen donor ligands. The principal forms in freshwater include $CrOH^{2+}$, $Cr(OH)_2^+$, and $Cr(OH)_4^-$. Most Cr (III) compounds are soluble in water only at low pH values. At pH above 5 to 6 generally Cr (III) hydroxide precipitates. However, Cr (III) can be formed, for example, with sulfite ions (SO_3^{2-}) which form a compound stable at pH 9 and above (Moore, 1991).

Cr (VI), on the other hand, is water soluble, always existing in solution as a component of a complex anion. The anionic species varies with pH, and may be chromate (CrO_4^{2-}), hydroxychromate ($HCrO_4^-$), or dichromate ($Cr_2O_7^{2-}$). At pH more than 6.5, most Cr^{6+} is present as the chromate ion (Moore, 1991).

3.2.2 Occurrence

Chromium principally occurs as mineral in the chromite spinel group. These minerals have the generalized formula (Mg, Fe)O(Cr, Al, Fe)$_2O_3$. Depending on the degree of substitution in the Fe, Al, and Cr series, the chromite contain from 13% to 65% Cr_2O_3 (Moore, 1991). Natural chromium ores are mainly chromite ($FeCr_2O_4$) and less frequently krokoite ($PbCrO_4$) (Gauglhofer and Bianchi, 1991).

3.2.3 Production

Chromium metal is produced on a large scale. The commercially important ore of chromium is chromite ($FeCr_2O_4$). The chromium ore is either reduced directly to ferrochrome with coal in an electric oven or oxidize to chromate with oxygen from the air in an alkaline melt. Ferrochrome is an iron-chrome alloy containing about 60 % chromium. Pure chromium is obtained by the reduction of chromium oxide with aluminum, by electrolysis, or via chromium iodine (Gauglhofer and Bianchi, 1991).

World production of chromite was 11.4 million tones in 1992, with chromium content of 3.3 million tones. Small amounts of crocoit ($PbCrO_4$) and chrome ochre (Cr_2O_3) are also mined (Lee, 1996).

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3.2.4 Consumption

About 80% of the chromium mined goes into metallurgical application, especially for the manufacture of stainless steel. About 15% are used in chromium chemical manufacture and the remainder is used in refractory applications.

Chromium metal is used extensively in non-ferrous alloy and for electroplating. Ferrochrome is used to make many ferrous alloys, including stainless steel and hard 'chromium' steel (Lee, 1996).

Chromium (VI) chemical is principally used for metal plating (which $H_2Cr_2O_7$), as dyes pigments, and leather tanning (WHO, 1988). Chromate is used for the oxidation of various organic materials, in the purification of chemicals, in inorganic oxidation, and in the production of pigments. In addition chromate is used as rust and corrosion inhibition, for example, in diesel engines. Dichromate is converted to chromium sulfate for tanning. Sodium dichromate $Na_2Cr_2O_7$ is widely used as an oxidizing agent (Kimbrough *et al*., 1999).

Chromium (II) and (III) chemicals are also manufactured but in small amount compound with Chromium (VI). Chromium dichloride ($CrCl_2$) is used as strong reducing agent in the laboratory. Chromic oxide ($Cr_2O_3.nH_2O$), is used as a pigment in paints and as a fecal maker in digestive studies. Chromium chloride ($[Cr(H_2O)_6]Cl_3$) or ($[Cr(H_2O)_4Cl_2]Cl$) is used in basic solution for leather tanning. Chromium sulfate ($CrSO_4$) and nitrate $Cr(NO_3)_3$ are used as coloring and printing dyes. A large percentage of chromic acid is used for plating (WHO, 1988).

3.2.5 Source of chromium in the environment

The global emission of chromium (10^6 kg/yr) into the atmosphere from antropogenic sources the total value is 30.5 as follows: coal combustion (11.0); steel and iron manufacturing (16.0); oil combustion (1.4); refuse incineration (0.84); and wood combustion (1.3). Whereas, the total value of lead emission from natural sources is 43.9, which is from different sources: windblown (27.0); volcano (15); wild forest fires (0.09); sea salt (0.7); continental biosphere-particulate (1.0); marine biosphere volatile (0.06); and continental biosphere-volatile (0.05) (Salbu and Steinner, 1995).

Worldwide emissions of total chromium input into soil (10^6 kg/yr) are between 484 to 1309. The values is 14.5-150 from agriculture and animal waste

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fertilizers; 2-18 from logging and wood waste; 8-44 from urban refuse and sewage/sludge; 149-446 from fly ash; 5.1-38 from atmospheric fallout; and 305-616 from the other and wastage of commercial product (Ross, 1994).

Worldwide emissions of chromium input to freshwater from different sources in unit of thousand metric ton/yr are as follows: atmospheric deposition (2.2-16); manufacturing process includes metal (15-58), chemical (2.5-24), pulp and paper (0.01-1.5), and petroleum products (0-0.2); domestic wastewater includes central (8.1-3.6), and noncentral (6-42); nonferrous metal (3-20), discharge of sewage sludge (5.8-32); and base metal mining and dressing (0-0.7) (Moore, 1991).

3.2.6 Concentration of chromium in the environment

3.2.6.1 Rock

Chromium containing rocks and ores are found in all regions of the world. In the igneous rock, which is serpentine, basalt, and granite, chromium level is 0.1-14 μ g/g, 3-5 μg/g, and 20-24 μg/g, respectively. Chromium concentrations in the sedimentary rocks is 5.7-7 μg/g of limestone, 8-10 μg/g of sandstone, and 20-23 μg/g of shale (Ross, 1994). Chromium content is 50 mg/kg of greywackes rocks, 76 mg/kg of mica schists rocks, 88 mg/kg of granulites rocks as well as of continental crust, and 317 mg/kg of ocean crust (Wedepohl, 1991). Chromium can also been found in coal about 5-10 mg/kg (Merian, 1984).

3.2.6.2 Soil

Chromium concentration of the soils is derived from the rocks. Most of chromium in unpolluted soils is in the trivalent from, and the total concentration of it in unpolluted soil ranged from approximately 30 μg/g to 300 μg/g (Katz and Salem, 1994). Background of chromium levels in upper limit of non -polluted soil is 15 μg/g in sandy soil, and 30 μg/g in loam soil (Greger, 1999). Smith *et al*. (1989) cited normal background total chromium concentration of 87, 74, and 57 μg/g for Japanese, Swedish, and United States soil, respectively. Serpentine soil commonly contains 500 to 5000 mg/kg of chromium (Reeves and Baker, 2000). When hexavalent chromium is present in soil at concentrations of more than a few tenths of a ppb, contamination with industrial waste should be considered (Katz and Salem, 1994). Greene *et al*.

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(1972) reported that chromium concentration (50-450 μg/g) in contaminated soil from Hudson County.

3.2.6.3 Water

Generally, chromium is found in seawater 0.3 μg/L mainly as chromate. The amounts increased with the depth. The surface seawater contain 0.02-0.14 μg Cr(III)/L and 0.28-0.36 μg/Cr (VI)/L and that the concentration of each species was 0.2 at depths of 5,500 and 1,000 m (Katz and Salem, 1994). Total chromium is generally detected at low concentrations in freshwater at about 1-10 μg/L. The chromium level in rivers and lakes is usually between 0.5 μg/L and 10 mg/L, most approximately 1μg/L. Nowadays up to 25 μg/L chromium may be found in drinking water; in exceptional case such as in beer and wine contain 300 to 450 μg/L of chromium (Hamilton and Wetterhahn, 1988). In most countries the recommended limit of 0.05 mg/L of chromium in drinking water is applied. A maximum value of 0.02 mg/L of Cr (VI) is fixed in Switzerland. Chromium (III) is not mentioned there (Gauglhofer and Bianchi, 1991).

3.2.6.4 Atmosphere

Urban air concentrations have been reported to contain chromium in the range from less than 10 ng/m^3, while in remote site levels range from 0.3 ng/m^3 up to 2 ng/m^3. Antarctic airborne chromium concentrations as approximately 0.005 ng/m^3, and those over the Atlantic Ocean were reported to range from 0.007 to 1.1 ng/m^3. Airborne chromium level over the continental United States range from background 24-hours average of 5.2 ng/m^3, to a high urban annual average of 156.8 ng/m^3 in Baltimore. Airborne concentrations in the emissions from the stack of Japanese factory were in the range of 30-40 ng/m^3. In the industrialized areas of Hong Kong, airborne chromium concentration was 2,788 and 3,328 ng/m^3 (Katz and Salem, 1994).

Chromium level in the non- industrial atmosphere is about 10 ng/m^3, whereas in the air of industrial cities it is up to 70 ng/m^3 and in smoke from coal fires up to 2μg/m^3. Estimates of atmospheric half-life for Cr^{6+} reduction to Cr^{3+} range from 16 hours to 4.8 days (Kimbrough *et al*., 1999).

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3.2.7 Fate and transport of chromium in environment

Due to the extremely high boiling point of chromium, gaseous chromium is rarely encountered. The atmospheric transformation and transport of chromium largely occurs in the liquid phase, by dissolved in droplets and solid phase, by particle-bound or, more generally, aerosol (Seigneur and Constantinou, 1995). Chromium entrained in aerosol may be removed from the atmosphere by both dry and wet deposition, both of which are particle size-dependent process. In dry deposition the particles settle and are captured by the soil or surface water via gravitational sedimentation, impaction, or interception (Kimbrough *et al*., 1999). Wet deposition is the process where aerosol particles are actively entrained or scavenged by air moisture such as rain, snow, fog, or dew. This process is dependent on the size of chromium-containing particles and the size of the scavenging rain droplets or snowflake. Chromium can also be introduced or reintroduced, into the air via the wind resuspension of chromium-containing soil particles. The particles with a diameter of less than 50 μm, the wind-resuspension process is induced by both mechanical and wind disturbances that provide sufficient energy to overcome gravitational forces and allow particles to be dispersed by the wind (Kimbrough *et al*., 1999).

The reduction of chromium (VI) is far more likely than oxidation in the atmosphere because of the presence and concentration of reducing agents as well as the acidity of the atmosphere. The few materials capable of oxidizing Cr^{3+} to Cr^{6+}, such as ozone, occur in concentration too low to produce measurable conversion in the atmosphere. Chromate may also react with other metallic species, precipitating as lead or zinc chromate (Seigneur and Constantinou, 1995).

Chromium enters natural water by weathering of chromium-containing rock, direct discharge from industrial operations, wet and dry deposition, and reaching from soils. Reduction of chromium (VI) can occur under a variety of conditions if a suitable reducing agent is available (Anderson *et al*., 1994). This process is favored under acidic conditions. Oxidation of chromium (III) to (VI) was only noted in one of the natural waters and sediment studied, with half-lives ranging from 2 to 9 years. However, the oxidation of chromium (III) can be inhibited by competing substance in natural water. Chromium thus can move as dissolved ions or attached to particulate, or both. Under neutral to basic solution, chromium (III) will tend to precipitate out, while

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under the acid conditions it will tend to solubilize. While chromate and dichromate ions are extremely water soluble at all pHs, they can precipitate with a number of divalent cations (Kimbrough *et al*., 1999).

Chromium input to soils and sediments from antropogenic source occurs in-directly due to atmospheric deposition but is more commonly due to the dumping of chromium-bearing of liquid and solid waste from such sources as chromate by products (mud), ferrochrome slag, or chromium painting baths. Once in the soil or sediment, chromium can undergo a variety of transformations such as oxidation, reduction, sorption, precipitation and dissolution. The manganese dioxide (MnO_2) is the most likely oxidant of chromium (III) in soils and oxic sediments but not in anoxic sediments (Kimbrough *et al*., 1999).

3.3 Metal accumulation by aquatic plants

Aquatic plants have several characteristics favorable for metal accumulation. First, in term of biomass, aquatic and wetland macrophytes are the predominant organisms in the highly productive, littoral ecosystem (Brix and Schierup, 1989). Second, leaves and epiphytes provide an expanded area to trap particulate matter, sorb metal ions, and accumulate and sequester pollutants (Levine *et al*., 1990). Third, rooted species can absorb metals through their roots and rhizomes as well as through their leaves (Heisey and Damman, 1982). Fourth, aquatic plants are stationary and constantly exposed to (and absorbing) contaminants such as metal (Say *et al*., 1981).

Several biological and nonbiological factors are known to affect metal accumulation by aquatic plants. The biological factor includes plant species, age, generation, and route and mode of metal accumulation. The nonbiological factors can be divided into environmental and chemical factors. Environmental factors are composed of temperature, light, season, pH, salinity and plant nutrients. Chemical factors are concentration, duration of exposure time, competing ions and complexing agents (Wang and Lewis, 1997).

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3.3.1 Lead in aquatic plants

Total lead is often found in high concentrations in aquatic plants, particularly those growing in freshwater and receiving mine or other industrial waste. Residues in macroscopic species in streams from an industrialized area of West Germany ranged from 100 to 5,300 mg/kg DW, whereas concentration of up to 16,000 mg/kg were found in aquatic bryophytes growing in streams receiving mine waste. Lead in aquatic mosses was as low as 22 mg/kg in unpolluted part of the Mawddach basin but increased to 462 mg/kg near mine discharge sites (Moore, 1991).

Lead accumulation by most aquatic plant species such as *Potamogeton crispus* L., *P. perfoliatus* Bennett, *Myriophyllum alterniflorum* DC., *Littorella uniflora* (L.) Asch., *Isoetes lacustris* L., *Distichilis spicata* L., *Deschampsia cespitosa* (L.) Beauv., *Salicornia virginica* L., and *Elodea canadensis* Michx. are very limited and does not exceed 40-fold the external lead supply level (Welsh and Denny, 1980; Chigbo *et al*., 1982; Gallagher and Kibby, 1980). The study of Qian *et al*. (1999) by exposing 12 aquatic plants, which were grown for 10 days in solution culture supplemented with 1 mg Pb/L as $Pb(NO_3)_2$. They indicated that aquatic plant roots accumulated larger concentrations of lead than shoots. Accumulation of lead was 1,882 mg/kg dry weight in roots and 64 mg/kg dry weight in shoots of smartweed (*Plygonum hydropiperoides* L.). Umbrella plant (*Cyperus alternifolius* L.), fuzzy water clover (*Marsilea drummondii*), and parrot's feather (*Myriophyllum brasiliense* Camb.) accumulated lead levels in roots (1,000-1,200 mg/kg DW) and in shoots (30-45 mg/kg DW). Monkey flower (*Mimulus guttatus* Fishch.), sedge (*Cyperus pseudovegetus* Steud.), smooth cordgrass (*Spartina alterniflora* Loisel), iris-leaved rush (*Juncus xiphioides* E. Mey.), and mare's tail (*Hippuris vulgaris* L.) were the poorest plant species in lead uptake and accumulation at both the root and shoot levels with lead concentration not exceeding 200 mg/kg dry weight in roots and 18 mg/kg dry weight in shoots.

3.3.2 Chromium in aquatic plants

Total chromium in naturally aquatic plants is generally low, less than 5 mg/kg dry weight, except near polluting sources, where residues of up to 50 mg/kg dry weight have been reported. Aquatic plants that accumulate chromium from the

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environment have been evaluated as potential chromium scavengers for the decontamination of ponds and streams (Katz and Salem, 1994). Rai *et al*. (1995) used aquatic plants to remove chromium from pond water, which contained 4.866 μM Cr for 15 days. They found chromium accumulations in *Hydrodictyon reticulatum* (Linn.) Lagerheim (8.32 μmol/g DW), *Spirodela polyrrhiza* (L.) Schleiden (7.61 μmol/g DW), and *Ceratophyllum demersum* L. (7.36 μmol/g DW).

Zurayk *et al*. (2001) exposed 12 aquatic plants grown for 21 days in solution culture supplemented with 1 mg/L of Cr as $K_2Cr_2O_7$. They demonstrated that, the accumulation of *Nasturtium officinale* R.Br., *Cardamine uliginosa* L., *Mentha longifolia* L., *Veronica becabunga* L., *Veronica lysimachioides* L., *Mentha aguatica* L., *Apium nodiflorum* L., *Mentha sylvestris* L., *Veronica anagalloides* L., *Potentilla reptans* L., *Veronica anagallis aquatica* L., and *Mentha pulegium* L. were 150.7, 133.1, 128.0, 120.3, 90.3, 76.8, 74.2, 59.6, 24.6, 21.9, 12.5, and 10.5 mg/kg dry weight, respectively. In water hyacinth, a plant commonly used as a metal bioindicator, was reported to accumulate between 50 and 150 mg/kg dry weight of chromium (Jana, 1988). Similar to lead, the accumulation of chromium as $K_2Cr_2O_7$ in roots is more than shoots with greatest difference 60-fold. (Qian *et al*., 1999).

3.3.3. Bioconcentration factor

The bioconcentration factor (BCF) is defined as the metal concentration in plant tissue (mg/kg) at harvest divided by the initial concentration of heavy metal in the external nutrient solution (mg/L) (Wang and Lewis, 1997). Besides, It also has been known as a bioconcentration ratio (Knowlton *et al*., 1983), a metal accumulation factor (Wang and Lewis, 1997), a concentration ratio (Twining, 1988), and an enrichment ratio (Satake *et al*., 1989). The BCF provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate (Zayed *et al*., 1998). Different metals have different BCF values in the phytofiltration system. The BCF ranges from several hundred for cationic species such as As to close to 10,000 for cationic species such as Pb and Cu (Raskin *et al*., 1997). In uncontrolled field studies where moderate concentrations of metals ions were present, the BCF can be larger than 1 million the BCF value. In laboratory studies, the BCF for a variety of

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metals and aquatic macrophytes can be large and it dependent on plant organ, exposure time and metal concentration (Wang and Lewis, 1997).

3.4 Toxicity of heavy metal on plants

Toxicity is the potential and capacity of a test material to cause adverse effects on living organism. It is a result of exposure concentration and duration time. Toxicity tests are desirable in water quality evaluations because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota (APHA, 1995).

Because the sensitivities of aquatic plant species are generally unpredictable, so phytotoxicity data are necessary in the environment hazard assessment process. Besides, phytotoxicity data have served a relatively minor role in regulatory concerning the environment hazard of most potential contaminants and they are considered in the development of water quality criteria to protect aquatic life (Lewis, 1995), and for the toxicity evaluation of municipal and industrial effluents (Vasseur *et al*., 1991). A variety of phytotoxicity tests have been conducted with freshwater green algae, duckweed, blue-green algae, diatoms, and rooted macrophytes. Aquatic vascular plants have been used less frequently than algae as test species. In one literature survey, only 7% of 528 reported phytotoxicity test used macrophytic species (Blank *et al*., 1984).

3.4.1 Rooted vascular plant toxicity test

Rooted submersed and emergent macrophytes are seldom used in toxicity test. Their large size and slow growth and the lack of established test method have contributed to this trend. Due to macrophytes are exposed to contaminants through their roots and entire plant surface and the recent regulatory focus on contaminated sediments thus, they will become important test species in the future. The test species may be cultured or obtained from natural or commercial sources. They are exposed in test chambers containing a nutrient-enriched medium. The test period ranges from several hours to six weeks. Generally, interesting parameters are biomass, growth, node and frond count, root and stem length, chlorophyll content, photosynthetic and enzyme activity, and seedling growth (Kay *et al*., 1984).

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3.4.2 The effects of heavy metal toxicity on plants

In most plant, exposure to elevated concentrations of heavy metals results in reduced vigor and growth inhibition. The growth of whole plants or of plant parts is frequently used as an easily measurable parameter to monitor the effects of various stresses. Growth changes are often the first and most obvious reaction of plant under stress. Plant growth under trace metal stress is concerned with the role of plant hormone (Prasad and Hagemeyer, 1999). After prolonged metal exposure sensitive plants develop visible symptoms of toxicity such as chlorosis and necrosis, respiration, water relation, reproduction, causes changes in certain organelles, disruption of membrane structure and functions, and causes death in extreme cases. Moreover, heavy metal interferes with photosynthesis and pigment (Prasad, 1997). Photosynthesis is affected by a broad range of heavy metals. Cu^{2+}, Cd^{2+}, $Ni^{2+,}$ and Hg^{2+} directly influence the photosynthetic electron transport process and inhibit CO_2 fixation. The effects are due to three major processes such as oxidative breakdown of chlorophyll and carotenoids, oxidative damage of proteins and membrane structures and substitution of metal cofactors (Dietz *et al*., 1999). Chlorophyll and carotenoids degradation are routinely observed in response to exposure of plants to elevated concentrations of various heavy metals. Heavy metals usually decreased the total chlorophyll and chlorophyll a/b ratio in higher plant. In general, carotenoids were less affected by heavy metals resulting in a lower chlorophyll/ carotenoids ratio in higher plant (Prasad and Hagemeyer, 1999).

3.4.3 Toxicity of lead on aquatic plants

Lead is unlikely to affect aquatic plants at levels that might be found in the general environment (WHO, 1989). Lead is considered to be a non-essential metal to plants. Aquatic plants such as *Elodea nuttallii* (Planch.) St. John, *Callitriche platyearpa* Kutz, *S. polyrrhiza*, and *Lemna gibba* L. were exposed to lead nitrate of up to 10^{-5} mol/L for 70 to 73 days. There was no observable toxicity, and growth rates were unaffected (Van der Werff and Pruyt, 1982). Brown and Rattigan (1979) reported that concentration of lead acetate which produced 50% damage to the aquatic plants, *Elodea canadensis* Michx. and *Lemna minor* L. was 136 mg/L and 16.3 mg/L, respectively. In a separate experiment, they exposed *E. canadensis* to lead for 24 hours

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in the dark, then measured oxygen evolution in light. Levels of 47.6 and 99 mg Pb/L reduced photosynthetic oxygen evolution by 50% and 90%, respectively. Kay *et al*. (1984) exposed the *Eichhornia crassipes* (Mart.) Solms (water hyacinth) to lead nitrate concentration of 0.5 to 5 mg/L for six weeks. There was no observed effect on root development, leaf color, development of new plantlets, flowering or total plant growth. Stanley (1974) found that the EC_{50} of *Myriophyllum spicatum* L. (Eurasian water milfoil) for root weight, shoot weight, root length, and shoot length were 363, 808, 767, and 725 mg/L, respectively. Visible symptoms of toxicity through unspecific reactions to lead are smaller leaves and stunted growth. Leaves may become chlorotic and reddish with necrosis and the roots may turn black (Mehra and Farago, 1994).

3.4.4 Toxicity of chromium on aquatic plants.

Chromium has been classified under the category of borderline elements as well as lead. So, some of the biological perturbation and symptoms due to chromium may be similar to lead (Sharma *et al*., 1995). Both Cr (III) and Cr (VI) are taken up by aquatic plants. Cr (III) is sparingly soluble and less toxic, while Cr (VI) being more soluble in water, is highly toxic to biota (Nieboer and Richardson, 1980). Cr^{6+} is generally only moderately toxic to algae and aquatic plants. Chromium is toxic to higher plants at total tissue concentration of about 0.1 mmol/kg dry weight (Mengel and Kirkby, 1982). On the other hand, chromium is toxic for plants at about 0.5-5 μg/mL in nutrient solution (ANRCP, 1998). Wang (1986a) reported that the EC_{50} for duckweed L. *minor* was as high as 35 mg/L, giving a maximum permissibly concentration of 3.5 mg/L for standard setting purposes. In a relative study, Wang (1986b) showed that these toxic effects were not ameliorated by the presence of particulate matter, either organic or inorganic, under the test conditions. Although it is likely that ligands do affect the toxicity of both Cr^{3+} and Cr^{6+} to aquatic plants under some conditions, no definitive data are available to confirm this point. Although chromium is not considered to be essential for plant growth and development, some studies have indicated that at low concentration about 1 μM, chromium can stimulate plant growth (Bonet *et al*., 1991). Moreover, chromium interferes with several metabolic processes, causing plants to exhibit reduced phytomass, chlorosis, photosynthetic impairing, stunting and finally plant death (Vajpayee *et al*., 1999).

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Mukherji and Roy (1978) concluded that chromium seemed to act principally on plant roots, resulting in intense growth inhibition. The initial symptoms of chromium toxicity appeared as severe wilting and chlorosis of plants (ANRCP, 1998). Death of plants occurred within 3 days of treatment with 30 or 60 mg Cr/L (WHO, 1988).

3.5 Phytoremediation

Phytoremediation is defined as the use of green plants to remove pollutants or to render them harmless. Phytofiltration is subset of the phytoremediation being developed, in which plant root (rhizofiltration) or seedling (blastofiltration) grown in aerated water precipitate and concentrate toxic metal from polluted effluents.

Blastofiltration may represent the second generation of plant-based water treatment technology. It takes advantage of the dramatic increase in surface to volume ratio that occurs after germination and the fact that some seedlings also ab/adsorb large quantities of toxic metal ions. (Raskin *et al*., 1997).

Rhizofiltration is suitable for remediating most metals (i.e., Pb, Cd, Ni, Cu, Cr and V), excess nutrients (such as NH_4NO_3) and radionuclide (such as U, Cs and Sr) contaminated water (Prasad and Hagemeyer, 1999). An ideal plant for rhizofiltration should have rapidly growing roots with the ability to remove toxic metals from solution over extended periods of time (Raskin *et al*., 1997).

The mechanisms of metal removal by plant roots or seedlings are not necessarily similar for different metals. First, sorption by the roots, which component does not require biological activity and will take place in dead roots. Second, biological process includes intracellular uptake, vascular deposition and translocation to shoots. Third, precipitation involves a release of root exudates (Salt *et al*., 1995).

Phytoremediation of metal- contaminated waters is readily achieved by use of aquatic plant because there is no problem of bioavailability and solubilization of the target element in the rhizosphere (Brooks and Robinson, 1998). Aquatic plants are represented by a variety of algal and macrophytic species, typically classified as submerged, floating, or emergent plant species, are widely distributed in various aquatic environments, from fresh to salt water, as well as the littoral through pelagic zones (Wang and Lewis, 1997). They are important in oxygen production, nutrient cycling, controlling water quality, sediment stabilization and providing habitat and

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shelter for aquatic life and wildlife. In addition, They are primary energy sources for most aquatic ecosystem (Lewis, 1995).

There has been considerable interest in using aquatic plants in the treatment of wastewater because of their fast growth rate and simple growth requirement. Aquatic plants have also been used frequently to remove suspended solids, nutrients, heavy metals, toxic organic and bacteria from acid mine drainage and agricultural, landfill and urban storm water runoff (Lewis, 1995). Aquatic higher plants such s water hyacinth (*E. crassipes*), duckweed (*L. minor*) and water velvet (*A. pinnata*) have also been utilized for water purification and they can remove various heavy metals from solution (Dushenkov *et al*., 1995).

3.6 *Hydrocotyle umbellata* (L).

H. umbellata is an exotic aquatic plant in Thailand and it is called 'Wan Kaew' It is a perennial of Umbelliferae family, which has 275 genera and 15genera belong to aquatic plant. In the genera of hydrocotyle, there are 75 species but three species are aquatic plants and they are almost cosmopolitan. They are distributed in marshes, ponds, lake margins, and other wet ground and propagation is by seeds and creeping stems (Cook, 1996). Classification and morphology of plant was according to Fernald (1950).

3.6.1Classification

Kingdom	Plantae
Division	Spermatophyta: Seed plants, Flowering plants
Class	Dicotyledonaea: Dicotyledon
Order	Myrtiflorae
Family	Umbelliferae: Parsley Family
Genus	Hydrocotyl
Species	*Hydrocotyle umbellata* L.

3.6.2 Morphology

Roots: Many fibrous roots emerge from the stem at the nodes, they hang free in the water column or root in the substrate.

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Stems: Freely branching and elongate, fleshy, stem float on the water surface or sometimes creeping onto the shorelines.

Leaves: Suborbicular to reniform, erect on long petioles attached the center of the blade with no notch, centrally peltate, coarsely low-crenate.

Peduncles: Erect and nearly equaling to overtopping leaves.

Flowers: Simple umbel terminal (rarely proliferating), loosely subglobose, becoming 1.3-3 cm in diameter, which is a group of flower on long stalks as tall or taller than the leaves. Individual flowers are very small, white to greenish-white and consist of 5 petals and 5 sepals.

Fruits: Reniform, with deeply notched base, 1-2 mm or high, 2-3 mm of broad, the remote dorsal ribs obtuse.

3.6.3 Importance

Wolverton and McCaleb (1987) found that *H. umbellata* is a more cold-tolerant plant. So it can be used in biological treatment systems for upgrade wastewater effluent and due to bridge the gap during the winter month when the water hyacinth growth is reduced or stopped. *H. umbellata* was found to be very efficient in the nitrification of NH_3, N, and in winter was more effective than any of the other plants studied (Reddy and De Busk, 1985). Moorhead and Reddy (1990) studied the effectiveness of water pennywort in the use of oxygen to reduce biological oxygen demand and ammonia and nitrogen concentrations in sewage. They found that it was very effective in the conversion of organic carbon into carbon dioxide and possibly effective in the reduction of nitrogen content in the treated sewage. Clough and DeBusk (1987) found that the pennywort was the most effective of the secondary treatment of domestic waste in removing BODs and nutrients during both warm and cool season. Water pennywort can also withstand cooler temperatures of water and air, and thus can also be combined with more warm-seasoned plants to maximize the treatment process (Moorhead and Reddy, 1990). Finally, *H. umbellata* have also been utilized for water purification and they can remove various heavy metals from solution (Dushenkov *et al*., 1995; Salt, 1995).

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Figure 3-1. *Hydrocotyle umbellata* L.

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CHAPTER IV
MATERIALS AND METHODS

4.1 Chemical reagents

The chemical reagents used in this study were listed, as follows:

Chemical name	Chemical formula	Source
Acetone	CH_3COCH_3	Fisher
Boric acid	H_3BO_3	J.T. Baker
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	APSFinechem
Copper sulfate	$CuSO_4.5H_2O$	Merk
Ethylenediaminetetraacetic acid	$Na_2EDTA.2H_2O$	Fluka
Ferric chloride	$FeCl_3$	Merk
Lead nitrate	$Pb(NO_3)_2$	Mallinckrodt
Magnesium sulfate	$MgSO_4.7H_2O$	Merck
Manganese chloride	$MnCl_2.4H_2O$	Ajeck
Molybdic acid	$Na_2MoO_4.2H_2O$	Merck
Nitric acid	HNO_3	BDH
Potassium dichromate	$K_2Cr_2O_7$	Fluka
Potassium dihydrogen phosphate	KH_2PO_4	Carlo Erba
Potassium nitrate	KNO_3	Merck
Sodium hydroxide	NaOH	Merck
Zinc sulfate	$ZnSO_4.7H_2O$	BDH

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4.2 Instruments

The instruments used in this study were as follows:

4.2.1 Flame atomic absorption spectrophotometer (FAAS)

The FAAS measurements were performed using a GBC 932 plus model. The operating parameters for measurement were listed bellow:

	Pb	Cr
Wavelength (nm)	217.0	357.9
Lamp current (mA)	5	6
Slit width (nm)	1.0	0.2
Flame type	Air acetylene	Air acetylene

4.2.2 UV Spectrophotometer

An aquarius model CECIL 7200 UV spectrophotometer was used to measure the absorption spectra of chlorophyll solution.

4.2.3 Refrigerated centrifuge

A HEROLAB refrigerated centrifuge, model D-69168 Weisloch, UniCer FR type was used to separate the residue from the supernatant after chlorophyll extraction.

4.2.4 pH meter

A pH meter of Hanna instruments model 8417 was used for pH measurement. Common standard buffers (Merck) of pH 4.00$\pm$0.02 (20 ºC) and 7.00$\pm$0.02 (20 ºC) were employed for the calibration.

4.2.5 Analytical balance

A Sartorius balance was used for weighing the fresh weight of the whole plant. A Precisa balance model 158 Am-FR was used for weighing the chemical and dry weight of plants.

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4.2.6 Hot air oven

A THECLO hot air oven of GCA Precision Scientific was used for drying glasswares and plant materials.

4.3 Preparation of modified Hoagland's nutrient solution

Modified Hoagland's nutrient solution was prepared as follows:

Solution No.	Compound	Stock solution	Stock solution/ liter water
1	KH_2PO_4	2.72 g/100 mL	0.1 mL
2	KNO_3	2.02 g/100 mL	0.5 mL
3	$Ca(NO_3)_2.H_2O$	4.72 g/100 mL	0.5 mL
4	$MgSO_4.7H_2O$	4.64 g/100 mL	0.2 mL
5	$Na_2EDTA.2H_2O$	0.242 g/100 mL	0.1 mL
6	$FeCl_3$	0.12 g/100 mL	0.1 mL
7	Trace elements		0.1 mL
	H_3BO_3	0.286 g/500 mL	
	$MnCl_2.4H_2O$	0.182 g/500 mL	
	$ZnSO_4.7H_2O$	0.022 g/500 mL	
	$CuSO_4.5H_2O$	0.008 g/500 mL	
	$Na_2MoO_4.2H_2O$	0.002 g/500 mL	

The stock solutions were prepared by dissolving the compounds listed above in distilled water. Trace elements were put into stock solution No 7. Modified Hoagland's nutrient solution was prepared by adding specified amount of each of the stock solution to approximately 900 mL of distilled water and bringing the volume to 1,000 mL.

4.4 Preparation of toxicant solutions

4.4.1 Chromium (VI)

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

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4.4.2 Lead (II)

The stock solution of lead (1,000 mg/L) was prepared by dissolving analytical grade of lead nitrate [$Pb(NO_3)_2$] 1.598 g in distilled water 1,000 mL. The concentration was expressed in term of lead ion (Pb^{+2}) in mg/L of solution.

The toxicant solutions were freshly prepared by dissolving the salts of heavy metals in distilled water. Stock solutions were acidified by adding a few drops of concentrated HNO_3 in order to reduce precipitation of the metals ions.

4.5 Preparation of glasswares

The glasswares used in all experiments were cleaned by washing and soaking in 10% nitric acid solution for two days. Then, they were rinsed with distilled water twice and dried before use.

4.6 Culture of plants

Aquatic plants, *Hydrocotyle umbellata* were obtained from the Jatujak market and grown in the greenhouse. Then, they were acclimatized in a modified Hoagland's nutrient solution under laboratory conditions (25$\pm$2 ºC, illuminated with a light intensity of 1,355 LUX at 12h/12h light and dark cycle) for one week prior to the experiment.

4.7 Experimental procedures

After acclimatization, plants were rinsed with distilled water and six plants of similar size and weight were blotted to remove all excess water and then weighed on an electronic balance. They were placed into a plastic bowl, which contained 500 mL each of 0, 20, 40, 60, 80 and 100 mg Pb/L and 0, 2, 4, 6, 8 and 10 mg Cr/L. The total of 72 plants (one replicate) were used in both lead and chromium experiments (36 plants for each metal). The controls were not exposed to metals. The experiment was done in triplicate. The pH of the solution was adjusted to 5.7 and 6.5 for lead and chromium treatments, respectively, at alternate days. The solutions were changed on day 6 in order to ensure a continuous exposure to equal concentration of heavy metals and to eliminate possible nutrient limitation. The plastic bowls were covered with a clear plastic film to prevent air and dust contamination. The experiments were carried

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out in the laboratory under control conditions (25±2 ºC, illuminated with a light intensity of 1,355 LUX at 12h/12h light and dark cycle). Plants from each bowl were separately harvested after 0, 3, 6, 9 and 12 days. Measurements of fresh weight, dry weight, chlorophyll content, and metal concentration in plants were performed.

4.8 Measurements

4.8.1 Relative growth

Plant samples were gently blotted and weighed both before and after treatment. Relative growth was calculated by the equation:

$$\text{Relative growth} = \frac{\text{Final Fresh weight (FFW)}}{\text{Initial Fresh weight (IFW)}}$$

4.8.2 Biomass productivity

The biomass productivity of each plant was determined by drying the samples in a hot air oven at 100 ºC for three days until the constant dry weight was obtained. Their dry weights were measured by an electronic balance. They were expressed as percentage decrease of biomass productivity relative to that of control.

4.8.3 Chlorophyll determination

Chlorophyll extraction and determination were performed according to American Society for Testing and Materials (1999) and MacKinney (1941). After treatment, plant leaves were weighed by an electronic balance. They were extracted by homogenizing in a tissue grinder with 15 mL of 90% acetone. The homogenates were filtered through a Whatman No.4 filter paper and put into a 50 mL centrifuge tube and centrifuged at 3000 rpm for 10 minutes at 4 ºC. Before recording the spectrum, the filtered extract was diluted 10 times with 90% acetone. The absorbance of the extract was measured at both 663 nm and 645 nm, where chlorophyll a and b can maximally absorb. The instrument was set at zero absorbance with a cuvette containing 3 mL of 90% acetone before the chlorophyll solution was made. The cuvette was rinsed with 90% acetone after each use. The concentrations of chlorophyll contents in mg/L were calculated by the formulae:

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Chlorophyll a = (12.7*A663)-(2.69*A645)

Chlorophyll b = (22.9*A645)-(4.68*A663)

Total chlorophyll = (8.02*A663)+(20.2*A645)

All of the experiments were done in the dark and cold condition in order to protect the break down of chlorophyll pigment and for easy extraction, respectively.

4.8.4 Median effective concentration (EC_{50})

The median effective concentration (EC_{50}) is toxicant concentration estimated to cause a specified effect in 50% of a designated proportion of test organism (APHA, 1995). The EC_{50} of metals on biomass and total chlorophyll content in *H. umbellata* of each metal concentration and exposure time were expressed as percentage decrease of biomass and total chlorophyll content relative to control. Biomass and total chlorophyll content were plotted against log 10 metal concentration, and the concentrations at which 50% biomass and total chlorophyll content decreased were calculated by fitting into a probit regresstion line (Finney, 1971).

4.8.5 Analysis of accumulation of toxicants

Digestion of plant samples was done using the nitric acid digestion method according to the Standard Method (APHA, 1995).

After treatments, plants were rinsed with distilled water three times and separated into roots and shoots. Then, they were dried at 80 ºC for three days in a hot air oven. Each dried sample was weighed and transferred into a 40 mL test tube and homogenized. At least 10 times of concentrated nitric acid relative to dry weight of plant sample were put into these test tubes. The solution was heated slowly until digestion was complete as shown by a light-colored, clear solution. Test tubes were washed down with distilled water and then the samples were filtered through a Whatman No. 4 filter paper. The filtrate was diluted to a 50 mL in a volumetric flask, made up to volume with distilled water and mixed thoroughly. Finally, the solutions were transferred into a plastic bottle and a few drops of concentrated nitric acid were added in order to preserve the sample and kept in a refrigerator at 4 ºC before the metal concentrations were determined by a flame atomic absorption spectrophotometer (FAAS).

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4.8.6 Average accumulation

The average accumulation was calculated by the following equation:

$$\text{Average accumulation} = \frac{\text{Metal concentration in biomass}}{\text{Test duration}}$$

In this study, the test duration was 3, 6, 9, and 12 days.

4.8.7 Bioconcentration factor (BCF)

The bioconcentration factor (BCF) was calculated as follows:

$$\text{Bioconcentration factor} = \frac{\text{Concentration of metals in oven dried plants}}{\text{Initial concentration of metals in medium}}$$

4.9 Statistical analysis

The mean numbers of relative growth, biomass production, chlorophyll content, and heavy metal content were calculated and subjected to the analysis of variance (ANOVA) using randomizing block design and Least Significant Difference method (LSD) on the SPSS for window program.

4.10 Observation of toxicity symptoms

After the exposure of *H. umbellata* to lead and chromium, the morphological changes such as the occurring of new seedling and toxicity symptoms were observed and photographed at the end of each test duration.

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CHAPTER V
RESULTS

5.1 Effects of heavy metals on fresh weight and dry weight of *Hydrocotyle umbellata*

The effect of lead on fresh weight of *H. umbellata* is shown in Table 5-1. The maximum fresh weight (8.26 g) was found in the control on day 12. The minimum weight (4.89 g) was found in 100 mg Pb/L treatment on day 12.

The effect of chromium on fresh weight of *H. umbellata* is shown in Table 5-2. The maximum fresh weight (7.62 g) was found in the control on day 12. The minimum weight (5.19 g) was found in 10 mg Cr/L treatment on day 12.

The effect of lead on dry weight of *H. umbellata* is shown in Table 5-3. The maximum dry weight (0.74 g) was found in the control on day 12. The minimum weight (0.51 g) was found in 100 mg Pb/L treatment on day 12.

The effect of chromium on dry weight of *H. umbellata* is shown in Table 5-4. The maximum dry weight (0.71 g) was found in the control on day 12. The minimum weight (0.52 g) was found in 10 mg Cr/L treatment on day 12.

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Table 5-1. Effect of lead on fresh weight of *H. umbellata.*

Lead	Fresh weight (g)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	7.19±0.00	7.23±1.05	7.44±0.16	7.78±0.19	8.26±0.06
20	7.42±0.00	7.53±0.29	7.39±0.02	7.04±0.09	6.94±0.10
40	7.22±0.00	7.18±0.05	6.93±0.06	6.80±0.17	5.97±0.51
60	7.33±0.00	7.12±0.10	6.39±0.24	6.23±0.48	5.72±0.29
80	6.92±0.00	6.76±0.35	5.60±0.57	5.42±0.56	5.10±0.29
100	7.09±0.00	6.87±0.39	5.40±0.46	5.22±0.39	4.89±0.35

Each value is the mean of triplicate± S.D.

Table 5-2. Effect of chromium on fresh weight of *H. umbellata.*

Chromium	Fresh weight (g)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	6.62±0.00	6.83±0.00	6.94±0.00	7.51±0.00	7.62±0.00
2	6.58±0.00	6.40±0.28	6.56±0.33	6.77±0.12	7.49±0.63
4	6.97±0.00	7.01±0.16	7.16±0.24	6.60±0.30	6.79±0.51
6	6.89±0.00	6.69±0.86	6.59±0.19	6.43±0.71	6.68±0.66
8	6.87±0.00	6.58±0.09	6.45±0.32	6.36±0.45	5.86±0.96
10	6.71±0.00	5.64±0.19	5.38±0.16	5.23±0.66	5.19±0.76

Each value is the mean of triplicate± S.D.

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Table 5-3. Effect of lead on dry weight of *H. umbellata.*

Lead	**Dry weight (g)**				
(mg/L)	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	0.61±0.00	0.66±0.02	0.68±0.09	0.70±0.00	0.74±0.00
20	0.62±0.00	0.65±0.00	0.64±0.03	0.64±0.01	0.63±0.01
40	0.69±0.00	0.63±0.01	0.60±0.02	0.60±0.03	0.59±0.02
60	0.68±0.00	0.61±0.02	0.59±0.00	0.58±0.00	0.57±0.01
80	0.64±0.00	0.59±0.06	0.58±0.01	0.56±0.03	0.55±0.02
100	0.67±0.00	0.57±0.05	0.55±0.04	0.53±0.04	0.51±0.03

Each value is the mean of triplicate± S.D.

Table 5-4. Effect of chromium on dry weight of *H. umbellata.*

Chromium	**Dry weight (g)**				
(mg/L)	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	1.00±0.00	0.60±0.00	0.65±0.01	0.69±0.02	0.71±0.01
2	1.05±0.00	0.58±0.01	0.63±0.02	0.64±0.00	0.66±0.01
4	0.99±0.00	0.58±0.01	0.65± 0.01	0.61±0.02	0.59±0.02
6	1.06±0.00	0.57±0.01	0.64±0.03	0.58±0.01	0.59±0.38
8	0.86±0.00	0.57±0.01	0.62±0.02	0.56±0.01	0.55±0.02
10	0.89±0.00	0.56±0.01	0.55±0.02	0.53±0.02	0.52±0.03

Each value is the mean of triplicate± S.D.

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5.2 Effects of heavy metals on relative growth of *H. umbellata.*

The effect of lead on relative growth of *H. umbellata* is shown in Table 5-5 and Figure 5-1. The relative growth of the control increased with increasing exposure time. The relative growth of treated plants was decreased as the exposure time and lead concentration were increased. At 40, 60, 80, and 100 mg Pb/L, the relative growth was significantly decreased ($P \leq 0.05$) from that of control. The relative growth at 6, 9, and 12 days decreased significantly ($P \leq 0.05$) from the first three days at 40, 60, 80, and 100 mg Pb/L. However, the relative growth was not significantly decreased ($P > 0.05$) at 20 mg Pb/L at all exposure times and all lead concentrations on day 3. The maximum relative growth (1.08) was found in the control on day 12. The minimum relative growth (0.71) was found in 100 mg Pb/L treatment on day 12.

The effect of chromium on relative growth of *H. umbellata* is shown in Table 5-6 and Figure 5-2. The relative growth of the control increased with increasing exposure time. The relative growth of treated plants was decreased as the exposure time and chromium concentration were increased. At 6, 8, and 10 mg Cr/L, the relative growth was significantly decreased ($P \leq 0.05$) from that of control. The relative growth on day 9 and 12 was significantly decreased ($P \leq 0.05$) from the first six days at all chromium concentrations. The relative growth was not significantly decreased ($P > 0.05$) on day 3 at all chromium concentrations. However, at 4 mg Cr/L the results showed stimulation in growth of plants on day 3. The maximum relative growth (1.09) was found in the control on day 12. The minimum relative growth (0.86) was found in 10 mg Cr/L treatment on day 12.

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Table 5-5. Effect of lead on relative growth of *H. umbellata.*

Lead	**Relative growth**				
(mg/L)	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	1.00±0.00	1.01±0.02	1.04±0.01	1.05±0.03	1.08±0.01
20	1.00±0.00	1.00±0.01	0.98±0.05	0.97±0.01	0.95±0.03
40	1.00±0.00	0.99±0.01	0.95±0.02	0.94±0.02	0.82±0.04
60	1.00±0.00	0.97±0.00	0.90±0.04	0.87±0.06	0.81±0.07
80	1.00±0.00	0.98±0.02	0.80±0.09	0.81±0.05	0.74±0.01
100	1.00±0.00	0.97±0.04	0.78±0.05	0.73±0.09	0.71±0.00

Each value is the mean of triplicate± S.D.

Table 5-6. Effect of chromium on relative growth of *H. umbellata.*

Chromium	**Relative growth**				
(mg/L)	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	1.00±0.00	1.00±0.00	1.05±0.00	1.08±0.00	1.09±0.00
2	1.00±0.00	1.00±0.00	1.01±0.02	1.05±0.02	1.06±0.01
4	1.00±0.00	1.01±0.02	1.02±0.02	1.06±0.01	1.08±0.02
6	1.00±0.00	1.00±0.03	1.00±0.02	1.04±0.01	1.05±0.03
8	1.00±0.00	0.99±0.00	0.98±0.01	0.94±0.02	0.92±0.06
10	1.00±0.00	0.99±0.01	0.97±0.01	0.92±0.03	0.86±0.07

Each value is the mean of triplicate± S.D.

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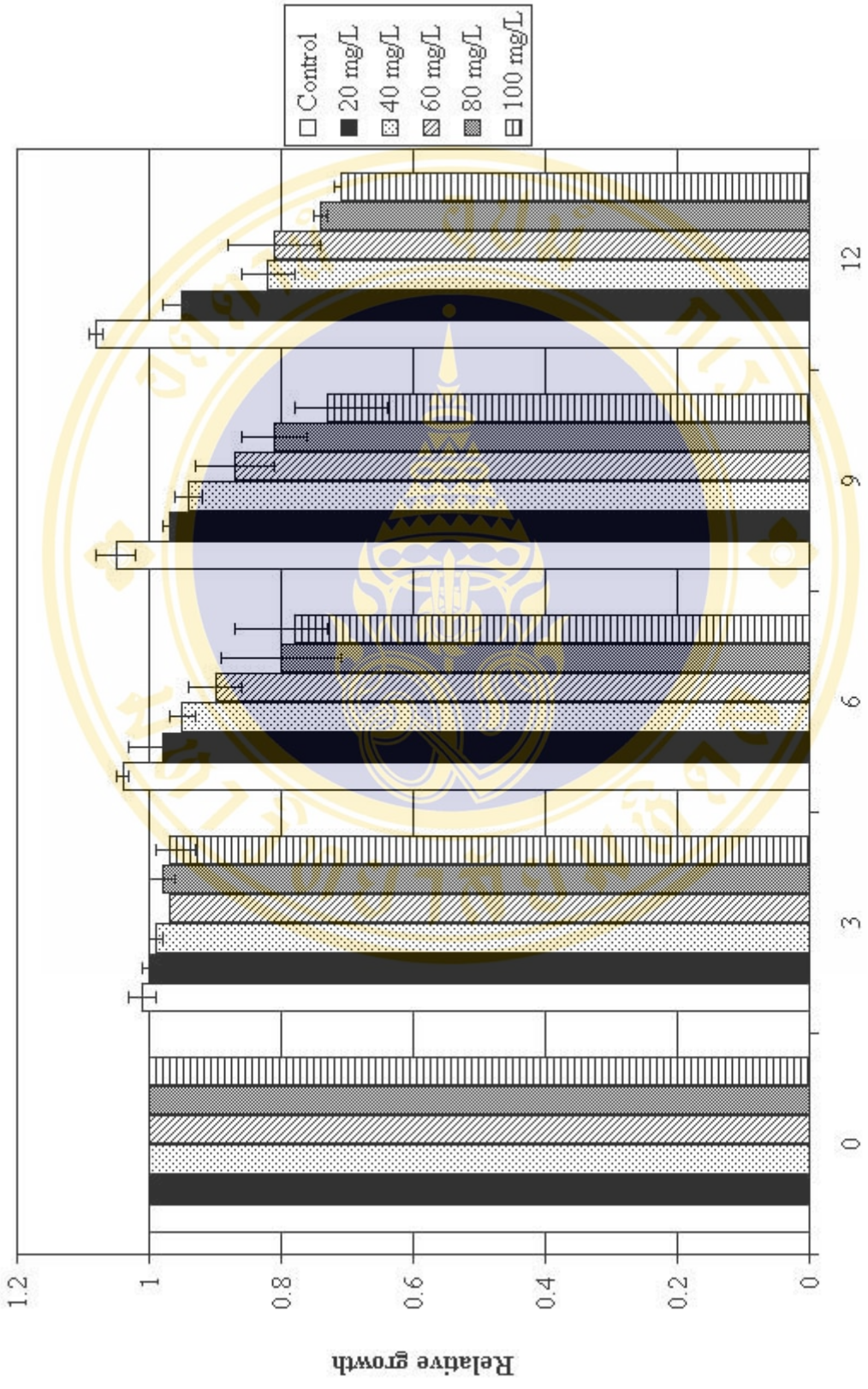

Figure 5-1. Effect of lead on relative growth of *H. umbellata*.

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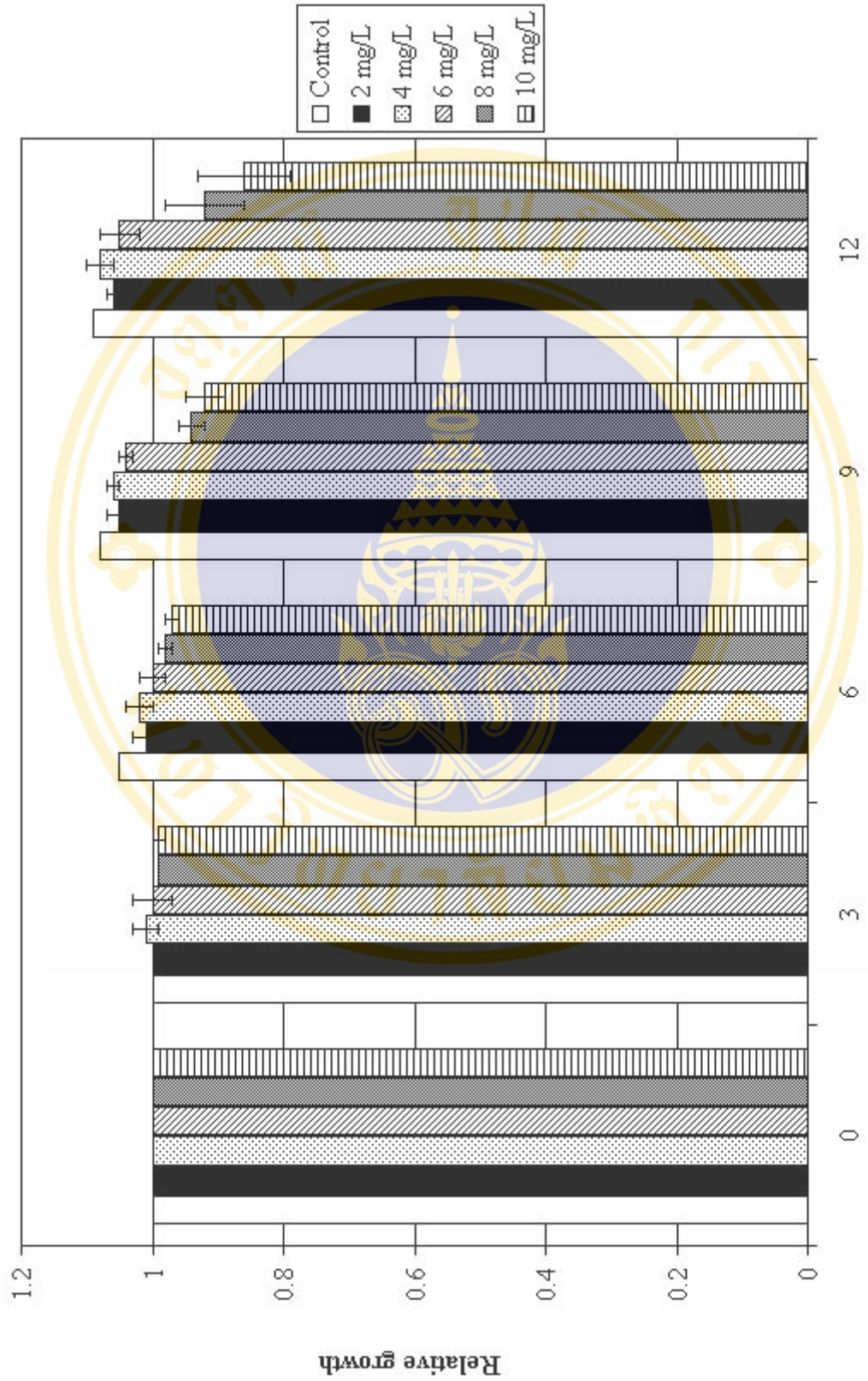

Figure 5-2. Effect of chromium on relative growth of *H. umbellata*.

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5.3 Effects of heavy metals on biomass productivity of *H. umbellata.*

The effect of lead on biomass productivity (% of control) of *H. umbellata* is shown in Table 5-7 and Figure 5-3. The biomass productivity was decreased as the lead concentration and exposure time were increased. At 20, 40, 60, 80, and 100 mg Pb/L, the biomass productivity was significantly decreased ($P \leq 0.05$) from that of control. The biomass productivity on 6, 9, and 12 days was significantly decreased ($P \leq 0.05$) from the first three days at all lead concentrations. The maximum biomass productivity (100%) was found in the control and the minimum biomass productivity (69.52%) was found in 100 mg Pb/L treatment on day 12.

The effect of chromium on biomass productivity (% of control) of *H. umbellata* is shown in Table 5-8 and Figure 5-4. The biomass productivity was decreased as the chromium concentration and exposure time were increased. At 2, 4, 6, 8, and 10 mg Cr/L, the biomass productivity was significantly decreased ($P \leq 0.05$) from that of control. The biomass productivity on 6, 9, and 12 days was significantly decreased ($P \leq 0.05$) from the first three days at all chromium concentrations. The maximum biomass productivity (100%) was found in the control and the minimum biomass productivity (70.59%) was found in 10 mg Cr/L treatment on day 12.

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Table 5-7. Effect of lead on biomass productivity of *H. umbellata.*

Lead	Biomass productivity (% of control)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
20	100±0.00	98.02±0.20	93.41±2.71	90.82±1.91	85.56±2.26
40	100±0.00	95.90±0.19	88.36±3.27	86.25±1.82	80.03±2.71
60	100±0.00	92.44±1.23	87.22±0.46	83.20±0.32	76.58±0.42
80	100±0.00	89.77±5.25	83.25±1.96	80.57±3.92	74.29±0.19
100	100±0.00	87.38±6.78	79.47±2.13	75.80±4.29	69.52±1.31

Each value is the mean of triplicate± S.D.

Table 5-8. Effect of chromium on biomass productivity of *H. umbellata.*

Chromium	Biomass productivity (% of control)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	100±0.00	100.00±0.00	100±0.00	100±0.00	100±0.00
2	100±0.00	97.44±0.88	95.67±3.74	91.69±0.27	88.81±1.19
4	100±0.00	96.56±1.87	93.02±0.95	86.99±2.25	83.24±2.75
6	100±0.00	96.29±1.00	91.50±2.35	83.12±1.47	79.73±0.38
8	100±0.00	95.12±2.15	88.85±0.53	80.23±0.88	74.64±3.13
10	100±0.00	93.26±1.88	86.28±3.29	75.09±2.34	70.59±4.37

Each value is the mean of triplicate± S.D.

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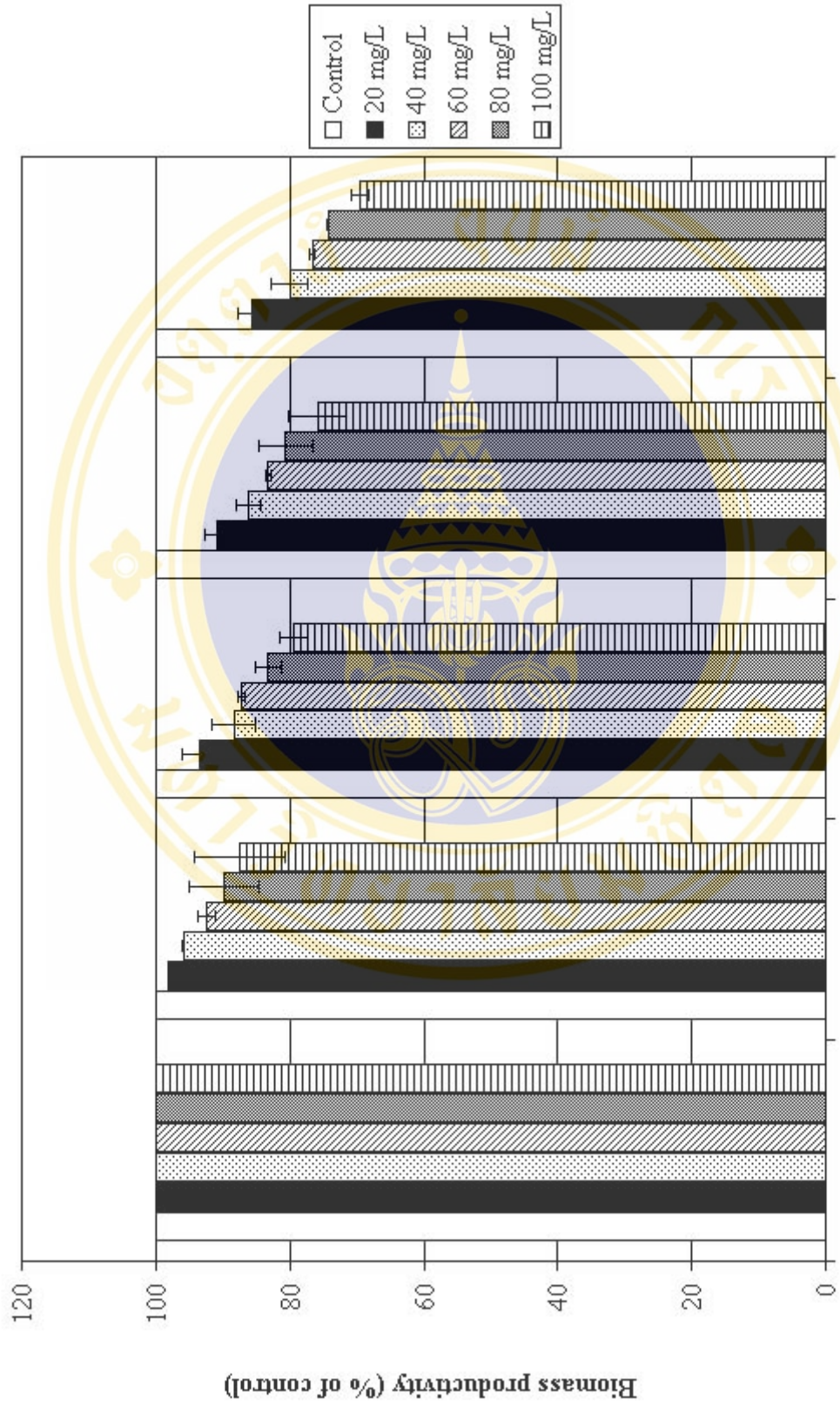

Figure 5-3. Effect of lead on biomass productivity of *H. umbellata*.

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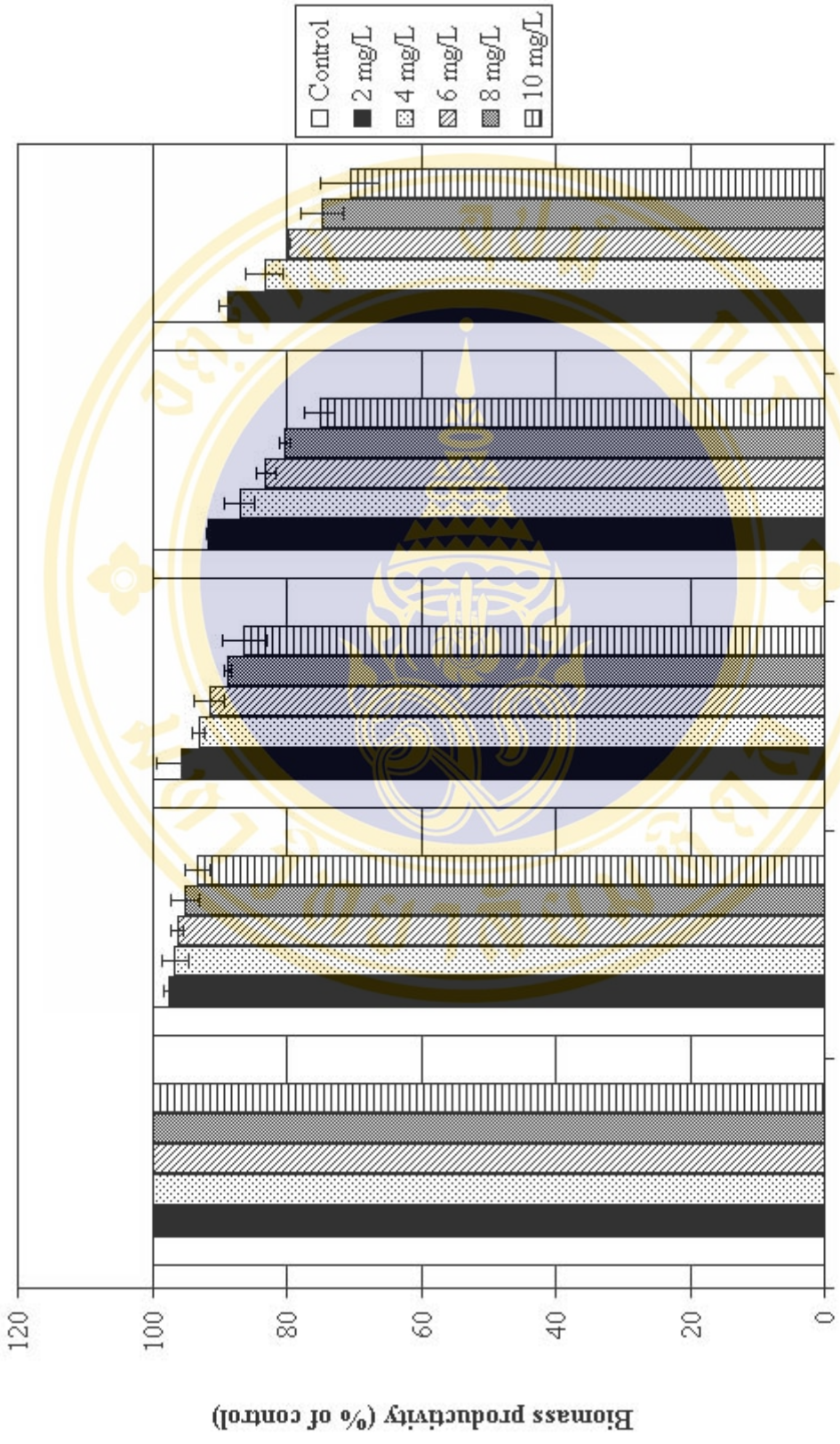

Figure 5-4. Effect of chromium on biomass productivity of *H. umbellata*.

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5.4 Effects of heavy metals on chlorophyll content of *H. umbellata.*

The effect of lead on chlorophyll content of *H. umbellata* is shown in Tables 5-9 to 5-11 and Figures 5-5 to 5-7. The chlorophyll contents of the control increased with increasing exposure time. At lead concentrations of 20, 40, 60, 80, and 100 mg Pb/L the chlorophyll contents were significantly decreased ($P \leq 0.05$) from that of control. Also, the chlorophyll contents on day 9 and 12 decreased significantly ($P \leq 0.05$) from the first six days for all concentrations. The highest total chlorophyll content (3.38 mg/g fresh weight) was observed in the control on day 12 and the lowest (2.25 mg/g fresh weight) was observed in 100 mg Pb/L treatment on day 12.

The effect of chromium on chlorophyll content of *H. umbellata* is shown in Tables 5-12 to 5-14 and Figures 5-8 to 5-10. The chlorophyll contents of the control increased with increasing exposure time. At chromium concentrations of 2, 4, 6, 8, and 10 mg Cr/L, the chlorophyll contents were significantly decreased ($P \leq 0.05$) from that of control. Also, the chlorophyll contents on day 9, and 12 decreased significantly ($P \leq 0.05$) from the first six days for all concentrations. The highest chlorophyll content (4.20 mg/g fresh weight) was observed in the control on day 12 and the lowest (2.30 mg/g fresh weight) was observed in 10 mg Cr/L treatment on day 12.

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Table 5-9. Effect of lead on chlorophyll a of *H. umbellata.*

Lead	Chlorophyll a (mg/g fresh weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	1.65±0.00	2.02±0.00	2.08±0.00	2.25±0.00	2.32±0.00
20	1.57±0.00	2.08±0.30	2.29±0.25	2.27±0.08	1.84±0.01
40	1.62±0.00	1.98±0.09	2.08±0.12	2.02±0.21	1.76±0.12
60	1.69±0.00	1.96±0.02	2.04±0.42	2.01±0.05	1.63±0.31
80	1.66±0.00	2.04±0.06	1.97±0.26	1.89±0.04	1.71±0.01
100	1.68±0.00	1.93±0.10	1.81±0.19	1.73±0.03	1.61±0.03

Each value is the mean of triplicate± S.D.

Table 5-10. Effect of lead on chlorophyll b of *H. umbellata.*

Lead	Chlorophyll b (mg/g fresh weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.75±0.00	1.04±0.00	1.08±0.00	1.02±0.00	1.06±0.00
20	0.77±0.00	0.94±0.32	1.00±0.17	0.95±0.20	0.93±0.23
40	0.69±0.00	0.91±0.11	1.11±0.10	1.02±0.22	0.99±0.23
60	0.84±0.00	0.89±0.01	1.07±0.37	0.82±0.05	0.96±0.04
80	0.72±0.00	0.79±0.04	1.05±0.22	0.74±0.01	0.79±0.10
100	0.70±0.00	0.77±0.05	0.69±0.08	0.65±0.02	0.65±0.02

Each value is the mean of triplicate± S.D.

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Table 5-11. Effect of lead on total chlorophyll content of *H. umbellata.*

Lead	Total chlorophyll (mg/g fresh weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	2.41±0.00	3.06±0.00	3.16±0.00	3.27±0.00	3.38±0.00
20	2.33±0.00	3.02±0.62	3.29±0.20	3.21±0.16	2.77±0.24
40	2.32±0.00	2.89±0.19	3.19±0.21	3.04±0.31	2.75±0.30
60	2.54±0.00	2.85±0.29	3.11±0.78	2.83±0.22	2.59±0.35
80	2.37±0.00	2.82±0.08	3.02±0.46	2.63±0.08	2.50±0.09
100	2.38±0.00	2.70±0.15	2.50±0.27	2.37±0.05	2.25±0.05

Each value is the mean of triplicate± S.D.

Table 5-12. Effect of chromium on chlorophyll a of *H. umbellata.*

Chromium	Chlorophyll a (mg/g fresh weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	1.43±0.00	2.29±0.00	2.25±0.00	2.31±0.00	2.89±0.00
2	1.37±0.00	2.08±0.28	1.98±0.01	1.96±0.10	2.53±0.43
4	1.45±0.00	2.09±0.16	1.94±0.04	1.45±0.23	2.34±0.18
6	1.68±0.00	1.98±0.18	1.94±0.02	1.70±0.16	2.27±0.18
8	1.58±0.00	1.94±0.03	1.91±0.06	1.96±0.02	1.98±0.47
10	1.63±0.00	1.85±0.02	1.84±0.05	1.72±0.06	1.67±0.15

Each value is the mean of triplicate± S.D.

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Table 5-13. Effect of chromium on chlorophyll b of *H. umbellata.*

Chromium	Chlorophyll b (mg/g fresh weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.63±0.00	1.05±0.00	1.20±0.00	1.24±0.00	1.31±0.00
2	0.74±0.00	0.75±0.10	0.70±0.01	0.89±0.23	1.04±0.02
4	0.65±0.00	0.75±0.16	0.69±0.11	1.33±0.22	0.99±0.13
6	0.58±0.00	0.70±0.04	0.67±0.07	1.02±0.46	0.91±0.05
8	0.64±0.00	0.70±0.05	0.63±0.01	0.76±0.01	0.80±0.25
10	0.64±0.00	0.66±0.03	0.60±0.06	0.61±0.04	0.63±0.03

Each value is the mean of triplicate± S.D.

Table 5-14. Effect of chromium on total chlorophyll content of *H. umbellata.*

Chromium	Total chlorophyll (mg/g fresh weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	2.06±0.00	3.34±0.00	3.45±0.00	3.55±0.00	4.20±0.00
2	2.11±0.00	2.83±0.38	2.68±0.21	2.86±0.32	3.58±0.62
4	2.10±0.00	2.84±0.22	2.63±0.13	2.78±0.07	3.33±0.29
6	2.26±0.00	2.68±0.22	2.61±0.09	2.72±0.30	3.18±0.23
8	2.22±0.00	2.64±0.18	2.54±0.07	2.72±0.01	2.78±0.71
10	2.28±0.00	2.53±0.04	2.44±0.84	2.33±0.06	2.30±0.16

Each value is the mean of triplicate± S.D.

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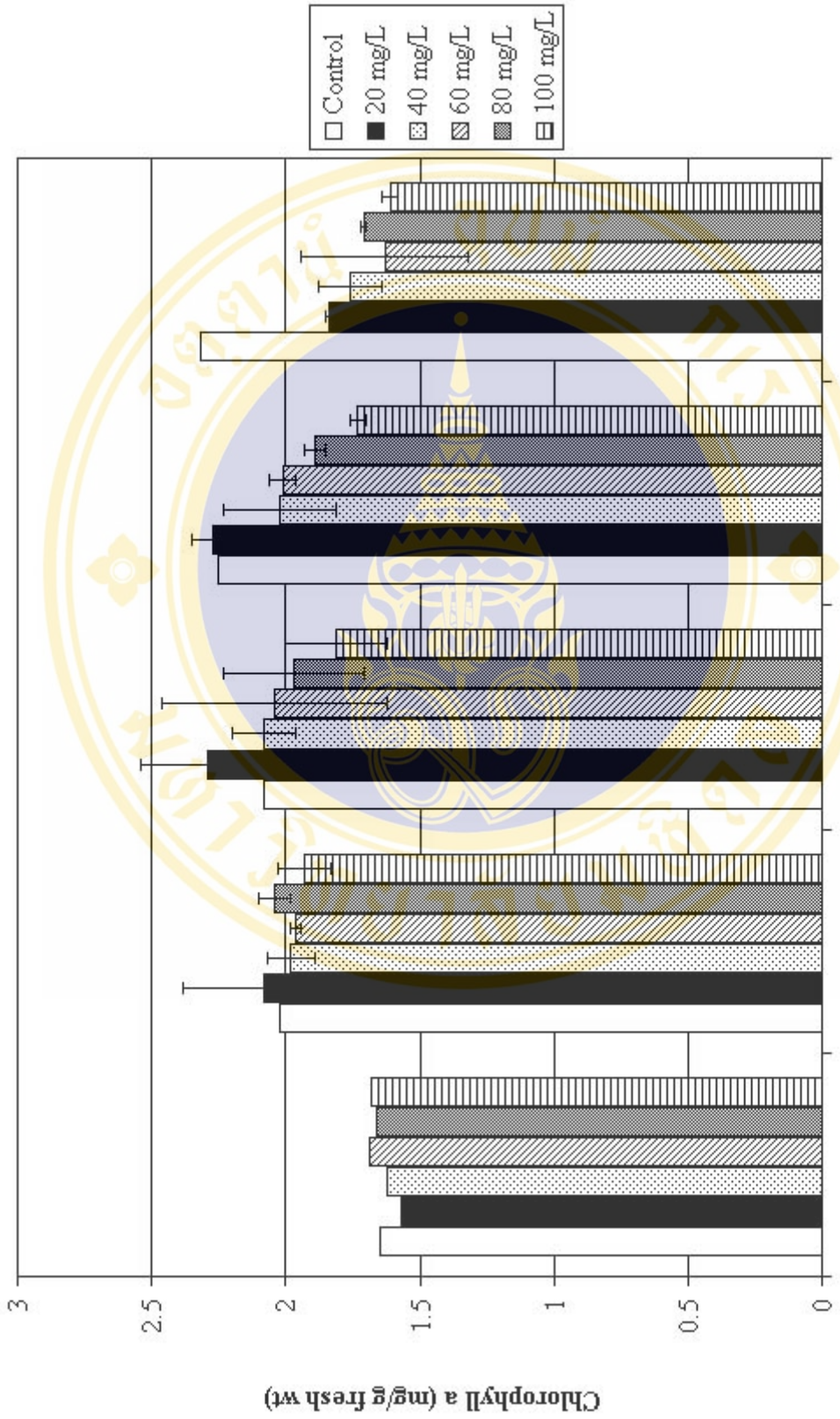

Figure 5-5. Effect of lead on chlorophyll a of *H. umbellata*.

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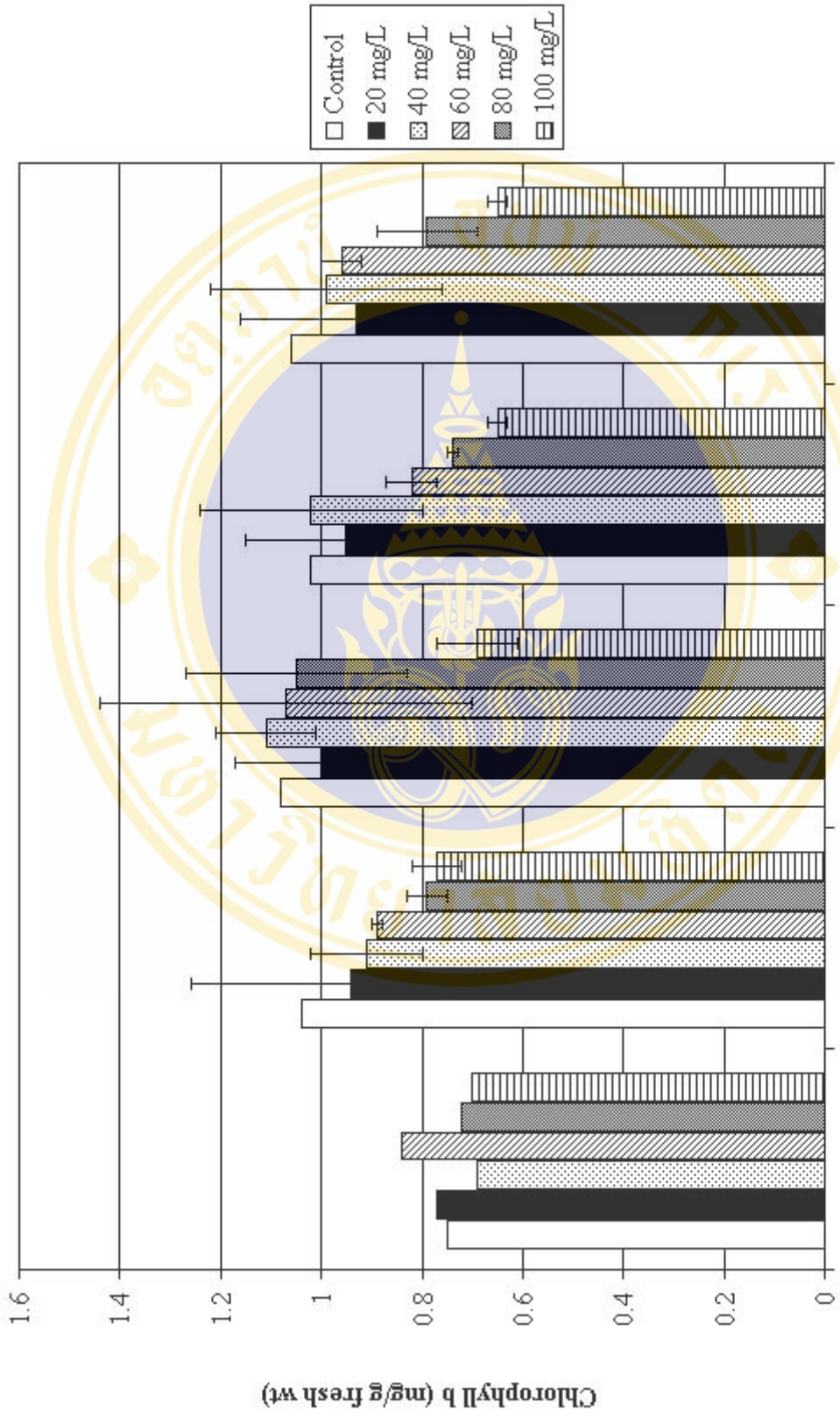

Figure 5-6. Effect of lead on chlorophyll b of *H. umbellata*.

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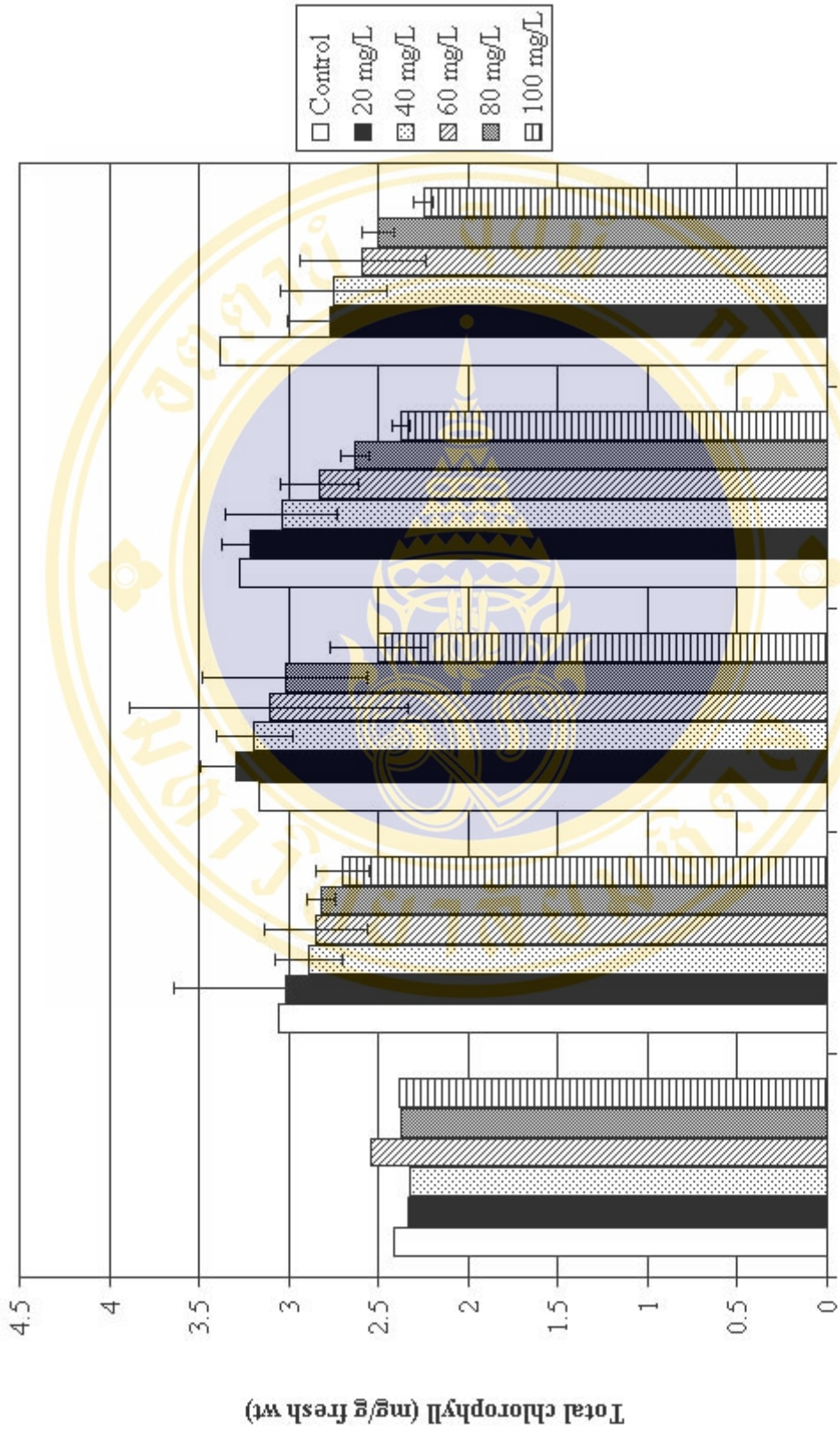

Figure 5-7. Effect of lead on total chlorophyll content of *H. umbellata*.

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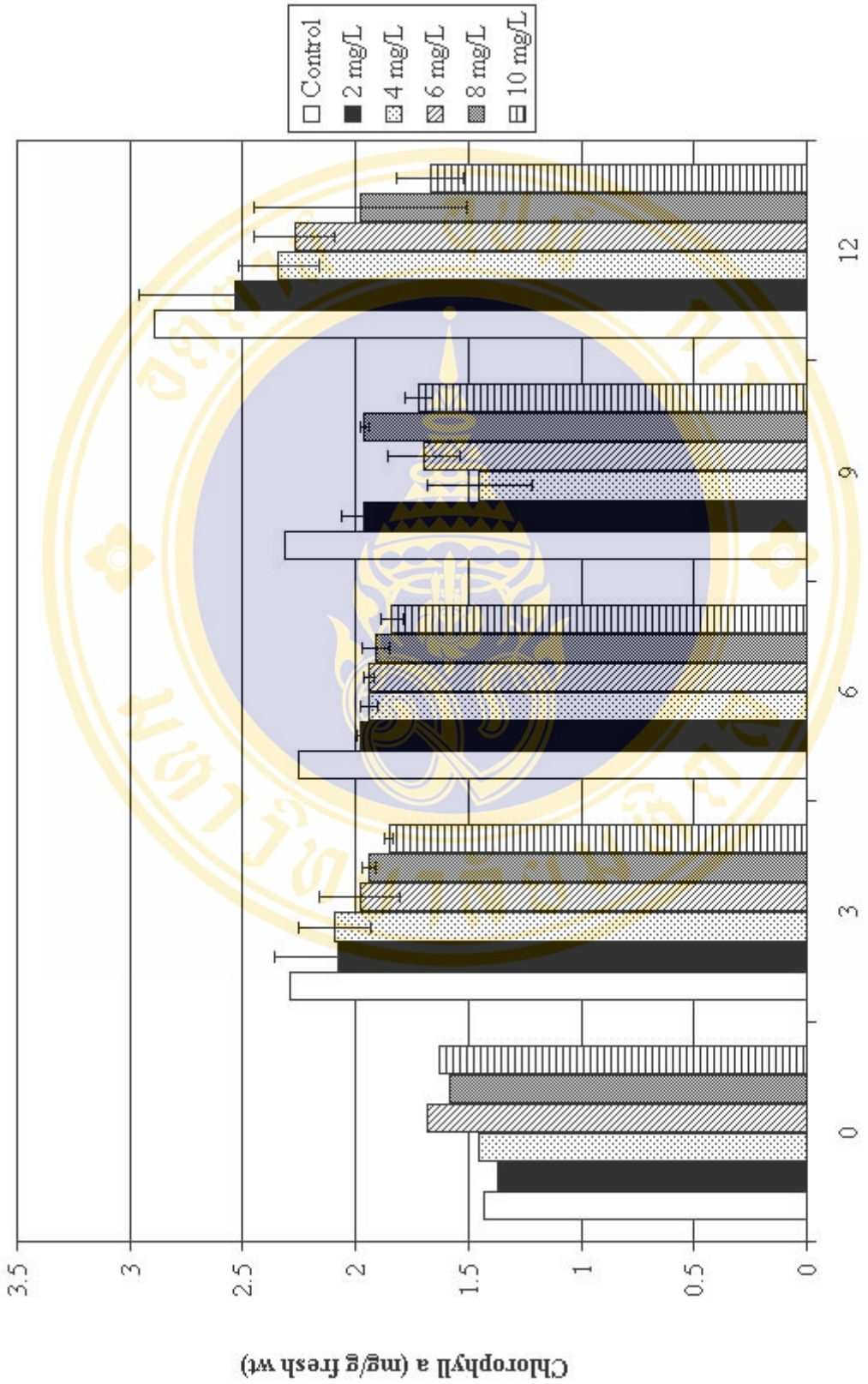

Figure 5-8. Effect of chromium on chlorophyll a of *H. umbellata*.

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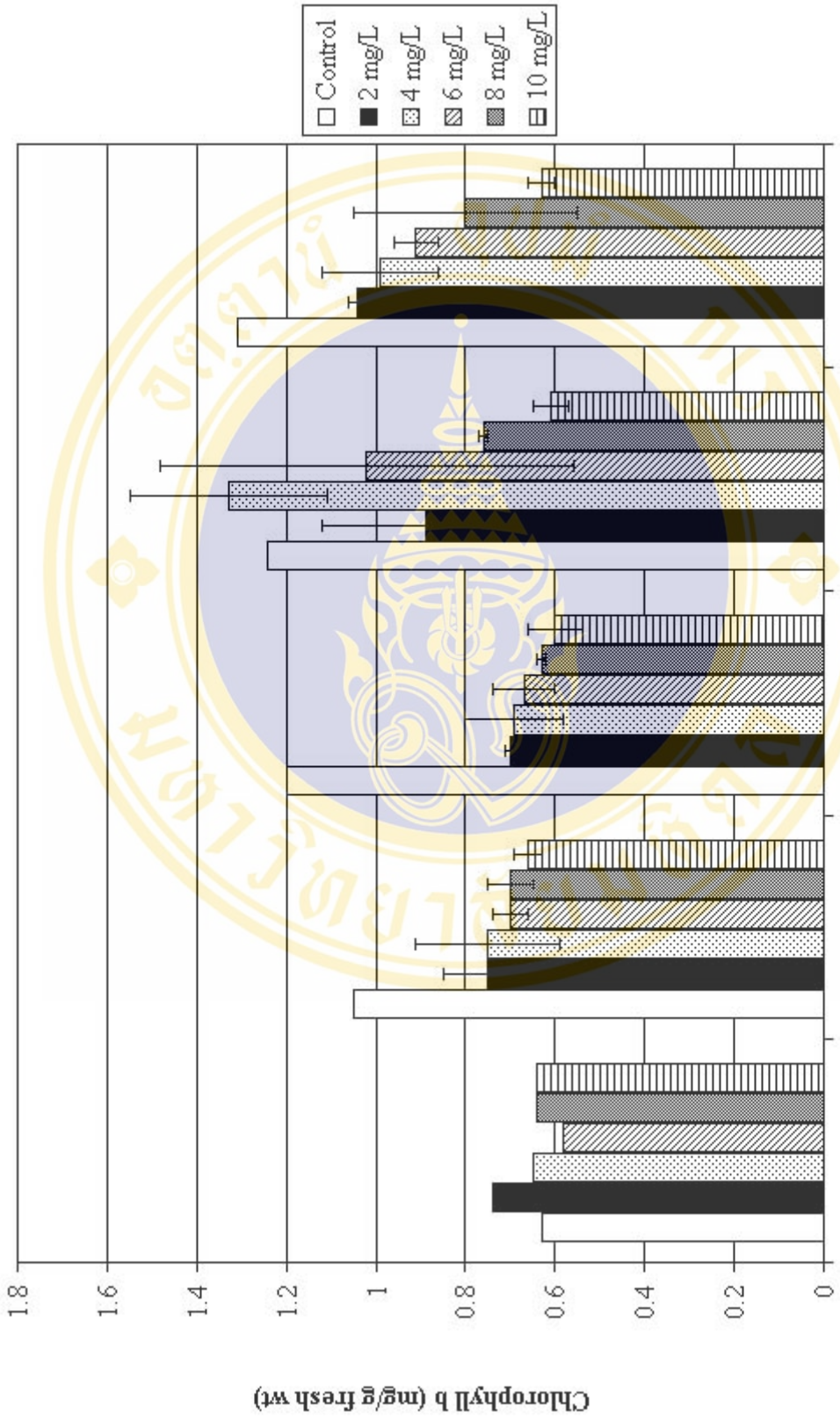

Figure 5-9. Effect of chromium on chlorophyll b of *H. umbellata*.

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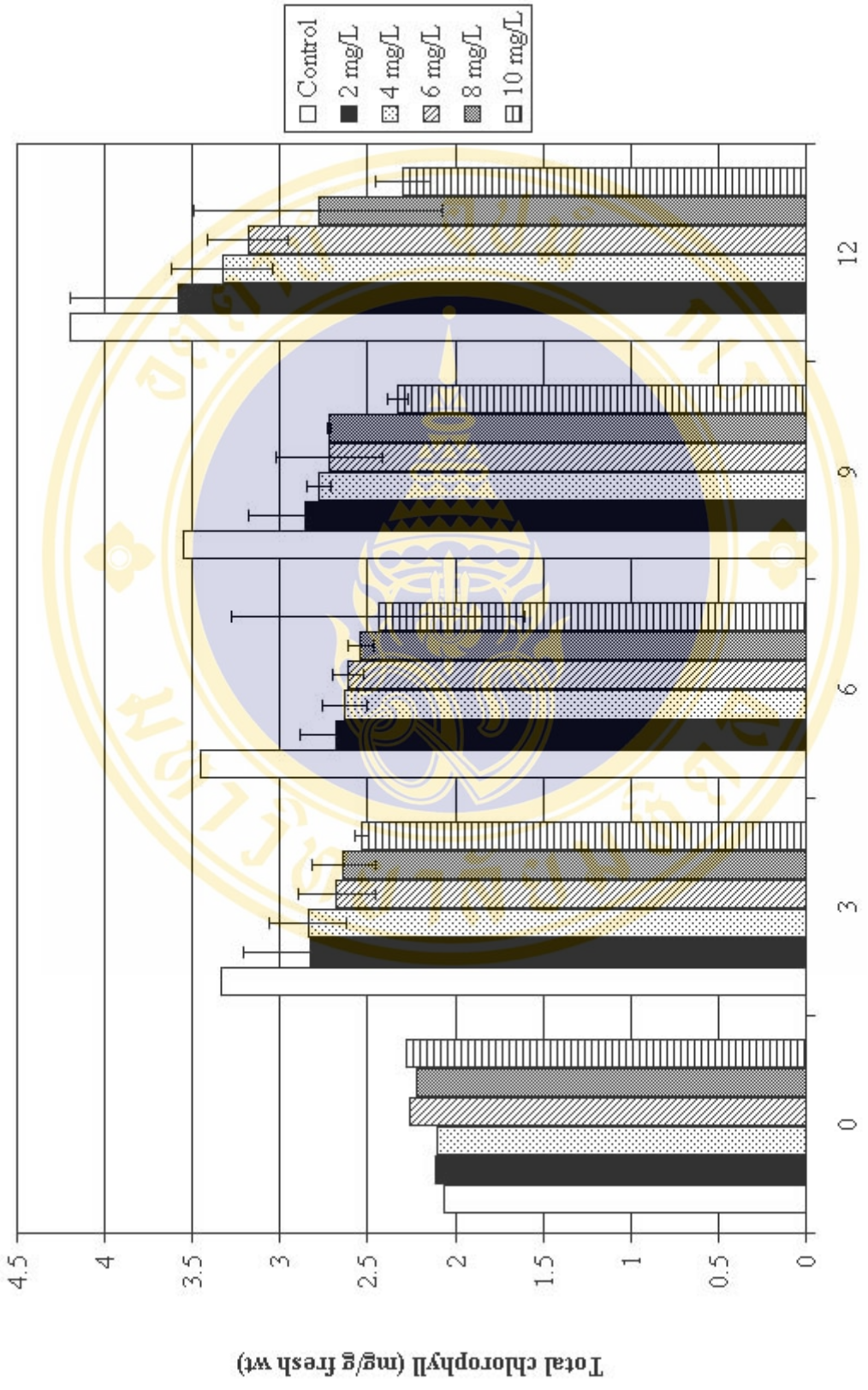

Figure 5-10. Effect of chromium on total chlorophyll content of *H. umbellata*.

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5.5 Median effective concentration (EC_{50})

The EC_{50} of lead and chromium on the biomass productivity and the total chlorophyll content of *H. umbellata* are shown in Table 5-15.

The EC_{50} of lead on biomass productivity on day 3, 6, 9, and 12 days were 386.81, 251.35, 213.41, and 164.23 mg/L, respectively, and for chromium, they were 84.43, 37.88, 20.56, and 17.41 mg/L, respectively.

The EC_{50} of lead on the total chlorophyll content at 3, 6, 9, and 12 days were 438.37, 303.21, 185.35, and 156.79 mg/L, respectively, and for chromium, they were 21.31, 17.69, 15.85, and 12.44, respectively.

The EC_{50}s of lead and chromium were decreased as the exposure time and metal concentrations were increased.

The EC_{50} of chromium was lower than that of lead during the same exposure time, suggesting that chromium was comparatively more toxic than lead.

Table 5-15. The median effective concentration (EC_{50}) of lead and chromium on biomass productivity and total chlorophyll content in *H. umbellata* at different exposure times.

Exposure time (days)	EC_{50} (mg/L)			
	Biomass productivity		Total chlorophyll content	
	Lead	Chromium	Lead	Chromium
3	386.81	84.43	438.37	21.31
6	251.35	37.88	303.21	17.69
9	213.41	20.56	185.35	15.85
12	164.23	17.41	156.79	12.44

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5.6 The accumulation of heavy metals by *H. umbellata*

Lead and chromium were not detected in control plants (shoots and roots) at all exposure times. The accumulation of lead in shoots of *H. umbellata* is shown in Table 5-16 and Figure 5-11. The lead content was significantly increased (P≤0.05) when the exposure time and lead concentration were increased. The highest lead content in shoots (663.03 mg/kg dry wt) was found at 80 mg Pb/L treatment on day 9. The lowest lead content (108.68 mg/kg dry wt) was found at 20 mg Pb/L treatment on day 3.

The accumulation of lead in roots of *H. umbellata* is shown in Table 5-17 and Figure 5-12. The lead content was significantly increased (P≤0.05) when the exposure time and lead concentration were increased. The highest lead content in roots (71,998.53 mg/kg dry wt) was found at 60 mg Pb/L treatment on day 9 and the lowest (25,267.17 mg/kg dry wt) was found at 20 mg Pb/L treatment on day 3.

The accumulation of chromium in shoots of *H. umbellata* is shown in Table 5-18 and Figure 5-13. The chromium content was significantly increased (P≤0.05) when the exposure time and chromium concentration were increased. The highest chromium content in shoots (21.97 mg/kg dry wt) was found at 10 mg Cr/L treatment on day 9 and the lowest (0 mg/kg dry wt) was found at 2 mg Cr/L treatment on day 3.

The accumulation of chromium in roots of *H. umbellata* is shown in Table 5-19 and Figure 5-14. The chromium content was significantly increased (P≤0.05) when the exposure time and chromium concentration were increased. The highest chromium content in roots (845.73 mg/kg dry wt) was found at 10 mg Cr/L treatment on day 9 and the lowest (270.89 mg/kg dry wt) was found at 2 mg Cr/L treatment on day 3.

Lead and chromium content (shoots and roots) are not linear in correlation to the concentration and exposure time. Higher lead and chromium contents was found in roots than shoots at all exposure times.

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Table 5-16. Lead accumulation in shoots of *H. umbellata.*

Lead	Lead accumulation (mg/kg dry weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20	0.00±0.00	108.68±5.55	145.09±13.89	245.85±6.53	278.15±29.53
40	0.00±0.00	121.19±39.10	161.34±6.58	324.23±71.27	405.57±83.30
60	0.00±0.00	180.73±48.63	227.42±36.74	385.49±90.65	440.04±52.09
80	0.00±0.00	229.96±10.02	296.93±9.16	663.03±39.57	561.51±55.34
100	0.00±0.00	255.83±59.15	356.95±9.79	618.43±60.24	507.89±99.37

Each value is the mean of triplicate± S.D.

Table 5-17. Lead accumulation in roots of *H. umbellata*.

Lead	Lead accumulation (mg/kg dry weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00± 0.00
20	0.00±0.00	25267.17±5433.58	28808.05±4804.93	38054.74±2556.69	34763.47±3056.20
40	0.00±0.00	29977.27±1756.06	32371.78±1182.10	55230.44±5033.59	37188.58±2955.07
60	0.00±0.00	33906.96±5525.92	34336.05±1007.50	71998.53±1687.59	43479.05±1676.73
80	0.00±0.00	36663.47±5740.72	38849.93±3860.12	57016.92±1968.83	44717.11±5249.66
100	0.00±0.00	61964.33±6425.85	58999.26±4351.97	50194.78±7569.74	48249.24±5661.85

Each value is the mean of triplicate± S.D.

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Table 5-18. Chromium accumulation in shoots of *H. umbellata.*

Chromium	Chromium accumulation (mg/kg dry weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.90±0.25	2.06±0.57	4.67±0.99
4	0.00±0.00	0.97±0.11	2.01±0.18	4.43±0.28	8.96±0.25
6	0.00±0.00	2.51±0.44	5.19±1.49	6.95±1.01	11.42±0.43
8	0.00±0.00	4.31±0.44	10.66±1.48	14.67±1.24	15.04±0.77
10	0.00±0.00	6.98±1.67	15.27±1.57	21.97±2.26	17.59±1.62

Each value is the mean of triplicate± S.D.

Table 5-19. Chromium accumulation in roots of *H. umbellata.*

Chromium	Chromium accumulation (mg/kg dry weight)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	270.89±29.29	321.58±66.45	378.57±41.48	456.69±17.23
4	0.00±0.00	393.44±46.80	482.99±20.21	577.32±40.17	720.59±28.94
6	0.00±0.00	433.85±83.91	530.49±95.87	633.60±95.65	772.64±46.98
8	0.00±0.00	487.63±19.19	583.05±23.66	709.89±59.07	841.87±54.23
10	0.00±0.00	528.77±4.98	675.17±91.01	845.73±53.48	810.20±57.40

Each value is the mean of triplicate± S.D.

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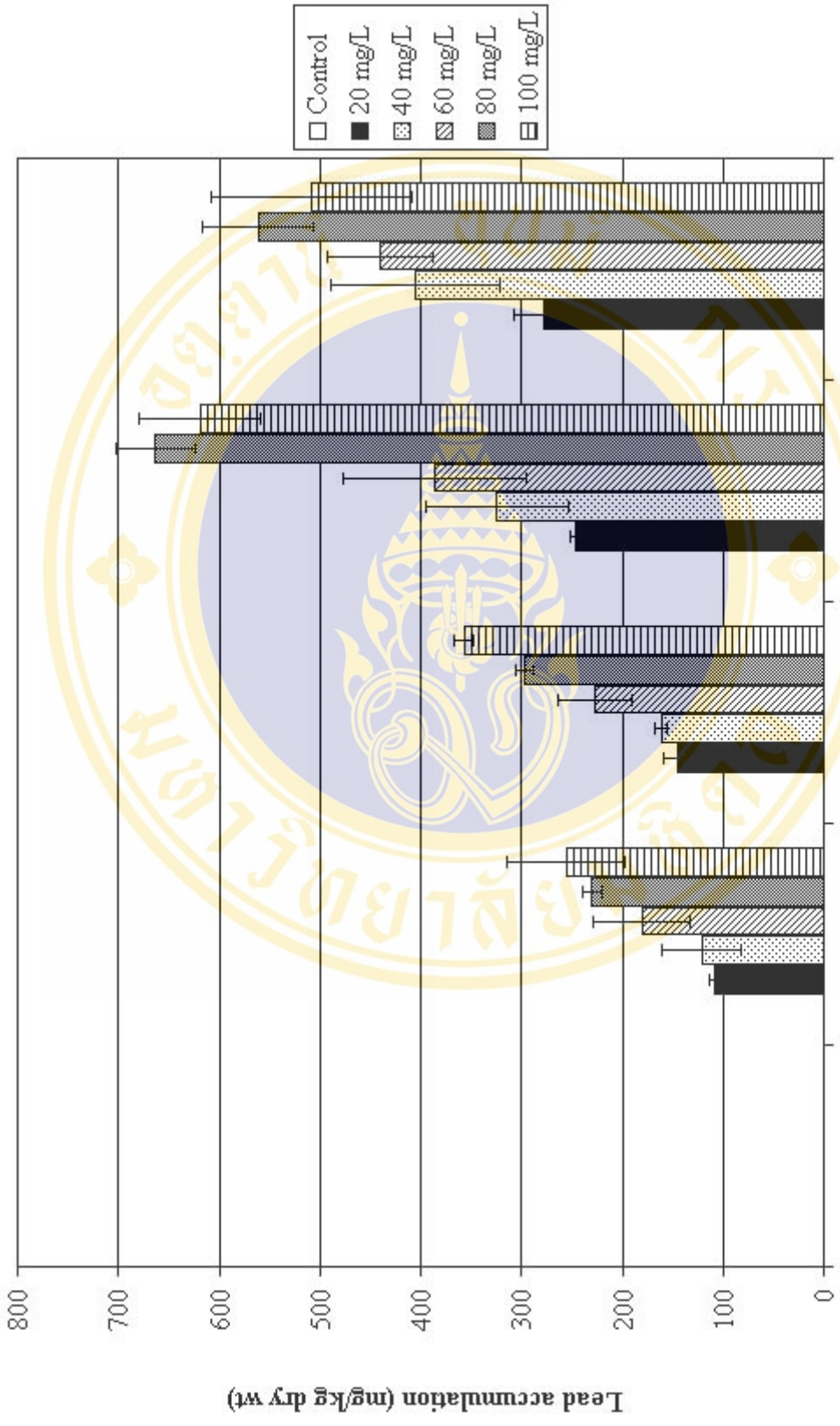

Figure 5-11. Accumulation of lead in shoots of *H. umbellata*.

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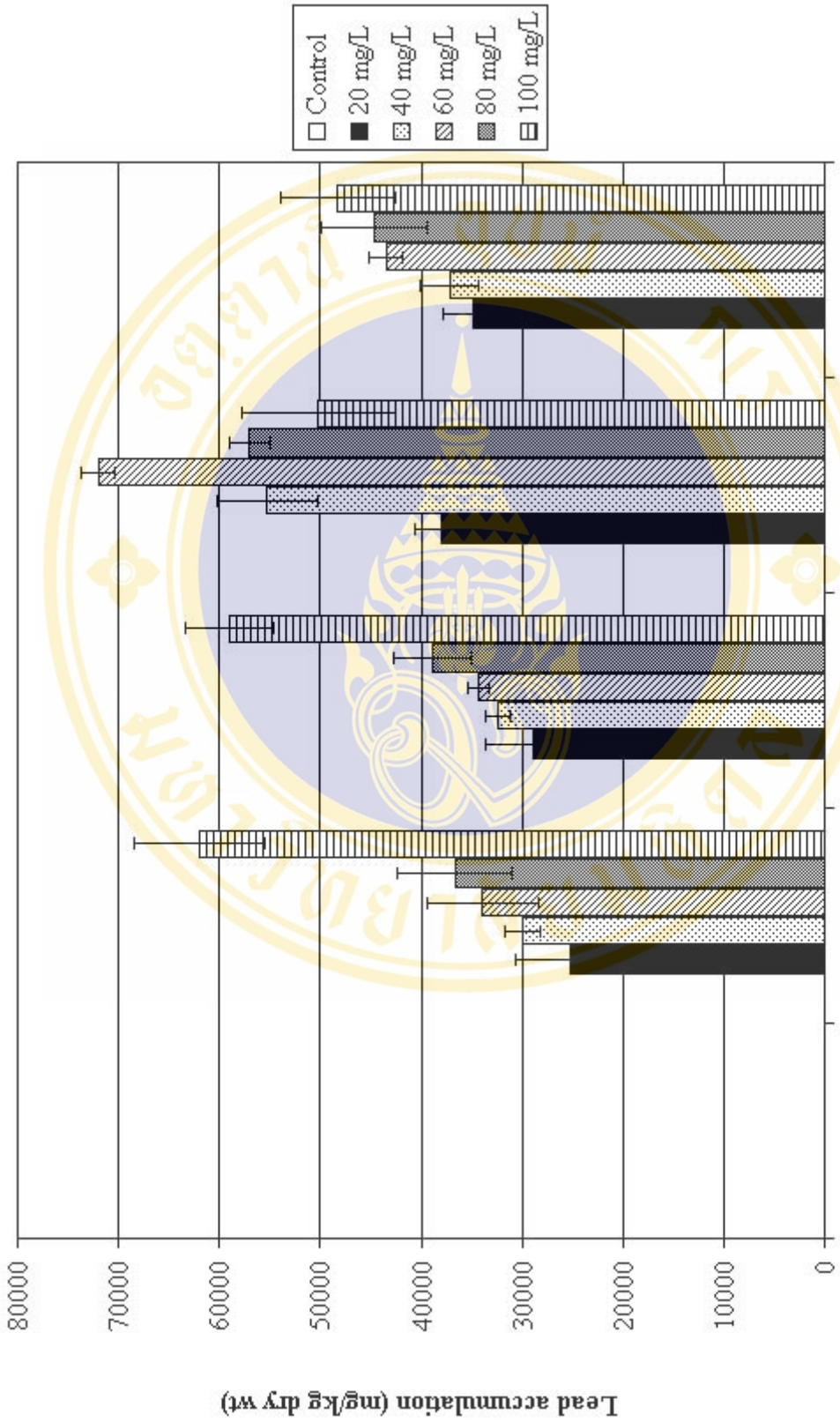

Figure 5-12. Accumulation of lead in roots of *H. umbellata*.

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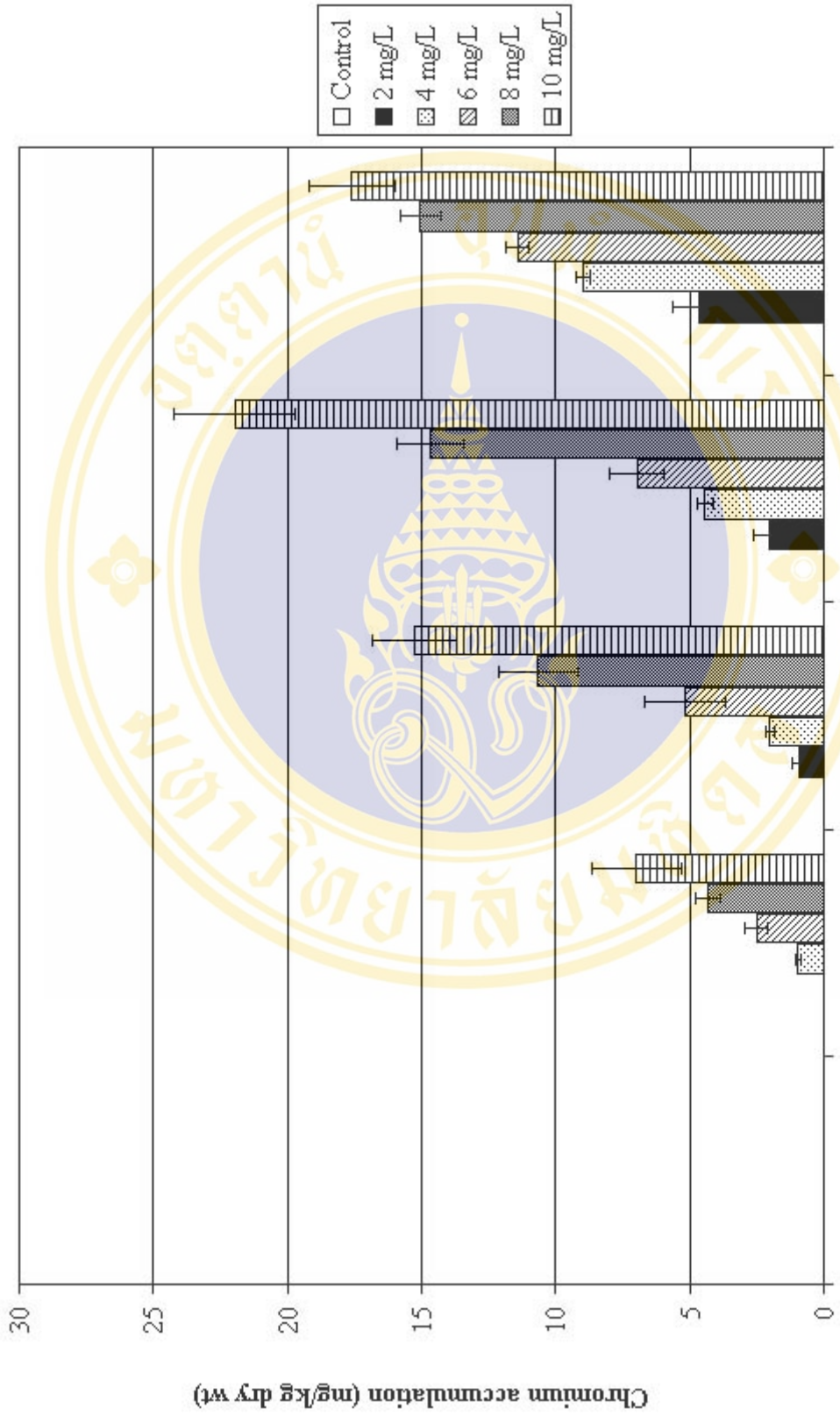

Figure 5-13. Accumulation of chromium in shoots of *H. umbellata*.

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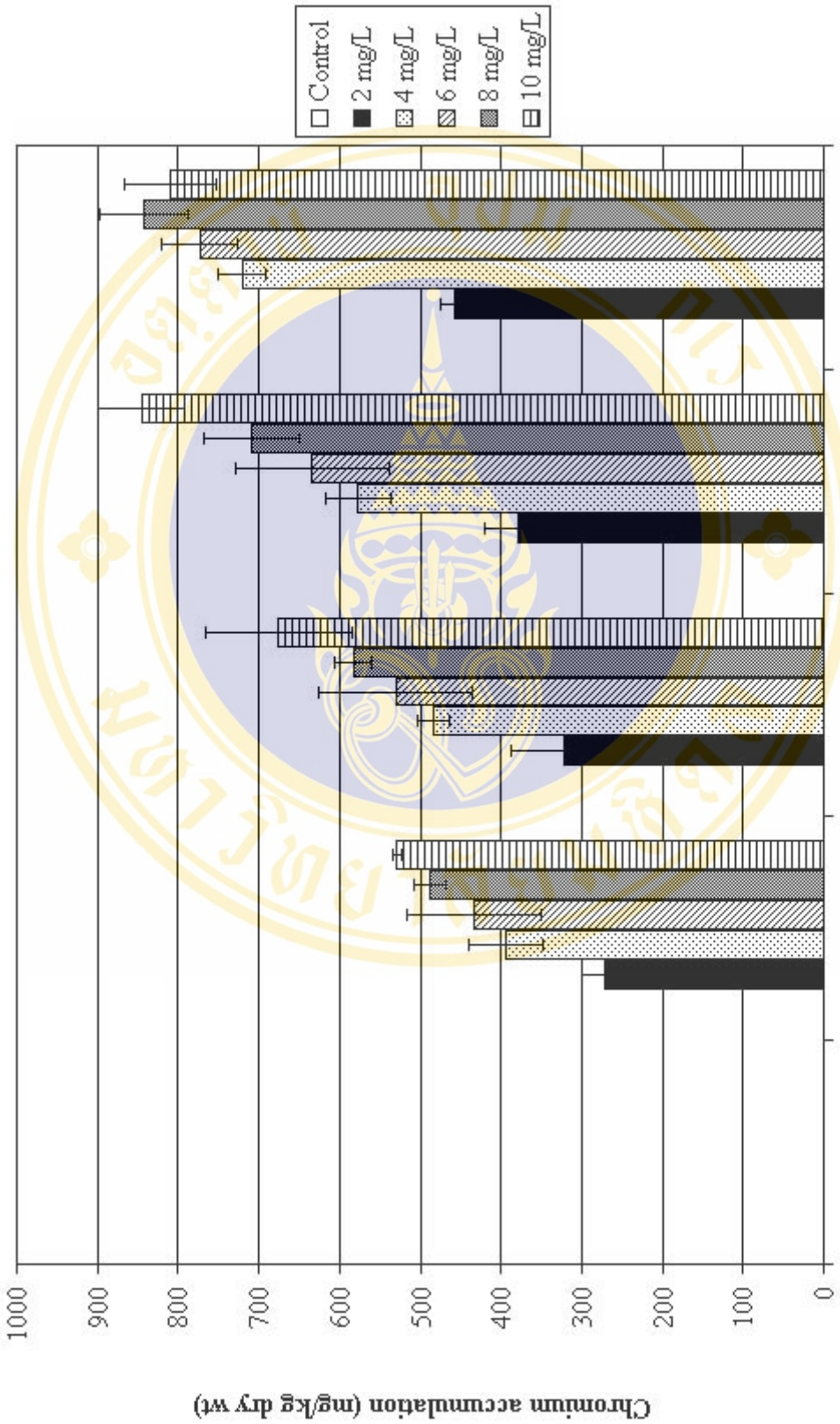

Figure 5-14. Accumulation of chromium in roots of *H. umbellata*.

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5.7 Average accumulation of heavy metal by *H. umbellata*

The average accumulations of lead and chromium in shoots and roots of *H. umbellata* in the control were zero. In general, the average accumulations of lead and chromium in roots were higher than those in shoots.

The average accumulations of lead in shoots of *H. umbellata* are shown in Table 5-20 and Figure 5-15. They were rapidly decreased on the first six days at all lead concentrations. But, they were increased again on day 9 at 40, 60, 80, and 100 mg Pb/L treatments especially at 80 mg Pb/L. Finally, on day 12, they were decreased. At the lowest lead concentration (20 mg Pb/L), the average accumulations of lead were 36.23 and 23.18 mg/kg dry wt.day after 3 and 12 days, respectively. At the highest lead concentration (100 mg Pb/L), the average accumulations of lead were 85.28 and 42.32 mg/kg dry wt.day after 3 and 12 days, respectively.

The average accumulations of lead in roots of *H. umbellata* are shown in Table 5-21 and Figure 5-16. They were rapidly decreased on the first six days and then slowly decreased until day 12 except at 20, 40 and 60 mg Pb/L, these values were slightly increased on day 9. At the lowest lead concentration (20 mg Pb/L), the average accumulations of lead were 8,422.39 and 2,896.96 mg/kg dry wt.day after 3 and 12 days, respectively. At the highest lead concentration (100 mg Pb/L), the average accumulations of lead were 20,654.78 and 4,020.77 mg/kg dry wt.day after 3 and 12 days, respectively.

The average accumulations of chromium in shoots of *H. umbellata* are shown in Table 5-22 and Figure 5-17. They were slowly increased on the first six days and then slowly decreased after day 6 until day 12. At the lowest chromium concentration (2 mg Cr/L), the average accumulations of chromium were zero and 0.39 mg/kg dry wt.day after 3 and 12 days, respectively. At the highest chromium concentration (10 mg Cr/L), the average accumulations of chromium were 2.33 and 1.47 mg/kg dry wt.day after 3 and 12 days, respectively.

The average accumulations of chromium in roots of *H. umbellata* are shown in Table 5-23 and Figure 5-18. They were rapidly decreased on the first six days and then slowly decreased on day 9 and 12. At the lowest chromium concentration (2 mg Cr/L), the average accumulations of chromium were 90.30 and 38.06 mg/kg dry wt.day after 3 and

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12 days, respectively. At the highest chromium concentration (10 mg Cr/L), the average accumulations of chromium were 176.26 and 67.51 mg/kg dry wt.day after 3 and 12 days, respectively.

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Table 5-20. Average accumulation of lead in shoots of *H. umbellata.*

Lead	Average accumulation (mg/kg dry wt.day)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20	0.00±0.00	36.23±4.63	24.18±0.93	27.32±0.74	23.18±2.46
40	0.00±0.00	40.40±2.19	26.89±6.52	36.03±7.92	33.80±6.94
60	0.00±0.00	60.24±12.25	37.90±8.11	42.83±10.07	36.67±4.34
80	0.00±0.00	76.65±16.67	49.93±1.53	73.67±10.35	46.79±4.61
100	0.00±0.00	85.28±3.26	59.32±9.86	68.71±6.69	42.32±5.11

Each value is the mean of triplicate± S.D.

Table 5-21. Average accumulation of lead in roots of *H. umbellata*.

Lead	Average accumulation (mg/kg dry wt.day)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20	0.00±0.00	8422.39±1811.19	4801.34±800.82	4228.31±284.08	2896.96±254.68
40	0.00±0.00	9992.42±585.35	5395.30±197.02	6136.72±559.29	3099.05±246.26
60	0.00±0.00	11302.32±1841.97	5722.67±167.92	7999.84±187.51	3623.25±139.31
80	0.00±0.00	12221.16±1913.57	6474.99±643.35	6335.21±218.76	3726.43±437.47
100	0.00±0.00	20654.78±2141.95	9833.21±725.33	5577.20±841.08	4020.77±471.82

Each value is the mean of triplicate± S.D.

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Table 5-22. Average accumulation of chromium in shoots of *H. umbellata*.

Chromium	Average accumulation (mg/kg dry wt.day)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.15±0.04	0.23±0.06	0.39±0.01
4	0.00±0.00	0.32±0.11	0.48±0.03	0.46±0.03	0.68±0.02
6	0.00±0.00	0.84±0.15	0.87±0.25	0.77±0.11	0.73±0.03
8	0.00±0.00	1.44±0.15	1.78±0.14	1.63±0.14	1.25±0.06
10	0.00±0.00	2.33±0.56	2.54±0.25	2.44±0.25	1.47±0.13

Each value is the mean of triplicate± S.D.

Table 5-23. Average accumulation of chromium in roots of *H. umbellata*.

Chromium	Average accumulation (mg/kg dry wt.day)				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	90.30±9.67	53.60±1.07	42.02±4.61	38.06±1.44
4	0.00±0.00	131.15±15.60	80.50±3.37	64.08±4.35	60.05±2.41
6	0.00±0.00	144.62±27.97	88.21±15.92	70.40±10.63	61.35±4.32
8	0.00±0.00	162.54±5.06	97.43±3.82	78.80±6.56	70.16±4.52
10	0.00±0.00	176.26±1.66	112.53±15.17	93.97±5.94	67.51±4.78

Each value is the mean of triplicate± S.D.

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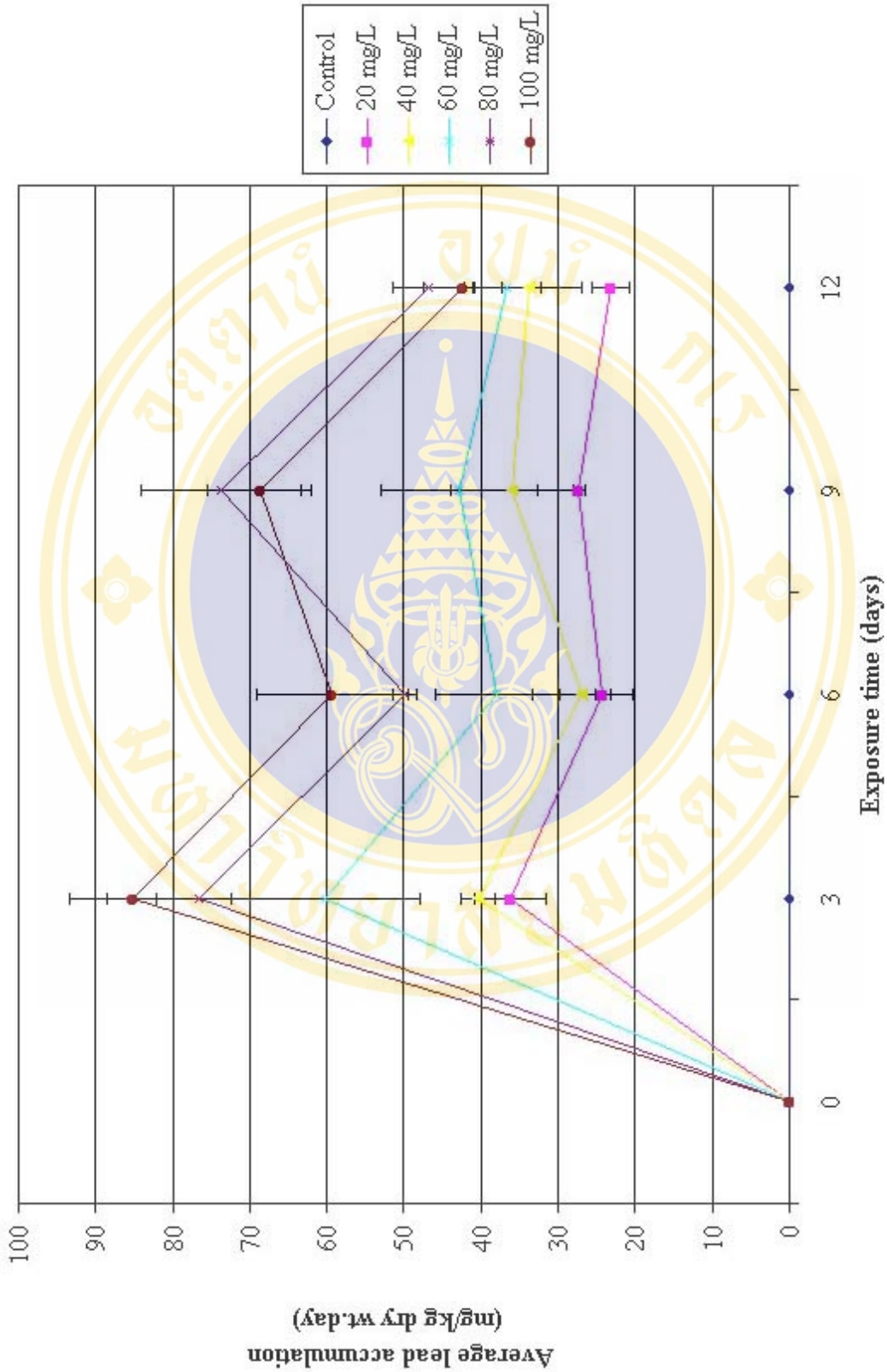

Figure 5-15. Average accumulation of lead in shoots of *H. umbellata*.

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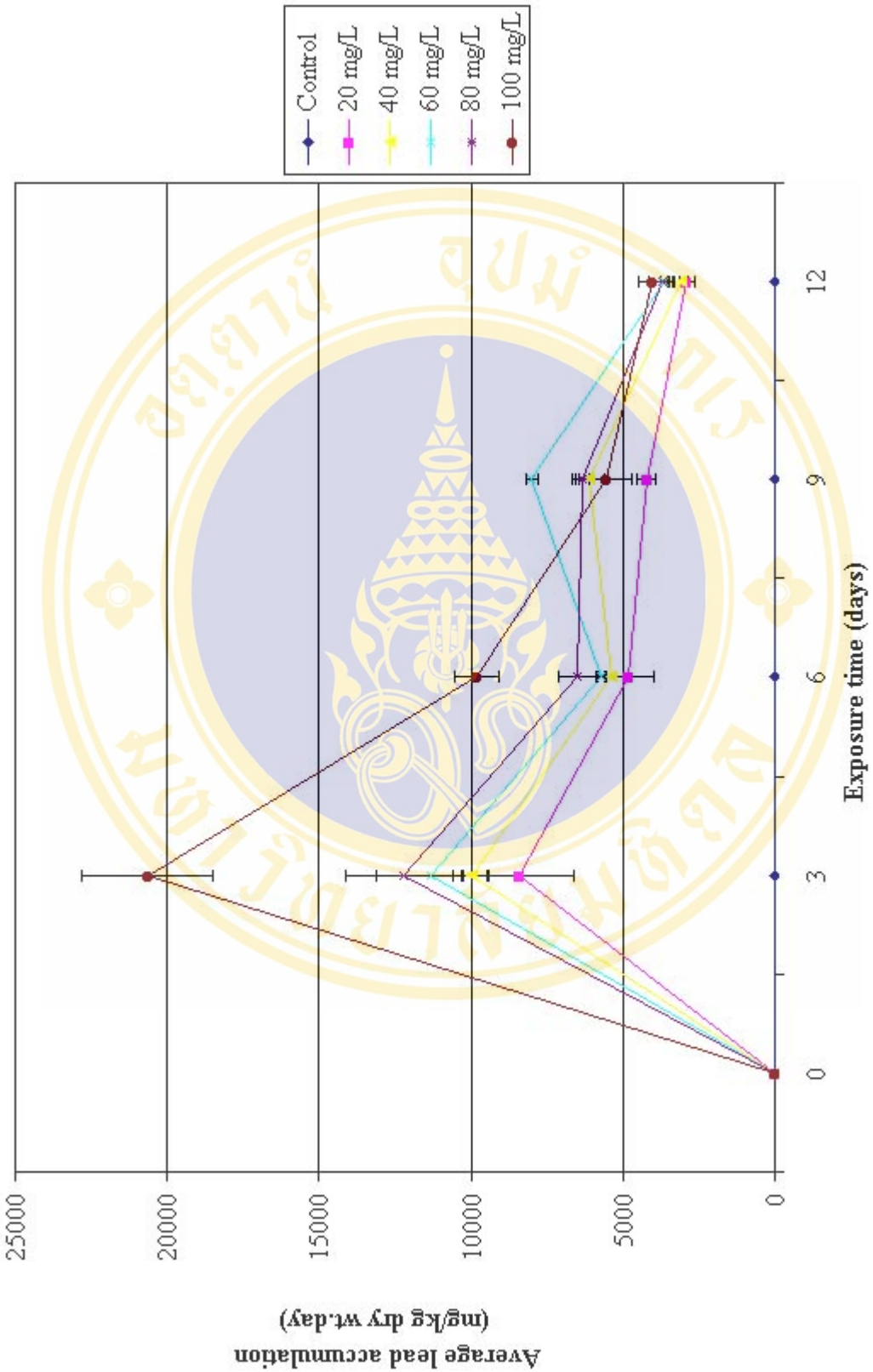

Figure 5-16. Average accumulation of lead in roots of *H. umbellata*.

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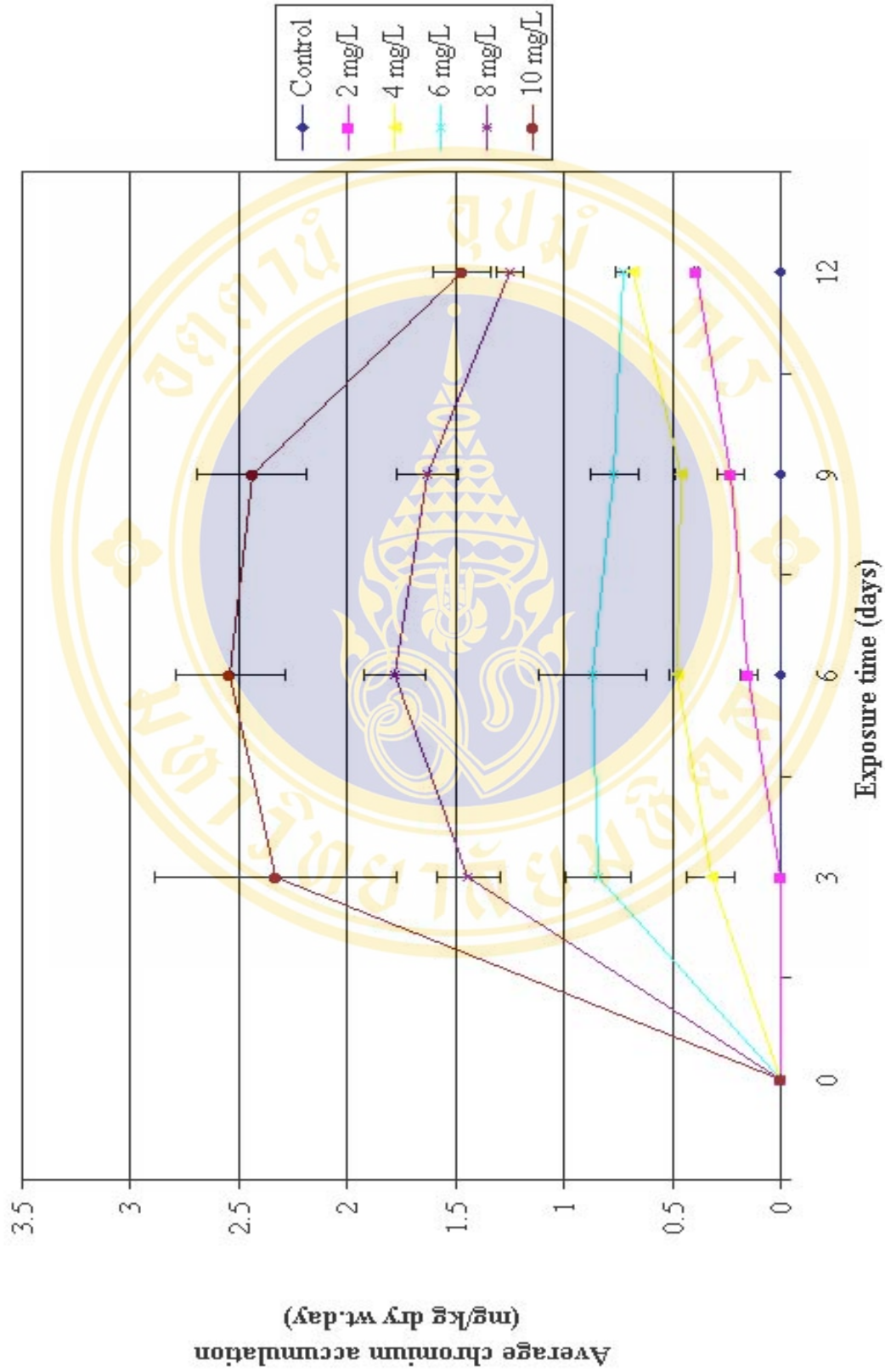

Figure 5-17. Average accumulation of chromium in shoots of *H. umbellata*.

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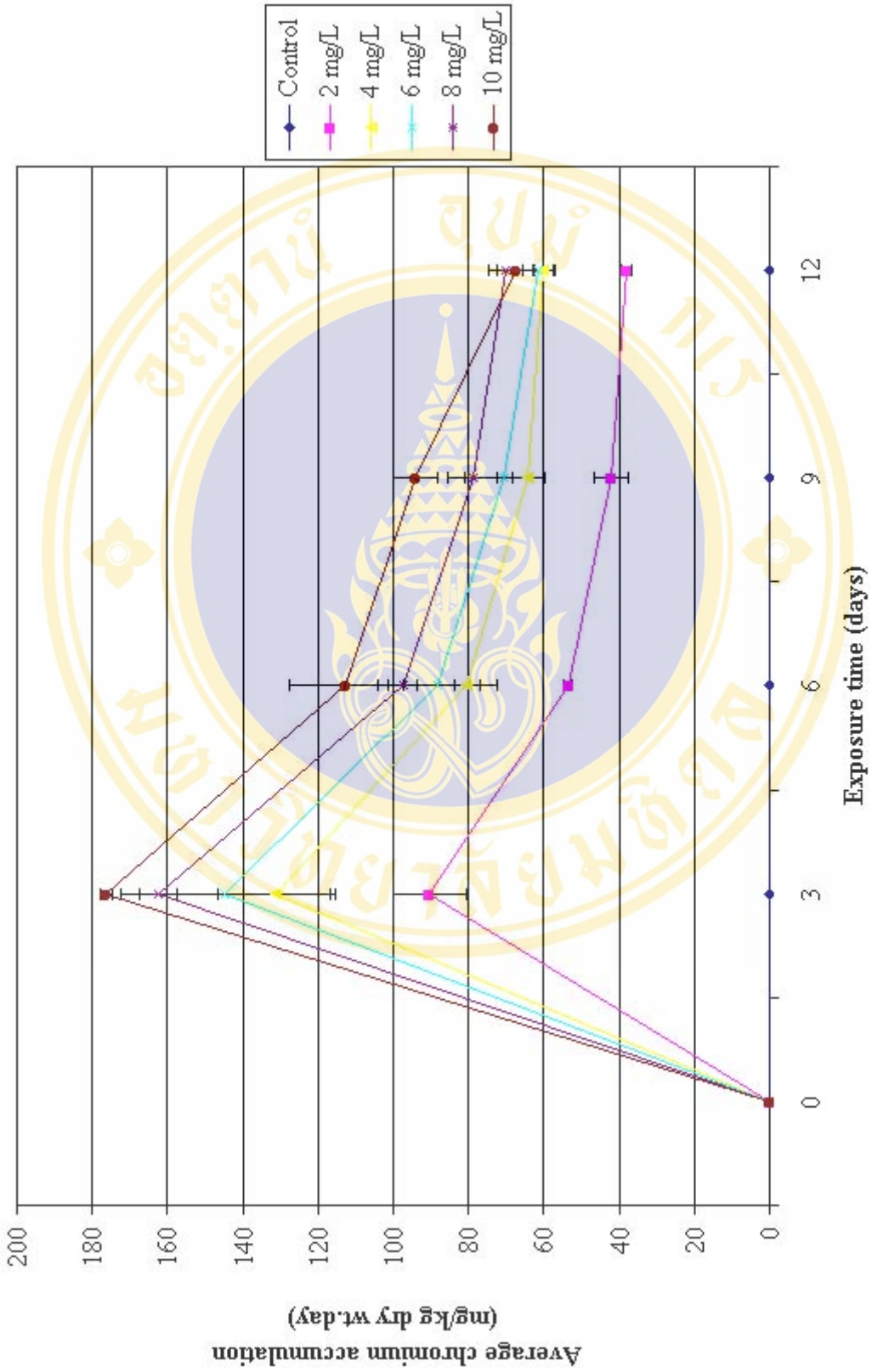

Figure 5-18. Average accumulation of chromium in roots of *H. umbellata*.

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5.8 Bioconcentration factor (BCF) of *H. umbellata*

The BCF values of lead and chromium in shoots and roots of *H. umbellata* in the control were zero. In general, the BCF values in roots were higher than those in shoots.

The BCFs of lead in shoots of *H. umbellata* are shown in Table 5-24 and Figure 5-19. The BCFs of lead in shoots were significantly decreased (P≤0.05) from that of 20 mg Pb/L when lead concentration was increased at each exposure time but they were significantly increased (P≤0.05) when the exposure time was increased at each lead concentration. The BCFs at 20, 40, 60, 80, and 100 mg Pb/L were 13.91, 10.14, 7.33, 7.02, and 5.08, respectively on day12.

The BCFs of lead in roots of *H. umbellata* are shown in Table 5-25 and Figure 5-20. The BCFs of lead in roots were significantly decreased (P≤0.05) when lead concentration were increased, except at 100 mg Pb/L at each exposure time, they were significantly increased (P≤0.05) when the exposure time was increased at each lead concentration. However, the results showed that the BCF value at 100 mg Pb/L was more than that at 80 mg Pb/L on day 3 and 6. On day 9, the BCF value was higher than that on day 12 at each concentration. The BCFs at 20, 40, 60, 80, and 100 mg Pb/L were 1,738.17, 929.71, 724.65, 558.96, and 482.49, respectively on day12.

The BCFs of chromium in shoots of *H. umbellata* are shown in Table 5-26 and Figure 5-21. The BCFs of lead in shoots were significantly increased (P≤0.05) when chromium concentration and exposure time were increased. However, the results showed that the BCF values significantly decreased (P≤0.05) when chromium concentration was increased on day 12. The BCFs at 2, 4, 6, 8, and 10 mg Cr/L were 2.34, 2.04, 1.46, 1.88, and 1.76, respectively on day 12.

The BCFs of chromium in roots of *H. umbellata* are shown in Table 5-25 and Figure 5-22. The BCFs of chromium in roots were significantly decreased (P≤0.05) when chromium concentrations in feed solutions were increased at each exposure time, indicating that the ability of *H. umbellata* to accumulate chromium was decreased when chromium concentration was increased. The BCFs at 2, 4, 6, 8, and 10 mg Cr/L were 228.34, 180.15, 122.71, 105.23, and 81.02, respectively on day 12.

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The BCF of lead was higher than that of chromium at the same duration, suggesting that the accumulation potential of lead is higher than that of chromium in *H. umbellata.*

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Table 5-24. Bioconcentration factor (BCF) of lead in shoots of *H. umbellata*.

Lead	Bioconcentration factor				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20	0.00±0.00	5.43± 0.69	7.25± 0.28	12.29± 0.99	13.91± 1.48
40	0.00±0.00	3.03± 0.16	4.03± 0.98	8.11± 1.78	10.14± 2.08
60	0.00±0.00	3.01± 0.61	3.79± 0.81	6.42± 1.51	7.33± 0.87
80	0.00±0.00	2.87± 0.63	3.71± 0.11	8.29± 1.16	7.02± 0.69
100	0.00±0.00	2.56± 0.10	3.56± 0.59	6.18± 0.64	5.08± 1.00

Each value is the mean of triplicate± S.D.

Table 5-25. Bioconcentration factor (BCF) of lead in roots of *H. umbellata*.

Lead	Bioconcentration factor				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20	0.00±0.00	1263.36±271.68	1440.40±240.23	1902.74±127.83	1738.17±152.81
40	0.00±0.00	749.43±73.9	809.29±29.55	1380.76±125.84	929.71±73.88
60	0.00±0.00	565.12±92.10	572.27±16.79	1199.98±28.13	724.65±27.86
80	0.00±0.00	458.29±71.76	485.62±48.25	712.71±24.61	558.96±65.96
100	0.00±0.00	619.64±64.26	589.99±43.52	501.95±75.70	482.49±56.62

Each value is the mean of triplicate± S.D.

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Table 5-26. Bioconcentration factor (BCF) of chromium in shoots of *H. umbellata*.

Chromium	Bioconcentration factor				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.45±0.13	1.03±0.28	2.34±0.00
4	0.00±0.00	0.24±0.03	0.50±0.05	1.11±0.07	2.07±0.06
6	0.00±0.00	0.42±0.06	0.87±0.25	1.16±0.17	1.90±0.07
8	0.00±0.00	0.54±0.07	1.33±0.18	1.83±0.16	1.88±0.10
10	0.00±0.00	0.70±0.17	1.53±0.16	2.20±0.23	1.76±0.16

Each value is the mean of triplicate± S.D.

Table 5-27. Bioconcentration factor (BCF) of chromium in roots of *H. umbellata*.

Chromium	Bioconcentration factor				
(mg/L)	Day 0	Day 3	Day 6	Day 9	Day 12
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	135.44±14.64	160.79±33.22	189.28±20.74	228.34±8.16
4	0.00±0.00	98.36±11.70	120.75±5.05	144.17±9.88	180.15±7.23
6	0.00±0.00	72.31±13.99	88.21±15.92	105.60±15.94	122.71±8.65
8	0.00±0.00	60.95±1.90	73.08±2.87	88.74±7.38	105.23±18.26
10	0.00±0.00	52.88±0.50	67.52±9.10	84.57±5.35	81.02±6.78

Each value is the mean of triplicate± S.D.

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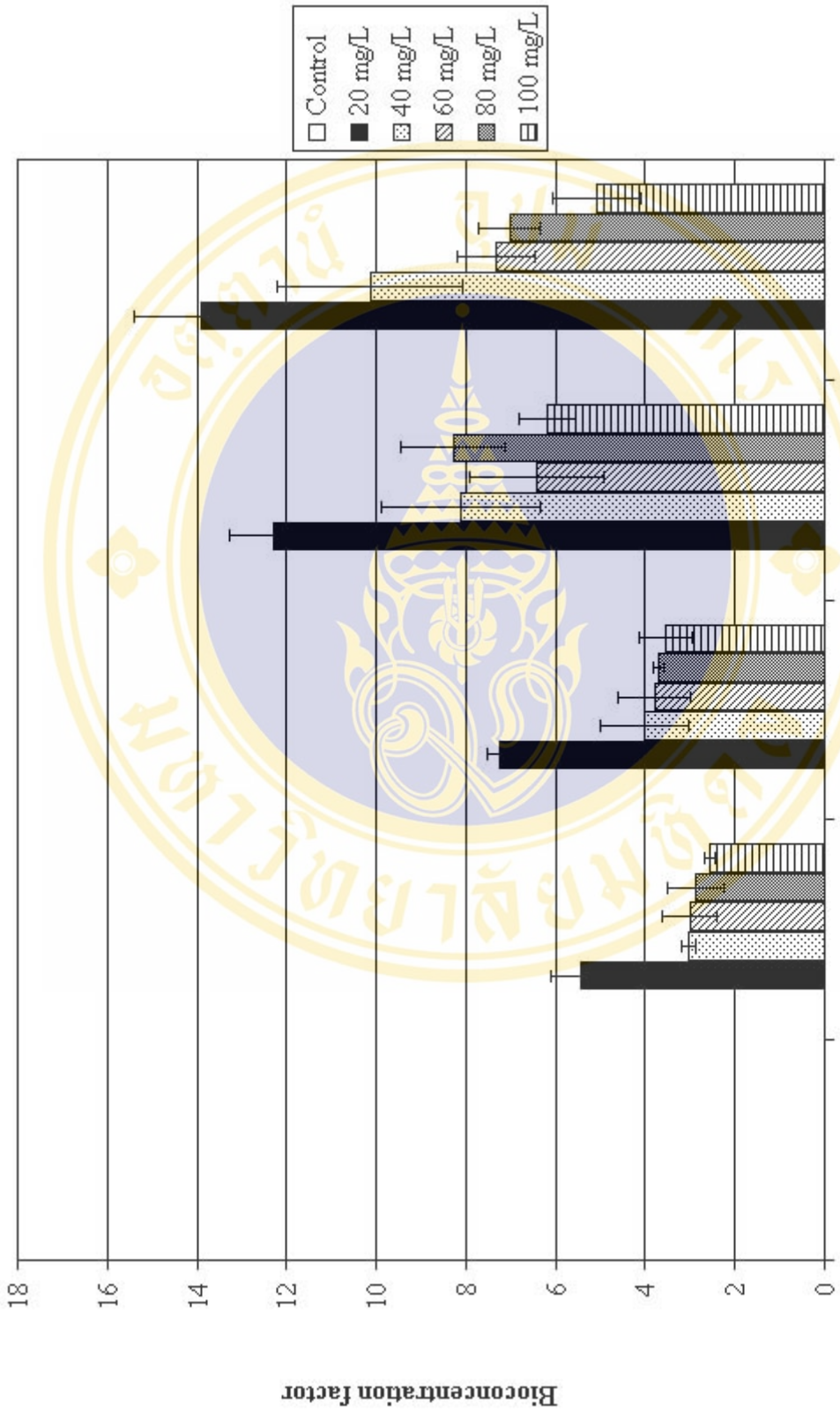

Figure 5-19. Bioconcentration factor (BCF) of lead in shoots of *H. umbellata*.

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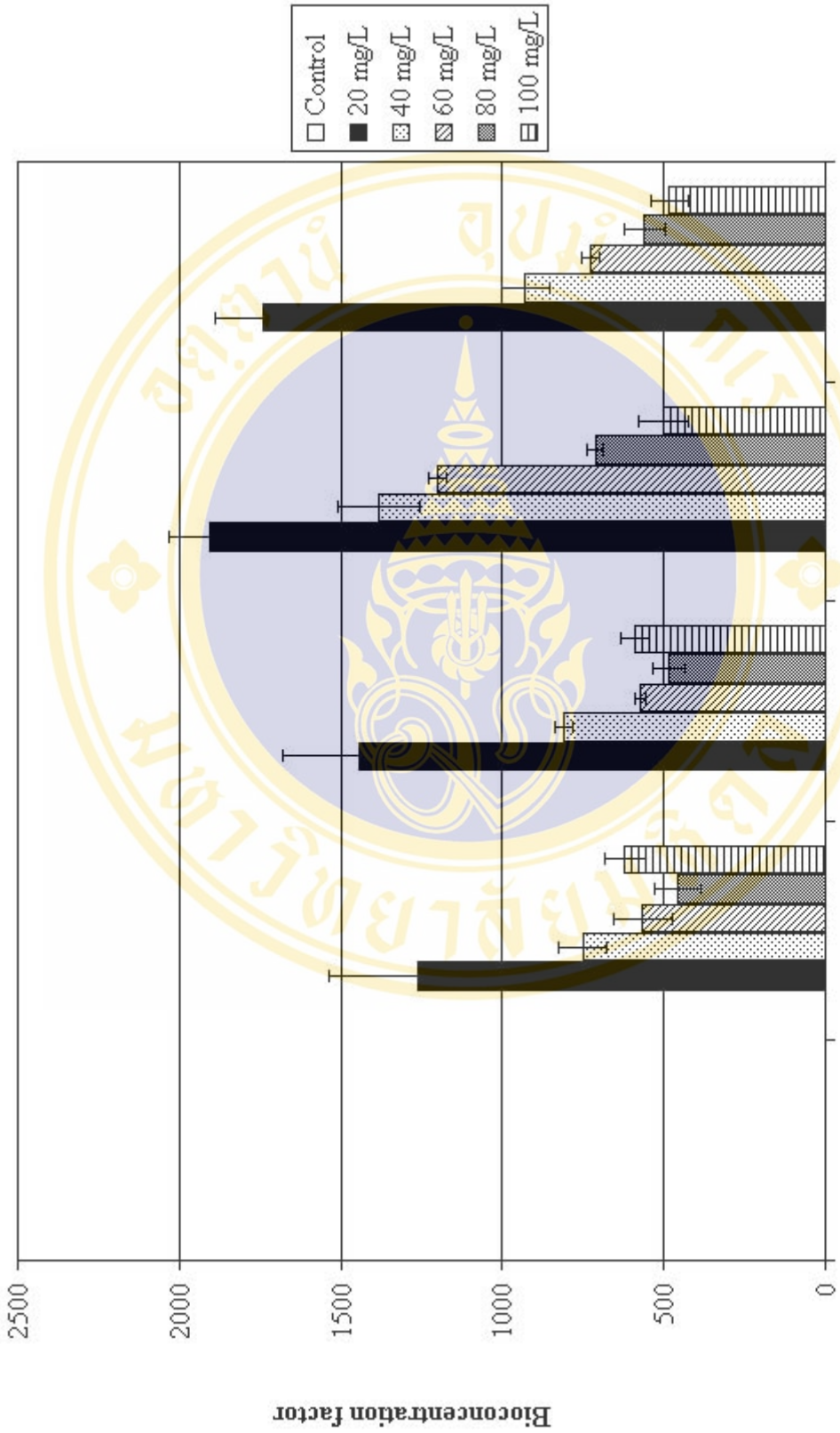

Figure 5-20. Bioconcentration factor (BCF) of lead in roots of *H. umbellata*.

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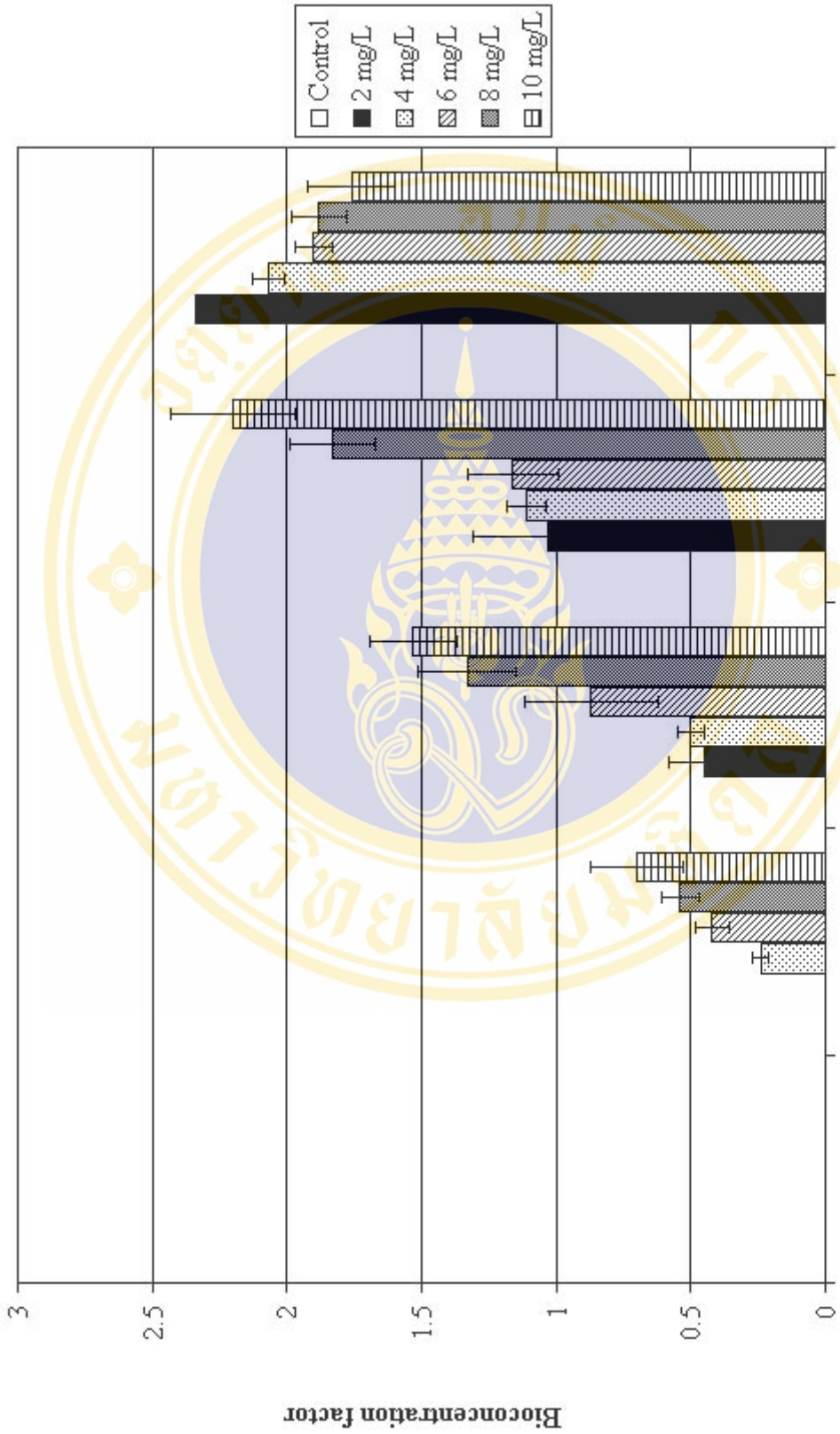

Figure 5-21. Bioconcentration factor (BCF) of chromium in shoots of *H. umbellata*.

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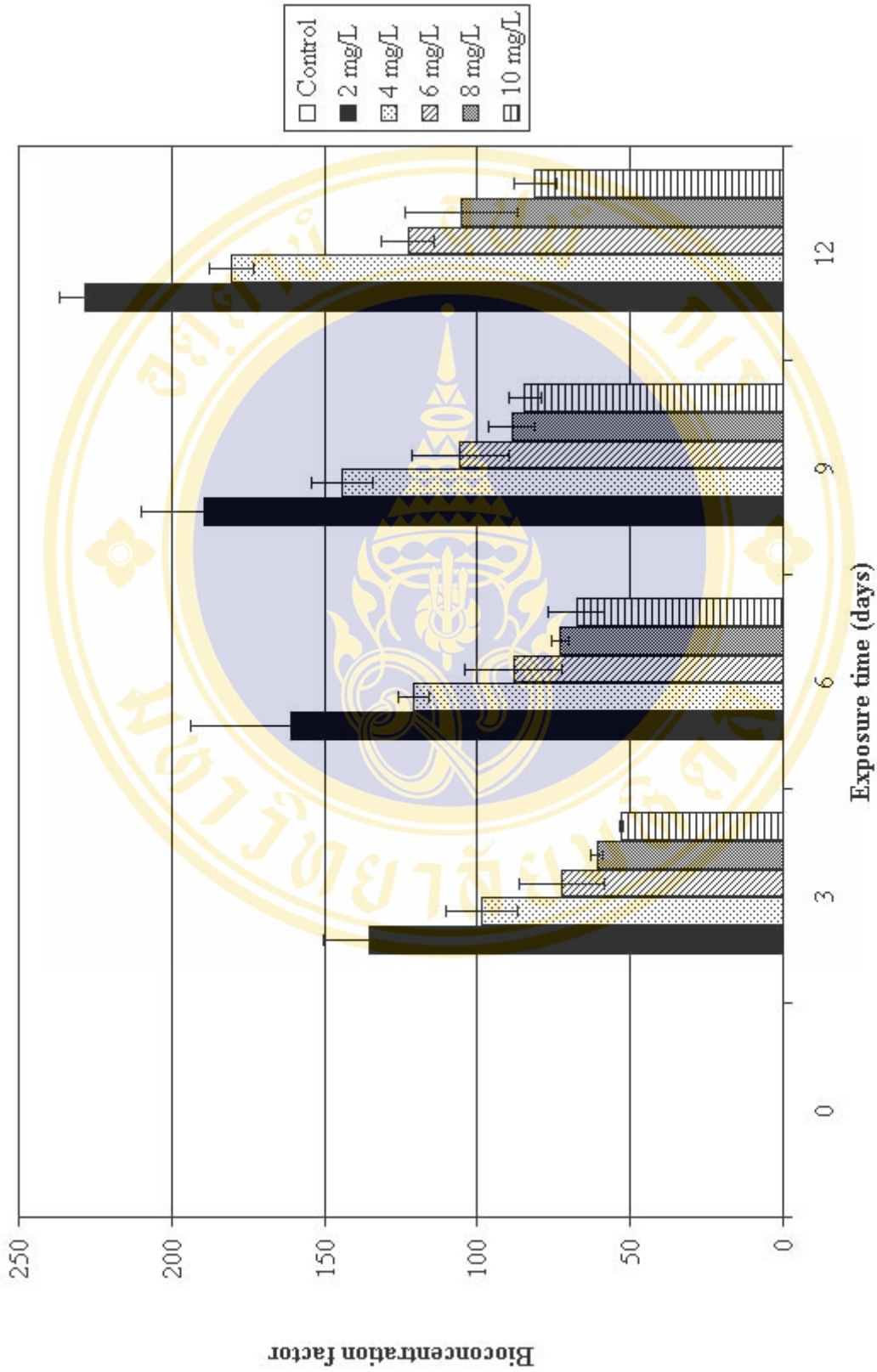

Figure 5-22. Bioconcentration factor (BCF) of chromium in roots of *H. umbellata*.

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5.9 Toxicity symptoms

5.9.1 New growth

The effect of lead on the numbers of plantlets of *H. umbellata*, exposed to lead at different concentrations and exposure times are shown in Table 5-28. They were significantly decreased ($P \leq 0.05$) from that of control when lead concentration and exposure time were increased. The highest number of plantlets (14) was observed in the control on day 12 and the lowest (zero) was observed in 40 and 100 mg Pb/L treatments on day 3.

The effect of chromium on the numbers of plantlets of *H. umbellata*, exposed to chromium at different concentrations and exposure times are shown in Table 5-28. They were significantly decreased ($P \leq 0.05$) from that of control when chromium concentration and exposure time were increased. However, at 2 mg Cr/L, the result showed a stimulation of new growth on day 3, 6 and 9. The highest number of plantlets (26) was observed in 4 mg Cr/L treatment on day 12 and the lowest (zero) was found in 10 mg Cr/L treatment on day 3.

However, the plantlets in control were larger in size than those of metal treatments.

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Table 5-28. Effects of lead on production of plantlets of *H. umbellata.*

Lead (mg/L)	Number of plantlets			
	Day 3	Day 6	Day 9	Day 12
Control	2	10	13	14
20	1	6	9	9
40	-	1	8	7
60	2	1	5	4
80	1	1	5	5
100	-	1	5	7

Table 5-29. Effects of chromium on production of plantlet of *H. umbellata.*

Chromium (mg/L)	Number of plantlets			
	Day 3	Day 6	Day 9	Day 12
Control	3	9	21	25
2	4	12	16	20
4	1	3	3	26
6	1	3	3	24
8	1	4	3	20
10	-	1	2	14

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5.9.2 Chlorosis

The chlorosis of *H. umbellata* leaves exposed to 100 mg Pb/L at different exposure times is shown in Figure 5-23. Control plants and plants exposed to 20 mg Pb/L were healthy throughout the experiment. On day 3, lead had no visible effects on plants at all concentrations. On day 6, the first chlorosis appeared in 100 mg Pb/L treatment (Figure 5-23D). On day 9, chlorosis appeared is 80 and 100 mg Pb/L treatments and it was more severe in 100 mg Pb/L treatment (Figure 5-23E). On day 12, slight chlorosis was found in 40 mg Pb/L treatment. Complete chlorosis, necrosis and withering of petiole were found in 100 mg Pb/L treatment (Figure 5-23F).

Toxicity symptom of roots treated with lead is shown in Figure 5-24. When the lead concentration and exposure time were increased, the root color was changed from light green to dark brown.

The necrosis of *H. umbellata* leaves exposed to 10 mg Cr/L at different exposure times are shown in Figure 5-25. Control plants and plants exposed to 2, 4, 6, 8 mg Cr/L were healthy throughout the experiment (Figures 5-25A to 5-25E). Complete necrosis and withering of petiole were found in 10 mg Cr/L treatment (Figure 5-25F).

Toxicity symptom of roots treated with chromium is shown in Figure 5-26. It was similar to that with lead. When the chromium concentration and exposure time were increased, the root color was changed from light green to dark brown.

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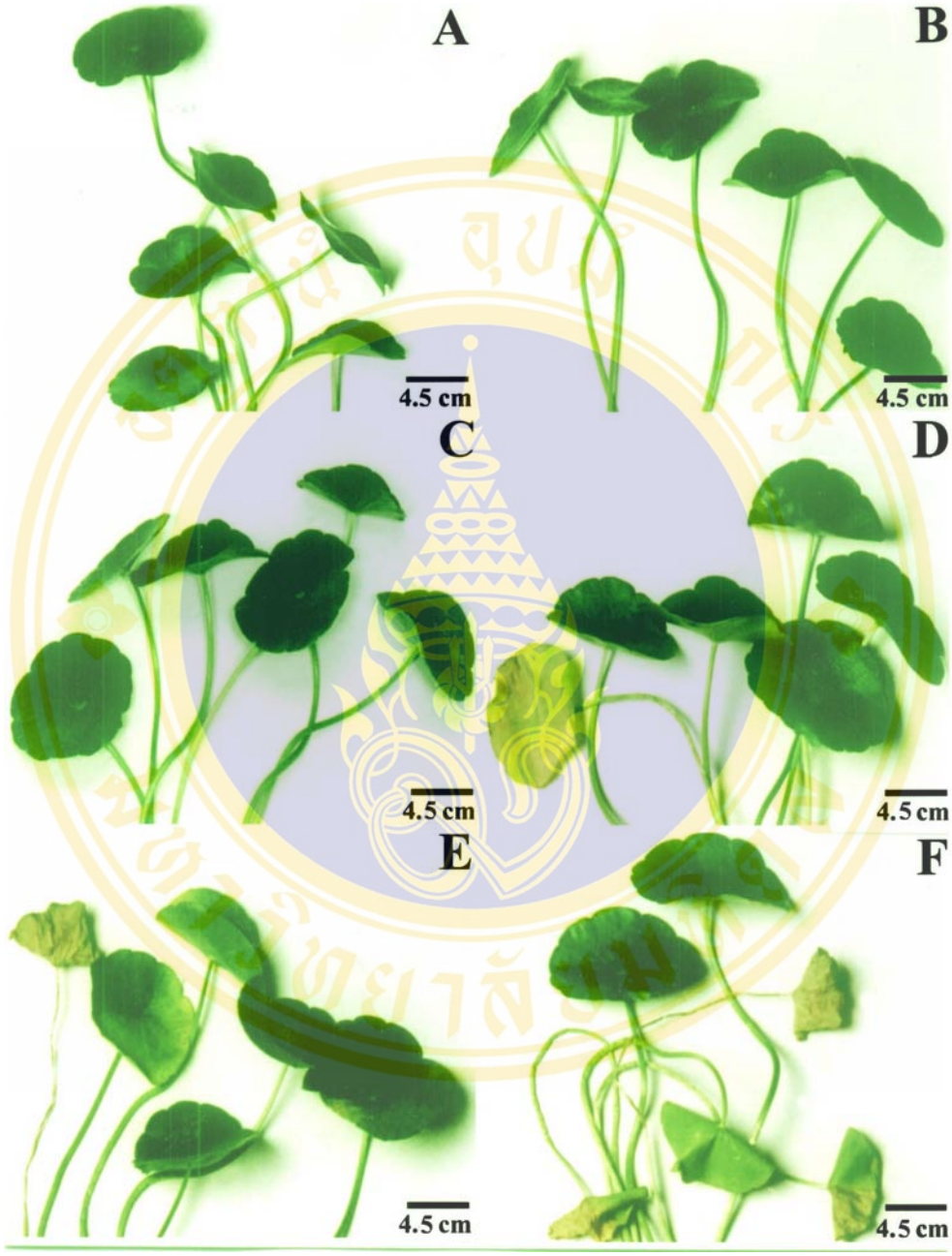

Figure 5-23. Shoots of *H. umbellata* treated with 100 mg Pb/L at different exposure times: Control (A), 0 day (B), 3 days (C), 6 days (D), 9 days (E), and 12 days (F).

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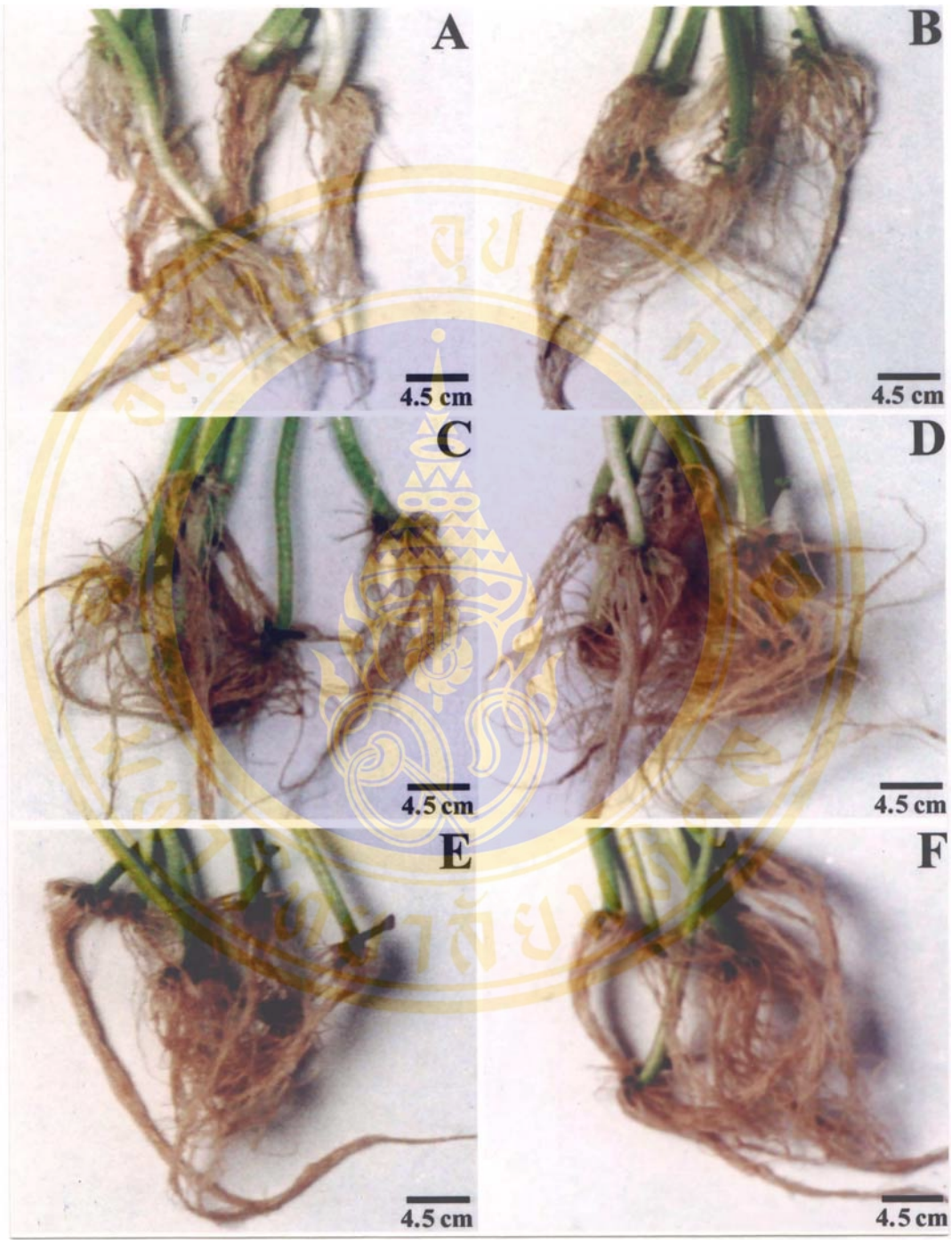

Figure 5-24. Roots of *H. umbellata* treated with 100 mg Pb/L at different exposure times: Control (A), 0 day (B), 3 days (C), 6 days (D), 9 days (E), and 12 days (F).

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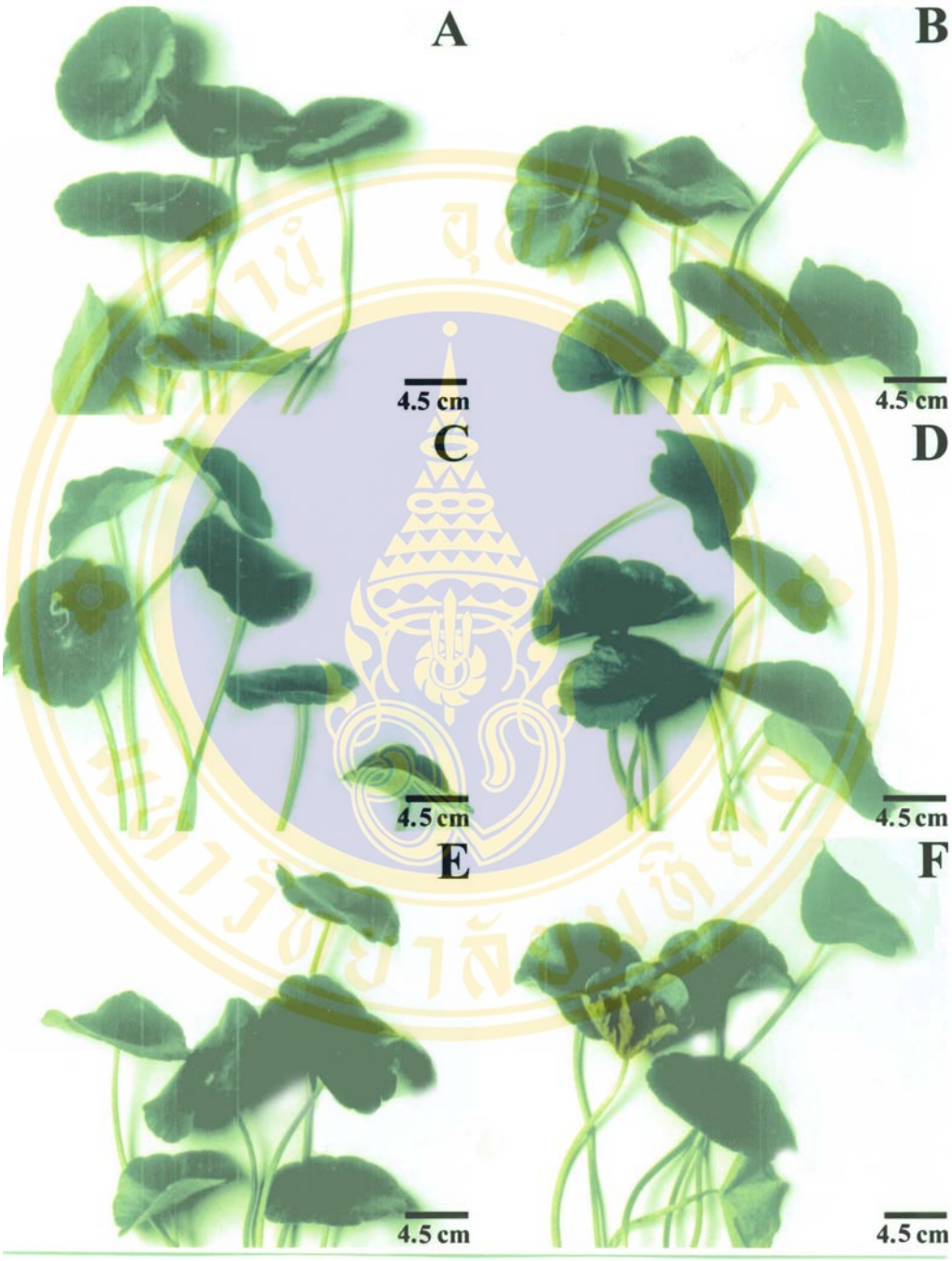

Figure 5-25. Shoots of *H. umbellata* treated with 10 mg Cr/L at different exposure times: Control (A), 0 day (B), 3 days (C), 6 days (D), 9 days (E), and 12 days (F).

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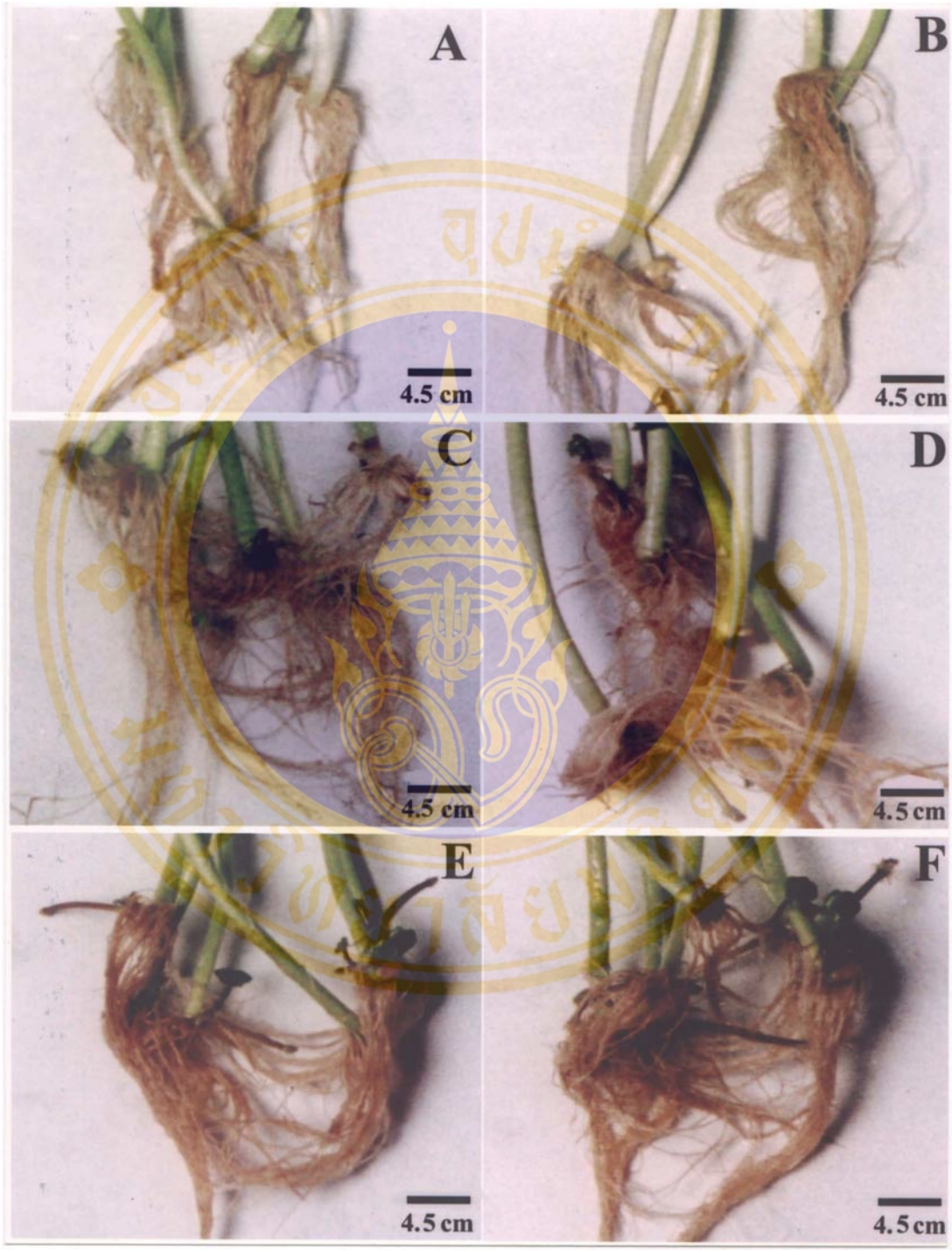

Figure 5-26. Roots of *H. umbellata* treated with 10 mg Cr/L at different exposure times: Control (A), 0 day (B), 3 days (C), 6 days (D), 9 days (E), and 12 days (F).

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CHAPTER VI
DISSCUSSION

6.1 Effects of heavy metals on relative growth and biomass productivity

In the present investigation, it is clear that the relative growth and biomass productivity of *Hydrocotyle umbellata* significantly decreased when the exposure time and heavy metals concentration were increased. A decrease in relative growth and biomass productivity might be attributed to a disruption of nitrogen metabolism, which is directly related to yields potential of the plants. Since nitrogen is one of the primary essential nutrients involved as a constituent of biomolecules such as nucleic acids, nitrogenous bases, coenzyme, and proteins, any deviation in these constituents would severely inhibit the growth and yield of plants (Chatterjee and Chatterjee, 2000). Both lead and chromium can disrupt nitrogen metabolism by reduced nitrate reductase activity (Gupta and Chandra, 1994; Vajpayee *et al*., 2000) as it is considered to be limiting factor for growth, development and protein content of plants (Solomonson and Barber, 1990) and sensitive to metals toxicity (Singh *et al*., 1997) and thus factors affecting this enzyme may influence plant growth, too (Srivastava, 1980). The inhibition of nitrate reductase in the presence of lead may be due to:

1) The reduced supply of NADH due to its oxidation by heavy metal or its reduced production due to swelling of the mitochondria (Gangenbach *et al*., 1993).
2) The possible direct effect of heavy metals on the enzyme as the metals has a high binding efficiency with –SH groups (Prasad and Prasad, 1987).
3) The lesser NO_3 supply to the site of the enzyme synthesis because lead treatment can create water stress to the plants and reduced synthesis of protein due to Pb^{2+} supply (Singh *et al*., 1997).

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Moreover, the increased peroxidase activity might be linked to a decline in growth rate and biomass as found in plants treated with various metals (Chakmak and Horst, 1991). Peroxidase activity levels have been found to show a significant positive correlation in plant tissue with level of lead. Because peroxidases have various physiological role in plant cells and participate in many reactions including lignification, cross linking of cell wall polysaccharides, oxidation of indole-3-acetic acid, reduction of cell elongation and phenol oxidation (Mocguot *et al*., 1996). Then a stimulation of this enzyme can cause alterations of the cell wall (Gaspar *et al*., 1985), and loss of membrane function (Samantary, 2002), and degradation of indole acetic acid (IAA) (Salin, 1988). Lane *et al*. (1978) indicated that the inhibiting effects of lead on growth might arise from the interference of lead with auxin hormone, which stimulates plant growth and multiplication. The primary cause of inhibition of cell growth arises from a lead -induced stimulation of IAA oxidation (Liu *et al*., 1994).

The formation of free radical species (all forms of active oxygen), which may be initiated directly or indirectly by metals, is one reason to explain the reduced plant growth, because it can cause severe damage to different cell components, particularly biological membranes (Van Asscheand and Clijsters, 1990; Dietz *et al*., 1999).

Chromium has been reported to induce oxidative stress in aquatic plants (Roy *et al*., 1992). Finally, the reason to explain the reduced growth by heavy metals is the nutrient uptake (Nichols *et al*., 2000). Sela *et al*. (1989) reported that Cr(VI) exposure influenced a decrease in the essential nutrients such as Ca, Mg, and K. The Cr(VI) was reported to induce similar decreases in *Azolla caroliniana* Willd growth (Wilson and Al-Hamdani, 1997). Similarly in soybeans treated in nutrient culture containing 0-5 mg Cr^{6+}/L showed decreased uptake of Ca, K, P, Fe, and Mn (WHO, 1988).

However, the results of the present investigation showed a stimulation in relative growth *of H. umbellata* at 4 mg Cr/L on day 3. The positive effect of chromium on plant growth was due to a limited substitution of chromium (VI) for molybdate (Warington, 1946). It was probably due to the reduction of Cr (VI) to Cr (III) which Cr (III) was suggested to be related to an influence on the levels of cytokinin or other plant growth hormones, interaction with nucleic acids or chromium induced increase in the levels of the

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polyamine spermine (Zayed and Terry, 2003). Although lower chromium concentration can stimulate plant growth and yield, which have been observed by several researchers (e.g., Bonet *et al*., 1991; Warington, 1946) but it is not an essential element (Huffman and Alloway, 1973).

6.2 Effect of heavy metals on chlorophyll content

The results of the effect of lead on total chlorophyll content of *H. umbellata* revealed that the total chlorophyll content significantly decreased from that of control at each exposure time except at 20 and 40 mg Pb/L on day 6. This can be explained by the study which showed that in a number of plant species, there was an increase in organic acid exudation as a metal resistance mechanism (Rengel, 1997). An increased in chlorophyll a value was observed in the Cd-treated cultures when they were preincubated with cysteine, glutamate or citrate (Prasad, 1999). At 20, 40, 60, and 80 mg Pb/L on day 6 the total chlorophyll content was higher than that on day 3. This can be explained that the toxicity of lead on total chlorophyll content may take a long time for the total chlorophyll content to be decreased. On the other hand, lower lead concentration is not enough to decrease the total chlorophyll content. However, at high lead concentration and long exposure time, the total chlorophyll content was significantly decreased.

The results of the effect of chromium on total chlorophyll content of *H. umbellata* showed that it was significantly decreased from that of control when chromium concentration were increased at each exposure time. However, at the same chromium concentration of 2, 4, 6, and 8 mg Cr/L the total chlorophyll content was increased when increased the exposure time. This may be due to the fact that at lower chromium concentration the amount of chromium translocated to the shoots is very low which leads to the increase of cytokinin hormone (Zayed and Terry, 2003). This hormone can stimulate the biosynthesis of chlorophyll (Dangswad, 2001), and the lower chromium concentration in the shoots was not enough to became toxic to chlorophyll content.

The results of the effect of lead and chromium on total chlorophyll content of *H. umbellata* revealed that chlorophyll a, b, and total chlorophyll content significantly decreased when the exposure time and heavy metals concentration were increased. This

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decline in chlorophyll content might be caused by an inhibition of an important enzyme of chlorophyll biosynthesis, δ-aminolevulinic acid dehydrogenase (δ-ALAD) and protochlorophyllide reductase (Ouzounidou, 1995). Vangronsveld and Clijster (1994). also showed a decrease of chlorophyll and a marked enhancement of protochlorophyllide reductase levels in the presence of sublethal concentration of Zn, Cd, and Hg. Two main inhibitory mechanisms of metal action on enzymes can be distinguished: 1) binding of metals to functional groups (such as SH-groups), 2) induction of metal deficiency in metalloenzymes and substitution of the toxic for the essential metal in the enzyme complex (Vangronsveld and Clijster, 1994).

δ-ALAD is a chloroplast enzyme, which is not membrane bound. It catalyses the pathway of porphobilinogen (PBG) synthesis from δ-aminolevulinic acid (ALA) (Prasad and Prasad, 1987). It is a metalloenzyme (Chen and Nelands, 1973) and its activity in plant is dependent on availability of magnesium, as is an essential cofactor for δ-ALAD. Therefore, chromium and lead may also reduce δ-ALAD activity by exchanging the Mg at the active site (Vajpayee *et al*., 2000; Singh *et al*., 1997). The effect of heavy metals on this enzyme is the interaction of the heavy metals with functional sulphydryl group of the enzyme, as lead possesses a strong affinity for the SH- ligand (Vangronsveld and Clijsters, 1994). Lead inhibited the activity of δ-ALAD by reducing tissue hydration, binding with –SH group of the enzyme (Singh, 1997). Vajpayee *et al*. (2001) reported that Cr(VI) causes toxicity to δ-ALAD by impairing δ-ALA utilization. Moreover, Vajpayee *et al.* (2000) revealed that chromium treatment above 1M inhibited δ-ALAD activity, and decreased chlorophyll contents of *Nymphaea alba* L. Depletion in chlorophyll content of Cr-treated plants might be attributed to reduced δ-ALAD activity, which results in lesser availability of PBG required for chlorophyll biosynthesis. Decreased total chlorophyll content showed a positive correlation with reduced δ-ALAD activity as reported earlier (Shibata and Ochiai, 1976).

The decrease in chlorophyll concentration may be the result of an inhibited photosynthetic electron transport (Bohner *et al*., 1980). Heavy metals are potential inhibitors of photosystem II but photosystem I was reported to be less sensitive. At least

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two potential metal-sensitive sites can be derived in the photosynthetic electron transport chain. The potential metal sensitive sites can be derived in the photosynthetic electron transport chain is the water-splitting enzyme at the oxidizing site of PS II and the NADPH- oxidoreductase at the reducing site of PS I (Clijsters and Van Assche, 1985). Lead inhibited photosynthetic electron transport associated with PS II, but had no effect on electron transport through PS I. The primary site of lead inhibition was on the oxidizing site of PS II, between the primary electron donor of PS II and the site of water oxidation (Miles *et al.*, 1972). Chromium toxicity is chiefly credited to its interference with electron in photosynthesis (Larcher, 1995). Nichols *et al*. (2000) found a reduction in the light harvesting capacity at wavelengths ranging between 400-450 and 650-700 nm of the plant with the increase in Cr (VI) concentration in the growth medium.

In addition, the decreased chlorophyll concentration may be the result of the decomposition of the chloroplast membrane with excess heavy metals (Sandmann and Boger, 1980), and through the induced Fe deficiency and nutrient imbalances leading to interrupted chlorophyll biosynthesis (Van Assche and Clijsters, 1990).

6.3 Effect of heavy metals on median effective concentration (EC_{50})

In the present study, the results showed that the EC_{50} values on the biomass productivity and the total chlorophyll content of chromium were lower than those of lead. This suggests that chromium is much more toxic than lead. Similarly, the results of elemental toxicity experiment has shown that chromium is about 10 times more toxic to macrophyte than lead (Brooks and Robinson, 1998). Lyngby and Brix (1984) reported that heavy metal toxicity to *Zostera marina* have been found to be in the order as follows: Hg>Cu>Cd>Zn>Cr>Pb. Gaur *et al*. (1994) studied the relationship between heavy metal accumulation and toxicity in *Spirodela polyrrhiza* and *Azolla pinnata* and demonstrate that depending on the metal concentration in the medium solution, the order of metal toxicity was Cd>Cu=Ni>Co>Cr>Zn>Pb for *S.polyrrhiza* and Cd>Cr>Co>Cu>Ni>Pb>Zn for *A. pinnata.*

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6.4 Heavy metal accumulation

The results of lead and chromium accumulation in *H. umbellata* indicated that heavy metal accumulation in plant significantly increased when the exposure time and heavy metals concentration were increased. Similarly, Lee *et al*. (1991) found that the amount of heavy metal accumulation increased proportionally as the metal concentration in water increased. Plant tissue concentrations of chromium increased slightly as the supply level increased from 0.1 to 1.0 mg/L (Zayed *et al*., 1998). Jain *et al*. (1990) found that lead and zinc content in *A. pinnata* and *L. minor* increased with increase in the initial concentration in the feed solutions and exposure time. Wang and Lewis (1997) reported that metal accumulation by aquatic macrophyte under controlled laboratory conditions is dependent on the metal concentration in water. This results showed that the accumulation of lead in root and shoot was higher than that of chromium. Similarly, Soltan and Rashed (2003) reported that heavy metal concentration in roots of water hyacinth in decreasing order was Mn, Zn, Pb, Cu, Cr, Ni, Co, and Cd.

However, the present investigation found that the heavy metal accumulation is not linearly in correlated with the increase in concentration. This is probably due to the fact that heavy metals are bound in the tissue, causing saturation that is governed by the rate at which the heavy metal is conducted away (Greger, 1999). Besides, it may be explained by the finding of Dushenkov *et al*. (1995), who reported that at higher lead concentration, more lead was removed from the solution than accumulated in the roots as a result of the formation of an amorphous white precipitate (lead phosphate as its major component) on the walls and at the bottom of the hydroponic container. This precipitation probably involves a release of root excudates and increase with exposure times (Salt *et al*., 1995). A markedly decrease in metal content for the plant growing in 10 mg Cr/L and 100 mg Pb/L solution compared to other concentration was paralleled by the wilting of the petiole. Wilting minimizes the uptake of metal by plant, where the process of uptake can take place through the cell membrane by diffusion and osmosis (Soltan and Rashed, 2003). In the present study, *H. umbellata* accumulated lead and chromium to the highest concentration of 726.61, and 21.97mg/kg dry weight in shoots, respectively, and 71,998.53, and 845.73 mg/kg dry weight in roots, respectively. The preferential

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accumulation of lead and chromium in the present study showed that they are accumulated in roots more than shoots. This may be due to the fact that heavy metals such as lead and chromium can be slowly translocated from roots to shoots. Baker *et al*. (1994) observed that Zn, Cd, Co, Mn, and Ni were readily transported to the shoot, whereas, Al, Cr, Cu, Fe, and Pb were predominantly immobilized in the roots. Mehra and Farago (1994) reported that plant roots are usually able to take up and accumulate large quantities of Pb^{2+} in culture solutions but translocation to aerial shoots is generally limited due to binding at root surface and cell wall. Since phosphate concentration in the phloem is high and Pb^{2+} with insoluble phosphate, so lead is not usually transported in the phloem. As the root structure contains sites with high exchange capacity, such as the carboxylic groups of hemicellulose and pectin substance and those of proteins, roots are an efficient cationic exchange barrier which can greatly reduce the movement of lead toward stem and leaves (Nicolo *et al*., 1995).

Transport of chromium up the roots is very slow, hence low levels of chromium are found in the tops of plants. Shewry and Peterson (1974) showed that less than 1% of the accumulated chromium was transported to the shoots. It was probably due to the reduction of Cr (VI) to Cr (III) which reduces its mobility from roots to shoots (Sen *et al*., 1987). The reduction of CrO_4^{2-} (hexavalent chromium) to Cr^{3+}(trivalent chromium) by plant roots take place mainly in plant tissues and does not require rhizosphere microbes. The reduction in roots may have been catalyzed by Fe (III)-reductase enzymes (Zayed *et al*., 1998). A resent study has shown that chromium application to Fe-deficient plants increased the activity of root associated Fe (III) reductase (Schmidt, 1996). Chromium (III) forms complexes with –COOH groups which inhibit the translocation of chromium (Vajpayee *et al*., 2000).

Similarly, Qian *et al*. (1999) found that accumulation of lead in roots and shoots of smartweed were 1,882 and 64 mg/kg dry weight, respectively. Umbrella plant, fuzzy water clover, and parrot's feather accumulated more lead levels in roots (1,000-1,200 mg/kg dry weight) than in shoots (30-45 mg/kg DW). The accumulation of chromium was higher in roots (2,980, 1767, and 1300 mg/kg dry weight in roots of smart weed, parrot's feather, and fuzzy water clover) than in shoot (less than 16 and 24 mg/kg dry weight).

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The results on the average accumulation of lead in shoots of *H. umbellata* showed that it decreased rapidly on the first six days and then increased again on day 9 at 40, 60, 80, and 100 mg Pb/L and then slowly decreased on day 12 which was similar to the average accumulation of lead in roots. This can be explained that on day 9, the increased amount of lead in roots by surface sorption led to the increase in the penetration of lead in root tissue and thus the increase in intergradent between lead concentration in the shoots and in the roots. Roots had higher lead concentration than shoots. So, the translocation of lead from roots to shoots was increased.

The results on the average accumulation of lead in roots of *H. umbellata* showed that it decreased rapidly on the first six days and increased again on day 9 at 40, 60, and 80 mg Pb/L and then slowly decreased on day 12. This can be explained that in the experiment, the solution was renewed on day 6, which may lead to the increased surface sorption. Surface sorption is a combination of such physical and chemical process as chelation, ion exchange and specific adsorption. This component does not require biological activity and will take place in dead roots (Salt *et al*., 1995). However, at 20 and 100 mg Pb/L on day 9, the average accumulation did not increase. This can be explained that the lowest lead concentration of the new solution was not enough to increase the surface sorption and at high lead concentration, it involves root-mediated precipitation from the solution more than that accumulate in root tissue or surface sorption. So, at 20 and 100 mg Pb/L, the average accumulation of lead in roots decreased rapidly on the first six days and then slowly decreased until day 12.

The results on the average accumulation of chromium in shoots of *H. umbellata* showed that it increased slowly throughout 12 days at 2 and 4 mg Cr/L but it increased slowly from day 3 to day 6 and then slowly decreased on day 9 and 12 at 6, 8, and 10 mg Cr/L. This can be explained that the translocation of chromium from roots to shoots it very slow and it take longer time for chromium to be saturated in the shoots. Hence, during 3 to 6 days, the average accumulation of chromium in the shoots was slowly increased at all chromium concentrations. But, at lower chromium concentration, the average accumulation was continuously increased up to day 12 because of the amount of chromium in the shoots was very low and not enough to reach the point of saturation. At

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higher chromium concentration (6, 8, and 10 mg Cr/L), the average accumulation was slowly decreased on day 9 to day 12. This may be due to the amount of chromium transport to shoots was enough to reach the point of saturation.

The results on the average accumulation of chromium in roots of *H. umbellata* showed that they decreased rapidly on the first six days and then slowly decreased on day 9, and 12. Similarly, Jain *et al*. (1990) found that the total amount of lead and zinc taken up during the multiple exposure time study increased as lead or zinc content in the feed solution was increased. However, with each subsequent exposure the percentage of lead or zinc taken up decreased. This behavior is compatible with the mechanism of the uptake in three stages. The first stage corresponds to a rapid surface binding a large amount of heavy metal is taken up in this stage and it is limited to the Donnan-free-space of the cell wall. The second stage is slower and it is the intracellular diffusion (penetration into the protoplast including the cell organelles) that governs the process. The slowest third stage result from the active accumulation of metal within the plant cells. This stage is dependent upon factors that affect the metabolism such as temperature and light intensity (Martin and Boaventura, 2002).

6.5 Bioconcentration factor (BCF)

Bioconcentration factor is defined as the metal concentration in the plants to the initial concentration of metal in the feed solutions. It provides an index of the ability of the plant to accumulate the heavy metal with respect to the heavy metal concentration in the substrate. In this study, the bioconcentration factor of *H. umbellata* in each group of lead treatment was higher than that in each group of chromium, suggesting that the uptake of lead was better that that of chromium. This result confirms with the earlier report. Rai *et al*. (1995) found that the preferential sequence of metal accumulation of *Hydrodictyon reticulatum, S. polyrrhiza, Chara corallina, Ceratophyllum demersum, Vallisneria spiralis, Bacopa monnieri, Alternanthera sessilis* and *Hygrorrhiza aristata* was Fe>Pb> Mn>Cr>Cu>Cd.

In general, when the metal concentration in water increases, the amount of metal accumulation in plants increases, whereas the bioconcentration factor values decrease

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because at low metal concentration per absorption area, low competition between ions at the uptake sites occurs while the opposite occurs at high concentration (Greger, 1999). The present study shows that there was a decrease in BCF of lead and chromium with the increase of metals in the feed solution. Similarly, Zayed *et al*. (1998) found that at the lowest supply level (0.1 mg/L), the BCF for lead and chromium in duckweed (458 and 420, respectively) were higher than those obtained at the highest supply level (10 mg/L) (63 and 287,respectively). Zhu *et al*. (1999) report that BCFs of water hyacinth were very high for Cd, Cu, Cr and Se at low heavy metal concentration and they were decreased as increasing the high heavy metal concentration.

Other species have exhibited very high BCFs of lead ranging from 8,064 for coontail, 7,174 for *Hydrorrhiza*, 2,521 for giant duckweed, and 2133 for musk-grass, respectively, which would also qualify them as good phytoremediator. By comparison, the BCFs of lead in duckweed was ranging from only 50 to 460. So, it appeared to be a poor accumulator of lead (Zayed *et al*., 1998).

The greatest BCFs of chromium were reported for coontail (15,330-31,400) and for water net (11,394) (Rai *et al*., 1995). In comparison, very low BCF of chromium in other aquatic plants was reported by Zurayk *et al*. (2001) that the BCFs of *Nasturtium officinale*, *Cardamine uliginosa*, *Mentha longifolia*, and *Veronica beccabunga* were 150, 133, 128, and 120 respectively. Chromium BCF values in duckweed ranged from 280 to 660 (Zayed *et al*., 1998).

From the view of phytoremediation, a good accumulator should have the ability to bioconcentrate the element in its tissue, for example, a BCF of over 1000 (a 100-fold compared on a fresh weight) (Zayed *et al*., 1998). Based on these criteria, the results showed that *H. umbellata* is a good accumulator of lead with the BCF 1902.74 but it is a poor accumulator of chromium with the BCF 228.34. Thus, *H. umbellata* is useful for the phytoremediation of lead pollution in waters. Moreover, *H. umbellata* has a rapid growth, very easy to harvest and it can be found throughout the aquatic.

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Table 6-1. Bioconcentration factor (BCF) of lead and chromium in various aquatic plants

Aquatic plants	Bioconcentration factor		Reference
	Lead	Chromium	
Hydrocotyle umbellata	1902.74	228.34	Present study
Ceratophylum demersum	8,064	15,330-31,400	(Garg and Chandra,1990)
Hydrodictyon reticulatum	5,000	11,394	Rai *et al*. (1995)
Spirodela polyrrhiza	2,521	7,920	,,
Chara corollina	2,133	2,081	,,
Vallisneria spiralis	1,777	1,993	,,
Bacopa monnieri	366	2,016	,,
Alternanthera sessilis	555	722	,,
Hygrorrhiza aristata	7,174	652	,,
Lemna minor	458	420	Zayed *et al*. (1998)
Eichhornia crassipes	3,225	-	(Muramoto and Oki, 1983)
Lemna polyrrhiza	1,000	-	(Sharma and Gaur, 1995)
Azolla pinnata	1,200	-	Jain *et al*. (1990)

6.6 Toxicity symptoms

Toxicity symptom of *H. umbellata* exposed to lead and chromium increased when the exposure time and heavy metal concentration were increased. The results showed that lead and chromium caused root color change from light green to dark brown. Might be due to the root surface was often covered with a dark brown layer, which may include mucilage and a diversity of microorganism (Vesk *et al*., 1999). Moreover, this metal caused wilting of petiole due to direct effect on root damage, which reduced water and nutrient uptake (Soltan and Rashed, 2003). Lead causes leaf chlorosis, which is lack of chlorophyll pigment. In addition, lead can affect chloroplast that may be responsible for a reduction in grana stacks, a reduction in the amount of stroma in relation to the

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lamellar system and striking change in chloroplast and cell death has been reported in the study with the hydrophyte, *C. demersum.* (Rebechini and Hanzely, 1974).

Chromium causes foliar symptoms of necrosis, which is a death of tissue. Due to high oxidation power of chromium, which causes the membrane damage. Its direct effects are mainly caused by alteration in the content of essential trace element and stomata closure (Vazquez *et al*., 1987). However, this study showed a number of new plantlet increase with increasing exposure time at the same metal concentration. This may be due to the older plants had reduced the level of dissolved metals in the culture medium, which the metals were bound to root of original plants (Kay *et al*., 1984).

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CHAPTER VII
CONCLUSION

The study of the accumulation and toxicity of lead and chromium in aquatic plant, *Hydrocotyle umbellata*, can be summarized as follows:

1. The study on the effects of lead and chromium on relative growth and biomass productivity of *H. umbellata* indicated that they were significantly decreased ($P \leq 0.05$) when the exposure time and heavy metal concentration were increased. However, the results showed stimulation in growth of *H. umbellata* at 4 mg Cr/L on day 3.
2. The study on the effects of chlorophyll contents of *H. umbellata* indicated that they were significantly decreased ($P \leq 0.05$) when heavy metal concentration were increased on day 9 and 12.
3. The median effective concentration (EC_{50}) of lead and chromium on the biomass productivity and total chlorophyll contents of *H. umbellata* showed a decrease when the exposure time were increased. The EC_{50} of chromium was lower than that of lead during the same exposure time, suggesting that chromium was comparatively more toxic than lead.
4. The accumulation of lead and chromium *by H. umbellata* indicated that they were significantly increased but it was not linear with the exposure time and heavy metal concentration. Clearly, lead and chromium were accumulated higher in the roots than in the shoots.
5. The average accumulation of lead and chromium in roots decreased sharply on the first 6 days and then slowly decreased on day 9 and 12. The average accumulation of chromium in shoots increased slowly and then rapidly decreased on day 12 whereas those of lead decreased rapidly on the first 6 days and increased again on day 9 and then decreased on day 12.

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6. The bioconcentration factor (BCF) of lead and chromium significantly decreased ($P \leq 0.05$) when initial lead and chromium concentration were increased at each exposure time, suggesting that the ability of *H. umbellata* to accumulate these metals was decreased with metal concentration were increased. However, The ability of *H. umbellata* to accumulate chromium in shoots was increased during 3 and 9 day and decreased on day 12. The BCFs of lead was higher than that of chromium at the same exposure time, suggesting that the accumulation potential of lead is higher than that of chromium in *H. umbellata.*
7. The toxicity symptoms of *H. umbellata* exposed to lead and chromium showed that there was a reduction in the production of new plantlets. Lead caused leaf chlorosis whereas chromium caused leaf necrosis. However, both lead and chromium caused withering of petiole. The color of roots was changed from light green to dark brown. The toxicity symptoms increased when heavy metals concentration and exposure time were increased.

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