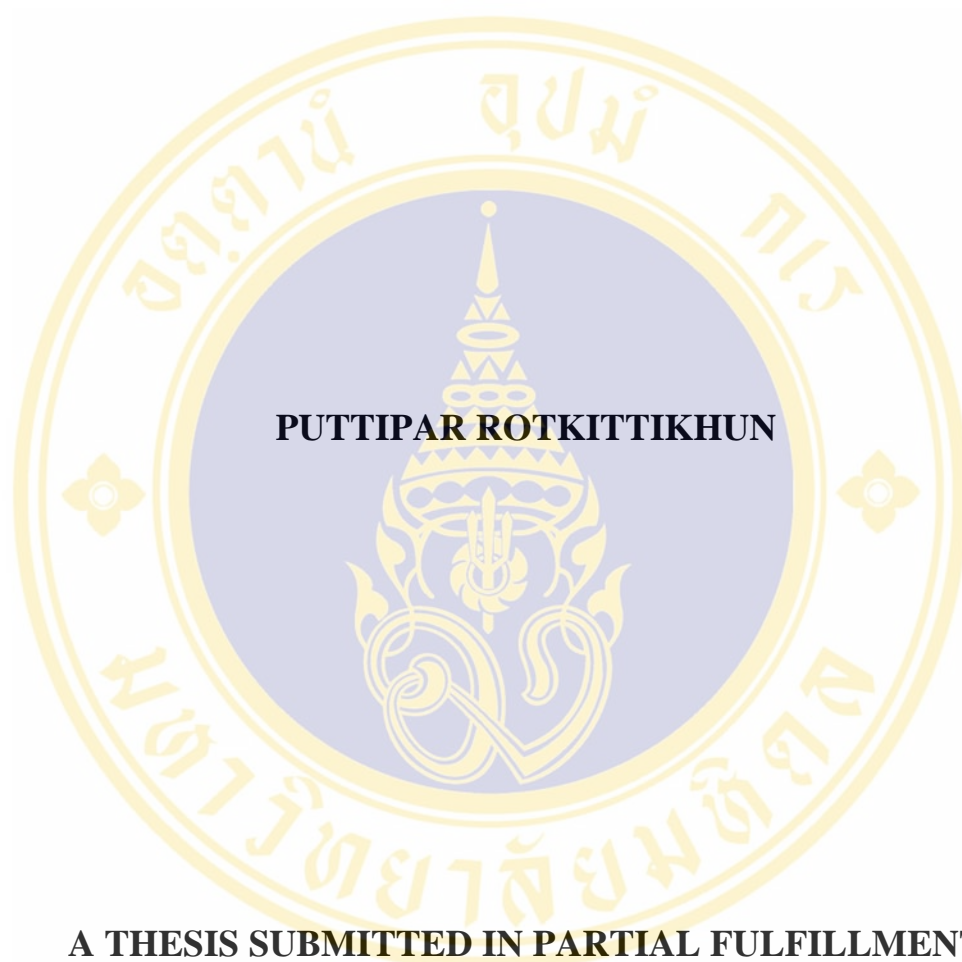


**PHYTOREMEDIATION OF LEAD CONTAMINATED SOIL
USING VARIOUS GRASS SPECIES**



**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY (BIOLOGY)
FACULTY OF GRADUATE STUDIES
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2007

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Thesis
Entitled

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USING VARIOUS GRASS SPECIES**

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PHYTOREMEDIATION OF LEAD CONTAMINATED SOIL USING VARIOUS GRASS SPECIES

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ABSTRACT

A field survey of terrestrial plants growing in the Bo Ngam lead mine area, Thailand, was conducted to identify species accumulating exceptionally high concentrations of lead. Lead concentrations in surface soil ranged from 325 to 142,400 mg kg⁻¹. The highest lead concentration in soil was found at the ore dressing plant area and lowest at a natural pond area. In different areas, the concentrations of lead in plants were different when comparing various study sites. A total of 48 plant species belonging to 14 families were collected from five sampling sites. Twenty-six plant species had lead concentrations more than 1000 mg kg⁻¹ in their shoots. Three species (*Microstegium ciliatum*, *Polygala umbonata*, *Spermacoce mauritiana*) showed extremely high lead concentrations in their shoots and roots.

A glasshouse study was conducted to compare growth performance, metal tolerance and metal uptake by two grasses, *Thysanolaena maxima* and four ecotypes of *Vetiveria zizanioides* and to study the effects of soil amendments to this lead mine soil. Both *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla ecotype) could tolerate high Pb concentrations in soil and had very good growth performance. Application of pig manure increased EC value and reduced DTPA-extractable Pb concentration in the soils. Pig manure application improved the growth of vetiver but did not improve *T. maxima*. The uptake of Pb of both species was reduced when soils were amended with pig manure. Application of inorganic fertilizer did not improve growth of vetiver but did improve that of *T. maxima*. Application of fertilizer increased the accumulation of *T. maxima*.

Experiments were conducted to evaluate lead tolerance and accumulation in *V. zizanioides* grown in hydroponics and a pot study and to examine the effects of lead on vetiver oil production. Elevated concentrations of lead in solution decreased the length of shoots and roots of plants. Vetiver grown in highly contaminated soils showed no apparent phytotoxicity symptoms. The results indicated that lead had an effect on vetiver oil production and composition by stimulating oil yield and the number of its constituents. The highest yield was found in plants grown in nutrient solution with 100 mg Pb L⁻¹ and the greatest number of total constituents was found in plants grown in soil spiked with 1000 mg Pb kg⁻¹. The predominant compound of vetiver oil was khusimol (10.7-18.1%).

KEY WORDS: PHYTOREMEDIATION / LEAD / *Vetiveria zizanioides* / *Thysanolaena maxima* / VETIVER OIL

122 P.

การบำบัดดินที่ปนเปื้อนด้วยสารตะกั่วโดยใช้หญ้าชนิดต่างๆ (PHYTOREMEDIATION OF LEAD CONTAMINATED SOIL USING VARIOUS GRASS SPECIES)

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บทคัดย่อ

จากการสำรวจพืชบริเวณเหมืองบ่องามเพื่อหาพืชที่มีการดูดซับสารตะกั่วสูงพบว่าดินบริเวณผิวดินมีความเข้มข้นของตะกั่วระหว่าง 325-142,400 มก/กก โดยพบความเข้มข้นสูงสุดที่บริเวณโรงแต่งแร่และต่ำสุดที่บริเวณบ่อน้ำธรรมชาติ เมื่อเปรียบเทียบความเข้มข้นของตะกั่วในพืชบริเวณต่างๆกันจะพบว่าพืชมีการดูดซับสารตะกั่วแตกต่างกัน จากการเก็บตัวอย่างพืชใน 5 บริเวณรวม 48 ชนิด จาก 14 สกุล พบว่ามี 26 ชนิดที่มีการสะสมตะกั่วในส่วนต้นสูงกว่า 1000 มก/กก และมีพืช 3 ชนิดที่มีค่าความเข้มข้นตะกั่วสูงมากทั้งในส่วนรากและส่วนต้น ได้แก่ *Microstegium ciliatum*, *Polygala umbonata* และ *Spermacoce mauritiana*

จากการศึกษาการเจริญเติบโต ความทนทานและการดูดซับสารตะกั่วของหญ้า 2 ชนิดคือ *Thysanolaena maxima* (ตองกง) และ *Vetiveria zizanioides* (หญ้าแฝก) 4 กลุ่มพันธุ์ และผลของวัสดุปรับปรุงดินต่อดินที่ปนเปื้อนด้วยสารตะกั่วพบว่าตองกงและหญ้าแฝกกลุ่มพันธุ์สุราษฎร์ธานีและสงขลามีความทนทานและสามารถเจริญเติบโตได้ดีในดินที่มีสารตะกั่วความเข้มข้นสูง การผสมขี้หมูจะลดค่า EC และค่าความเข้มข้นของตะกั่วที่สกัดโดย DTPA ของดินรวมทั้งลดการดูดซับสารตะกั่วของพืชทั้งสองชนิด ขี้หมูช่วยเพิ่มการเจริญเติบโตของหญ้าแฝกแต่ไม่ช่วยเพิ่มการเจริญเติบโตของตองกง การใส่ปุ๋ยอินทรีย์ช่วยเพิ่มการเจริญเติบโตและการดูดซับสารตะกั่วของ ตองกงแต่ไม่ช่วยเพิ่มการเจริญเติบโตและการดูดซับสารตะกั่วของหญ้าแฝก

จากการศึกษาความทนทานและการดูดซับสารตะกั่วของ *V. zizanioides* และผลของสารตะกั่วต่อการผลิตน้ำมันหญ้าแฝกพบว่าความยาวของรากและใบของหญ้าแฝกลดลงเมื่อความเข้มข้นของตะกั่วในสารละลายสูงขึ้นแต่ผลจากการศึกษาในดินไม่พบความเป็นพิษของสารตะกั่วต่อพืช สารตะกั่วมีผลในการเพิ่มปริมาณและองค์ประกอบของน้ำมันหญ้าแฝก ปริมาณน้ำมันสูงสุดพบในพืชที่ปลูกในสารละลายตะกั่ว 100 มก/ล จำนวนองค์ประกอบของน้ำมันหญ้าแฝกมากที่สุดพบในพืชที่ปลูกในดินที่มีความเข้มข้นตะกั่ว 1000 มก/กก องค์ประกอบหลักของน้ำมันหญ้าแฝกคือ khusimol (10.7-18.1%)

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LIST OF ABBREVIATION



$\mu\text{g L}^{-1}$	microgram per liter
mg L^{-1}	milligram per liter
mL	milliliter
L	liter
μM	micromolar
M	molar
mg kg^{-1}	milligram per kilogram
mg	milligram
g	gram
kg	kilogram
kg ha^{-1}	kilogram per hectare
t ha^{-1}	ton per hectare
mm	millimeter
cm	centimeter
m	meter
km	kilometer
s	second
min	minute
h	hour
d	day
DW	dry weight
SD	standard deviation
$^{\circ}\text{C}$	degree celsius

CHAPTER I

INTRODUCTION

Soil pollution, a very important environmental problem, has attracted considerable public attention in recent decades (Garbisu and Alkorta, 2001). Metalliferous mining and processing, including the dumping of wastes, often produces severe heavy metal pollution (Baker et al., 1994). The land surface is damaged and soil is contaminated with high concentration of heavy metal. Heavy metal polluted soils usually lack established vegetation cover due to the toxic effects of pollutants or recent physical disturbance. Barren soils are more prone to erosion and leaching which spread pollutants in the environment (Salt et al., 1995). The solutions to rehabilitation of the mine land are revegetation with metal tolerant plant species and removing heavy metal from land by plants.

In recent times the emerging field of phytoremediation has seen studies conducted on the use of green plants to remove heavy metals from the environment in a cost effective and ecologically manner (Pulford and Watson, 2003). This has included techniques such as phytoextraction, the use of hyperaccumulating plants to remove metals from soil by concentrating them in the harvestable parts (Kumar et al., 1995; Huang and Cunningham, 1996; Garbisu and Alkorta, 2001). There are many reports of hyperaccumulating plants (Cunningham and Berti, 1993; Shen and Liu, 1998). A hyperaccumulator has been defined as a plant that can accumulate cadmium $>100 \text{ mg kg}^{-1}$, copper, lead $>1,000 \text{ mg kg}^{-1}$, zinc $>10,000 \text{ mg kg}^{-1}$ in their shoot dry matter (Reeves and Brooks, 1983). In accumulator plants, the metal concentrations in shoots invariably greater than that in roots, showing a special ability of the plant to absorb and transport metals and store them in their above-ground part (Baker and Brooks, 1989; Baker et al., 1994).

Amongst the substances that contribute anthropogenic to pollution of the biosphere, trace metals are one of the most toxic and recalcitrant, with lead the most

widespread. Kanchanaburi Province, 230 km west of Bangkok, Thailand, is rich in minerals, especially lead, with total reserves of 7.7 billion tons (Department of Primary Industries and Mines, 1998). There are several lead mines in the area of Thong Pha Phum District of that province that have been in operation for 40-60 years; several of them have been closed down due to the expiration of concession.

Plant species are very diverse in metal mining areas in the tropics. Hence, such areas have a good potential to provide suitable species for phytoremediation of lead contaminated land. The large scale phytoremediation of metal pollutants from soils requires plant species that have high biomass, are rapidly growing and accumulate metals. The concentration of metal in the harvestable plant tissue must be significantly higher than in the soil.

Some soils are so heavily contaminated that removal of metals using plants would take an unrealistic amount of time. The normal practice is to choose drought-resistant, strong root system, fast growing crop or fodder which can grow in metal-contaminated and nutrient deficient soils. Phytostabilization is the use of metal-tolerant plant species to immobilize heavy metals through absorption and accumulation by roots, adsorption onto roots, or precipitation within the rhizosphere. This process reduces metal mobility and also reduces bioavailability for entry into the food chain (Wong, 2003). Phytostabilization techniques are most appropriate for immobile materials and large surface areas. The technique is currently acceptable for remediation at mining sites (Cunningham et al., 1995). Selection of appropriate plant species would be very important to ensure self-sustainable vegetation cover (Wong, 2003).

Vetiver grass technology has become one of the leading biological systems for soil and water conservation, embankment stabilization and land restoration (Truong, 2000; Chen et al., 2000; Pang et al., 2003). There is a wealth of evidence to show that vetiver grass is extremely tolerant to the hostile soil conditions and that it is widely used as a natural effective, and low-cost option to vegetate and stabilize heavy metal-contaminated land (Truong and Baker, 1998). Owing to its fast growth rate, extensive and proliferating root system, and its tolerance to heavy metals, vetiver grass has been used in the restoration of mining wastes, particularly contaminated tailings (Truong, 1999). Furthermore, the root of vetiver plants contains oil producing

cells. The vetiver oil is used extensively in perfumery, cosmetics, and biomedical utilization (Anderson, 1970; Maffei, 2002; Wong, 2003; Massardo et al., 2006). By planting vetiver grass in a lead mine area, it could serve the dual purpose of stabilizing the mine site and at the same time producing oil with a high commercial value.

Thysanolaena maxima is one of the most widely distributed species in Bo Ngam lead mine (PbCO_3) in Thong Pha Phum district, Kanchanaburi Province, Thailand. It can attain a very good height (2-3 m) and produces large biomass even when growing in extremely high lead content (10 to 100 g Pb kg^{-1}) soils (Rotkittikhun et al., 2006). However, the use of *T. maxima* in phytoremediation technology is not widely recognized owing to the lack of detailed investigations of its capacity to absorb contaminants, its tolerance and practical field application.

In the first part of this study the lead concentrations in plant samples in Bo Ngam lead mine area, Kanchanaburi Province were investigated to search for potential lead hyperaccumulators. The second part involved the laboratory study on the lead accumulation of some grass species (*V. zizanioides* and *T. maxima*) with high potential for phytostabilization and the effect of soil amendment (pig manure and fertilizer) on the growth performance and lead accumulation. The third part demonstrated the lead tolerance and accumulation in vetiver grass and the effects of lead on vetiver oil production grown in hydroponics and pot study.

CHAPTER II

OBJECTIVES

The overall objectives of the present study were to search for potential lead accumulators and lead excluders from plant species grown naturally in the Bo Ngam lead mine area, Kanchanaburi province, Thailand, and to use these plants in the rehabilitation of lead mine area. Hence, a field survey was conducted to investigate the lead accumulating capacity of plant species in Bo Ngam lead mine area.

Bo Ngam lead mine was dominated by several grass species especially *Thysanolaena maxima* which could attain the height of 2-3 m and they were very tolerant to lead at high concentration. Hence a glasshouse study was conducted to investigate the efficiency of *T. maxima* in phytostabilization of lead by comparing with the common grass species, *Vetiveria zizanioides*. Vetiver grass is extremely tolerant to heavy metals and it is widely used to vegetate and stabilize heavy metals contaminated land. In order to improve the quality of lead mine soil used in the study, various soil amendments were performed.

In addition, vetiver grass is also well known for its oil production in the roots. The vetiver oil is used extensively in perfumery, cosmetics, and biomedical utilization. By planting vetiver grass to vegetate a lead mine area, it could serve the dual purpose of stabilizing the mine site and at the same time producing oil with a high commercial value. Hence, hydroponics and pot experiments were conducted to investigate the effects of lead on growth and oil production in *V. zizanioides*.

The specific objectives of this study were as follows:

1. To estimate the lead accumulating capacity of plant species in Bo Ngam lead mine area and choose potential accumulators that would be suitable for phytoremediation.
2. To compare the accumulation of lead and plant growth in grasses, *T. maxima* and four ecotypes of *V. zizanioides* under laboratory conditions.

3. To evaluate the effects of fertilizer and pig manure amendments on the revegetation of lead mine using *T. maxima* and *V. zizanioides*.
4. To evaluate the extent of lead tolerance and accumulation in vetiver grass grown in hydroponics and in pot trials and to investigate any effects of lead on vetiver oil production.



CHAPTER III

LITERATURE REVIEW

1. Heavy Metals

Heavy metals are natural elements that are found at various high background levels (Table 1) at different places throughout the world due to various concentrations in the bedrock. Thus, for example, Ni, Cr and Co are abundant in serpentine soils whereas Zn, Pb and Cd are high in calamine soils. Heavy metals are persistent and cannot be deleted from the environment. Thus, a problem arises when their availability is high due to high background levels or to human activity.

Table 1. Background levels in natural water and sediment (Förstner and Wittmann, 1979) and the upper limit of non-polluted soil (Temmerman et al., 1984; Greger, 1999).

Metal	Natural water ($\mu\text{g L}^{-1}$)		Soil ($\mu\text{g L}^{-1}$)		Sediment ($\mu\text{g L}^{-1}$)	
	Seawater	Freshwater	Sandy soil	Loam	Lake	Sea
Cd	0.01-0.07	0.07	1	1	0.14-2.5	0.02-0.43
Cr	0.08-0.15	0.5	15	30	7-77	11-90
Co	0.04	0.05	5	15		0.1-74
Cu	0.04-0.1	1.8	15	25	16-44	4-250
Hg	0.01	0.01	0.15	0.15	0.004-0.2	0.001-0.4
Mn	0.2	< 5	500	800		390-6700
Mo	10	1	5	5		0.2-27
Ni	0.2-0.7	0.3	1	1	34-55	2-225
Pb	0.001-0.015	0.2	50	50	14-40	7-80
Zn	0.01-0.62	10	100	150	7-124	16-165

Heavy metals are enriched in the environment by human activities of different kinds. Results of these activities end up in outlets and wastes where heavy metals are transported to the environment by air, water and deposits, thereby increasing the metal concentrations in the environment. For example, the metal concentrations in river waters has been shown to be increased several thousands fold by effluents from mining wastes. Metals supplied to the environment are transported by water and air, ultimately reaching the soil and sediment where they become bound. However, the time taken for them to become bound may be fairly long and it has been shown that the bioavailable fraction of metals in soil is high at the beginning of the binding period, but decrease with time. Thus, there is probably a bigger problem with anthropogenically supplied metals, with high levels of bioavailable metals, than with high background levels originating from bedrock, with slow weathering.

1.1 Sources of heavy metal contaminants in soils

Although heavy metals are ubiquitous in soil parent materials, the major anthropogenic sources of metals to soils and the environment are (Alloway, 1995):

- a) Metalliferous mining and smelting
- b) Agricultural and horticultural materials
- c) Sewage sludges
- d) Fossil fuel combustion
- e) Metallurgical industries- manufacture, use and disposal of metal commodities
- f) Electronics- manufacture, use and disposal of electronic commodities
- g) Chemical and other manufacturing industries
- h) Waste disposal

(a) Metalliferous mining and smelting

Metals utilized in manufacturing are obtained from either the mining of ore bodies in the earth's crust, or the recycling of scrap metal. Ores are naturally occurring concentrations of minerals at sufficiently high concentration of metals to render them economically worthwhile exploiting. With increasing demand for metals and improvements in mineral extraction technology, ore bodies with progressively lower metal contents are being mined. These lower grade ore bodies are usually larger

in extent and require a higher proportion of rock to be mined per ton of metal extracted and consequently much greater quantities of waste are produced, especially tailings.

(b) Agricultural and horticultural materials

Agricultural practices constitute very important non-point sources of metals which make significant contributions to their total concentrations in soils in many parts of the world, especially in regions of intensive farming. The main sources are:

- impurities in fertilizers: Cd, Cr, Mo, Pb, U, V, Zn
- sewage sludge: especially Cd, Ni, Cu, Pb, Zn (and many other elements)
- manures from intensive animal production, especially pigs and poultry: Cu, As, Zn
- pesticides: Cu, As, Hg, Pb, Mn, Zn
- refuse derived composts (not widely used in agriculture): Cd, Cu, Ni, Pb, Zn
- desiccants: As
- wood preservatives: As, Cu, Cr
- corrosion of metal objects (galvanized metal roofs and wire fences: Zn, Cd)

(c) Sewage sludges

Sewage sludge is the residue produced from the treatment of domestic and industrial waste-waters and large amounts are produced worldwide. Sewage sludges are a significant source of plant nutrients and organic matter and some specially treated sludges, such as those containing lime or cement kiln dust.

(d) Fossil fuel combustion

In general, fossil fuel combustion results in the dispersion of a wide range of heavy metals, which can include: Pb, Cd, Cr, Zn, As, Sb, Se, Ba, Cu, Mn, U and V, over a very large area, although not all these elements are present in significant concentrations in all types of coal and petroleum. The metals accumulate in the coal and petroleum deposits as they formed and are either emitted into the environment as airborne particles during combustion, or accumulate in the ash which may itself be transported and contaminate soils or water, or, may be leached in situ. The combustion of petrol (gasoline) containing lead additives has been the largest source

of this metal in the environment and has affected soil over a high proportion of the earth's terrestrial surface

(e) Metallurgical industries

Metallurgical industries can contribute to soil pollution in several ways: (I) by the emission of aerosols and dusts which are transported in air and eventually deposited on soils or vegetation; (II) by liquid effluents which may pollute soils at times of flooding; (III) by the creation of waste dumps in which metals become corroded and leached into the underlying soil.

(f) Electronics

A large number of heavy metals are used in the manufacture of semi-conductors, cables, contacts and other electrical components, these include: Cu, Zn, Au, Ag, Pb, Sn, Y, W, Cr, Se, Sm, Ir, In, Ga, Ge, Re, Sn, Tb, Co, Mo, Hg, Sb, As and Cd. Environmental pollution can occur from the manufacture of the components, their accidental contact with soils and their disposal in waste.

(g) Chemical and other industrial sources

Other significant sources of heavy metal pollution of soils and the environment can be either the manufacture and/or use and disposal of the following:

Chlorine manufacture, Hg

Batteries, Pb, Sb, Zn, Cd, Ni, Hg

Pigments and paints, Pb, Cr, As, Sb, Se, Mo, Cd, Ba, Zn, Co

Catalysts, Pt, Sm, Sb, Ru, Co, Rh, Re, Pd, Os, Ni, Mo

Polymer stabilizers, Cd, Zn, Sn, Pb (pollution from incineration of plastics)

Printing and graphics, Se, Pb, Cd, Zn, Cr, Ba

Medical uses, Ag, As, Ba, Cu, Hg, Sb, Se, Sn, Pt, Zn

Additives in fuels and lubricants, Se, Te, Pb, Mo, Li

(h) Waste disposal

The disposal of household, municipal and industrial wastes can lead to soil pollution with heavy metals in various ways. The landfilling of municipal solid waste can lead to several metals including Cd, Cu, Pb, Sn, Zn being dispersed into soil, groundwaters and surface waters in leachates if the landfill is not managed properly.

2. Lead

In nature lead is a ubiquitous, non-essential element. Lead is ranked at about the 36th place of the element in the earth's crust (Ewers and Schlipkötter, 1991) in an average concentration of about 15 mg kg⁻¹ (Kabata-Pendias and Pendias, 2000), and in soils remote from man concentrations range from 5-25 mg kg⁻¹. Natural mobilization of lead into the environment occurs principally from the erosion of lead-containing rocks and through gaseous emissions during volcanic activity. In the last fifty years great amounts of lead have been extracted, concentrated and used by man, and re-emitted into the environment.

2.1 Chemical and physical properties

Lead (Pb) has an atomic number of 82, an atomic mass of 207.19, and is a member of Group IVB of the Periodic Table. Two oxidation states (Pb(II) and Pb(IV)) are stable but the environmental chemistry of the element is dominated by the plumbous ion, Pb²⁺. Elemental lead is a dense (11.34 g cm⁻³) bluish-grey color metal which melts at 327 °C and boils at 1740 °C (WHO, 1989). Lead is very soft, malleable and ductile. The most important lead minerals are galena (PbS), cerussites (PbCO₃), anglesite (PbSO₄), and minim (Pb₃O₄). Galena occurs mostly in deposits, which also contain zinc minerals and small amounts of copper, iron, cadmium, and other metals. Natural lead is a mixture of four stable isotopes: 204 Pb (1.4%), 206 Pb (24.1%), 207 Pb (22.1%), and 208 Pb (52.4%). Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements: 206 Pb for the uranium series, 207 Pb for the actinium series, and 208 Pb for the thorium series. Forty other isotopes of lead, all of which are radioactive, are recognized.

Lead readily alloys with other metals: the Pb/Sb alloy is chiefly used to make battery plates but is also used in shotgun pellets and Pb/Sn alloys are often used as solder. Lead metal, in combination with PbO₂, is used to fabricate the lead-acid accumulator battery. Other inorganic compounds are widely used, e.g., the yellow chromate is used in road markings, and many paints contain lead oxides or lead soaps to promote polymerization. There is an extensive organic chemistry of Pb(IV) compounds, especially tetra-alkyl and tetra-aryl compounds.

2.2 Uses of lead

In 1982, the total consumption of refined lead was about 502 million tons worldwide (World Metal Statistics, 1983; Ewers and Schlipkötter, 1991). About 40% of all lead consumed are used for the production of lead-acid batteries. The production of tetramethyllead (TML) and tetraethyllead (TEL), which are used as antiknock agents in gasoline, accounts for approximately 10% of the world lead consumption. In the 1970s a maximum of TML and TEL (more than 250 000 t/a in the USA) was produced, since then consumption has decreased again (Nriagu, 1989). Lead based pigments are used as a protective coating for steel structures, for paints used on highways and for other exterior uses. The use of lead for interior paint in houses has been prohibited in many countries. Lead chemicals are used in glassware and ceramics and as stabilizers in plastics. Lead sheets, cable sheathing, solder, ammunition, bearing alloys, type metal, tubes, weights and ballast, and low melting alloys together account for about 20% of the lead used. PbO_2 and lead tetraacetate are oxidizing agents. In recent years lead consumption has been decreasing partially because leaded gasoline is used less, because one tries to substitute heavy batteries, and because lead tubes for many applications are not allowed any longer (Saager, 1987). Especially the production of TML and TEL is decreasing since about 1980 because of the detection of increased levels of lead in blood (particularly in children) and because catalysts reducing exhausts to avoid smog are destroyed in the presence of lead.

A large proportion of lead waste is recovered and recycled in secondary lead smelters. Concerning lead storage batteries the recovery is more than 90% in some countries. The recovery of lead sheets, pipes, and cable sheathing may vary between 50 and 90%. Lead from alloys is recovered to a substantially lesser degree (Ewers and Schlipkötter, 1991).

2.3 Occurrence and cycle in the environment

2.3.1 Occurrence in the lithosphere

The terrestrial abundance of lead indicates a tendency for lead to concentrate in the acid series of magmatic rocks and argillaceous sediments in which the common

lead concentrations range from 10 to 40 mg kg⁻¹, while in ultramafic rocks and calcareous sediments its range is from 0.1 to 10 mg kg⁻¹ (Table 2).

Table 2. Lead in main soil-parent rocks (Kabata-Pendias and Pendias, 2000).

Rock Type	Pb (mg kg ⁻¹)
Magmatic Rocks	
Ultramafic rocks	0.1-1.0
Mafic rocks	3-8
Intermediate rocks	12-15
Acid rocks	15-24
Acid rocks (volcanic)	10-20
Sedimentary rocks	
Argillaceous sediments	20-40
Shales	18-25
Sandstones	5-10
Limestones, dolomites	3-10

The average abundance of lead in the earth's crust is estimated at about 15 mg kg⁻¹. In the terrestrial environment, two kinds of lead are known: primary and secondary. Primary lead is of geogenic origin and was incorporated into minerals at the time of their formation, and secondary lead is of a radiogenic origin from the decay of U and Th. The ratio of lead of various origins is used for dating the host materials.

The natural lead content of soil is inherited from parent rocks. The natural lead occurrence in the top horizons of different soils from various countries show that

amount range from 3 to 189 mg kg⁻¹, while mean values for soil types range from 10 to 67 mg kg⁻¹ and average 32 mg kg⁻¹ (Kabata-Pendias and Pendias, 2000).

2.3.2 Occurrence in the hydrosphere

About 17,000 tons of lead is estimated to enter the rivers and oceans from natural sources, and the deep water of the oceans contains 0.03-0.05 µg L⁻¹ (Alloway, 1995). Fresh waters, prior to industrialization, probably contained about 0.6 µg L⁻¹, and even today rivers and lakes in areas where no lead working is carried out often contain less than 1 µg L⁻¹ although generally the lead content of lakes and rivers lies within the range 1-10 µg L⁻¹.

2.3.3 Occurrence in the atmosphere

Until the early part of the present century, lead smelting and refining were the major sources of the lead emitted to atmosphere, but, with more rigid methods of control and the emergence of other sources, these processes are now minor contributors, at least in a qualitative sense. Smelting and refining processes still emit about 200-250 tons a year to the atmosphere, and thus remain important sources of local contamination. Their influence on local lead levels depends to a large extent upon the height of the chimney, the type of trapping device used in the stack and the topography of the surrounding countryside. Local contamination, however, may be extensive and widespread. The combustion of coal and oil releases very little lead into the atmosphere, and there is no doubt that the single most important source of lead in the atmosphere is leaded petrol. In the general atmosphere, lead from petrol accounts for 90% or more of the total amount present.

The lead which is emitted from the exhaust pipes of motor cars is mainly in the inorganic form although some of the organic lead escapes combustion, and further quantities evaporate from the car engine and petrol tank. Lead alkyls also evaporate during their manufacture and transfer, and some is inevitably lost in the same way from filling stations. The proportion of the ambient atmospheric lead which is in the organic form is not known with any certainty and estimates lie in the range 1-20%. Little is known of the chemical species of lead which are present in the atmosphere

but the indications are that the lead emitted from cars is initially in the form of halides which are then converted to oxides, sulphates and carbonates.

2.4 Reactions of lead with soil components

The natural lead content of soils is strongly related to the composition of bedrock, and lead is reported to be the least mobile among the other heavy metals. The relatively low lead concentrations in natural soil solutions support this statement. Although the lead species can vary considerably from one soil type to another, it may be concluded that lead is associated mainly with clay minerals, Mn oxides, Fe and Al hydroxides, and organic matter. However, in some soil, lead may be highly concentrated in Ca carbonate particles or in phosphate concentrations. Phosphate added to a soil contaminated with lead slightly decreases its easily soluble fraction. In soils heavily polluted with lead, the formation of pyromorphite, $Pb_5Cl(PO_4)_3$, is observed. The solubility of lead can be greatly decreased by liming. A high soil pH may precipitate lead as hydroxide, phosphate, or carbonate, as well as promote the formation of Pb-organic complexes, which are rather stable. Increasing acidity may increase the lead solubility, but this mobilization is usually slower than the accumulation in the organic-rich layer of soils. The characteristic localization of lead near the soil surface in most soil profiles is primarily related to the surficial accumulation of organic matter. Organic matter should be considered as an important sink of lead in polluted soils (Kabata-Pendias and Pendias, 2000).

2.5 Toxic effects of lead

The toxic effects of lead are directed principally against the blood, the nervous system and the kidney.

2.5.1 Toxic effects on human

Exposure to lead mainly occurs through inhalation of air and ingestion of lead in food, paint, water, soil, or dust. Lead accumulates in the body in blood, bone, and soft tissue. Because it is not readily excreted, lead can also affect the kidneys, liver, nervous system, and other organs. Excessive exposure to lead may cause anemia, kidney disease, reproductive disorders, and neurological impairments such as

seizures, mental retardation, and/or behavioral disorders. Even at low doses, lead exposure is associated with changes in fundamental enzymatic, energy transfer, and other processes in the body. Fetuses and children are especially susceptible to low doses of lead, often suffering central nervous system damage or slowed growth. Recent studies show that lead may be a factor in high blood pressure and subsequent heart disease in middle-aged white males. Lead may also contribute to osteoporosis (brittle bone disease) in post-menopausal women. EPA's health-based national air quality standard for lead is $1.5 \mu\text{g m}^{-3}$ measured as an annual maximum quarterly average concentration.

2.5.2 Toxic effects to plants

Lead has been reported to have a wide range of effects in plants, including disruption of cell membranes and mitosis, inhibition of plant growth, ATP (adenosine triphosphate) synthesis and structural protein formation, reduction of photosynthesis, water absorption and transpiration rates, increase in generation time, and decrease in pollen germination and seed viability (Pang et al., 2003).

In barley (*Hordeum vulgare*) leaves treated with lead ranging from 0.04 to 4.0 mM for 8 to 24 h, there were reduction of Chl b and number of grana (Woźny et al., 1995; Prasad and Strzalka, 1999). *Chlamydomonas reinhardtii* suffered a 50% reduction of photosynthesis by exposure to 5 μM Pb. Severe chloroplast damage was accompanied by the accumulation of lead in thylakoid membranes (Irmer et al., 1986; Barceló and Poschenrieder, 1999).

There is some evidence based on experimental work with maize and soybeans, that both photosynthesis and transpiration are inhibited by the presence of high lead levels in plants (Bazzar et al., 1974; Purves, 1985).

3. Lead mine area

3.1 General information on lead mine area

Kanchanaburi Province, 230 km west of Bangkok, Thailand, is rich in minerals, especially lead, with total reserves of lead are 7.7 billion tons (Department of Primary Industries and Mines, 1998). There are several lead mines in the area of

Thong Pha Phum District of this province that have been in operation for 40-60 years; several of them have been closed down due to the expiration of concessions.

Mineral deposits in Thong Pha Phum area of Kanchanaburi province are lead and zinc. Primary ore is lead sulfide (PbS) from hydrothermal process deposited in Ordovician limestone in the form of Stratabound deposits. Secondary ore is lead carbonate (PbCO₃) and lead sulphate (PbSO₄) due to chemical alteration with host rock and oxidation process along contact zone with limestone and air by hydrothermal process.

3.1.1 Bo Ngam lead mine

Bo Ngam lead mine is located in Thong Pha Phum district, consisting of one plot of expired mining concession (its request for renewal has been submitted by Lead Concentrate (Thailand) Co., Ltd.) and one plot of mining concession of Phol and Son Co., Ltd. The topography is mountainside and valley from the south to north. The area is rather flat with elevation of 900 m above MSL. Small streams flow through valleys, a part of Huai watershed and a part of Ta Nao Sri Range. Currently, this area is classified as one of the most potential lead-zinc areas.

3.1.2 Klity ore dressing plant

Klity ore dressing plant of Lead Concentrate (Thailand) Co., Ltd. is located at Thong Pha Phum district, Kanchanaburi province. Its operation was started in 1967. Ore from Bo Ngam mine (6 km off in the north) was processed.

3.2 Mining activities

3.2.1 Bo Ngam area

1. Mining: Open pit mining method was performed in the pit. By using backhoe to excavate lead carbonate ore (PbCO₃) found in the lateritic overburden of depth up to 5 m, it was transported to washing plants nearby. Stripping ratio was approximately 10:1. Concentrate gained from washing process would be more than 25% lead content. It was concentrated to 60% lead content at Klity ore dressing plant before it was transported further for smelting to achieve lead metal of 99.99% lead content (Fig. 1).

2. Wastewater and tailing system: Sedimentation process within tailing pond was used for the separation of water and slime. Treated water was pumped back for reuse in washing process.

3. Current activity: Mining operation ceased since 26 July 1996, due to the expiration of concession. Rehabilitation process is ongoing at this area.

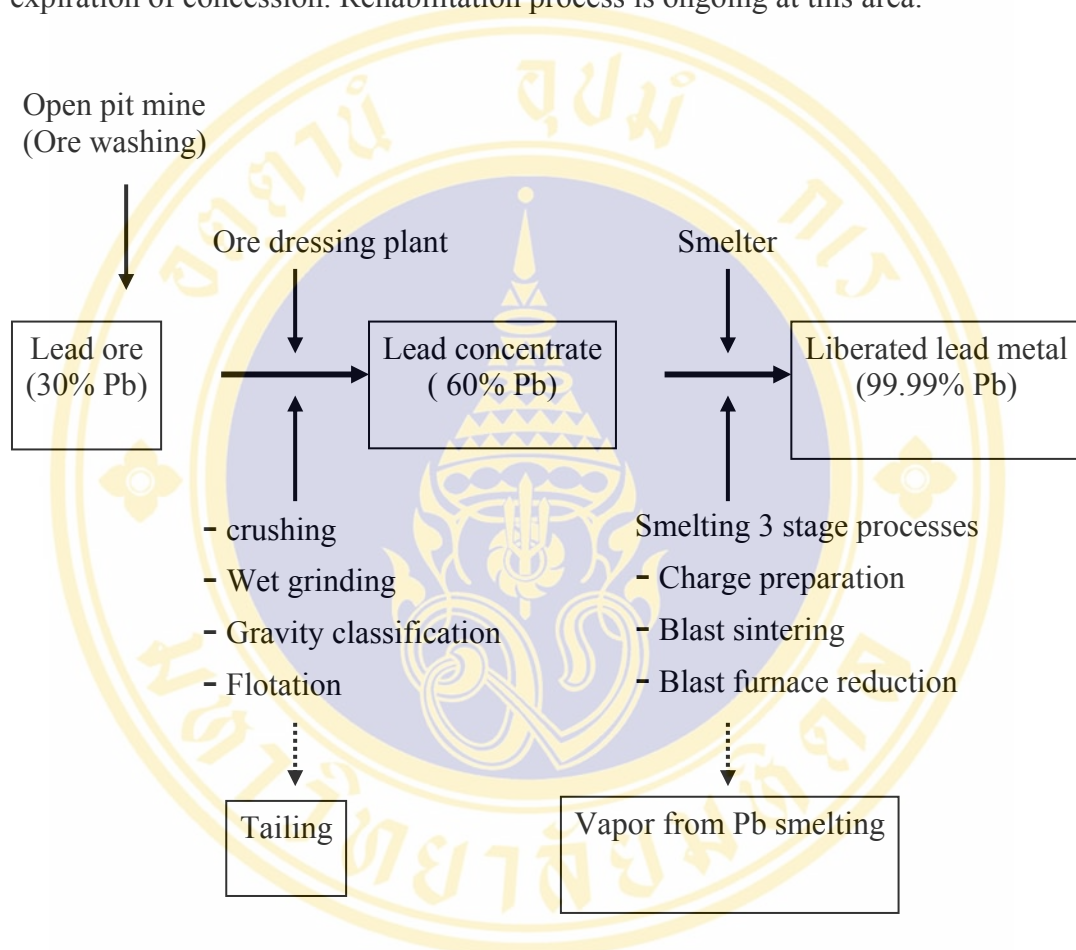


Figure 1. Mining process at Bo Ngam lead mine.

3.2.2 Klity area

1. Ore dressing plant: Ore concentrated from Bo Ngam area was ground by jaw crusher before passing to shaking table and further grinding by rod mill to reduce ore size for flotation.

2. Wastewater and tailing system: Sedimentation process was used to separate water and slime in the tailing pond before discharging to other area. Improvement of tailing pond system had been made after the tailing pond incident collapsed in 1998.

Three tailing ponds were constructed to fulfill the requirement. Circulation water was used in the process.

3. Current activity: Klity ore dressing plant ceased operation since 23 April 1998. The dressing plant license expired on 4 October 2000 without extension. Most of equipments and related buildings were already demolished and transported out of the area. Only two ball mills were left at the site, waiting for resale. Rehabilitation of tailing ponds were conducted by backfilling with clay overlaid with soil together with grass and tree planting. Some ponds have been left for community use.

4. Soil degradation

Mining activities generate a large amount of waste rocks and tailings which deposited at the surface (Wong, 2003). Mining causes major damage to whole ecosystem. In both surface and deep mining the original vegetation is inevitably destroyed, and the soils usually lost or buried by wastes (Bradshaw, 1997). The waste rocks and tailing are often very unstable and will become sources of pollution. Mine tailings can cause the high incidence of erosion agents (wind and water run-off). The erosion processes in mine tailings can pose risks in two ways: (1) the structural stability of the tailing may be seriously affected by intensive erosion processes or adverse seismic and/or meteorological situations, provoking in some cases its collapse (Conesa et al., 2006). There are many examples of tailing collapse, among them the tailing accident of Aznalcollar (South Spain) in 1998 that was considered one of the worst ecological disasters in Europe at recent times; (2) release of metals from mine sites takes place mainly through acid mine drainage and erosion of waste dumps and tailings deposits (Salomons, 1995). These pollutant effects can reach local and, in some cases, regional scales and affect urban or agricultural zones (Conesa et al., 2006). Restoration of a vegetation cover can fulfill the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings (Wong, 2003). Restoration involves returning it to its original state; rehabilitation only a partial return. Reclamation implies ending up in some new state where either structure or function is different from the original; this is quite likely where the soil mineral material has been totally replaced (Fig. 2). Soil development and the restoration of mine working can be left to natural processes. But in most situations the process of

natural succession is slow, and it is common for 50 or 100 years to elapse before satisfactory vegetation cover develops, particularly on mine wastes. The revegetation of these areas is often difficult and slow due to the extreme soil properties, which include physical conditions, gross lack of nutrients and toxicity from residual heavy metals (Bradshaw, 1997). Therefore, the success of revegetation should overcome these major problems. The constraints related to plant establishment and short or long term treatments are presented in Table 3.

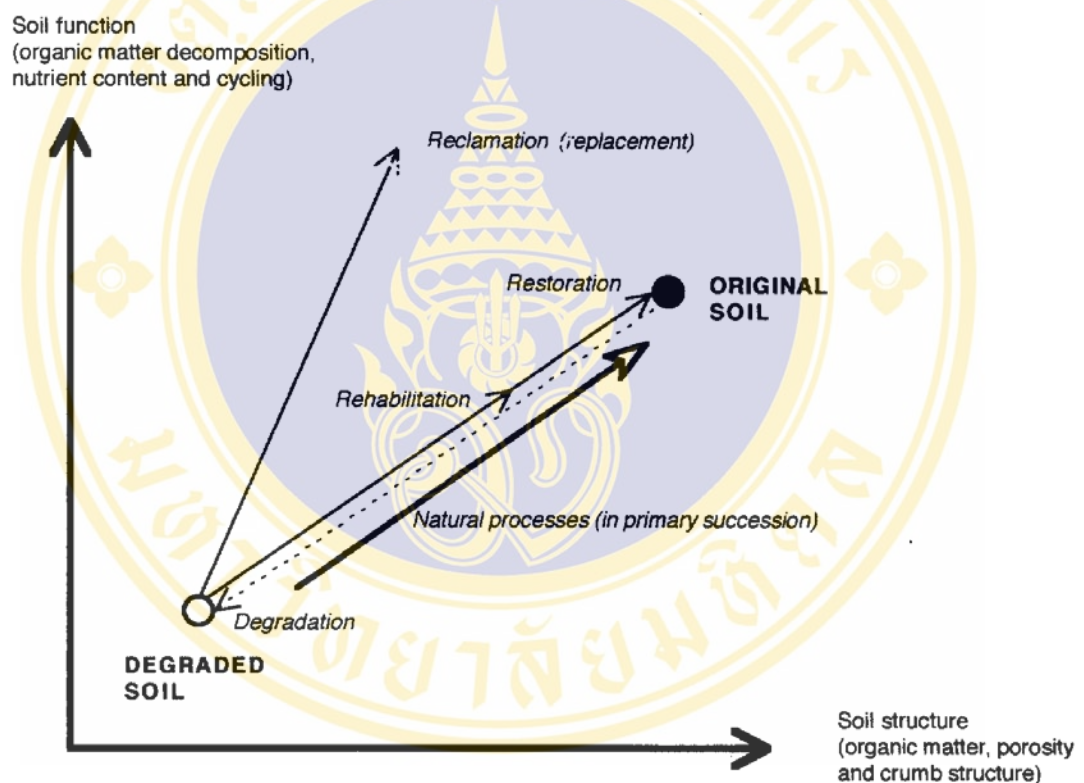


Figure 2. The contrasting approaches to the restoration of soils in land degraded by mining (Bradshaw, 1997).

4.1 Physical problems

Soil structure is one of the major problems in mining area. Mine soil is compact and lack of organic matter. Soil bulk densities of more than 1.8 normally inhibit root growth completely (Ayerst, 1978; Bradshaw, 1997). The only solution is to loosen compacted materials by ripping or scarifying. The accumulating organic matter and the associated microbial activity will prevent the return of compacted

conditions and will lead to progressive lowering of bulk density. Some mine wastes are coarse and open textured, initial establishment may require the addition of fine material. With tree planting the fine material can be placed in small pockets into which the trees are planted. The plants will subsequently root into the deeper layers where there are adequate supplies of water (Coppin and Bradshaw, 1982; Bradshaw, 1997).

In mine areas, fine sand or silt materials can be liable to surface erosion by wind or water. The most valuable solution is a mulch of chopped straw or the equivalent, spread on the surface. Water is another problem for restoration in mine area. Excess water can lead to saturation of the soil, anaerobiosis and death of vegetation. The solution can be surface drainage or a system of subsoil drains. Where drought is a problem, the application of organic mulch can be very effective. The accumulation of organic matter will ameliorate overall conditions. For arid conditions long term survival will, however, be dependent on the use of appropriate drought-tolerant species.

4.2 Nutritional problems

Deficiencies in nutrients such as phosphorus, potassium, magnesium and calcium are effectively permanent. Natural processes cannot overcome these deficiencies except where the nutrients are present but locked up in unweathered minerals. In this case they can be released by natural weathering but this can take a very long time. The solution is normally necessary to add the missing nutrient by the use of fertilizer. Nitrogen is a different problem. Nitrogen is required in the greatest amounts. It has a special place in soils because it does not occur in a mineral form, and is therefore absent from the primary materials from soils. Therefore it cannot be provided by fertilization. The crucial step is to select one or more N-fixing species which are appropriate to the expected end use. The legumes such as *Trifolium repens* and *T. pretense* are ideal, capable of fixing over 100 k N ha⁻¹ year⁻¹ (Dancer et al., 1977; Palmer and Iverson, 1983; Bradshaw, 1997). Another solution is the application of organic matter which provides a continuous source of nutrients, e.g., it provides most of the N reserve in soils and comprises typically 5% N which is mineralized at about 2% per year (Harris et al., 1996; Wong, 2003).

Table 3. The major problems of mined land and their short and long-term treatments (Bradshaw, 1983; Bradshaw, 1997).

Limiting factor	Variable	Problem	Immediate treatment	Long-term treatment
Physical	Structure	Too compact	Rip or scarify	Vegetation*
		Too open	Compact or cover with fine material	Vegetation*
	Stability	Unstable	Stabilizer, mulch or nurse*	Regrade or vegetation*
Nutrition	Moisture	Too wet	Drain	Drain
		Too dry	Organic mulch or nurse*	Tolerant species*
	Macronutrients	Nitrogen deficiency	Fertilizer	Legume* or other N-fixer*
		Other deficiency	Appropriate fertilizer	Fertilizer or tolerant species*
		Micronutrients	Deficient	Fertilizer
Toxicity	pH	Too high	Pyritic waste or organic matter	Weathering or tolerant species*
		Too low	Lime	Lime or tolerant species*
	Heavy metals	Too high	Organic matter or tolerant cultivar*	Inert covering or tolerant cultivar*
Salinity	Salinity	Too high	Gypsum, irrigation, or tolerant species*	Weathering or tolerant species*

*Indicates natural solution found as part of normal processes of succession.

4.3 Toxicity problems

Toxicities can be one of the most intractable problems in mine restoration. The commonest toxicity is low pH due to the weathering and oxidation of sulphide minerals. Many metal ores are sulphides and often have large quantities of iron sulphide (pyrite) associated with them (Bradshaw, 1997). Pyrite weathers only slowly, so that acid can be produced in the soil material for many decades, maintaining pH as low as 3 (Bradshaw, 2000). The solution to get rid of acidity is the application of lime or another way is the selection of acid tolerant species. In the mining for heavy metals, residual metals can be found in most of the waste materials produced. Metal toxicity would restrict the growth of all but the most tolerant plants. Metal toxicity can be reduced by application of organic matter, phosphate fertilizer and to a limited extent by lime. A combination of these and the introduction of metal tolerant plant is the most economical approach that most closely follows natural processes (Gunn, 1995; Bradshaw, 2000).

5. Remediation technologies for metal contaminated soils

Selection of an appropriate remediation technology to alleviate the problems caused by soil pollution is based on several criteria, such as characteristics of the contaminated land, form and concentration of contaminants, as well as availability and effectiveness of the remediation technologies. In most cases, the probable end use of the remediated land also plays a major role in the selection of an appropriate technology, as most conventional methods render the soil infertile and unsuitable for agricultural purposes (Saxena et al., 1999).

5.1 Conventional remediation technology

Conventional technologies available for water and soil remediation can be broadly classified based on whether they are employed in situ or ex situ. The most conventional technologies commonly used for remediation are:

(a) Soil flushing

The process involves physical separation by vertical or horizontal leaching using a liquid (water or an aqueous solution containing chelators) which is followed by collection and treatment of the leachates in basins or trench infiltration systems.

(b) Pneumatic Fracturing

The process involves injecting pressurized air into the soil to develop cracks in low permeability areas, thereby enhancing the extraction efficiencies of other in situ technologies.

(c) Solidification/stabilization

In these processes, the contaminant is physically enclosed in a stabilized mass or through chemical interactions induced between the stabilizing agent and the contaminant.

(d) Vitrification

This technology utilized thermal energy to melt the soil to enable physical or chemical stabilization.

(e) Electrokinetics

The contaminants are mobilized as charged species towards polarized electrodes placed in the soil. The migrated contaminants can be removed or treated in situ.

(f) Chemical reduction/ oxidation

This process the contaminants are chemically converted into less hazardous, more stable, less mobile and/or inert forms.

(g) Soil washing

This process involves separation of contaminants adsorbed to fine soil particles using an aqueous solution, through size separation, gravity separation, or attrition scrubbing.

(h) Excavation, retrieval and off-site disposal

This technology requires removal and transportation of the contaminated soil to an off-site treatment and disposal facility.

In general, the conventional technology termed as pump-and-treat and dig-and-dump techniques are limited in their applicability to small areas and have their own limitations. At sites where the concentration of contaminants are high, the use of conventional technology is not economically viable (Saxena et al., 1999).

5.2 Phytoremediation

In the last decade, an emerging low-cost, ecologically friendly alternative to the conventional remediation technologies has gained a great deal of interest in both public and private sectors (Saxena et al., 1999). Phytoremediation is a relatively recent invention, the act of phytoremediation is an age-old practice which uses plants to cleanse nature (Cunningham et al., 1997; Prasad and Freitas, 2006). Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless (Raskin et al., 1997). Phytoremediation is being developed as a potential remediation solution for contaminated sites. Soil and water contaminated with metals pose a major environmental and human health problem that is still in need of an effective and affordable technological solution. Nonradioactive As, Cd, Cu, Hg, Pb and Zn and radioactive Sr, Cs and U (referred to here as toxic metals) are the most environmentally important metallic pollutants. The phytoremediation of metals is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove toxic metals, including radionuclides, from soil and water (Raskin et al., 1994; Salt et al., 1995; Cunningham et al., 1995; Cunningham et al., 1996; Raskin et al., 1997).

Phytoremediation 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. The metals targeted for phytoremediation include Pb, Cd, Cr, As and various radionuclides. The harvested plant tissue, rich in accumulated contaminant, is easily and safely processed by drying, ashing or composting. The volume of toxic waste produced as a result is generally a fraction of that of many current, more invasive remediation technologies, and the associated costs are much less. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues (Raskin et al., 1997).

The use of plants for remediating heavy metal contaminated soils has multifold advantages as follows: (1) large scale application, as plant can be sown or planted in large areas, (2) growing plants is relatively inexpensive, (3) plants provide an aesthetic value to the landscape of contaminated sites, (4) phytoremediation process is environmentally friendly and ecologically safe, (5) some plant species used for phytoremediation can have potential economic returns which would offset the cost

of the technology, (6) plants concentrate the contaminant within their tissues, thereby reducing the amount of hazardous waste, and (7) concentrated hazardous waste requires smaller reclamation facilities for extracting the heavy metals.

Apart from the direct advantages, plants provide indirect benefits to the contaminated sites such as: (1) increased aeration of the soil which in turn enables microbial degradation of organic contaminants and microbe-assisted uptake of metal contaminants, (2) reduced top soil erosion due to plant stand, (3) enhancement of rhizospheric micro-fauna and flora for maintaining a healthy ecosystem (Saxena et al., 1999). At sites contaminated with metals, plants are used to either stabilize or remove the metals from the soil and ground water through phytoextraction and phytostabilization (USEPA, 1998).

5.2.1 Phytoextraction

Phytoextraction refers to the uptake and translocation of metal contaminants in the soil by plant roots into the aboveground portions of the plants (Fig. 3). Certain plants, called hyperaccumulators, absorb unusually large amounts of metals in comparison to other plants. One or a combination of these plants is selected and planted at a particular site based on the type of metals present and other site conditions. After the plants have been allowed to grow for some time, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfill, but the volume of ash will be less than 10% of the volume that would be created if the contaminated soil itself were dug up for treatment. Metals such as Ni, Zn, and Cu are the best candidates for removal by phytoextraction because it has been shown that they are preferred by a majority of the approximately 400 known plants that uptake and absorb unusually large amounts of metals.

The success of phytoextraction depends on two components, the contaminated soil and the plant species (Barceló and Poschenrieder, 2003; Ernst, 2005). The decisive soil factors are the degree of contamination, the metal availability for uptake into roots (bioavailability), the nutrient status and the water holding capacity of the soil (Ernst, 1996). The plant characteristics are the ability to explore the soil by the

roots and their associated (microorganisms), the genetically steered uptake efficiency by the roots via general and/or metal-specific transporters (Clemens et al., 2002; Ernst, 2005), the translocation of the metals from the root to the shoot, the accumulation in shoots without hampering the basic plant metabolism, the growth form, and the metal- resistance of the plant species or ecotype (Ernst, 1996; Lasat, 2002; Ernst, 2005).

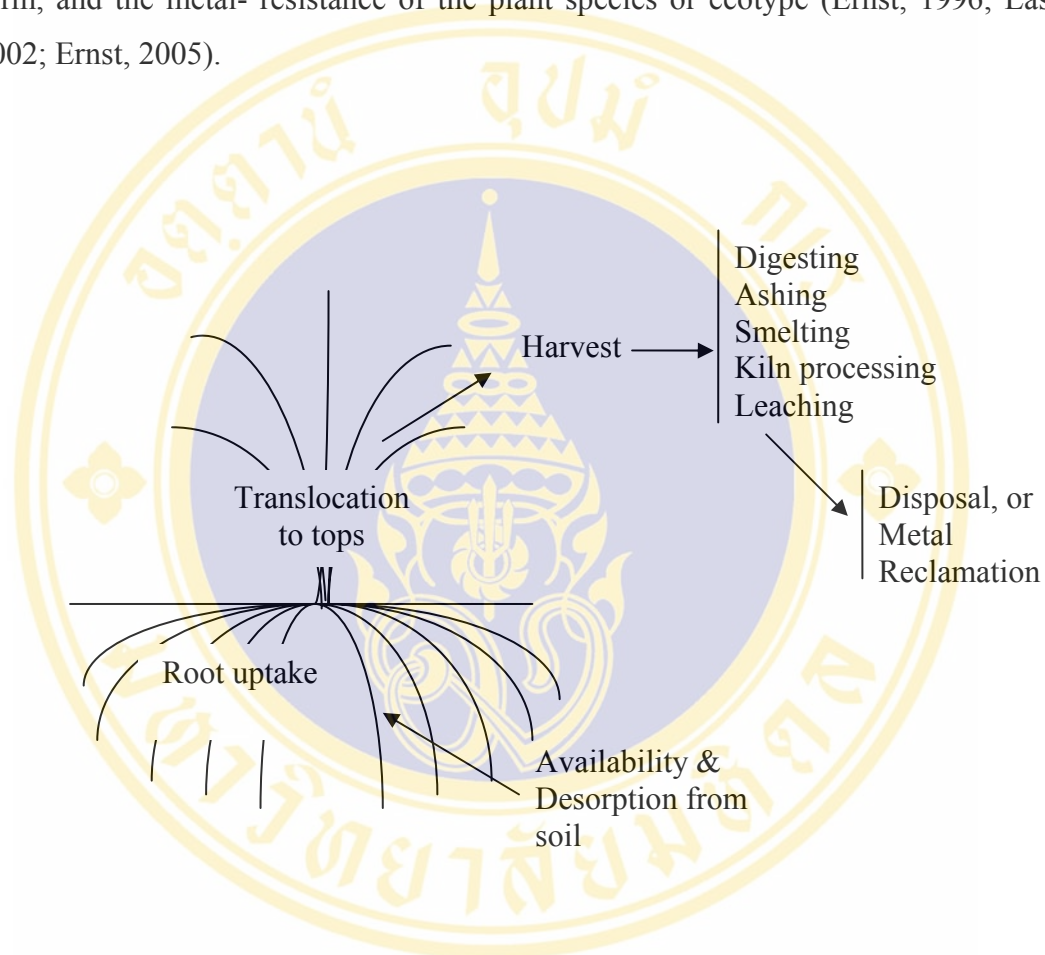


Figure 3. Phytoextraction schematic (Cunningham et al., 1995).

5.2.1.1 Factors influencing heavy metal availability

(a) pH

The chemical forms of heavy metals in soil are affected by modification to the soil pH. An increase in pH results in higher adsorption of Cd, Zn, Cu to soil particles and reduce the uptake of Cd, Zn, Pb by plants (Kuo et al., 1985; Sazena et al., 1999). On the other hand, acidification increases the metal absorption by plants through a reduction of metal adsorption to soil particles (Brown et al., 1994; Chaney et al., 1995).

(b) Redox potential

The redox potential of the soil is a measure of the tendency of the soil solution to accept or donate electrons. As the redox potential decreases, heavy metal ions are converted from insoluble to soluble forms, thus increasing bioavailability (Kabata-Pendias and Pendias, 1984). Therefore, lower pH and Eh of the soil would enhance the mobility of most metals.

(c) Cation Exchange Capacity (CEC)

The cation exchange capacity of the soil is a measure of the ability of the soil to retain metal ions. The cation exchange capacity increases with increasing clay content in the soil while the availability of the metal ions decreases (Kabata-Pendias and Pendias, 1984; Saxena et al., 1999).

(d) Soil type

The bioavailability of heavy metals in the soil also depends on the texture of the soil. A gradient of metal ion availability exists in varying soil types with the availability being lowest in clay soils, followed by clay loam, and finally loams and sand. The complexation of heavy metals with organic matter, humic acid in particular, has been well documented (Friedland, 1989). High organic matter content enhances the retention of the metals, drastically reducing the metal availability.

(e) Chelates

The addition of synthetic chelating agents to contaminated soil was shown to substantially increase the metal solubility in the soil (Salt et al., 1995; Cunningham and Ow, 1996; Kabata-Pendias and Pendias, 2000).

(f) Plant associated factor

1. Metal-chelating molecules can be secreted into the rhizosphere to chelate and solubilize soil-bound metal. Only iron-chelating compounds, termed phytosiderophores, have been studied well in grass species. These phytosiderophores are released in response to iron deficiency and can, in principle, mobilize Cu, Zn and Mn from soil. Mugineic and deoxymugeneic acids from barley and corn and avenic from oats are probably the best studied plant phytosiderophores (Kinnerseely, 1993; Raskin et al., 1997).

2. Roots can reduce soil-bound metal ions by specific plasma membrane bound metal reductase, which may increase metal availability.

3. Plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from the roots. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants (Crowley et al., 1991; Raskin et al., 1997; Lasat, 2002).

4. Roots can employ rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) to increase the bioavailability of metals.

5.2.1.2 Metal accumulation in plants

Metal uptake in relation to the external concentration of the metal may differ in different genotypes of plants. Baker (1981) proposed some basic strategies shown in Fig. 4. There are some plants, called excluders that have low uptake of the metal at quite high external metal concentrations. These plants have some kind of barrier to avoid uptake, but when metal concentrations become too high, this barrier loses its function, probably due to toxic action by the metal, and the uptake massively increases. Other plants, called accumulators, have high accumulation of metals at very low external metal concentrations. These plants have certain detoxification mechanisms within the tissue, which allow the plant to accumulate high amounts of metals. At high external metals concentration, however, these plants do not increase their uptake, probably due to competition between metal ions at the site of uptake. The third type of uptake characteristic is found in the indicator plants. These plants have a tissue concentration reflecting the external metal concentration, increasing uptake linearly with increasing metal concentration in the external medium (Greger, 1999).

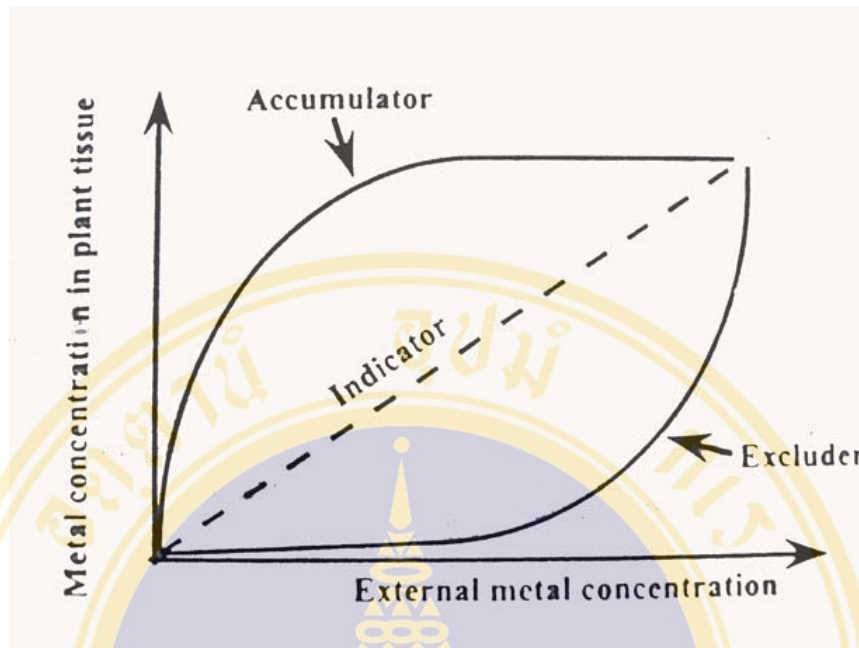


Figure 4. Plants with three different uptake characteristics (Baker, 1981).

5.2.1.3 Metal absorption by roots

Toxic metal elements enter the plants primarily via root absorption of the cation from the soil solution. Since root cells of many plant species have plasma membrane potential ranging from -100 to -200 mV (Huang, et al., 1992; Blaylock and Huang, 2000) there exists a very large electrical chemical potential gradient across the plasma membrane. The electrical chemical potential gradient across the plasma membrane of root cells can drive these metals into root cells. Furthermore, the metal activity of the listed elements in the cytoplasm of root cells must be maintained at very low levels because of the chemical characteristics of cytoplasm. The negative membrane potentials and the low intracellular metal activity could result in a large driving force for the influx of these metals into root cells. There is circumstantial evidence in the literature suggesting that Zn, Cu and Ni are transported into the root cell via a common transporter (Kochian, 1993). Using radiotracer techniques, plant physiologists have studied voltage-gated cation channels in the plasma membrane of root cells, and identified strongly voltage-gated Ca channels operating in the root-cell plasma membrane (Marshall et al., 1994; Blaylock and Huang, 2000). The voltage-gated channels are characterized by increased Ca transport activities as the transmembrane electrical potential is depolarized. These authors also found that the

activity of the voltage-gated divalent cation channels was rapidly inhibited by Cu, Cd, and Pb. The inhibition of the channel activity by Pb, Cu, and Cd could arise from the blockage of the channel by these metals, or competitive transport of Pb and Cd through the cation channels. Tomsig and Suszkiv (1991) have detected permeation of lead through a Ca channel. They also found that voltage-gated lead transport was blocked by nifedipine (a Ca channel blocker) and stimulated by a Ca channel agonist. These results suggested that Pb, Cd, and other divalent cations can be transported into root-cell via voltage-gated cation channels (Blaylock and Huang, 2000).

5.2.1.4 Mechanism of translocation

The mechanisms of metal translocation seem to be very similar to those for essential nutrients such as Fe or Ca; in as much as the transport from the root to the shoot takes place principally through the xylem. Prezemeck and Haase (1991) suggested phytochelatin-mediated metal binding in the xylem sap as a possible mechanism for metal translocation. However, Salt et al. (1995) found that the translocation of Cd through the xylem sap was not dependent on the production of phytochelatins in roots. Several types of compounds have been proposed to be involved in metal absorption and translocation in accumulator plant species (Saxena et al., 1999). Among these, low molecular weight chelators such as citrate (Lee et al., 1977) and free histidine in *Alyssum lesbiacum* (Krämer et al., 1996) have been reported to play an important role. The presence of Pb-EDTA complexes in the xylem exudates of *Brassica juncea* suggests a potential involvement of chelators in metal transport within the plants (Vassil et al., 1998). The mechanisms by which this may occur still remain unclear, as the accumulation of chelators such as organic acids upon metal exposure, might in part reflect disturbances in metabolic status of the plant (Godbold et al., 1984). Apart from these factors, heavy metal ion translocation within the plant is also influenced by other factors such as transpiration rate, root uptake, radial transport, and xylem loading (Salt et al., 1995).

5.2.1.5 Phytoextraction of lead

In phytoextraction of lead-mined soil, soil lead is taken into plant roots, translocated into the top of the plant, and removed by plant harvesting. Several

technical parameters affect the efficiency of this process. First, the low solubility of lead in soils causes lead to be unavailable for plant uptake. Often at a site, the total lead level is quite high, but the fraction of lead availability for root uptake is exceeding low (Cunningham and Berti, 2000). Predominant insoluble Pb compounds in the soil include Pb phosphates, Pb carbonates, and Pb (hydr)oxides. Table 4 presents some of the Pb minerals expected in soils and their respective formation constants. Second, the lead translocation in plants to harvestable plant portions is rather poor. Finally, lead is toxic to the plant tissue. Along with these technical concerns, there are still a number of regulatory, economic and logistical questions that must be answered adequately before phytoextraction is accepted as a cost-effective and viable remediation technology. At present, no lead-contaminated site has been fully remediated using phytoextraction.

In the current concept of phytoextraction, the removal process will only be practical, relatively timely, and effective if the plant has a high biomass and can accumulate at least 1% Pb in its above ground tissue. At one time, achieving such high lead concentrations in plant tissue was thought to be a remote possibility at best.

Table 4. Formation constants of lead compounds (Lindsay, 1979; Blaylock and Huang, 2000).

$\text{Pb}_3(\text{PO}_4)_2 + 4\text{H}^+ \leftrightarrow 3\text{Pb}^{2+} + 2\text{H}_2\text{PO}_4^-$	-5.26	
$\text{Pb}_4\text{O}(\text{PO}_4)_2 + 6\text{H}^+ \leftrightarrow 4\text{Pb}^{2+} + 2\text{H}_2\text{PO}_4 + \text{H}_2\text{O}$	2.24	
$\text{Pb}_5(\text{PO}_4)_3\text{OH} + 7\text{H}^+ \leftrightarrow 5\text{Pb}^{2+} + 3\text{H}_2\text{PO}_4 + \text{H}_2\text{O}$	-4.14	hydroxypyromorphite
$\text{Pb}_5(\text{PO}_4)_3\text{Br} + 6\text{H}^+ \leftrightarrow 5\text{Pb}^{2+} + 3\text{H}_2\text{PO}_4 + \text{Br}^-$	-19.49	bromopyromorphite
$\text{Pb}_5(\text{PO}_4)_3\text{Cl} + 6\text{H}^+ \leftrightarrow 5\text{Pb}^{2+} + 3\text{H}_2\text{PO}_4 + \text{Cl}^-$	-25.05	chloropyromorphite
$\text{Pb}_5(\text{PO}_4)_3\text{F} + 6\text{H}^+ \leftrightarrow 5\text{Pb}^{2+} + 3\text{H}_2\text{PO}_4 + \text{F}^-$	-12.98	fluoropyromorphite
$\text{PbCO}_3 + 2\text{H}^+ \leftrightarrow \text{Pb}^{2+} + \text{CO}_2 + \text{H}_2\text{O}$	4.65	cerussite
$\text{PbSO}_4 \leftrightarrow \text{Pb}^{2+} + \text{SO}_4^{2-}$	-7.79	anglesite
$\text{PbS} \leftrightarrow \text{Pb}^{2+} + \text{S}^{2-}$	-27.51	galena

The discovery of other metal-hyperaccumulating plants promoted the search for hyperaccumulators of lead with similar tissue concentrations. The literature defines a lead hyperaccumulating plant as one that can accumulate at least 1,000 mg

Pb kg⁻¹ or 0.1% in leaf dry matter (Baker, 1994). The lead hyperaccumulators are particularly rare which are reported only 14 taxa (Baker et al., 2000) (Table 5). Despite their fascinating biochemistry, many hyperaccumulators have extremely low biomass and slow growth rates. Additionally, because these plants are mostly uncultivated, their agronomic, breeding and disease and pest control requirements are not optimized. Thus far, these constraints have made it difficult to translate the hyperaccumulating phenotype of these plants directly into a remediation strategy (Cunningham and Berti, 2000).

Table 5. Some hyperaccumulators of lead.

Species	Locality	Maximum shoot conc. (mg kg ⁻¹)	References
<i>Thlaspi caerulescens</i>	England	2740	Shimwell and Laurie, 1972
<i>T. rotundifolium</i> ssp. <i>cepaifolium</i>	Italy	8200	Reeves and Brooks, 1983
<i>Minuartia verna</i>	U.K.	20000	Barry and Clark, 1978
<i>Agrostis tenuis</i>	U.K.	13490	Willian et al., 1977
<i>Festuca ovina</i>	U.K.	11750	Barry and Clark, 1978

5.2.2 Phytostabilization

Some soils are so heavily contaminated that removal of metals using plants would take an unrealistic amount of time. In-place inactivation stabilizes soil both chemically and physically through the use of soil amendments and a vegetative cover (Cunningham and Berti, 2000). Phytostabilization techniques are most appropriate for immobile materials and large surface areas. The technique is currently acceptable for remediation at mining sites (Cunningham et al., 1995).

Phytostabilization is the use of certain plant species to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants or rhizosphere. This process reduces the mobility of the contaminant and prevents migration to the ground water or air, and it reduces bioavailability for entry into the food chain. This technique can be used to reestablish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations in surface soils or physical disturbances to surficial materials. Metal-tolerant species can be used to

restore vegetation to the sites, thereby decreasing the potential migration of contamination through wind erosion and transport of exposed surface soils and leaching of soil contamination to ground water. Figure 5 shows the process of phytoextraction and phytostabilization.

5.2.2.1 Soil Amendment

Soil amendments alter the existing heavy metals chemistry in the soil and reduce the biological availability of heavy metals by inducing the formation of very insoluble forms. Soil amendments should (1) be inexpensive, (2) be easy to handle and apply, (3) be safe to the workers handling the amendment, (4) be compatible with and nontoxic to the plants selected for revegetation (5) be readily available or easy to produce, and (6) not cause additional environmental impact to the site. Some amendment may have secondary benefits such as supplying plant nutrients or increasing soil moisture holding capacity (e.g. phosphate fertilizers, organic materials). The mechanisms by which different amendments alter the form of metal contaminants in soil may vary with different soil amendments and contaminants, and in some cases, are largely speculative (Berti and Cunningham, 2000). The soil amendments for lead phytostabilization are shown in Table 6. Phosphate amendments include phosphoric acid, phosphate fertilizers, or byproducts high in phosphates may enhance the formation of highly insoluble forms of lead. Phosphate addition may produce pyromorphites, which are Pb phosphate minerals that are extremely insoluble even under very acidic conditions (Ma et al., 1993; Zhang et al., 1997; Berti and Cunningham, 2000). Hydrous Fe oxide materials have a great capacity to adsorb lead and reduce the availability. Organic materials such as sewage sludge and refuse or manure compost can be used for reducing metal availability and to certain extent as a slow release nutrient source (Wong, 2003). Table 7 shows the organic matter and nutrient content of some common organic materials which could be used to lower metal availability, in addition to remediating the physical and chemical properties of the spoils, and the provisions of plant nutrients (Bradshaw and Chadwick, 1980; Wong, 2003). In addition to organic amendments, inorganic amendments are used to improve substrate characteristics. These include quarry waste, pulverized refuse, pulverized fuel ash, etc.

Table 6. Soil amendments proposed for phytostabilization and their suggested mode of lead inactivation (Cunningham and Berti, 2000).

Amendment type		Suggested mode of inactivation
Phosphate materials	H ₃ PO ₄ , apatite, calcium orthophosphate, Na ₂ HPO ₄ , KH ₂ PO ₄ , and other phosphate fertilizers	Formation of pyromorphites (e.g., chloropyromorphite)
Hydrous iron oxides	Iron rich, corroded steel shots	Sorption of Pb on oxide surface exchange sites, or formation of Pb-Fe compounds
Inorganic clay minerals	Synthetic zeolites, aluminosilicate byproducts from burning of coal refuse	Sorption of Pb on mineral surface exchange sites, or incorporation into the mineral structure
Organic materials	Manures, composts, sludges, and other biosolids	Sorption of Pb on exchange sites, or incorporation into the organic material

Table 7. Nutrient and organic matter contents of some organic soil amendments (% dry solids) (Bradshaw and Chadwick, 1980; Wong, 2003).

Material	Nitrogen	Phosphorus	Potassium	Organic matter
Farmyard manure	0.6	0.1	0.5	26
Poultry manure	2.3	0.9	1.6	68
Sewage sludge	2.0	0.3	0.2	45
Domestic refuse	0.5	0.2	0.3	65
Straw	0.5	0.1	0.8	95

5.2.2.2 The role of plants

Plants play an important role in phytostabilization by physically stabilizing the soil with dense canopies and rooting system to stabilize the soil against rain impact and erosion or leaching, as well as restrict off-site migration of the contaminants (Cunningham and Berti, 2000). Currently, the role of plants in phytostabilization does not extend to chemically altering the form of the contaminants. Plants chosen for phytostabilization should be poor translocators of metal contaminants to aboveground

plant tissue that could be consumed by humans or animals. Plants should also be tolerant of the soil metals level as well as the other initial site conditions (e.g. soil pH, salinity, soil structure, water content). The plants must grow quickly to establish ground cover. Additionally, the plants must be easy to establish and care for, and have a relatively long life or be able to self-propagate (Berti and Cunningham, 2000).

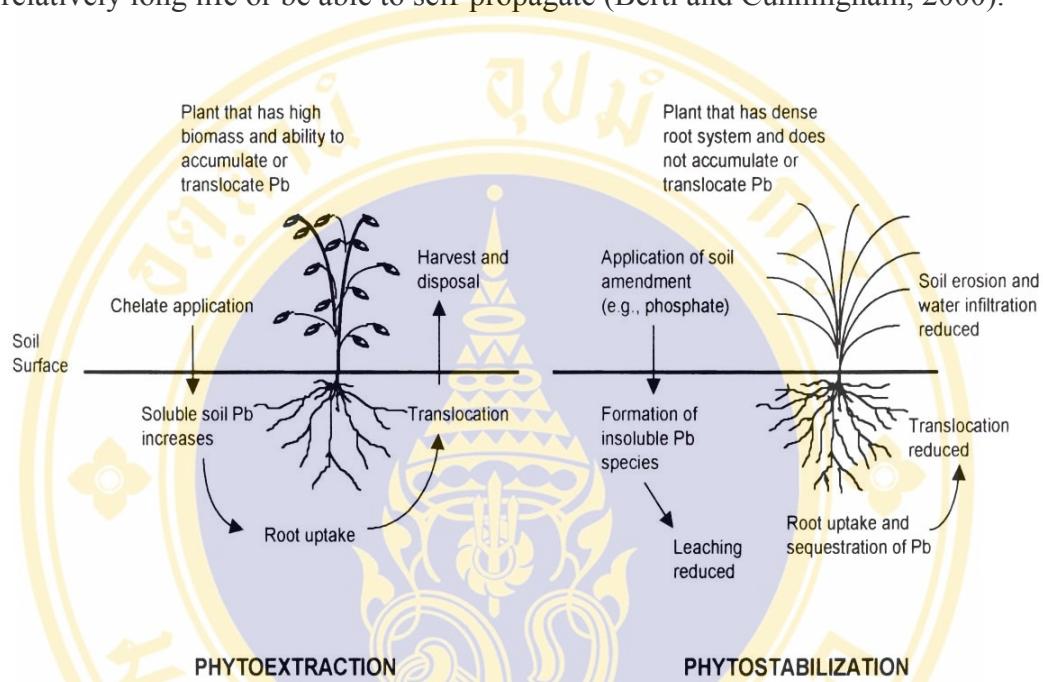


Figure 5. Comparison of the process involved in phytoextraction and phytostabilization (Cunningham and Berti, 2000).

The stabilization of metalliferous mine wastes in the UK utilized local metal-tolerant plant species. By extensive fertilization and planting of the endemic metal-tolerant varieties, they were able to stabilize the site and establish excellent vegetative cover. Based on these results, three cultivars of different grasses were made commercially available: *Agrostis tenuis* cv Goginan for acid lead/zinc wastes, *Festuca rubra* cv Merlin for calcareous lead/zinc wastes, and *Agrostis tenuis* cv Parys for copper wastes (Salt et al., 1995).

Vetiver grass (*Vetiveria zizanioides*) has a massive finely structured and deep root system capable of reaching 3-4 m in the first year. In Australia vetiver has been successfully used to stabilize mining overburden and highly saline, sodic and alkaline (pH 9.5) tailing of coal mines (Radloff et al., 1995; Truong and Baker, 1998).

For gold mine tailings in Australia, fresh gold tailing are typically alkaline (pH 8-9), low in plant nutrients, and very high in free sulphate (830 mg kg^{-1}), sodium and total sulfur (1-4%). Vetiver established and grew very well on these tailings without fertilizers, but growth was improved by the application of 500 kg ha^{-1} of diammonium phosphate. Due to high sulfur content, old gold mine tailings are often extremely acidic (pH 2.5-3.5), high in heavy metals and low in plant nutrients. Revegetation of these tailings is very difficult and very expensive and the bare soil surface is highly erodible. Field trial was conducted on two old gold tailing sites (pH 2.7-3.6; sulphate 0.37-3.6%; total sulfur 1.31-3.75). Results from both sites indicated that with adequate supplies of nitrogen and phosphorus fertilizers (300 kg ha^{-1} of DAP), excellent growth of vetiver was obtained at the site pH = 3.6 without any liming. But the addition of 5 t ha^{-1} of agricultural lime significantly improved vetiver growth. In another site (pH = 2.7) although vetiver survived without liming, the addition of lime (20 t ha^{-1}) and fertilizer (500 kg ha^{-1} of DAP) improved vetiver growth greatly (Truong, 1999).

Recently, it has been found that vetiver grass was the best plant species (in terms of biomass production and coverage) when compared with other three grass species (*Paspalum notatum*, *Cynodon dactylon* and *Imperata cylindrica*) (Shu et al., 2001) and has better growth performance than the two legume species (*Sesbania rostrata* and *S. sesban*) used for revegetating Pb/Zn mine tailing in South China (Yang et al., 2003). Recent research also indicated that *V. zizanioides* has higher tolerance to acid mine drainage (AMD) from a Pb/Zn mine and wetland microcosms planted with this grass can effectively adjust pH and remove SO_4^{2-} , Cu, Cd, Zn, Pb, and Mn from AMD (Shu, 2003; Prasad, 2006). Xia (2004) reported that vetiver was the best plant species (in terms of survival rate, biomass production and coverage) when compared with *P. notatum*, *Stenotaphrum secundatum*, *Pennisetum glaucum* and *P. purpureum* for rehabilitation of the degraded ecosystem of an oil shale mined land of Moaming Petro-Chemical Company located in Guangdong Province, China.

6 Plants

6.1 *Vetiveria zizanioides* (L.) Nash (Vetiver grass)

The classification of vetiver grass is as follows:

Kingdom Plantae -- Plants

Subkingdom Tracheobionta -- Vascular plants

Superdivision Spermatophyta -- Seed plants

Division Magnoliophyta -- Flowering plants

Class Liliopsida -- Monocotyledons

Subclass Commelinidae

Order Cyperales

Family Poaceae -- Grass family

Genus *Vetiveria* – vetiver grass

Species *Vetiveria zizanioides* (L.) Nash

6.1.1 Morphological characteristics of vetiver grass

(I) Culm

Vetiver grass is a prolific tiller growing naturally in clumps with thin, long, and erect leaves. The vetiver clumps may grow densely tufted in a big cluster or scattering over the nearby spaces. The base of the clump is dense which makes it obviously distinctive from other types of grass. With the bases of the leaves laying on top of one another, the culm base looks flat. The genuine culm is a tiny shoot hidden in the leaf sheath at the culm base near the soil. The growth of vetiver is determined by tillering which occurs regularly by producing new shoots on the sides, making the clump bigger and bigger. Normally, vetiver grass has a short culm with unclear joints and pedicels. Aerial branching and culm rising slightly above the ground is not commonly found in fertile conditions, but with vetiver tillers grown in bags, in plots with old tillers or in critical conditions.

(II) Leaf

Vetiver leaves will sprout from the bottom of the clump. Each blade is narrow, long and coarse. The edge of the blade is parallel and the apex is acute. Particularly on old leaves, the edge and midrib are spinulose. Normally, the base and the middle of the blade have few spines whereas the apex has numerous spines. All spines are pointing diagonally towards the apex. The ligule at the base of the leaf is observable in a shrinkingly bending form with short silky hair which sometimes cannot be noticed.

(III) Roots

Vetiver roots are important and the most useful part. Most grass have fibrous roots which spread out from the underground part of the culm and hold the soil in an horizontal pattern. The roots that penetrate vertically into the soil are not deep. In contrast, the root system of vetiver grass does not expand horizontally but penetrates vertically deep into the soil, whether it be the main roots, secondary roots or fibrous roots.

(IV) Inflorescence/spikelets

Vetiver inflorescence is erect and it appears in the form of a panicle. The panicle and the stalk which is round and long is about 100-150 cm high above the ground. However, for a mature culm, the stalk can be as high as 200 cm. The inflorescence or the panicle alone is about 20-40 cm high and can spread out at a maximum width of 10-15 cm. The inflorescence of *V. zizanioides* are mostly purple, the color which is an ordinary attribute of this species. The spikelet appears in pairs with similar features and size, except for the base of the stalk which has 3 spikelets. Each pair consists of both sessile and pedicelled spikelets. The sessile spikelet is at the middle, whereas, the pedicelled one is at the tip. Each spikelet is similar in appearance to a spindle. The edge is parallel and oval. The cuneate apex is 1.5-2.5 mm. wide and 2.5-3.5 mm. long. The surface of the back of the spikelet is rough and consists of minute spines, especially at the edge which can be clearly seen. The lower part of the spikelet is smooth.

(V) Seed and seedling

After breeding, the sessile spikelet which is a hermaphrodite flower produces seeds. Each seed is light brown and in spindle shape. The surface is smooth and the apex and base is round. The inner texture is like sticky flour which turns stiff when exposed to strong wind, concentrated sunlight or other critical climatic conditions. Stiffness disables the seed to enlarge, thus impairing its chance to germinate. Since the seed can germinate only within a limited period of time and some ecotypes which are imported do not seed at all, the vetiver grass cannot spread like a serious weed.

There are two species of vetiver grass in Thailand; *Vetiveria zizanioides* Nash, syn. *Chrysopogon zizanioides* (L.) Roberty and *Vetiveria nemoralis* A. Camus. *V. zizanioides* is commonly found in nature grows in various conditions and adapts

suitably to those conditions while *V. nemoralis* or local vetiver has a limited scope of distribution. It is found only in Southeast Asia covering the countries of Thailand, Laos, Cambodia, Vietnam and Malaysia. Moreover, there are no records of its uses. *V. nemoralis* is commonly found in dry areas or in soil conditions with good water drainage potential in every region of Thailand, especially in dipterocarp forests. However, there are only a few in the South.

V. zizanioides is a kind of plant that can suitably and rapidly adapt to the environment. Some ecotypes of *V. zizanioides* in Thailand are as follows (Fig. 6):

1) Sri Lanka: grows well in areas with laterite soil, cool climate and shade; produces 10 culms per clump; each clump is rather loose and has a small base with a diameter of 11 cm and a height of 101 cm; the shoot is round and the internode fastly elongates; the mature leaf is small; the lower surface of the leaf is a little bit white similar to that of *V. nemoralis*; the spikelet is purple and starts yielding about one month after planting; multiplication is simple in areas with high humidity and little sunlight; not resistant to the rotten base disease.

2) Kamphaeng Phet 2: grows well in areas ranging from sandy soil to laterite soil; produces 18 culms per clump; each clump is rather loose and broadly tufted with a diameter of 8 cm and a height of 94 cm; the shoot is round and small; the internode fastly elongates; the leaf is dark green while the lower surface is white; the spikelet is reddish purple and starts appearing about half a month after planting; gives good fresh weight; contains more nutritional value as animal feeder than other ecotypes, both in terms of protein content (5.2% dry weight) and digestible dry material; appropriate time for cutting is 4 weeks.

3) Surat Thani: grows well in loose clay soil and laterite soil; produces 22 culms per clump; each clump is loose and broadly tufted with a diameter of 13 cm and a height of 108 cm; the shoot is plump; the internode rapidly elongates; the leaf is light green while the lower surface is white; the spikelet is reddish purple and can be seen after one month of planting.

4) Songkhla 3: grows well in areas ranging from loose clay soil to laterite soil; produces 24 culms per clump; each clump is loose with a diameter of 13 cm and a height of 112 cm; the shoot is round and plump; the internode fastly elongates; the

leaf is light green while the lower surface is white; the spikelet is reddish purple and starts yielding about one and a half months after planting.



Figure 6. Some ecotypes of *V. zizanioides* in Thailand; (A) Sri Lanka, (B) Kamphaeng Phet, (C) Surat Thani, (D) Songkhla.

The vetiver grass has rather unique morphological and physiological characteristics. Vetiver grass is a fast-growing plant that tolerates various extreme soil conditions including prolonged drought, flood, submergence, extreme temperature (-10 to 48 °C), and a wide range of soil acidity and alkalinity (pH from 3 to 10.5)

(Wong, 2003). It has been widely known for its effectiveness in erosion and sediment control (Truong and Baker, 1998). Furthermore, vetiver also shows a very strong resistance to salt, and soil EC_{se} values resulting in 90 and 50% biomass yield relative to control are 6.4 and 20.9 dS m⁻¹ (Xia, 2001). The previous studies have shown that vetiver grass can grow well in soils contaminated with multiple elements at high concentrations such as those found at coal, cadmium, and gold mining sites (Truong and Baker 1998; Roongtanakiat and Chairroj, 2002) and can accumulate relatively high concentrations of lead in root (Chantachon et al., 2004). Vetiver is sterile and noninvasive; it does not compete with native vegetation and, as a nurse plant, it fosters the voluntary return of native plants (Prasad, 2006).

6.1.2 Vetiver oil

Vetiver root tissues contain oil-producing cells, responsible for its characteristic odour (Peyron, 1989; Massardo et al., 2006). These secretory cells are localized in the first cortical layer outside the endodermis of mature roots (Kartusch and Kartusch, 1978). Essential oil can be detected in the inner bark within the cortical layer, where lysigen lacunae are also observed, providing the true storage vessel for the essential oil of vetiver root (Viano et al., 1991).

Vetiver root aromatic oil has been used as a repellent for the control of termites (Zhu et al., 2001). In addition, vetiver grass has been cultivated for their odorous roots that contain the essential oil used extensively in perfumery, cosmetics, and biomedical utilization (Guenther, 1950; Anderson, 1970; Maffei, 2002; Wong, 2003; Massardo et al., 2006). The volatile oil from vetiver roots is a complex mixture of sesquiterpene alcohols and hydrocarbons, and is also a very viscous oil with an extremely low volatility (Lavania, 2003). Slow evaporation rate of vetiver oil coupled with its pleasant aroma makes it a perfume by itself. Currently, the main vetiver oil producing countries are Haiti, Indonesia and Réunion island (Chomchalow, 2000). Vetiver roots 15-18 months old are most suitable to realize high concentration and good quality of essential oil.

Numerous studies have been published since 1940 on the essential oils of *V. zizanioides* (Champagnat et al., 2006). Most have reported on the structure determination and synthesis of some compounds of vetiver oils (Marshall and

Anderson, 1967; Marshall and Johnson, 1968, 1970; Anderson, 1970; Nigam and Komae, 1968). Weyerstahl et al. (1996, 2000) only studied the constituents of Haitian vetiver oil. Chemical composition of vetiver oil is extremely complex, containing some 100 sesquiterpene-type compounds and their derivatives (Lavania, 2003).

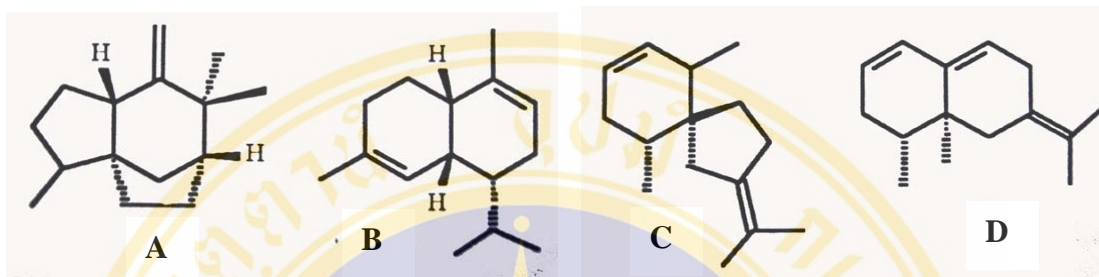
A typical analysis of vetiver oil indicates three major fractions: the lower boiling or sesquiterpene hydrocarbon fraction, the intermediate fraction containing the bulk of oxygenated derivatives, and the third fraction represented by khusimol fraction, which contains the main alcohol component in the vetiver oil (Lemberg and Hale, 1978; Massardo et al., 2006). Champagnat et al. (2006) reported that the main hydrocarbons in vetiver oil were β -vetivenene, β -vetispirene, α -amorphene and khusimene. The three major ketones were α -vetivone, β -vetivone and khusimone. The major alcohols were khusimol and vetiselinol (Fig. 7). Three primary alcohols, which are the major constituents, are responsible for the base note of vetiver oil (Weyerstahl et al., 2000). Demole et al. (1995) and Lavania (2003) reported that α -vetivone, β -vetivone and khusimol can be considered as the 'finger print' of vetiver oil.

Martinez et al. (2004) evaluated different methods of oil extraction from Brazilian vetiver roots and found that the pretreatments of the vetiver roots before hydrodistillation did not significantly increase the yield and rate of extraction. Supercritical carbon dioxide extraction resulted in high yield (3.2%) in significantly less time than the other methods (Table 8). But higher extraction yield obtained from supercritical carbon dioxide extraction presented the large amounts of nonvolatile compounds, such as fatty acids and waxes, so in terms of volatile oil, hydrodistillation resulted in higher yields.

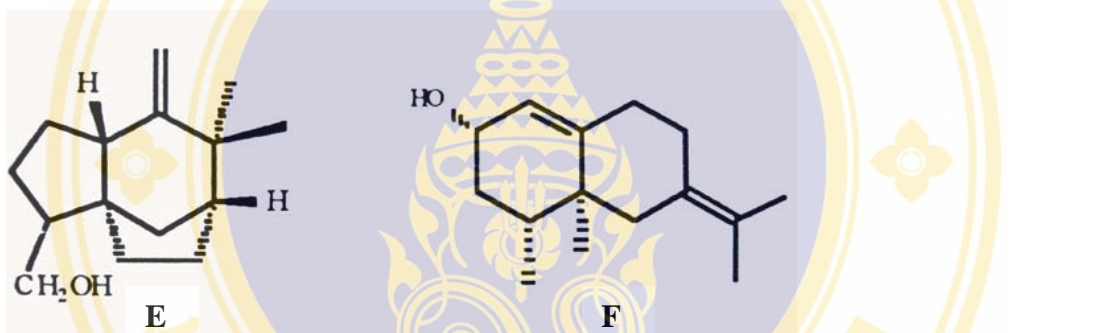
However, there are relatively few papers reporting the effects of environmental conditions on vetiver oil yields and composition. Lemberg and Hale (1978) and Champagnat et al. (2006) studied on the composition of vetiver oils of different geographical origins. Champagnat et al. (2006) indicated that no meaningful differences were observed comparing the composition of vetiver oil from nine geographical origins. Adams et al. (2003) reported on the oil composition in relation to DNA-fingerprints and found that all of the cultivars (13 cultivars) had khusimol

(14-31%), (*E*)-isovalencenol (10-16%), β -vetivone (2-6%) and α -vetivone (3-6%) as main components.

Hydrocarbons



Alcohols



Ketones

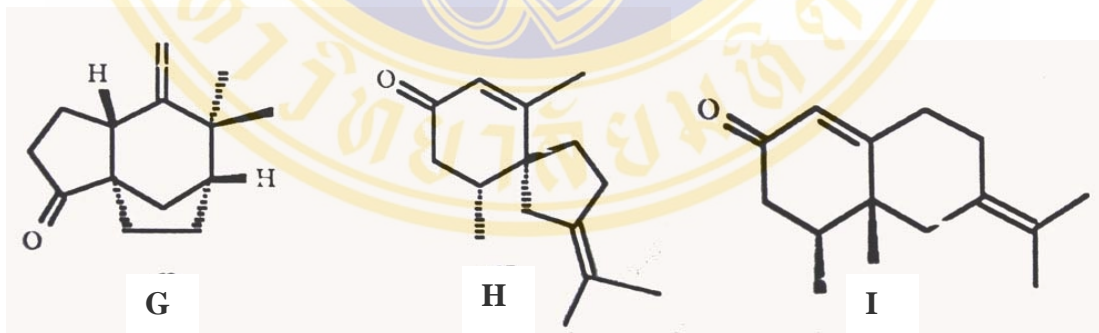


Figure 7. Structure formula of: (A) khusimene, (B) α -amorphene, (C) β -vetispirene, (D) β -vetivenene, (E) khusimol, (F) vetiselinenol, (G) khusimone, (H) β -vetivone, (I) α -vetivone (Champagnat et al., 2006).

Table 8. Yields and times of extraction from Brazillian vetiver roots with different methods (Martinez et al., 2004).

Method	Extraction time (h)	Yield (%)
Hydrodistillation (HD)	16	1.8 ± 0.1
HD-N ₂	16	1.8 ± 0.1
HD-NaOH	16	1.8 ± 0.1
HD-enzymes	16	1.9 ± 0.1
HD-NaOH and enzymes	16	1.7 ± 0.1
SFE (200 bar, 40 °C)	1	3.2 ± 0.2

In addition, the correlations between vetiver oil production and cultivation conditions have been investigated (Adams et al., 2004; Pripdeevech et al., 2006). Adams et al. (2004) reported that a major difference between the oils of the cleansed and the non-cleansed, normal, vetiver was the very low oil yield from the cleansed (meristem tissue cultured) plant. Pripdeevech et al. (2006) indicated the similar results that the higher yield was obtained in plants grown in normal soil added with microbes. This may be due to the intracellular bacteria that were involved with the essential oil cells in the glands of vetiver roots. Dethier et al. (2004) reported that soil fertilization increased vetiver oil yields dramatically without changing the composition. Massardo et al. (2006) state that vetiver oil production is closely related to the metabolic activity of plant roots, which is affected by changes in environmental temperatures. During winter the drop in temperature causes a decrease in plant metabolic activities and hence a decrease in oil production (Massardo et al., 2006).

6.2 *Thysanolaena maxima* (Tiger grass)

The classification of *T. maxima* is as follows:

Kingdom Plantae -- Plants

Subkingdom Tracheobionta -- Vascular plants

Superdivision Spermatophyta -- Seed plants

Division Magnoliophyta -- Flowering plants

Class Liliopsida -- Monocotyledons

Subclass Commelinidae

Order Cyperales

Family Poaceae -- Grass familyGenus *Thysanolaena*Species *Thysanolaena maxima* (Roxb.) O. Kuntze

T. maxima occurs from Indo-China and China and throughout Southeast Asia. It can thrive at low to medium elevations but can grow faster in higher elevation from about 2500 to 5000 feet (Rhind, 1945). Normally, it grows in open areas particularly in logged-over areas, mountain slopes and ravines. *T. maxima* is a strongly tufted, very robust perennial grass with erect or slightly spreading solid bamboo-like culms up to 3.5 m tall. Leaf-sheath is hairy along outer margin; ligule is a scarious membrane, 1-2 mm long; leafblade is lanceolate-acuminate, 30-65 cm x 3-7.5 cm, base broad and rounded or subcordate, margins scaberulous, conspicuously glaucous beneath. Inflorescence a terminal huge and drooping panicle, up to 140 cm long, well exerted, branches divided and subdivided into many branchlets; spikelets are awnless, short pedicelled, falling with part of the pedicel, often in pairs on a common peduncle, 2-flowered; lower glumes clasping. Caryopsis is subglobose to ovoid, 0.6 mm long, reddish-brown in color. Young leaves and stem tips are used to feed cattle and buffaloes. Its large inflorescences are used in making brooms. The grass is occasionally planted for ornamental purposes and as a hedge.



Figure 8. *Thysanolaena maxima* (Roxb.) O Kuntze.

CHAPTER IV

MATERIALS AND METHODS

1. Uptake and accumulation of lead by plants from the Bo Ngam lead mine

1.1 Site description

Bo Ngam lead mine is located in Thong Pha Phum District, Kanchanaburi Province, situated in between north latitude $14^{\circ}55'$ - $14^{\circ}60'$ and east longitude $98^{\circ}55'$ - $98^{\circ}60'$. The annual temperature is 26.7°C and annual rainfall is 1507.8 mm (the highest in June and lowest in January, November and December). The area of mine is 47.84 km^2 . Lead in this area is in the form of cerussite (PbCO_3).

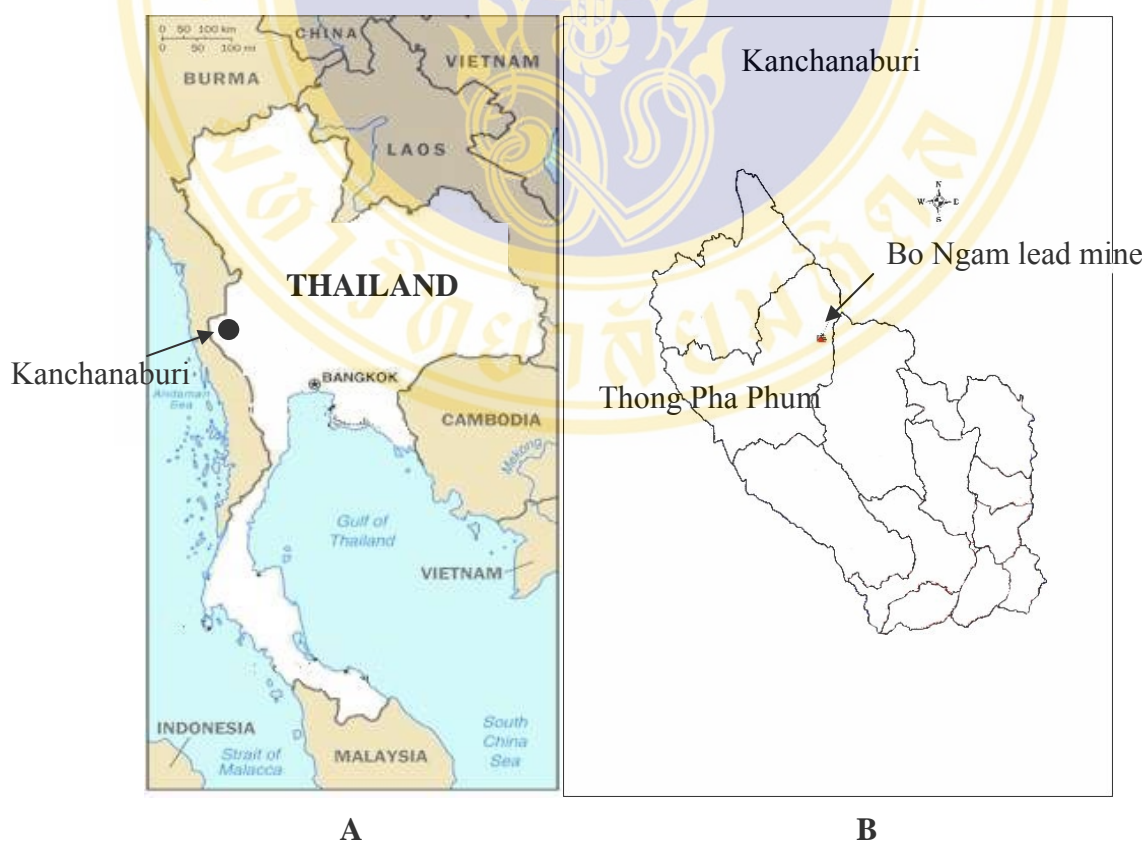


Figure 9. (A) The map of Thailand showing Kanchanaburi province. (B) Bo Ngam lead mine area.

1.2 Soil sampling and analysis

Five sampling sites were selected in Bo Ngam lead mine.

Site 1 Open pit area (N 14°59'49.9'' E 98°57'29.4'')

Site 2 Stockpile area (N 14°59'48.9'' E 98°57'25.3'')

Site 3 Ore dressing plant area (N 14°57'09.3'' E 98°55'20.8'')

Site 4 Tailing pond area (N 14°57'07.2'' E 98°55'21.9'')

Site 5 Natural pond area (N 14°56'56.1'' E 98°55'22.5''; uncontaminated site)

Samples of soil were collected from five sites during October 2003 to October 2004. The soil samples were collected at the surface (0-10 cm), subsurface (10-40 cm) and around the plant roots (Fig. 10). After collection, they were dried at 60 °C for 48 h, then ground into fine powder and sieved through a 0.28 mm nylon sieve. Surface and subsurface soil samples were characterized for: pH and electrical conductivity (EC) by using pH meter and EC meter accordingly, organic matter by Walkley-Black titration (Walkley and Black, 1934), total N by Kjeldhal method (Black, 1965), available P by Bray II method (Bray and Kurtz, 1945), available K by atomic absorption spectrophotometer after extracted with NH₄OAc (International Center for Agricultural Research in the Dry Areas (ICARDA), 2001) and texture using the hydrometer method (Allen et al., 1974). Lead concentrations were determined in the laboratory of the Biology Department, Faculty of Science, Mahidol University, and also verified by the laboratory of the Department of Primary Industries and Mines, Bangkok.



Figure 10. Soil samples collected from surface and subsurface.

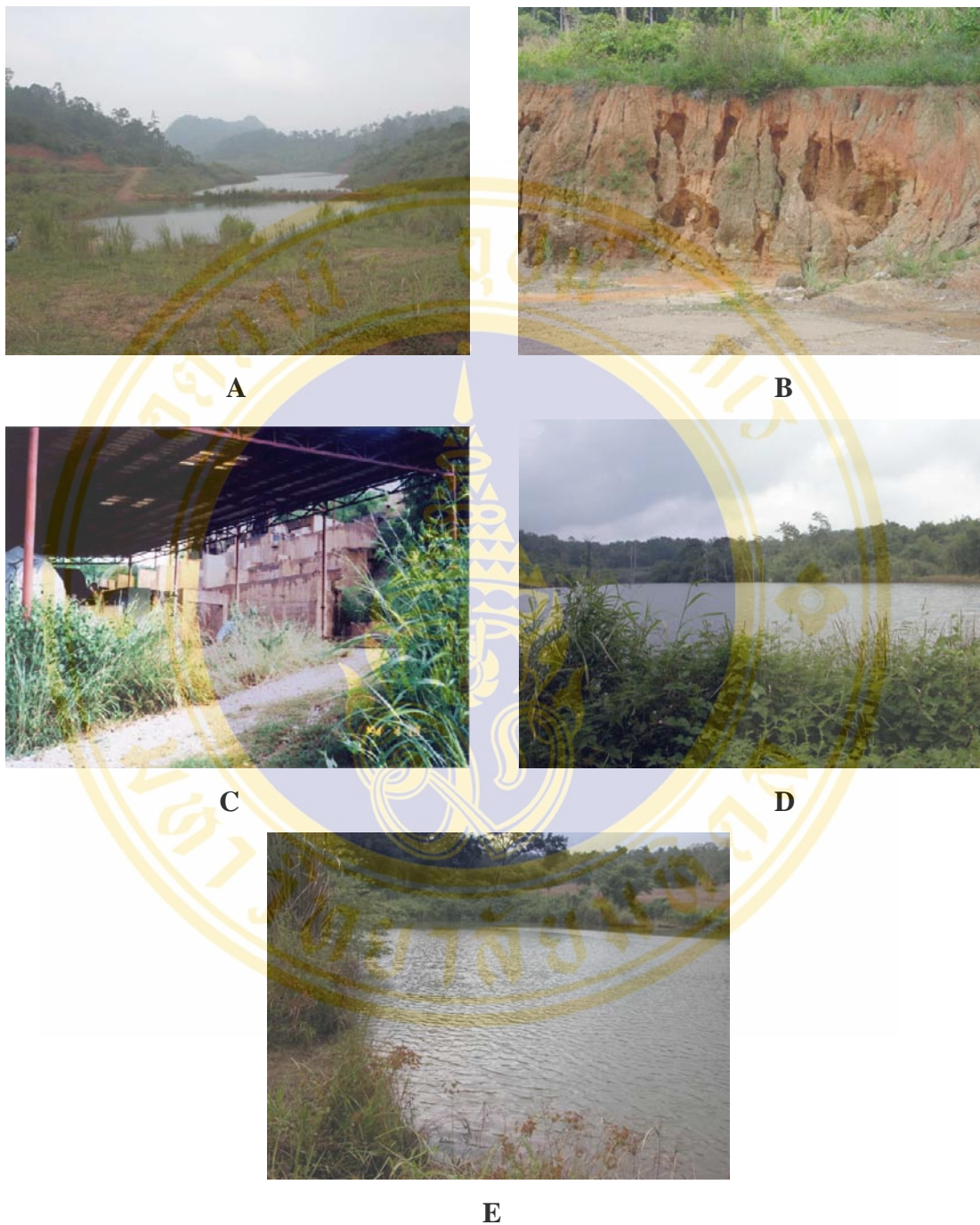


Figure 11. Five sampling sites at Bo Ngam lead mine; (A) Open pit area, (B) Stockpile, (C) Ore dressing plant area, (D) Tailing pond area, (E) Natural pond area.

To determine the concentration of lead in soil, 0.5 g of soil sample was digested with HNO_3 (APHA, 1998). After digestion, lead concentration was measured by a flame atomic absorption spectrophotometer (Varian SpectraA 55 B). The extractable lead in soil was determined by the sequential extraction method which included two phases: phase 1, exchangeable fraction extracted by 0.01 M $\text{Ca}(\text{NO}_3)_2$ and phase 2, acid-soluble fraction extracted by 0.11 M CH_3COOH (Shiowatana et al., 2001).

1.3 Plant sampling and analysis

Plant samples were collected from the same location as the soil samples. For herbs, the whole plants were taken, but for shrubs only shoots were collected. At least three individuals of each plant species were randomly collected within the sampling area. Plant samples for identification only were kept in a plant press and separated from those used for analysis. Plant classification followed that of Smitinand (1980). Plant identification was confirmed by the Department of Botany, Kasetsart University, Thailand.

After collection, soil around the plant roots was separated and analyzed for lead concentration, then each plant sample was thoroughly washed in running tap water for 5 min, and with a solution of phosphate-free detergent for 15 s, then with tap water for another 15 s. Samples were carefully rinsed with deionized water twice and separated into shoots and roots. They were then oven dried at 60 °C for 48 h, ground into fine powder and sieved through a nylon sieve. The concentrations of lead in shoots and roots of plants were determined using 0.5 g plant sample digested with HNO_3 . The lead concentration was measured by a flame atomic absorption spectrophotometer. Plant samples were sent to two independent laboratories for lead analysis: the laboratory of the Department of Soil Science, Ministry of Agriculture and Cooperatives, and the Central Instrument Facilities, Faculty of Science, Mahidol University. The lead analyses from the two independent laboratories showed similar results especially those samples with unusually high lead content.

1.4 Shoot/root quotient

Shoot/root quotient was calculated as follows:

$$\text{Shoot/root quotient} = \frac{\text{Heavy metal concentration in plant shoot (mg kg}^{-1}\text{)}}{\text{Heavy metal concentration in plant root (mg kg}^{-1}\text{)}}$$

Shoot/root quotient > 1 indicates preferential partitioning of metals to the shoot (Baker and Whiting, 2002).

1.5 Extraction coefficient

Extraction coefficient was calculated as follows:

$$\text{Extraction coefficient} = \frac{\text{Lead concentration in plant root (mg kg}^{-1}\text{)}}{\text{Lead concentration in soil (mg kg}^{-1}\text{)}}$$

Extraction coefficient can be used to evaluate the ability of plant to accumulate the heavy metal (Chen et al., 2004).

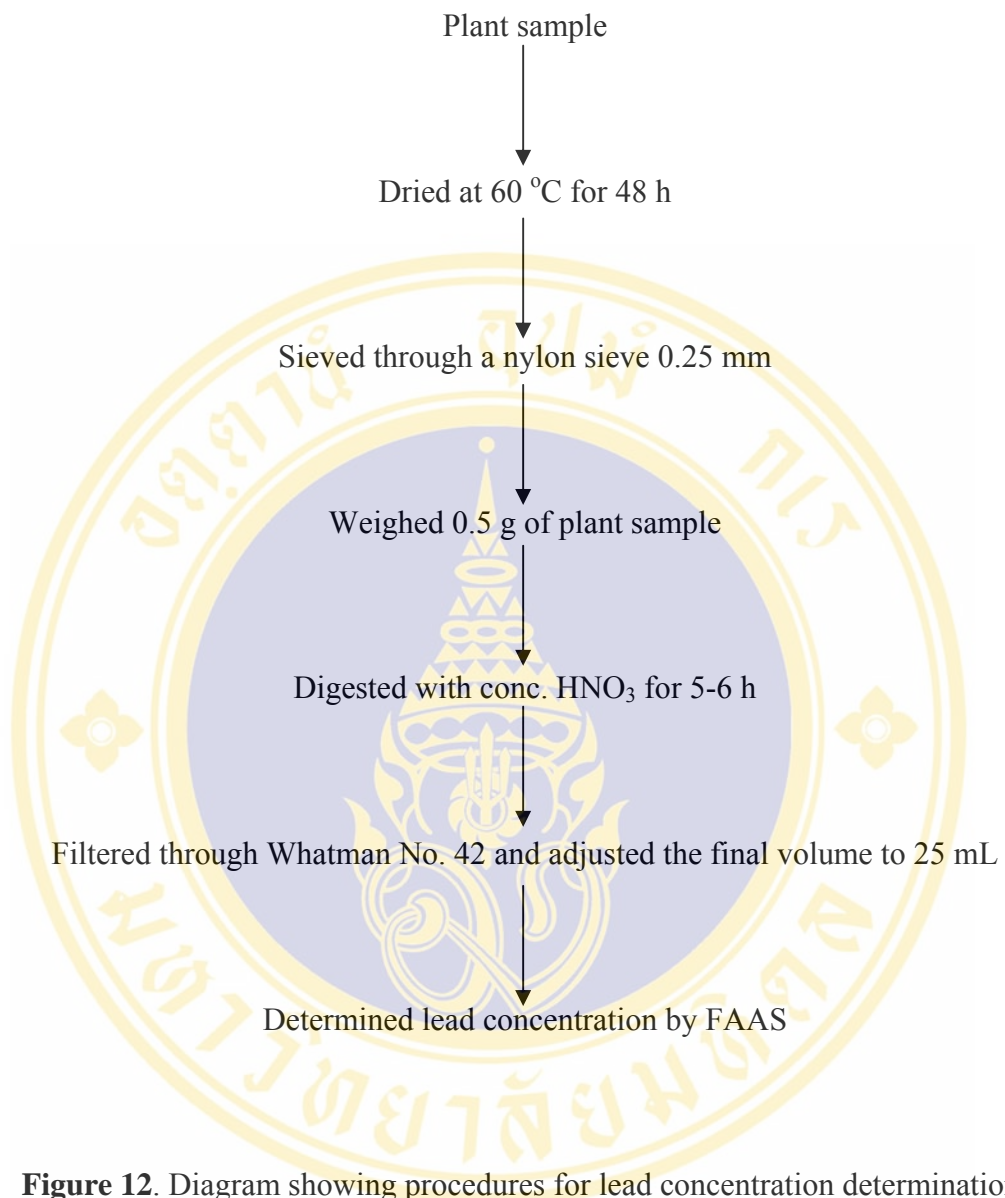


Figure 12. Diagram showing procedures for lead concentration determination.

2. Growth and lead accumulation by the grasses *Vetiveria zizanioides* and *Thyrsanolaena maxima* in lead-contaminated soil amended with pig manure and fertilizer: A glasshouse study

The study comprised two experiments: Experiment I was designed to assess lead tolerance and accumulation in *V. zizanioides* and *T. maxima* grown in soil spiked with various concentrations of lead. Experiment II was designed to test the effects of pig manure and inorganic fertilizer amendments on lead mine soil using *V. zizanioides* and *T. maxima*.

2.1 Plant materials

Four ecotypes of *V. zizanioides* (three from Thailand: Surat Thani, Songkhla, Kamphaeng Phet, and one from Sri Lanka) were obtained from the Land Development Department, Kanchanaburi Province, Thailand. They were grown in the nursery at Mahidol University, Kanchanaburi, Thailand. The native grass, *T. maxima* were collected from the field site about 60 km from Bo Ngam lead mine, Kanchanaburi, Thailand.

2.2 Soil preparation

2.2.1 Experiment I

Natural soil was collected from 0 to 20 cm depth from a suburban area of Kanchanaburi, where native grasses including *T. maxima* were growing. It was air-dried and sieved through a 2 mm mesh sieve. Standard soil characterizations were performed (Department of Soil Science, Ministry of Agriculture and Cooperatives, Bangkok, Thailand). Lead was added to give nominal concentrations of 100, 1000 and 10000 mg Pb kg⁻¹ by mixing with 0.125, 1.25 and 12.5 g of 2PbCO₃ · Pb(OH)₂ per kg soil. Soil without addition of lead served as the control. The fertilizers applied to the soil consisted of 120 mg N kg⁻¹ of dry soil as NH₄NO₃, 80 mg P kg⁻¹ of dry soil and 100 mg K kg⁻¹ of dry soil as KH₂PO₄. The total and DTPA-extractable concentration of lead in the soil, electrical conductivity (EC) and soil pH after four weeks equilibration were determined. Soils were measured for pH and EC before the addition of lead using a glass-electrode pH meter and EC meter respectively. Organic matter was determined by Walkley–Black titration (Walkley and Black, 1934), total N

by the Kjeldhal method (Black, 1965), total P by Bray II method (Bray and Kurtz, 1945), total K by atomic absorption spectrophotometer after digestion with Na_2CO_3 and texture was assessed using the hydrometer method (Allen et al., 1974) (Table 13). Total concentrations of lead and other metals in soil were determined using 0.5 g soil sub-samples digested with HNO_3 (APHA, 1998). After digestion, lead concentration was determined by flame atomic absorption spectrophotometry (FAAS Varian Spectra AA 55B) and other metals (Fe, Zn, Mn, Cu, Cd and Ni) were determined by ICP-OES (ERAN 6000). DTPA-extractable lead content was determined by FAAS (ICARDA, 2001) (Tables 13 and 14).

2.2.2 Experiment II

Lead mine soil was collected from the open pit mine area at Bo Ngam lead mine, Kanchanaburi at 0–20 cm depth. Soil was air-dried, sieved through a 2 mm mesh sieve and mixed well. Pig manure was supplied from a pig farm in Kanchanaburi, air-dried for four weeks and sieved through a 2 mm mesh. Soil and pig manure characterizations were investigated using similar methods as described in Experiment I.

2.3 Plant growth experiment

2.3.1 Experiment I

A glasshouse pot trial was designed to test the lead accumulation and growth of *T. maxima* and the four ecotypes of *V. zizanioides*. Soil was placed in plastic pots (17 cm in diameter, 20 cm in height) which had two pieces of plastic screen at the bottom to retain the soil. Plants were selected, pruned (shoots were 20 cm and roots were 5 cm in length) and then transplanted into the pots (2 plants/pot) containing various concentrations of lead: 113, 192, 707, 10750 mg kg^{-1} of dry soil (Table 14). There were four replicates for each treatment. The pots were placed in the glasshouse under controlled conditions and the soil moisture content was maintained at 40% of the water holding capacity by weighing and adding deionized water every two days (temperature 20–30 °C, day length 13–14 h). After 60 d, the plants were harvested. Plant samples were washed thoroughly with tap water, rinsed with deionized water

and divided into shoots and roots. They were oven-dried at 60 °C for 48 h to a constant weight and the dry weight yield was recorded.

2.3.2 Experiment II

A glasshouse experiment was designed to test the growth and accumulation of *T. maxima* and the four ecotypes of *V. zizanioides* in soil amended with fertilizer or pig manure as follows:

Treatment 1: Soil only (S)

Treatment 2: Soil + 20% pig manure (S + 20)

Treatment 3: Soil + 40% pig manure (S + 40)

Treatment 4: Soil + fertilizer 75 mg kg⁻¹ (S + F1)

Treatment 5: Soil + fertilizer 150 mg kg⁻¹ (S + F2)

Pig manure was mixed with soil at different percentages (20% and 40% w/w). In addition, soil was also amended with two levels of fertilizer addition (N:P:K = 15:15:15): 75 and 150 mg kg⁻¹ of dry soil. Soil without fertilizer and pig manure served as the control. The total and DTPA-extractable concentration of lead in the soil, EC and soil pH after four weeks' equilibration were determined. The DTPA-extractable of lead contents were determined using flame atomic absorption spectrophotometry (FAAS) after extraction with 0.005 M DTPA. The procedures for planting, harvest and analysis of lead were similar to those described in Experiment I.

2.4 AB-DTPA method (Ammonium Bicarbonate-DTPA Method)

The AB-DTPA is a multi-element soil test for alkaline soils. The extracting solution was 1 M in the ammonium bicarbonate (NH₄HCO₃), and 0.005 M DTPA (diethylenetriamine-pentaacetic acid). A 0.005 M DTPA solution was obtained by adding 1.97 g DTPA to 800 mL deionized water. Approximately 2 mL 1:1 ammonium hydroxide (NH₄OH) was added to facilitate dissolution and to prevent effervescence when bicarbonate was added. When most of the DTPA was dissolved, 79.06 g ammonium bicarbonate (NH₄HCO₃) was added and stirred gently until dissolved. The pH was adjusted to 7.6 with ammonium hydroxide. The solution was diluted to 1 L volume with deionized water. After that, 10 g air-dry soil were added into a 125 mL flask. 20 mL extracting solution was added, and the flask was shaken

on a reciprocal shaker for 15 minutes at 180 cycles/minute with flasks kept open. The extracts were then filtered through Whatman No. 42 filter paper and determined the metal concentrations by the flame atomic absorption spectrophotometer.

2.5 Water holding capacity

To determine soil water holding capacity (WHC), triplicate soils were placed into cores with a mesh net bottom. They were saturated with distilled water and allowed to drain for 18 h while the top was sealed to prevent evaporation. At the end of the period, the soil in the cores was weighed and recorded. Soils were then dried overnight at 80 °C for 18 h followed by drying at 104 °C for 2 h and reweighed. The soil moisture content prior to drying was defined as 100% WHC or field capacity (Angle et al., 2003)

2.6 Growth and metal accumulation

Plant growth and survival were determined as percentage survival, plant height and dry biomass. To determine lead accumulation, dried plant samples were ground to a fine powder and sieved through nylon mesh. The concentrations of lead in shoots and roots of plants were determined using 0.5 g plant sub-samples digested with HNO₃ (APHA, 1998). Lead concentration was measured by FAAS. The root/shoot ratio is a highly representative indicator of environmental stress that is encountered by plants (Chiu et al., 2006). It was calculated as follows:

$$\text{Root/shoot ratio} = \frac{\text{Dry weight of root (g)}}{\text{Dry weight of shoot (g)}}$$

2.7 Statistical analysis

The data for height, biomass, heavy metal concentrations of soil and plant and root/shoot ratios of plants under different treatments were analyzed using the SPSS statistical package by one-way analysis of variance (ANOVA) to compare the means of different treatments. Where significant F values were obtained, differences between individual means were tested using the least significant different test (LSD) at the $p = 0.05$ significance level.

3. Lead tolerance and accumulation in the grass *Vetiveria zizanioides* and the effects of lead on vetiver oil production

3.1 Plant materials

Vetiver plants (*Vetiveria zizanioides* 'cv. Monto') were kindly provided by Dr. Paul Truong, Veticon Consulting, Brisbane, Australia. The plants were pre-cultured in potting-mix in a glasshouse under semi-controlled conditions (12-h photoperiod; 25-30 °C) for 18 months. They were then pruned to uniform size (shoots 20 cm, and roots 5 cm).

3.2 Experimental design

3.2.1 Hydroponics study

This experiment was designed to assess lead tolerance, lead accumulation and oil production in vetiver grown in a standard nutrient solution spiked with increasing concentrations of lead. Forty-eight uniform-sized vetiver plants were selected and acclimated in modified Hoagland's solution and placed in a glasshouse. The composition of the nutrient solution was: 400 μM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 200 μM NH_4NO_3 , 400 μM KNO_3 , 100 μM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1000 μM MES, 554.4 μM NaOH, 10 μM KH_2PO_4 , 9.2 μM H_3BO_3 , 13.6 μM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 13.6 μM HBED, 0.03 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 1.8 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.3 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.32 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ adjusted to pH 5. Seven days after installation, lead treatments were applied. Nominal concentrations of lead of 10, 50 and 100 mg L^{-1} were created in each container (20 x 18 x 12.5 cm^3) of 3.5 L modified Hoagland's solution. All treatment solutions were aerated. Lead was supplied as stock lead nitrate [$\text{Pb}(\text{NO}_3)_2$] solution, prepared in deionized water. The treatment solutions were changed every 7 days. There were 3 replicates (2 plants/replicate) for each unit. Plants were set up in the glasshouse under semi-controlled growth conditions. Six plants from each treatment were harvested after 5 and 7 weeks.

3.2.2 Pot study

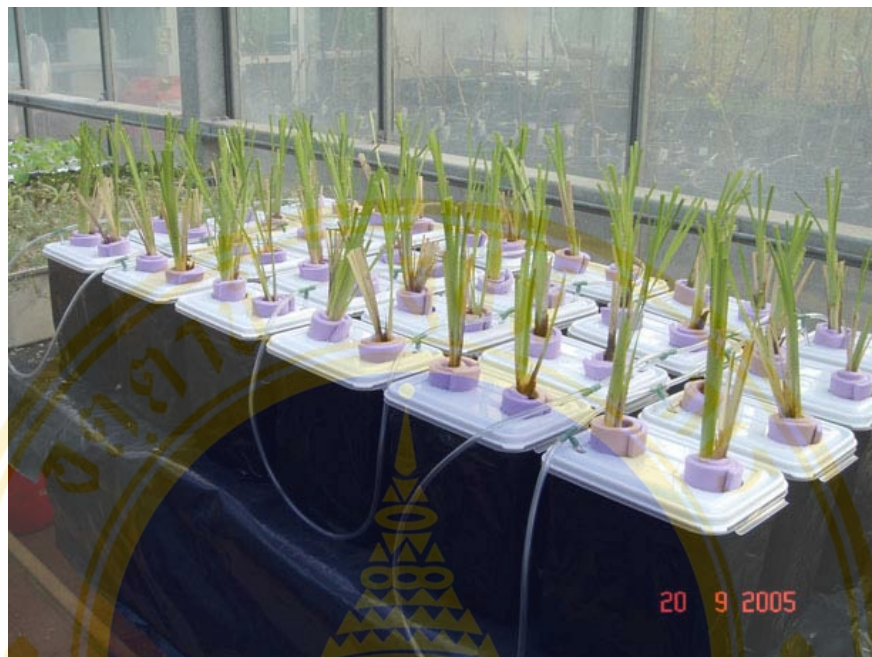
A glasshouse pot trial was designed to test lead tolerance, lead accumulation and oil production in vetiver grown in soil with varying concentrations of applied lead. The mixture of potting mix and sand was used for the experiment (sand: potting

mix 3:1) was air-dried and sieved through a 2 mm mesh. Lead (as lead carbonate) was added to give nominal concentrations of 100, 500 and 1000 mg Pb kg⁻¹. Soil without lead addition served as the control. Soils before and after the addition of lead were measured for pH and EC using a glass pH electrode and EC meter, respectively. Background levels of heavy metals in the soil were measured by an Inductive Coupled Plasma Atomic Emission Spectrometer, ICP-AES (Varian Vista Ax) using *aqua regia* digestion (Table 21). The total and diethylenetriamine-pentaacetic acid (DTPA)-extractable concentrations of lead in the soil after four week's equilibration were determined by ICP-AES. Lead was extracted with 0.005 M DTPA [1.79 g DTPA + 79.06 g NH₄HCO₃ + 2 mL NH₄OH dissolved in 800 mL deionized H₂O, and made up to 1 L, pH 7.6] (ICARDA, 2001).

Forty-eight uniform-sized plants of vetiver were selected for use in this glasshouse experiment. 6 kg of sieve soil was placed into each 17 cm diameter plastic pot which had two pieces of plastic screen at the bottom to retain the soil. Plants were transplanted into the pots containing increasing concentrations of lead (0, 100, 500 and 1000 mg kg⁻¹ of dry soil). There were 3 replicates (2 plants/replicate) for each treatment. The pots were placed in the glasshouse under semi-controlled conditions and watered daily. Fertilizer (N:P:K = 16:3.5:10) was added 6 g/ pot at monthly intervals. Six plants from each treatment were harvested after 2 and 3 months.

3.3 Measurements of the plant sizes and lead concentrations

After growth periods of 5 and 7 weeks for the hydroponic experiment, and 2 and 3 months for pot experiment, plants were harvested and the shoot and root length were measured, then plants divided into shoots and roots. They were washed with tap water, 5% extran and rinsed with deionized water. The concentrations of lead in shoots and roots of plants were determined using 0.5 g plant sub-sample digested with HNO₃ (APHA, 1998) and lead concentrations in the digests were measured by ICP-AES.



A



B

Figure 13. Experimental containers containing *V. zizanioides* exposed to different lead concentrations: (A) Hydroponics study, (B) Pot study.

3.4 Extraction and analysis of vetiver oil

3.4.1 Oil extraction

Fresh roots of vetiver were ground with liquid nitrogen. Then approximately 500 mg ground root was immersed into 3 mL of ethanol and placed on a shaker for 3 weeks at room temperature. The isolated oil was dehydrated by adding anhydrous sodium sulphate and then stored in an amber-colored glass bottle.

3.4.2 Gas chromatography

Samples were analyzed by GC-FID using an autosystem XL gas chromatograph (Perkin Elmer) equipped with BPX5 capillary column (0.5 μm film thickness, 0.25 mm i.d., 30 m length). The oven temperature program was maintained at 70 $^{\circ}\text{C}$ for 4 min, and then raised to 280 $^{\circ}\text{C}$ at the rate of 4 $^{\circ}\text{C}/\text{min}$. Injector and detector temperatures were 250 $^{\circ}\text{C}$ and 300 $^{\circ}\text{C}$, respectively. The carrier gas was helium (1 mL/min). Aliquots (1 μL) of each sample were injected and in 1:20 split ratio.

3.4.3 Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were carried out on a Agilent 6890N, fitted with DB5 (30 m x 0.25 mm i.d., 0.25 μm film thickness) linked on-line with a Agilent Mass selective detector (5973); ionization voltage 70 eV.

3.4.4 Qualitative and quantitative analyses

Vetiver oil constituents were identified using GC-MS by comparison of their mass spectra with those stored in the Wiley 7 library or with mass spectra from the literature (Adams, 2001). Further identification was made by comparison of their retention indices with those in the literature (Weyerstahl et al., 2000; Adams, 2001; Adams et al., 2004; Massardo et al., 2006). The retention indices were determined in relation to a homologous series of *n*-alkanes ($\text{C}_9\text{-C}_{20}$) under the same operating conditions. The oil yield was calculated relative to the dry matter. Percentage data shown are mean values of three replicates from the experiment.

3.5 Statistical analysis

The data for shoot and root lengths, plant lead concentrations and oil production of plants under different treatments were all analyzed using the SPSS statistical package by one-way analysis of variance (ANOVA) to compare the means of different treatments. Where significant F -values were obtained, differences between individual means were tested using least significant different test (LSD) at the $p = 0.05$ significance level.



CHAPTER V

RESULTS

1. Uptake and accumulation of lead by plants from the Bo Ngam lead mine

1.1 Soil characterization

The soil characteristics from five sampling sites are presented in Table 9. The soil textures of most sites were sandy loam or loam except for the soils around the pond area which were clay or clay loam. The soil pH of most sites ranged between 6 and 7. The highest electrical conductivity (EC) value (1.14 dS m^{-1}) was found in soil at site 3 and the lowest (0.16 dS m^{-1}) in soil at site 5. The percentages of total nitrogen from all sites were quite similar (0.09-0.13). The available phosphorus was highest in soil from the natural pond area. The available potassium from soil at sites 4 and 5 (pond area) was higher than that from sites 1 and 2. Similarly, the organic matter of soil from sites 4 and 5 was higher than that of soil from sites 1 and 2.

1.2 Lead concentration in soil

Total and sequential-extractable concentrations of lead in soils from sites 1-5 are shown in Table 10. In general, surface soil (0-10 cm) had higher lead than subsurface soil (10-40 cm) except for sites 1 and 4. Total lead concentration in surface soil can be divided into four levels: extremely high ($142,400 \text{ mg kg}^{-1}$) at site 3 (ore dressing plant area); very high from site 1 ($88,000 \text{ mg kg}^{-1}$) and site 2 ($65,000 \text{ mg kg}^{-1}$); moderately high at site 4 ($6,420 \text{ mg kg}^{-1}$) and low (325 mg kg^{-1}) at site 5 (natural pond area). Total lead contents for the studied sites were in the following order: site 3 > site 1 > site 2 > site 4 > site 5. Extractable lead concentrations from subsurface soils were higher than those from surface soils except at sites 2 and 5. Extractable lead contents for the studied sites were in the following order: site 1 > site 3 > site 2 > site 4 > site 5. Soils from sites 1 and 3 contained extremely high total and extractable lead

Table 9. Characteristics of soil from Bo Ngam lead mine.

Site	Depth	pH	EC (dS/m)	Total N (%)	Available P (ppm)	Available K (ppm)	OM (%)	Soil Texture			
								Sand (%)	Silt (%)	Clay (%)	Texture
1	0-10 cm	7.7	0.2	0.11	3	20	0.2	75	23	2	SL
	10-40 cm	7.8	0.22	0.12	3	20	0.2	67	27	6	SL
2	0-10 cm	7.6	0.18	0.09	3	50	0.2	61	29	10	SL
	10-40 cm	6.9	0.2	0.1	3	50	0.2	41	47	12	L
3	0-10 cm	6.7	1.14	0.13	1	120	0.6	41	41	18	L
	10-40 cm	7	1.05	0.12	1	100	0.5	39	39	22	L
4	0-10 cm	6.8	0.22	0.11	2	170	1	31	31	38	CL
	10-40 cm	7	0.22	0.09	2	100	0.9	25	29	46	C
5	0-10 cm	6.9	0.2	0.1	8	130	1	35	25	40	C
	10-40 cm	7.3	0.16	0.11	97	130	0.7	33	25	42	C

contents. Lead concentrations in soils around the plant roots were different in different plant species. Soil around the plant roots from site 3 had extremely high lead concentration (170,670-177,500 mg kg⁻¹), while that from site 5 had much lower concentration of lead (117-392 mg kg⁻¹) (data not shown here).

Table 10. Lead concentration in soil at sites 1-5, Bo Ngam lead mine.

Site	Total soil Pb (mg kg ⁻¹)		Extractable Pb (mg kg ⁻¹)	
	Surface	Subsurface	Surface	Subsurface
1 Open pit area	88000	109400	47940	56030
2 Stockpile area	65000	56100	23500	10670
3 Ore dressing plant area	142400	120850	25720	43350
4 Tailing pond area	6420	10 360	2400	4200
5 Natural pond area	325	86	16	6

1.3 Lead in plants

A total of 48 plant species belonging to 14 families was collected from five sampling sites (Fig. 14A). There were 22 herbs, 17 grasses, five undershrubs, two shrubs, and two climbers (Fig. 14B). Twenty-six plant species (>50% of the total) had lead concentrations of more than 1000 mg kg⁻¹ in their shoots, with the highest concentration (28,370 mg kg⁻¹) in *Spermacoce mauritiana* (Table 11). Three species (*Microstegium ciliatum*, *Polygala umbonata*, *Sp. mauritiana*) showed extremely high lead concentrations in their shoots (12,200-28,370 mg kg⁻¹) and roots (14,580-128,830 mg kg⁻¹; Table 11). Only eight plant species were found at site 5 and lead concentrations in plant shoots ranged between 23 and 394 mg kg⁻¹.

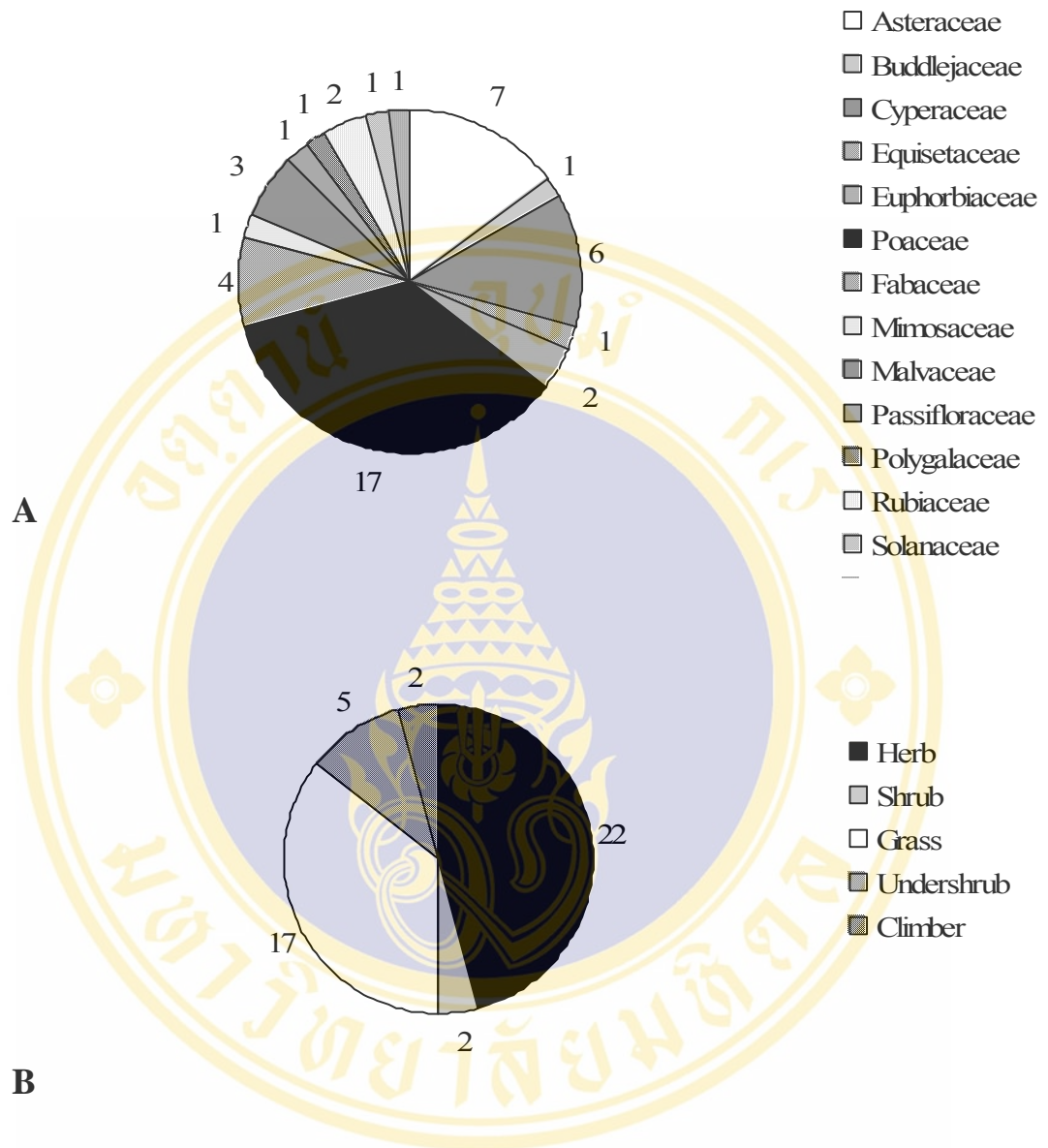


Figure 14. (A) Diagram showing numbers of plant species belonging to 14 families. (B) Diagram showing types of plant samples collected.

Table 11. Average lead concentrations in soil (attached to plant root), shoot and root of plant (mg kg^{-1} dry weight) from sites 1-4.

Family	Scientific name	Site	Type	Pb conc. in soil	Pb conc. in shoot	Pb conc. in root
Asteraceae	<i>Acmella oleracea</i>	4	Herb	458	217	250
	<i>Ageratum conyzoides</i>	2, 4	Herb	38776	3183	4446
	<i>Chromolaena odoratum</i>	1, 2, 3	Herb	118967	3520	9870
	<i>Conyza sumatrensis</i>	1, 2, 4	Herb	37606	2613	986
	<i>Crassocephalum crepidioides</i>	2, 4	Herb	40622	1906	7903
	<i>Sonchus arvensis</i>	1, 2, 3	Herb	134740	4830	2857
Buddlejaceae	<i>Buddleja asiatica</i>	1, 2	Shrub	85640	2187	1765
Cyperaceae	<i>Cyperus difformis</i>	2	Herb	95500	1310	6000
	<i>Pycnus flavidus</i>	4	Herb	4630	217	950
	<i>Fimbristylis bisumbellata</i>	4	Herb	138	28	42
	<i>Fimbristylis dichotoma</i>	4	Herb	24670	412	3620
	<i>Cyperus sp.</i>	2	Herb	20330	1233	642
	<i>Cyperus compactus</i>	4	Herb	1060	86	593
Equisetaceae	<i>Equisetum debile</i>	1, 2	Herb	78890	1505	21025
Euphorbiaceae	<i>Euphorbia heterophylla</i>	2	Herb	31830	6700	15130
	<i>Euphorbia hirta</i>	2	Herb	96750	7020	5380
Poaceae	<i>Bracharia sp.</i>	2	Grass	122170	1340	2850
	<i>Dactyloctenium aegyptium</i>	2	Grass	112000	8100	5930
	<i>Echinochloa colona</i>	4	Grass	1210	107	689
	<i>Eleusine indica</i>	1, 2, 4	Grass	48110	3782	10417
	<i>Neyraudia reynaudiana</i>	1	Grass	96830	857	10920
	<i>Imperata cylindrica</i>	1, 2, 3	Grass	106640	1430	10923
	<i>Melinis repens</i>	2	Grass	20500	1330	5750
	<i>Microstegium ciliatum</i>	3	Grass	175170	12200	128830

Table 11. Average lead concentrations in soil (attached to plant root), shoot and root of plant (mg kg^{-1} dry weight) from sites 1-4 (Cont.).

Family	Scientific name	Site	Type	Pb conc. in soil	Pb conc. in shoot	Pb conc. in root
	<i>Panicum maximum</i>	4	Grass	3520	920	954
	<i>Paspalum conjugatum</i>	1, 2, 4	Grass	44302	3344	3879
	<i>Paspalum scrobiculatum</i>	4	Grass	315	52	50
	<i>Pennisetum polystachyon</i>	2, 3, 4	Grass	104860	6205	24705
	<i>Phragmites vallatoria</i>	1	Grass	111670	403	17170
Poaceae	<i>Sporobolus indicus</i>	2, 3, 4	Grass	109333	5860	9299
	<i>Thysanolaena maxima</i>	2	Grass	96390	369	10720
Fabaceae	<i>Aeschynomene indica</i>	2	Undershrub	132330	4680	2450
	<i>Aeschynomene americana</i>	2, 4	Undershrub	41573	327	1557
	<i>Cruddasia insignis</i>	2	Climber	23250	802	2230
	<i>Vigna umbellata</i>	1, 2	Herb	64750	2875	10330
Mimosaceae	<i>Mimosa pudica</i>	1, 2	Herb	74170	1807	6305
Malvaceae	<i>Hibiscus surattensis</i>	2	Herb	81330	523	1200
	<i>Sida rhombifolia</i>	3	Undershrub	175000	9070	99670
	<i>Urena lobata</i>	2	Undershrub	21670	922	6389
Passifloraceae	<i>Passiflora foetida</i>	2	Herb	46500	347	754
Polygalaceae	<i>Polygala umbonata</i>	3	Climber	175500	21670	14580
Rubiaceae	<i>Spermacoce mauritiana</i>	3	Herb	172500	28370	78330
Solanaceae	<i>Solanum nigrum</i>	2	Shrub	120080	694	2320
Verbenaceae	<i>Stachytarpheta jamaicensis</i>	4	Undershrub	325	10	58

1.4 Extraction coefficient and shoot/root quotient

The average extraction coefficients of plants are presented in Fig. 15. The highest extraction coefficient (0.47) was found in *Acmella oleracea* and the lowest (0.004) were found in *Phragmites vallatoria* and *Thysanolaena maxima*. Figure 16 shows the average shoot/root quotients of plant species collected from sites 1-4. The highest shoot/root quotient (5.84) was found in *Conyza sumatrensis*. Only 12 species showed a shoot/root quotient higher than 1 (Fig. 16). Figure 17 shows plant species collected from sites 1-4, with their lead concentrations in shoot and shoot/root quotients. There were 11 species that showed $>1000 \text{ mg kg}^{-1}$ lead in shoot and shoot/root quotient >1 (Table 12).

Table 12. Plant species showed $> 1000 \text{ mg kg}^{-1}$ lead in shoot and shoot/root quotient > 1 .

Family	Species
Asteraceae	<i>Ag. conyzoides</i>
	<i>C. odoratum</i>
	<i>Co. sumatrensis</i>
	<i>S. arvensis</i>
Buddlejaceae	<i>B. asiatica</i>
Cyperaceae	<i>Cyperus sp.</i>
Euphorbiaceae	<i>E. hirta</i>
Poaceae	<i>D. aegyptium</i>
	<i>So. indicus</i>
Fabaceae	<i>Ae. indica</i>
Polygalaceae	<i>P. umbonata</i>

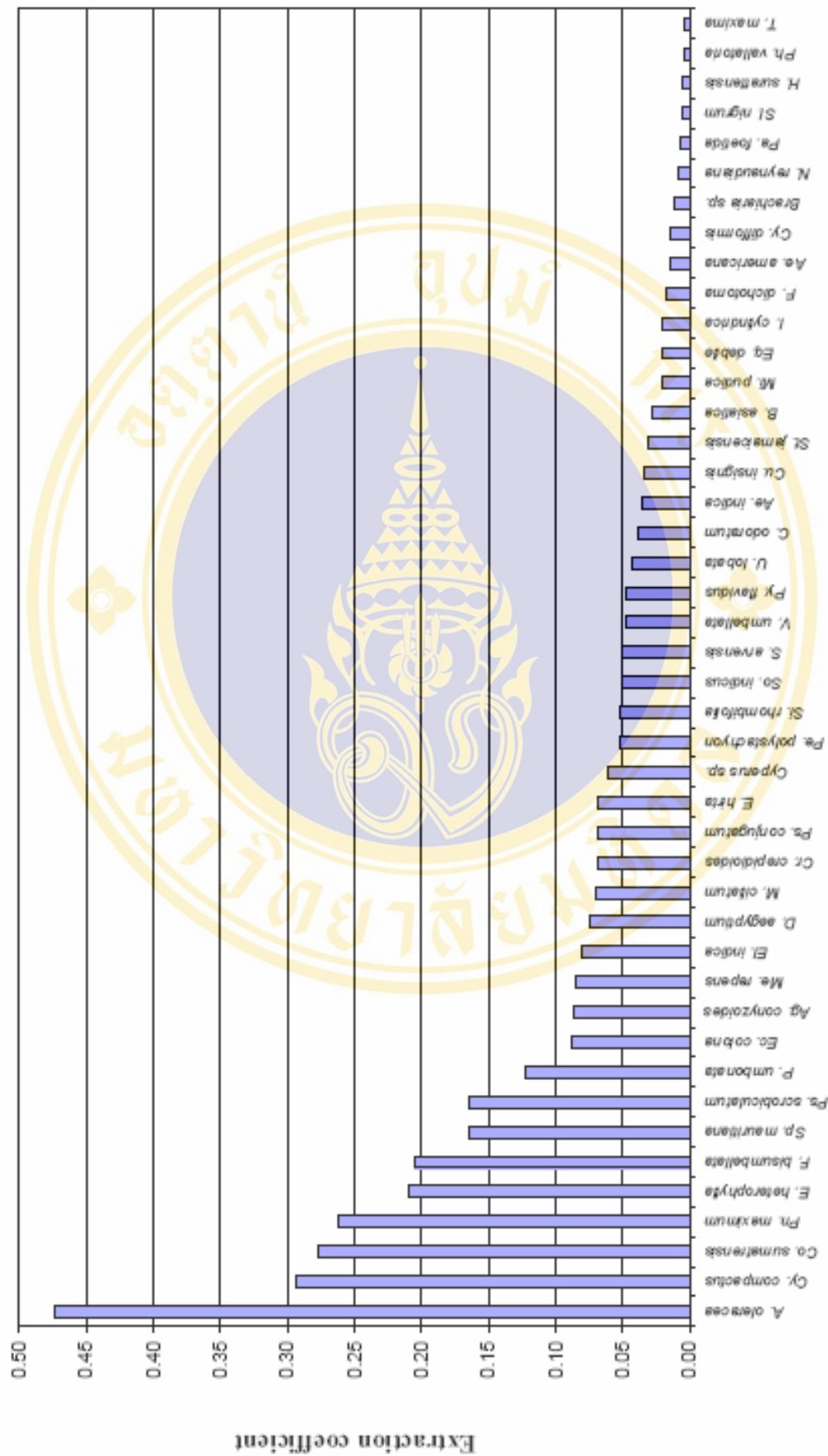


Figure 15. The average extraction coefficients of plants from sites 1-4.

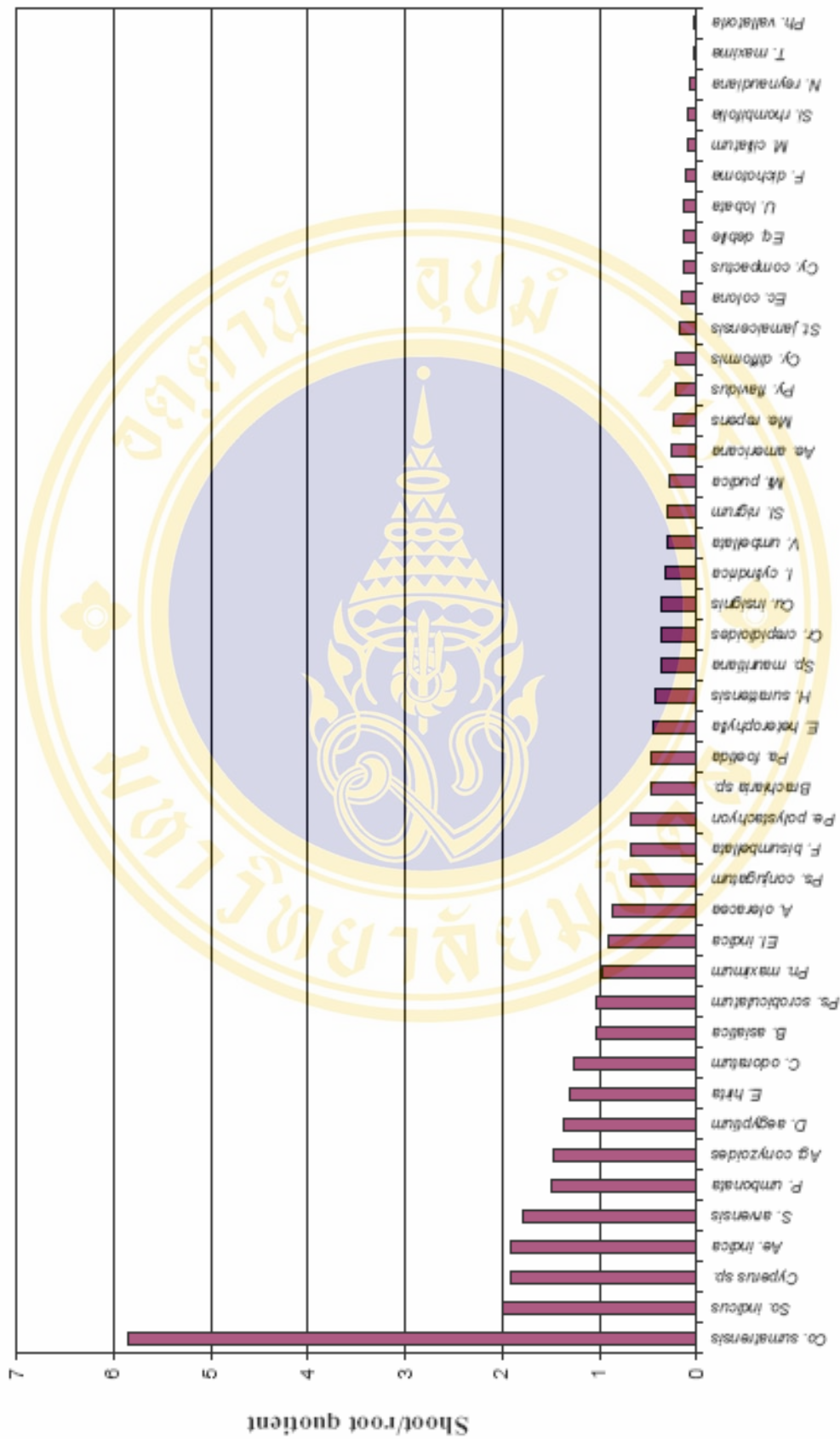


Figure 16. The average shoot/root quotients of plants from sites 1-4.

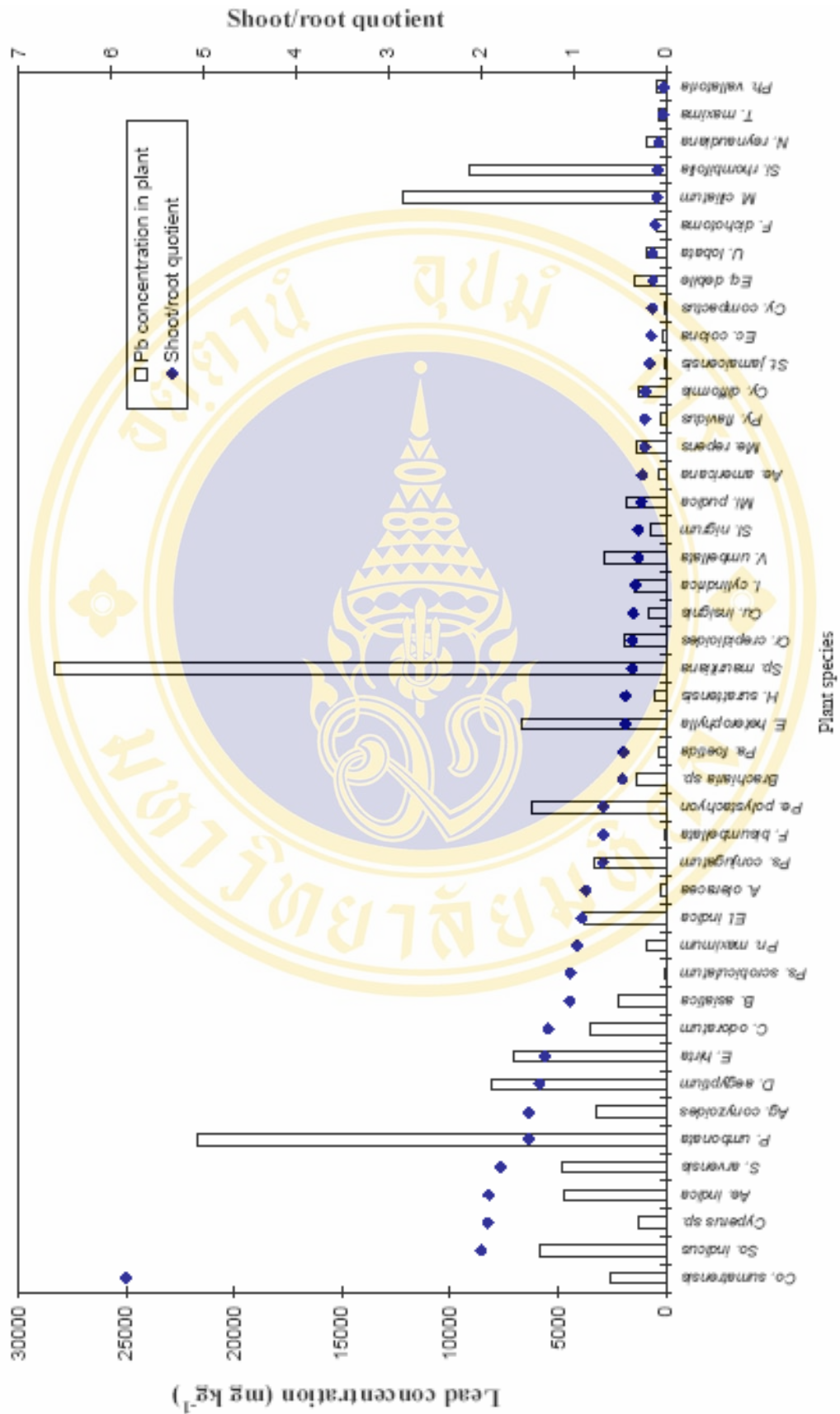


Figure 17. Lead concentrations and shoot/root quotients in plants from sites 1-4.

2. Growth and lead accumulation by the grasses *Vetiveria zizanioides* and *Thyrsanolaena maxima* in lead-contaminated soil amended with pig manure and fertilizer: A glasshouse study

2.1 Experiment I: Lead tolerance and accumulation in *T. maxima* and *V. zizanioides*

2.1.1 Soil characteristics

The general properties of the soil are presented in Table 13. It was near neutral pH (6.8), with an EC value of 0.41 dS m⁻¹. The soil texture was clay with low organic matter content (0.5%). The soil contained low levels of total N, P and K (400, 400 and 900 mg kg⁻¹). Background total lead concentration in the soil was slightly elevated, 113 mg kg⁻¹. After the application of lead, total and DTPA-extractable lead concentrations were in the range of 113–10,750 mg kg⁻¹ and 16.5–1065 mg kg⁻¹, respectively. The results showed that the addition of lead to the soils decreased pH, especially at the highest application dosage (Table 14).

2.1.2 Growth performance

The growth performance data (survival rate, height and biomass) of *T. maxima* and *V. zizanioides* are shown in Table 15. *T. maxima*, *V. zizanioides* (Surat Thani) and *V. zizanioides* (Songkhla) showed the best survival (100% in all treatments). *V. zizanioides* (Kamphaeng Phet) had the lowest survival. The height of the plants was significantly decreased with increases in lead concentration ($p \leq 0.05$). *T. maxima* attained the highest biomass in 100 mg Pb kg⁻¹ treatment and remained rather similar with increase in lead while in *V. zizanioides*, there was 50–60%, reduction in biomass when lead concentration was increased.

Table 13. Physical and chemical properties of soil, lead mine soil and pig manure used for the pot experiment.

Parameter	Soil	Lead mine soil	Pig manure
pH	6.75	7.07	6.5
EC(dS m ⁻¹)	0.41	0.23	4.1
Organic Matter (%)	0.5	0.2	29.65
Soil Texture	Clay	Loam	
Element concentrations	Total		
N (mg kg ⁻¹)	400	200	22400
P (mg kg ⁻¹)	400	300	34000
K (mg kg ⁻¹)	900	600	8300
Ca (mg kg ⁻¹)	1300	1100	31800
Mg (mg kg ⁻¹)	400	300	11300
Fe (mg kg ⁻¹)	33592	22584	314
Zn (mg kg ⁻¹)	<208	<208	843
Mn (mg kg ⁻¹)	503	1238	449
Cu (mg kg ⁻¹)	27.5	62.09	82.6
Cd (mg kg ⁻¹)	0.43	0.55	0.5
Ni (mg kg ⁻¹)	24.35	20.51	10.1
Pb (mg kg ⁻¹)	113	9017	10

Table 14. pH, EC, total and extractable of Pb (mean \pm SD, $n = 3$) in the different treatments before plant growth experiment.

Treatment	pH	EC (dS m ⁻¹)	Total Pb (mg kg ⁻¹)	Extractable Pb (mg kg ⁻¹)
Experiment I				
1 (0 mg kg ⁻¹)	6.75 \pm 0.05	0.41 \pm 0.03	113 \pm 17.6	16.5 \pm 0.2
2 (100 mg kg ⁻¹)	6.4 \pm 0.15	0.38 \pm 0.02	192 \pm 28.4	43.7 \pm 0.6
3 (1000 mg kg ⁻¹)	6.3 \pm 0.3	0.39 \pm 0.02	707 \pm 15.3	253 \pm 6
4 (10 000 mg kg ⁻¹)	5.3 \pm 0.2	0.37 \pm 0.02	10750 \pm 624	1065 \pm 13.2
Experiment II				
S	7.07 \pm 0.12	0.23 \pm 0.02	9020 \pm 954	263 \pm 11.5
S+20	7 \pm 0	3.13 \pm 0.03	8730 \pm 983	183 \pm 2.9
S+40	7 \pm 0	3.96 \pm 0.06	5650 \pm 522	143 \pm 2.9
S+F1	7.13 \pm 0.15	1.04 \pm 0.14	10 600 \pm 1500	367 \pm 17.6
S+F2	7.03 \pm 0.06	1.76 \pm 0.06	11 920 \pm 1290	403 \pm 5.8

2.1.3 Root/shoot ratio

Root/shoot ratio is an indicator of environmental stress. The root/shoot ratios of *T. maxima* and *V. zizanioides* grown in different treatments are presented in Fig. 18. *T. maxima* showed the highest values in all treatments. However, statistical analysis (LSD test) showed that means were not significantly different for each group ($p > 0.05$).

2.1.4. Lead accumulation in plants

Lead accumulation in shoots and roots of *T. maxima* and four ecotypes of *V. zizanioides* was similar (Table 16). Plants accumulated higher lead concentrations in roots (4100–5900 mg kg⁻¹) than shoots (248–422 mg kg⁻¹). Lead concentrations in the same part (shoots or roots) of plants under different treatments were generally in the descending order of 10000 > 1000 > 100 \approx 0 mg kg⁻¹ treatment.

Table 15. Survival (%), height (cm), and biomass (g dry weight/ pot) (mean \pm SD, $n = 4$) of *T. maxima* and four ecotypes of *V. zizanioides* grown on different lead concentrations for a period of 60 days.

	Treatment	<i>T. maxima</i>	<i>V. zizanioides</i> (Surat Thani)	<i>V. zizanioides</i> (Songkhla)	<i>V. zizanioides</i> (Kamphaeng Phet)	<i>V. zizanioides</i> (Sri Lanka)
Survival	1	100	100	100	100	100
	2	100	100	100	75	100
	3	100	100	100	50	100
	4	100	100	100	50	75
Height	1	37.8 \pm 12.2 a [*] -d [#]	59.5 \pm 6.1 a-c	69 \pm 24.4 a-b	54.8 \pm 7 b-c	89.5 \pm 15.0 a-a
	2	28.1 \pm 14.3 b-d	57.3 \pm 3.7 a-c	68.5 \pm 13.9 a-b	60.7 \pm 14.0 a-c	84.4 \pm 18.7 a-a
	3	26.8 \pm 5.1 b-c	44.4 \pm 5.8 b-b	45.88 \pm 25.3 b-b	54.5 \pm 9.2 b-a	48.3 \pm 23.8 b-ab
	4	24 \pm 4.6 b-d	39.3 \pm 6.9 c-b	46.5 \pm 6.8 b-a	26.3 \pm 3.2 c-cd	28.3 \pm 8.7 c-c
Biomass	1	6.5 \pm 0.8 b-c	7.0 \pm 1.4 a-c	8.1 \pm 1.5 a-b	12.5 \pm 3.9 a-a	8.9 \pm 1.2 a-b
	2	7.4 \pm 1.9 a-c	7.0 \pm 1.6 a-c	8.6 \pm 1.3 a-b	12.0 \pm 5.1 a-a	8.6 \pm 2.3 a-b
	3	6.3 \pm 2.4 b-b	4.0 \pm 1.4 b-c	5.6 \pm 2.2 c-b	7.7 \pm 2.4 b-a	7.6 \pm 1.5 b-a
	4	6.4 \pm 1.3 b-a	4.8 \pm 2.4 b-b	6.7 \pm 2.7 b-a	7.3 \pm 3.1 b-a	4.5 \pm 1.9 c-b

Data with different letters (*) in the same column and same item, or (#) in the same row indicate a significant difference at 5% level according to LSD test

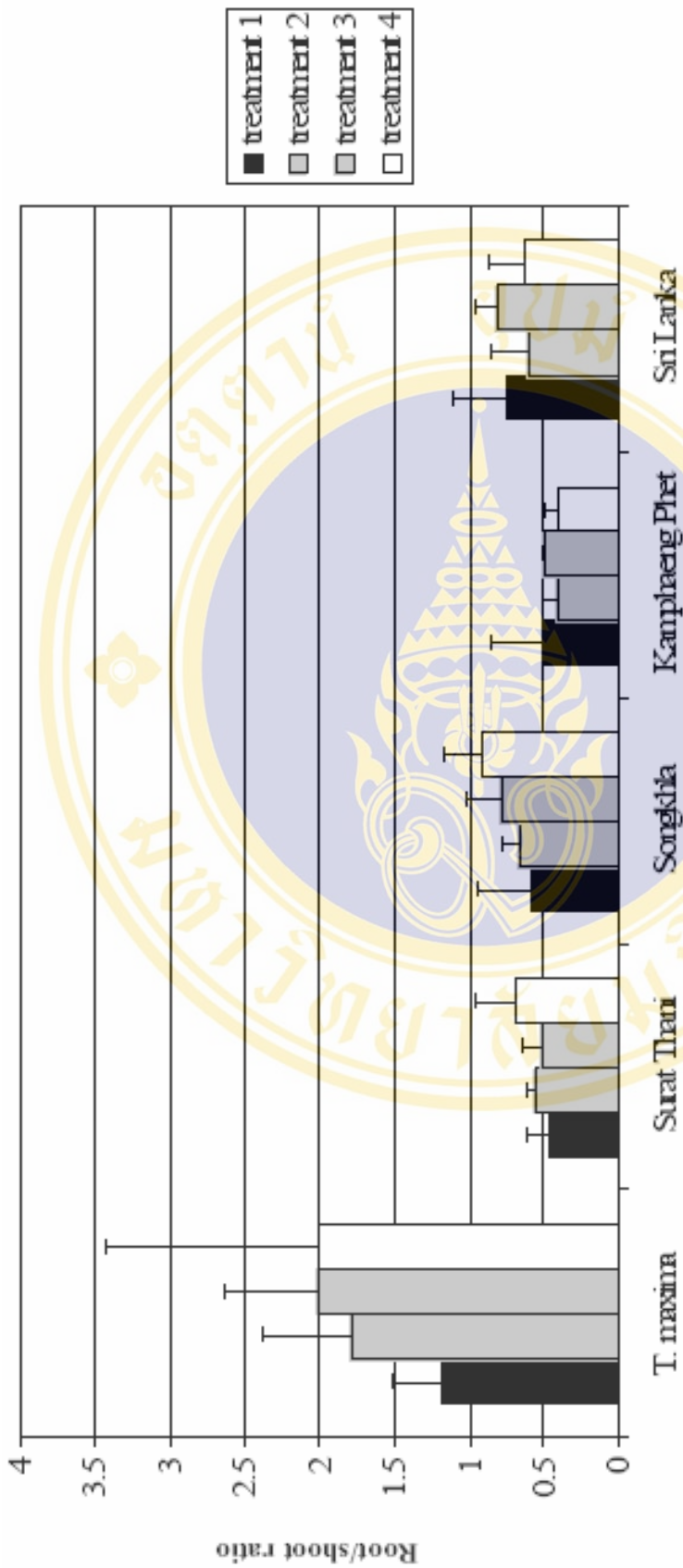


Figure 18. Effects of application of lead on the root/shoot ratio for *T. maxima* and four ecotypes of *V. zizanioides*. The root/shoot ratio was not significantly different in each group at 5% level according to LSD test ($n = 4$).

Table 16. Lead concentrations (mean \pm SD, $n = 4$) in soils and plants of *T. maxima* and four ecotypes of *V. zizanioides*.

	Metal concentration (mg kg ⁻¹)			
	Treatment 1	2	3	4
<i>T. maxima</i>				
Soil	81.6 \pm 6.0 c	153 \pm 31.2 c	638 \pm 84.9 b	7900 \pm 708 a
Shoot	16.2 \pm 9.5 c	20 \pm 4.1 c	140 \pm 118 b	357 \pm 61.3 a
Root	45.0 \pm 23.51 c	106 \pm 75.4 c	509 \pm 268 b	4290 \pm 719 a
<i>V. zizanioides</i> (Surat Thani)				
Soil	79.6 \pm 6.0 c	145 \pm 16.5 c	689 \pm 83.3 b	7830 \pm 1220 a
Shoot	12.5 \pm 5.0 c	16.2 \pm 9.4 c	142 \pm 54.5 b	359 \pm 2601 a
Root	18.7 \pm 2.5 c	41.1 \pm 9.4 c	474 \pm 74.3 b	4940 \pm 1080 a
<i>V. zizanioides</i> (Songkhla)				
Soil	89.5 \pm 5.8 c	148 \pm 18.8 c	739 \pm 147 b	8820 \pm 211 a
Shoot	12.5 \pm 2.9 c	23.7 \pm 8.5 c	214 \pm 143 b	422 \pm 387 a
Root	39.9 \pm 30.3 c	55.0 \pm 7.1 c	569 \pm 242 b	4170 \pm 827 a
<i>V. zizanioides</i> (Kamphaeng Phet)				
Soil	88.5 \pm 4.25 d	190 \pm 24.2 c	867 \pm 107 b	6510 \pm 282 a
Shoot	11.2 \pm 6.3 c	15.0 \pm 5.0 c	102 \pm 25.1 b	287 \pm 53.0 a
Root	39.8 \pm 20.3 c	48.3 \pm 7.8 c	775 \pm 24.9 b	5950 \pm 1200 a
<i>V. zizanioides</i> (Sri Lanka)				
Soil	100 \pm 13.4 c	170 \pm 20.3 c	834 \pm 172 b	8420 \pm 1240 a
Shoot	16.2 \pm 7.5 bc	15.0 \pm 4.1 c	69.8 \pm 7.0 b	248 \pm 271 a
Root	63.6 \pm 32.9 c	56.0 \pm 10.2 c	628 \pm 310 b	5770 \pm 1390 a

Data with different letters in the same row indicate a significant difference at 5% level according to LSD test

2.2 Experiment II: Effects of pig manure and inorganic fertilizer amendments on *V. zizanioides* and *T. maxima*

2.2.1 General properties of lead mine soil and pig manure

The general properties of lead mine soil and pig manure are presented in Table 13. The lead mine soil and pig manure were near neutral (pH 7.1 and 6.5, respectively). The EC value of lead mine soil was low (0.23 dS m^{-1}), while that of pig manure was high (4.1 dS m^{-1}). The pig manure contained much higher levels of organic matter, total N, P and K (140, 110, 110 and 10 times, respectively, higher than those in the lead mine soil). In terms of heavy metals, total concentrations of Mn, Cd, Ni and Pb in lead mine soil were higher while total Zn and Cu were higher in pig manure. The addition of pig manure or fertilizer to lead mine soil did not change the pH value but significantly increased the EC value, especially at the highest application dosage of pig manure (S + 40, $\text{EC} = 3.96 \text{ dS m}^{-1}$) (Table 14). The pig manure reduced total and extractable lead, while these were increased with fertilizer application.

2.2.2 Growth performance

The growth performance (survival rate, height and biomass) of *T. maxima* and *V. zizanioides* is presented in Table 17. *V. zizanioides* (Surat Thani) and *V. zizanioides* (Songkhla) showed best survival (100% in all treatments) while *V. zizanioides* (Kamphaeng Phet) had the lowest survival. The addition of pig manure decreased the survival of *T. maxima* and *V. zizanioides* (Sri Lanka). The results in terms of height and biomass of *T. maxima* showed that they were significantly decreased with the application of pig manure ($p \leq 0.05$) in comparison to those grown in pure lead mine soil or amended with fertilizer. For *V. zizanioides*, application of pig manure significantly increased the height and biomass of plants ($p \leq 0.05$). *V. zizanioides* (Sri Lanka) showed the maximum height and biomass in the 20% pig manure treatment. However, the addition of fertilizer did not significantly increase the height and biomass of vetiver grass ($p > 0.05$).

Table 17. Survival (%), and height (cm) (mean \pm SD, $n = 4$) of *T. maxima* and four ecotypes of *V. zizanioides* grown on Bo Ngam lead mine soil in different treatments for a period of 60 days.

	Treatment	<i>T. maxima</i>	<i>V. zizanioides</i> (Surat Thani)	<i>V. zizanioides</i> (Songkhla)	<i>V. zizanioides</i> (Kamphaeng Phet)	<i>V. zizanioides</i> (Sri Lanka)
Survival	S	100	100	100	50	100
	S+20	75	100	100	100	100
	S+40	75	100	100	25	75
	S+F1	100	100	100	75	100
	S+F2	100	100	100	50	100
Height	S	35.1 \pm 9.9 b* -bc [#]	36.6 \pm 9.7 d-bc	37.9 \pm 10.4 c-b	33.8 \pm 3.9 d-c	63.5 \pm 6.0 b-a
	S+20	23.8 \pm 2.5 c-c	63.3 \pm 3.9 b-b	66.0 \pm 8.0 a-b	60.0 \pm 20.2 b-b	86.0 \pm 17.4 a-a
	S+40	23.3 \pm 3.1 c-c	69.6 \pm 3.1 a-b	68.8 \pm 13.7 a-b	83.5 \pm 0 a-a	80.5 \pm 16.2 a-a
	S+F1	35.5 \pm 14.7 b-c	49.1 \pm 8.5 c-b	53.5 \pm 13.8 b-ab	52.2 \pm 13.6 c-ab	56.9 \pm 13.3 b-a
	S+F2	40.5 \pm 2.7 a-c	59.1 \pm 17.6 b-a	43.5 \pm 14.0 c-bc	48.8 \pm 3.9 c-b	60.3 \pm 19.0 b-a
Biomass	S	5.7 \pm 1.4 c-ab	3.5 \pm 1.1 b-c	4.6 \pm 1.4 c-bc	6.3 \pm 0.3 c-ab	6.7 \pm 1.3 c-a
	S+20	5.5 \pm 1.1 c-b	7.8 \pm 2.1 a-ab	8.0 \pm 2.0 a-ab	9.2 \pm 3.4 a-a	8.9 \pm 1.4 a-a
	S+40	4.5 \pm 1.1 d-a	7.5 \pm 1.3 a-a	7.0 \pm 3.5 b-a	5.4 \pm 0 d-a	8.0 \pm 5.1 ab-a
	S+F1	7.0 \pm 2.5 b-a	4.2 \pm 1.5 b-b	5.5 \pm 0.9 c-ab	7.9 \pm 0.9 b-a	7.1 \pm 2.4 bc-a
	S+F2	8.1 \pm 3.7 a-a	3.9 \pm 2.0 b-c	5.1 \pm 0.7 c-bc	6.9 \pm 0.4 c-ab	6.9 \pm 2.3 bc-ab

Data with different letters (*) in the same column and same item, or (#) in the same row indicate a significant difference at 5% level according to LSD test

2.2.3 Root/shoot ratio

Figure 19 shows the effects of pig manure and fertilizer application on the root/shoot ratio. *T. maxima* plants grown in lead mine soil amended with pig manure showed the highest root/shoot ratio (2.33), indicating that they had suffered the greatest stress while in fertilizer application, they were significantly lower ($p \leq 0.05$). In contrast, *V. zizanioides* (Surat Thani) grown in soil amended with fertilizer showed the lowest ratio.

2.2.4 Lead accumulation in plants

Lead concentrations in roots and shoots of *T. maxima* and *V. zizanioides* are presented in Table 18. In the unamended soil, Surat Thani and Songkhla ecotypes of vetiver showed the highest lead accumulation in shoot, while the Kamphaeng Phet ecotype showed the highest lead accumulation in root. Both grass species accumulated highest lead concentrations in the roots. The results showed that lead concentrations in both the shoot and root tissues of *T. maxima* and vetiver in soil amended with pig manure were significantly decreased ($p \leq 0.05$) (Table 18). *T. maxima* grown in soil amended with 75 mg kg^{-1} fertilizer showed the maximum lead accumulation both in shoot and root tissues. In vetiver, the application of 150 mg kg^{-1} fertilizer significantly increased the lead concentrations in root tissues ($p \leq 0.05$).

3. Lead tolerance and accumulation in the grass *Vetiveria zizanioides* and the effect of lead on vetiver oil production

3.1 Hydroponic study

3.1.1 Speciation

GEOCHEM-PC program (Parker et al., 1993) was used to calculate the lead speciation in the hydroponic solution. In the 10, 50 and 100 mg L^{-1} Pb treatments, 95.2%, 92.8% and 88.2% Pb, respectively were calculated to be in the free form. The remaining lead was present as complexes or in solid form with sulphate, nitrate and phosphate.

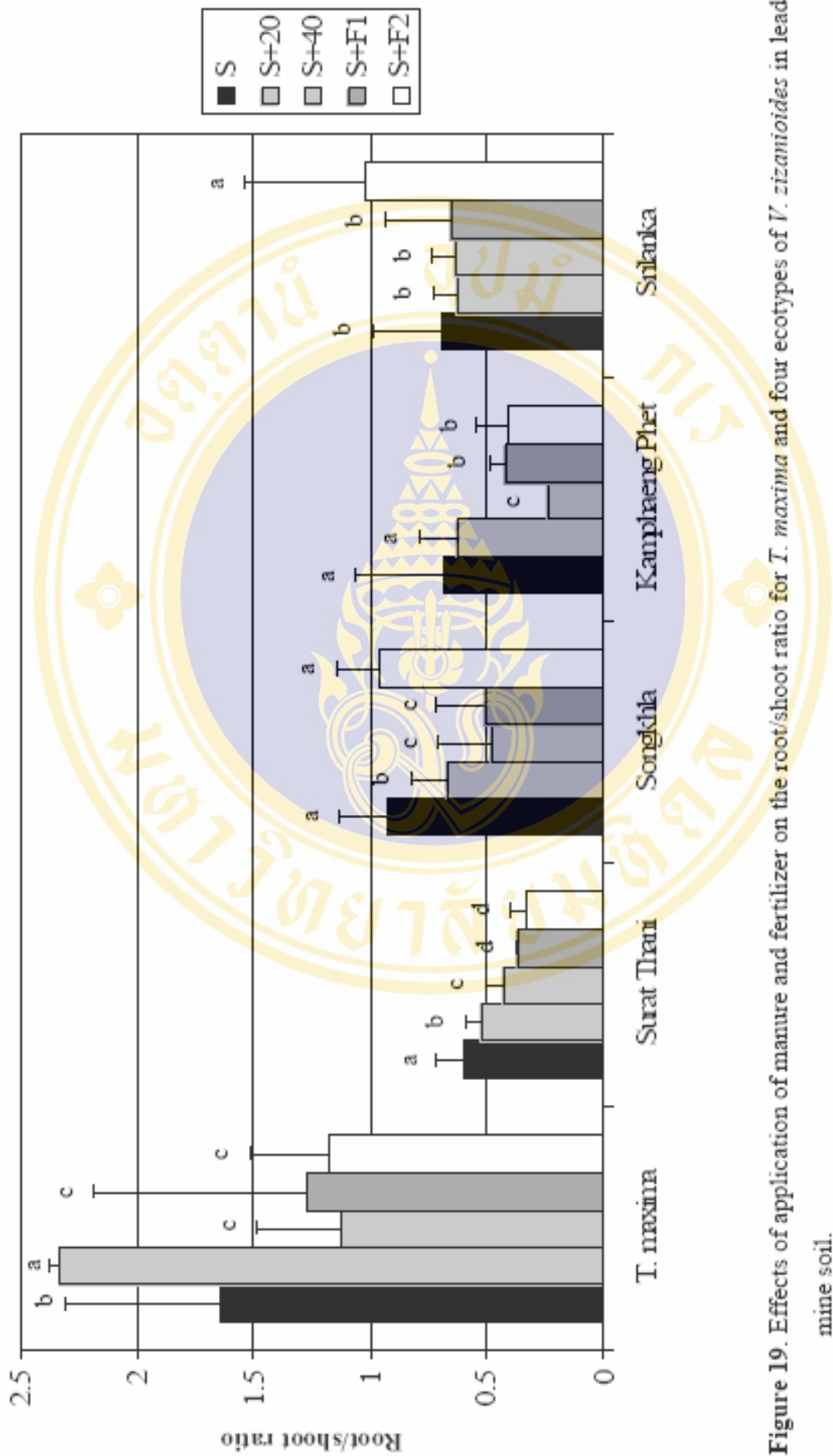


Figure 19. Effects of application of manure and fertilizer on the root/shoot ratio for *T. maxima* and four ecotypes of *V. zizanioides* in lead mine soil.

Table 18. Lead concentrations (mean \pm SD, $n = 4$) in soils and plants of *T. maxima* and four ecotypes of *V. zizanioides* grown on lead mine soil under different treatments for a period of 60 days.

	S	Metal concentration (mg kg ⁻¹)					S+F2
		S+20	S+40	S+F1	S+F2		
<i>T. maxima</i>							
Soil	7470 \pm 1830 b	7550 \pm 1100 b	6190 \pm 592 c	10 610 \pm 551 a	10880 \pm 1330 a		
Shoot	19.9 \pm 4.1 c	13.3 \pm 2.9 c	15.0 \pm 0.1 c	56.1 \pm 50.1 a	37.4 \pm 9.6 b		
Root	271 \pm 84.8 b	115 \pm 75.3 c	146 \pm 28.8 c	565 \pm 410 a	277 \pm 18.8 b		
<i>V. zizanioides</i> (Surat Thani)							
Soil	8160 \pm 1280 c	8200 \pm 425 bc	6590 \pm 449 d	9020 \pm 3320 b	12160 \pm 1040 a		
Shoot	128 \pm 61.5 a	11.2 \pm 2.5 d	15.0 \pm 7.0 d	39.9 \pm 10.8 c	59.8 \pm 20.3 b		
Root	347 \pm 120 c	236 \pm 221 d	126 \pm 49.6 e	475 \pm 179 b	705 \pm 188 a		
<i>V. zizanioides</i> (Songkhla)							
Soil	7870 \pm 843 c	8100 \pm 541 c	6210 \pm 1010 d	9220 \pm 208 b	11240 \pm 1330 a		
Shoot	124 \pm 75.9 b	18.7 \pm 4.7 c	23.7 \pm 8.5 c	43.6 \pm 4.7 c	179 \pm 200 a		
Root	388 \pm 154 b	234 \pm 51.5 c	200 \pm 83.8 c	193 \pm 47.7 c	712 \pm 538 a		
<i>V. zizanioides</i> (Kamphaeng Phet)							
Soil	8090 \pm 301 c	7350 \pm 753 d	6690 \pm 0 e	10570 \pm 917 b	12610 \pm 1120 a		
Shoot	90.2 \pm 106 a	33.6 \pm 13.1 c	54.8 \pm 0 b	102 \pm 62.6 a	54.8 \pm 35.1 b		
Root	759 \pm 509 b	462 \pm 310 c	214 \pm 0 d	759 \pm 226 b	911 \pm 6.7 a		
<i>V. zizanioides</i> (Sri Lanka)							
Soil	8870 \pm 1390 c	8330 \pm 858 d	6320 \pm 477 e	10510 \pm 1390 b	11250 \pm 458 a		
Shoot	34.9 \pm 10.8 c	26.2 \pm 7.5 c	8.3 \pm 2.9 d	59.9 \pm 27.4 b	138 \pm 57.8 a		
Root	459 \pm 386 b	138 \pm 63.3 c	107 \pm 20.5 c	504 \pm 155 b	686 \pm 195 a		

Data with different letters in the same row indicate a significant difference at 5% level according to LSD test

3.1.2 Plant growth

Table 19 shows the shoot and root lengths of vetiver plants grown in the nutrient solution. Plants grown in control and 10 mg L⁻¹ Pb treatments showed significantly longer shoot and root lengths when compared with those in treatments with added lead ($p \leq 0.05$). Root length was progressively decreased with increasing concentrations of lead. There was little difference between shoot and root length of plants grown for 5 weeks and 7 weeks. Visual sign of phytotoxicity (red-tipped leaves) were observed in plants grown in the 50 and 100 mg L⁻¹ Pb treatments.

Table 19. Length of shoots and roots (mean \pm SD, $n=3$) of vetiver grown in nutrient solutions and soils containing different lead concentrations.

Hydroponic study	5 weeks		7 weeks	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
Control	66.0 \pm 7.1 a	37.7 \pm 0.9 a	73.3 \pm 3.3 a	37.3 \pm 13.9 a
10 mg L ⁻¹	59.1 \pm 6.0 a	10.6 \pm 0.6 b	62.7 \pm 4.8 ab	11.8 \pm 0.8 b
50 mg L ⁻¹	46.7 \pm 0.7 b	11.1 \pm 0.6 b	52.9 \pm 8.8 bc	9.17 \pm 1.0 b
100 mg L ⁻¹	48.9 \pm 3.2 b	7.25 \pm 2.2 c	46.3 \pm 8.0 c	7.08 \pm 0.1 b
Pot study	2 months		3 months	
	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)
Control	94.5 \pm 3.9 a	R	108.5 \pm 8.7 a	R
100 mg kg ⁻¹	93.5 \pm 9.0 a	R	120.5 \pm 1.3 a	R
500 mg kg ⁻¹	92.8 \pm 2.9 a	R	119.7 \pm 21.6 a	R
1000 mg kg ⁻¹	96.8 \pm 11.4 a	R	108.8 \pm 18.3 a	R

Data with different letters in the same column indicate a significant difference at 5% level according to LSD test.

^R Reached the base of the pot

3.1.3 Lead accumulation

Lead concentrations in shoots and roots of vetiver are presented in Table 20. They increased with increasing lead concentration in the solution. Much higher lead concentrations were found in roots than shoots (175-fold in plants grown in 100

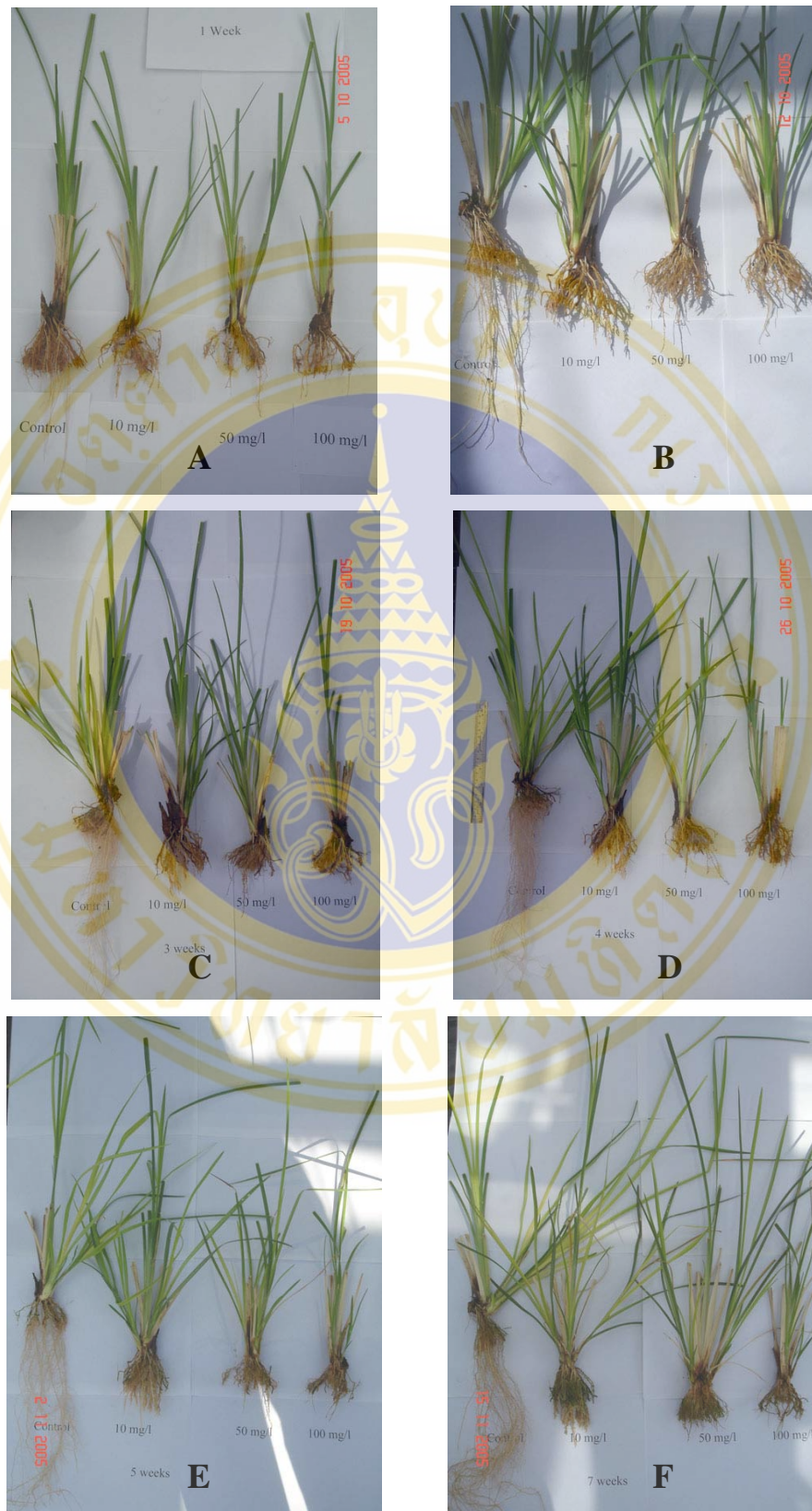


Figure 20. *V. zizanioides* grown in nutrient solution with different lead concentrations (0, 10, 50 and 100 mg L⁻¹) at various exposure times: (A) 1 week, (B) 2 weeks, (C) 3 weeks, (D) 4 weeks, (E) 5 weeks, (F) 7 weeks.

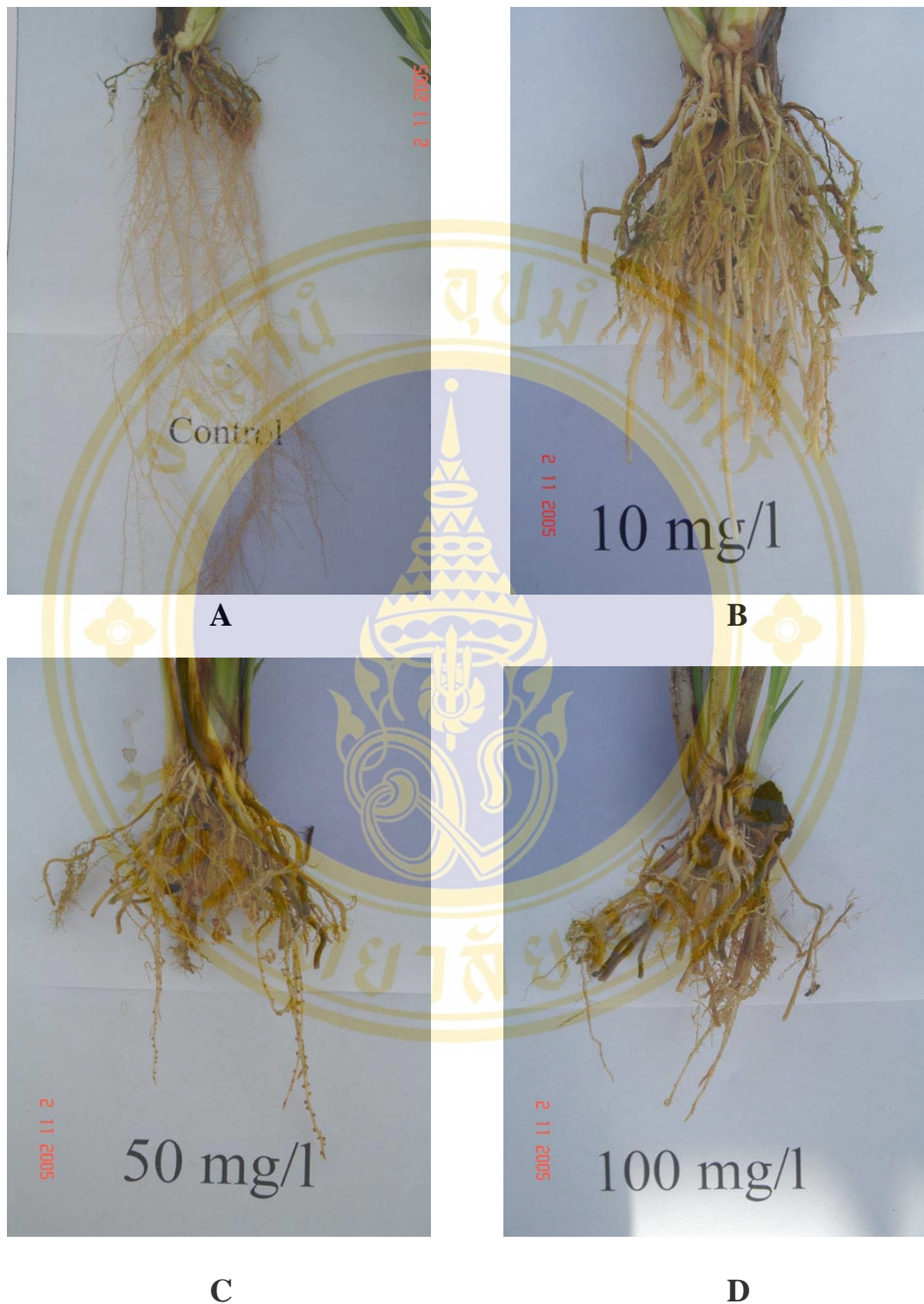


Figure 21. Roots of *V. zizanioides* grown in nutrient solution with different lead concentrations for 5 weeks: (A) Control, (B) 10 mg L⁻¹, (C) 50 mg L⁻¹, (D) 100 mg L⁻¹.



Figure 22. Phytotoxicity symptom in vetiver plants grown in 50 and 100 mg L⁻¹ treatments.

Table 20. Lead concentration (mean \pm SD, $n = 3$) in shoots and roots of vetiver grown in nutrient solutions and soils containing different lead concentrations.

Hydroponic study	5 weeks		7 weeks	
	Shoot (mg kg ⁻¹)	Root (mg kg ⁻¹)	Shoot (mg kg ⁻¹)	Root (mg kg ⁻¹)
Control	5.44 \pm 2.0 c* ^a #	5.47 \pm 2.0 d-a	3.5 \pm 1.5 d-a	2.66 \pm 1.2 c-a
10 mg L ⁻¹	78.3 \pm 5.9 b-a	8140 \pm 2325 c-a	87.4 \pm 4.2 c-a	5920 \pm 698 b-a
50 mg L ⁻¹	78.6 \pm 6.2 b-b	14200 \pm 618 b-a	112.4 \pm 11.7 b-a	14060 \pm 2095 a-a
100 mg L ⁻¹	111.8 \pm 23.3 a-a	19530 \pm 2520 a-a	143.9 \pm 22 a-a	16860 \pm 2155 a-a
Pot study	2 months		3 months	
	Shoot (mg kg ⁻¹)	Root (mg kg ⁻¹)	Shoot (mg kg ⁻¹)	Root (mg kg ⁻¹)
Control	0.54 \pm 0.5 c-a	4.2 \pm 2.5 c-a	0.19 \pm 0.3 c-a	1.70 \pm 1 c-a
100 mg kg ⁻¹	2.19 \pm 0.3 c-b	43.5 \pm 18.1 c-a	4.26 \pm 1.0 c-a	29.8 \pm 7.0 c-a
500 mg kg ⁻¹	13.6 \pm 4.4 b-a	315.4 \pm 131 b-a	20.4 \pm 5.9 b-a	295.5 \pm 92.8 b-a
1000 mg kg ⁻¹	25.0 \pm 1.9 a-b	585.1 \pm 103.5 a-a	37.6 \pm 1.5 a-a	628.9 \pm 88 a-a

Data with different letters (*) in the same column and same item, or (#) in the same row indicate a significant difference at 5% level according to LSD test

mg L⁻¹ treatment for 5 weeks). Plants treated with 100 mg L⁻¹ accumulated high concentrations of lead in their shoots (112-144 mg kg⁻¹) and roots (16,860-19,530 mg kg⁻¹).

3.2 Pot study

3.2.1 Soil characteristics

The general properties of the soil used in this experiment are summarized in Table 21. The soil pH was 7.1, with an EC value of 0.45 dS m⁻¹. The background total lead concentration in the soil was 3.5 mg kg⁻¹.

Table 21. Physical and chemical characteristics of soil used in the pot experiment (mean ± SD, *n* = 3).

Parameters	
pH	7.11 ± 0.09
EC (dS m ⁻¹)	0.45 ± 0.13
Elements	Concentration
Cd (mg kg ⁻¹)	2.66 ± 0.42
Cu (mg kg ⁻¹)	1.70 ± 0.69
Fe (mg kg ⁻¹)	3480 ± 634
Mn (mg kg ⁻¹)	57.8 ± 12.1
Ni (mg kg ⁻¹)	3.84 ± 0.54
Pb (mg kg ⁻¹)	3.48 ± 0.86
Zn (mg kg ⁻¹)	6.58 ± 1.22

3.2.2 Plant growth

The vetiver plants in the pot experiment grew well, and there were no visual signs of phytotoxicity in any of the treatments. There was no significant difference between treatments (*p* > 0.05) (Table 19). Plant roots in every treatment grew well and reached the base of the pots.

3.2.3 Lead accumulation

Lead concentrations in shoots and roots are presented in Table 20. Plants accumulated highest lead concentrations in the roots. Lead concentrations in plants increased with increasing lead in soil. The highest lead accumulations in shoot (38 mg kg^{-1}) and root (629 mg kg^{-1}) were measured in vetiver grown in the 1000 mg kg^{-1} treatment for 3 months.

3.3 Oil production

The highest oil yields found in plants grown in nutrient solution (100 mg L^{-1}) for 5 and 7 weeks were 1.29 and 1.22 %, respectively (Table 22). The lowest oil yield (0.42%) was produced by plants grown in nutrient solutions (10 and 50 mg L^{-1}) for 7 weeks. For the pot experiment, there was little difference between treatments and between the two growth periods.

3.4 Oil constituents

Total constituents of vetiver oil in different treatments are presented in Table 23. The highest total number of oil constituent (143 compounds) was found in vetiver plants grown in soil spiked with $1000 \text{ mg Pb kg}^{-1}$, followed by plants grown in solution with 100 mg L^{-1} (129 compounds).

Table 24 shows the percentage of 5 major compounds of vetiver oil in the descending order: khusimol (alcohol) > (*E*)-isovalencenol (alcohol) > vetiselinol (alcohol) > α -vetivone (ketone) > β -vetivone (ketone). From the hydroponics study, the percentage of vetiselinol increased with increasing lead in solution but did not relate to exposure time. Khusimol and (*E*)-isovalencenol percentages decreased when the lead concentration in solution was increased. For β -vetivone, the percentage decreased with increasing in exposure time but did not relate to lead concentration in solution. The percentage of α -vetivone increased with an increase in lead concentration but did not correlate with the exposure time. From the pot study, with an increase in lead concentration, khusimol showed a decrease in percentage, while vetiselinol, (*E*)-isovalencenol, and α -vetivone showed an increase. β -vetivone percentage was decreased with the increase in growth period but did not relate to lead concentration.

Table 22. Comparison of oil yields (dry weight basis) from various lead treatments (mean \pm SD, $n = 3$).

Oil yield % (dry weight basis)					
	Hydroponic study			Pot study	
	5 weeks	7 weeks		2 months	3 months
Control	0.49 \pm 0.13 b* [#] -a [#]	0.55 \pm 0.25 b-a	Control	0.58 \pm 0.22 a-a	0.81 \pm 0.29 a-a
10 mg L⁻¹	0.64 \pm 0.07 b-b	0.42 \pm 0.01 b-c	100 mg kg⁻¹	0.65 \pm 0.02 a-b	0.96 \pm 0.17 a-a
50 mg L⁻¹	1.18 \pm 0.28 a-a	0.42 \pm 0.09 b-b	500 mg kg⁻¹	0.72 \pm 0.09 a-b	0.64 \pm 0.08 a-b
100 mg L⁻¹	1.29 \pm 0.35 a-a	1.22 \pm 0.33 a-a	1000 mg kg⁻¹	0.59 \pm 0.06 a-b	0.94 \pm 0.11 a-ab

Data with different letters (*) in the same column and same item, or (#) in the same row indicate a significant difference at the 5% level according to LSD test

Table 23. Total constituents of vetiver oils from plants in various lead treatments.

Treatment			No. of constituent
Hydroponic	5 weeks	Control	65-89
		10 mg L ⁻¹	71-84
		50 mg L ⁻¹	97-117
		100 mg L ⁻¹	92-129
	7 weeks	Control	47-92
		10 mg L ⁻¹	74-94
		50 mg L ⁻¹	76-87
		100 mg L ⁻¹	66-87
Pot	2 months	Control	87-120
		100 mg kg ⁻¹	86-101
		500 mg kg ⁻¹	73-104
		1000 mg kg ⁻¹	120-143
	3 months	Control	80-98
		100 mg kg ⁻¹	76-93
		500 mg kg ⁻¹	72-87
		1000 mg kg ⁻¹	82-89

Khusimol was the largest component of vetiver oil (10.7-18.1%). In our hydroponics study, the highest percentage of khusimol was in control plants (7 weeks) followed by plants treated with 10 mg Pb L⁻¹ (5 weeks), while in the pot study, 100 mg kg⁻¹ treatments (2-3 months) yielded the highest percentage. For the second highest component, (*E*)-isovalencenol, 10 mg L⁻¹ treatments (5 weeks) yielded the highest percentage while in the pot study, the highest percentage was produce in 500 mg kg⁻¹ treatment (2 months).

Table 24. Comparison of major compounds of vetiver oil from plants grown in various lead treatments.

Treatment		Essential oil constituents (%)					
		Vetiselinenol	Khusimol	(E)-Isovalencenol	β-Vetivone	α-Vetivone	
Hydroponics study	5 weeks						
	Control	5.0	16.3	11.9	4.0	3.9	
	10 mg L ⁻¹	6.9	17.7	15.6	3.5	4.4	
	50 mg L ⁻¹	7.4	15.0	15.1	3.7	4.3	
7 weeks	100 mg L ⁻¹	7.7	15.3	14.0	3.7	4.9	
	Control	4.9	18.1	12.7	2.4	3.6	
	10 mg L ⁻¹	6.7	14.4	11.6	2.5	3.5	
	50 mg L ⁻¹	6.9	14.8	13.2	2.7	4.4	
Pot study	100 mg L ⁻¹	7.5	13.8	12.0	2.6	4.0	
	Control	2.7	15.6	10.7	3.6	1.6	
	100 mg kg ⁻¹	4.7	16.4	13.3	3.7	3.0	
	500 mg kg ⁻¹	6.1	15.8	14.4	3.7	3.7	
3 months	1000 mg kg ⁻¹	6.3	10.8	10.3	3.5	4.1	
	Control	8.6	13.6	12.3	1.9	2.6	
	100 mg kg ⁻¹	7.1	16.3	11.4	1.7	2.4	
	500 mg kg ⁻¹	8.4	13.2	12.4	2.0	2.9	
		1000 mg kg ⁻¹	9.3	10.7	13.6	1.9	2.3

Data shown are mean values of three plant replicates.

CHAPTER VI

DISCUSSION

1. Uptake and accumulation of lead by plants from the Bo Ngam lead mine

Metalliferous soils in the Bo Ngam lead mine area had the lowest pH of 6, the highest EC value of 1.14 dS m^{-1} , and were invariably deficient in essential plant nutrients, particularly N, P and K. It is well known that acidity affects plant growth directly and indirectly. Normal plant growth can be achieved in the pH range of 5-7 (Shu et al., 2001). Plant species also differ considerably in their ability to tolerate salt content in the root medium. In general, all species survive in EC range of $0\text{-}2 \text{ dS m}^{-1}$ (Shu et al., 2001). Hence, it appears that acidity and salinity were not constraints on vegetation establishment in the Bo Ngam lead mine area. But the toxic effects of residual quantities of lead and extremely low nutrients can strongly restrict plant growth.

All plants uptake metals to varying degrees from the substrates in which they are rooted. Baker (1981) proposed two basic strategies by which higher plants can tolerate the large amounts of metals in their environment: (1) exclusion, whereby transport of metals is restricted, and low, relatively constant metal concentrations are maintained in the shoot over a wide range of soil concentration; and (2) accumulation, whereby metals are accumulated in the upper plant parts at both high and low soil concentration. Baker and Whiting (2002) suggested that accumulators can be characterized by a leaf:root metal concentration ratio of more than 1, because of the ability to translocate metal from root to shoot, whereas in excluders, the ratio is less than 1. According to these criteria, among the 48 plant species sampled at Bo Ngam lead mine, 12 species were accumulators and 36 species were excluders. Most excluders (14 species) belonged to the grass family (Poaceae) although three species, *Dactyloctenium aegyptium*, *Sporobolus indicus* and *Paspalum scrobiculatum* were accumulators. *So. indicus* and *D. aegyptium* showed extremely high lead concentrations in their shoots ($>8000 \text{ mg kg}^{-1}$). Deram and Petit (1997) also found

lead hyperaccumulation in the grass *Arrhenatherum elatius* growing on calamine soils in France. These are of great interest because there have been no other convincing reports of the phenomenon in the Poaceae (Baker et al., 2000).

Hyperaccumulation of lead is particularly rare, due to the low solubility of most lead compounds, and the ready precipitation of lead by sulphate and phosphate at the root system (Baker et al., 2000). Baker et al. (2000) reported 14 taxa and six families, mostly from metalliferous soil. Two species of *Thlaspi* were reported to contain high lead concentration, *T. rotundifolium* ssp. *cepaefolium* (8200 mg kg⁻¹) from a lead/zinc mining area of Cave del Predil, northern Italy (Reeves and Brooks, 1983), and *T. caerulescens* (2740 mg kg⁻¹) colonizing a lead mine district in the Pennines, England (Shimwell and Laurie, 1972). Barry and Clark (1978) recorded shoot lead values of 130 mg kg⁻¹ to 11,750 mg kg⁻¹ in *Festuca ovina*. The present study found a total of 26 species of plants belonging to 11 families that had lead concentrations greater than 1000 mg kg⁻¹ dry weight in their shoots. Exceptionally high concentrations of lead in the shoots were found in plant samples from the ore dressing plant area with the soil lead of 142,400 mg kg⁻¹, such as *Sp. mauritiana* (28,370 mg kg⁻¹; Rubiaceae), *P. umbonata* (21,670 mg kg⁻¹; Polygalaceae). The rather erratic nature of lead values found in this kind of environment raises the question of the relative importance of entry via the root system and direct deposition on or into the leaves, e.g., from wind-blown soil particles (Baker et al., 2000). Careful study of this point is still required.

The standard for hyperaccumulator has not been defined scientifically. At present, there are four criteria: (1) the concentrations of heavy metal in plant shoots reach hyperaccumulating level (lead >1000 mg kg⁻¹; Baker et al., 1994), (2) the concentration of heavy metal in its above-ground part is 10-500 times more than that in usual plants (lead 5 mg kg⁻¹; Shen and Liu, 1998), (3) the metal concentrations in shoots are invariably greater than that in roots or shoot/root quotient >1 (Baker and Whiting, 2002), and (4) extraction coefficient >1 (Chen et al., 2004). In the present study, the concentrations of lead in most plant species collected were higher than that in usual plant. Comparing with the absolute concentrations of hyperaccumulator (lead >1000 mg kg⁻¹ in shoot dry matter), 26 species of plants were found. When shoot/root quotient >1 was used as a criterion, 11 species were hyperaccumulator. But when the

extraction coefficient > 1 was considered, no sample could be regarded as hyperaccumulator. If the three criteria were used (accumulation in shoot 10-500 times more than usual plant, shoot accumulation $>1000 \text{ mg kg}^{-1}$, and shoot/root quotient >1), there would be 11 plant species. These plants belonged to seven different families, such as Asteraceae (*Ageratum conyzoides*, *Chromolaena odoratum*, *Co. sumatrensis*, *Sonchus arvensis*), Buddlejaceae (*Buddleja asiatica*), Cyperaceae (*Cyperus* sp.), Euphorbiaceae (*Euphorbia hirta*), Poaceae (*D. aegyptium*, *So. indicus*), Fabaceae (*Aeschynomene indica*) and Polygalaceae (*P. umbonata*). Plant species that are suited for phytoremediation should have wide distribution, high above-ground biomass, high bioaccumulation, and high propagation rate (Visoottiviset et al., 2002). The plant species with high lead accumulation are not necessarily those occurring with the greatest frequency at the contaminated sites. For example, *P. umbonata* could accumulate $21,670 \text{ mg kg}^{-1}$ Pb in their shoot, but they were rarely found. From the 11 species of accumulator, about five species could be considered as possible phytoremediators of lead contaminated soil (Table 25). These were *C. odoratum* (herb), *S. arvensis* (herb), *Co. sumatrensis* (herb), *B. asiatica* (shrub), and *Cyperus* sp. (herb). These species were commonly found at lead-contaminated sites, had high above-ground biomass and high propagation rates. *C. odoratum* and *B. asiatica* are perennial shrubs that can attain the height of 3-4 m. It is generally preferable to use a perennial since phytoremediation will never take just a single year and the use of a perennial will prevent the need to annual planting.

2. Growth and lead accumulation by the grasses *Vetiveria zizanioides* and *Thyrsanolaena maxima* in lead-contaminated soil amended with pig manure and fertilizer: A glasshouse study

The choice of plants is a crucial aspect for the practical application of phytostabilization based techniques on mine-degraded soils (Tordoff et al., 2000; Bleeker et al., 2002; Freitas et al., 2004; Rizzi et al., 2004). In terms of stabilizing metal-contaminated sites, a lower metal concentration in shoots is preferred, in order to prevent metal potentially entering the ecosystem through the food chain (Pichtel et al., 2000; Taylor and Percival, 2001; Yang et al., 2003). From the present study, at the

Table 25. Plant species most suitable for lead contaminated areas.

Plant species	Criteria					
	Pb concentration in shoot	Ability for propagation	Frequency of distribution	Life cycle	Amount of shoot biomass	Height (m)
<i>Chromolaena odoratum</i>	Fair (3,730 mg/kg)	Excellent	Excellent	Fair (~2 years)	Excellent	1-3
<i>Conyza sumatrensis</i>	Excellent (6,380 mg/kg)	Excellent	Excellent	Good (~1 year)	Good	0.3-2
<i>Sonchus arvensis</i>	Excellent (7,700 mg/kg)	Excellent	Excellent	Good (~1 year)	Good	0.4-1.5
<i>Buddleja asiatica</i>	Fair (3,800 mg/kg)	Excellent	Excellent	Good (~1 year)	Excellent	2-4
<i>Cyperus</i> sp.	Fair (1,233 mg/kg)	Excellent	Excellent	Good (~1 year)	Excellent	0.2-0.6

highest application dosage of lead (10750 mg kg^{-1} as Pb), *T. maxima* and *V. zizanioides* (Surat Thani and Songkhla) could grow well and showed no toxicity symptoms indicating that they could tolerate the high lead concentrations in the soil, especially *T. maxima* which had the better growth performance. Lead concentrations in shoots and roots of both species were in the range of $248\text{--}422 \text{ mg kg}^{-1}$ and $4100\text{--}5900 \text{ mg kg}^{-1}$, respectively. The results on tolerance and accumulation of lead by vetiver grass were rather similar to the previous studies. Chen et al. (2004) and Chantachon et al. (2004) had shown that *V. zizanioides* could grow well at $5000\text{--}10000 \text{ mg Pb kg}^{-1}$ as $\text{Pb}(\text{NO}_3)_2$ and there were no visual signs of phytotoxicity. *V. zizanioides* has a massive finely structured and deep root system and it has been known for its effectiveness in erosion control and the ability to tolerate extreme soil conditions including heavy metal contamination (Wong, 2003). Truong and Baker (1998) have reported the successful use of *V. zizanioides* to stabilize mining overburden and highly saline, sodic, magnesian and alkaline (pH 9.5) tailings of coal mine and highly acidic (pH 2.7) and high arsenic tailings of gold mines in Australia. Nowadays vetiver grass has been widely used as an alternative method for rehabilitation of mine tailings in several countries, including China (Pang et al., 2003).

Our previous study has shown *T. maxima* is one of the most widely distributed species at Bo Ngam lead mine (Rotkittikhun et al., 2006). It has the ability to withstand very high soil lead concentrations (up to $10\text{--}100 \text{ g kg}^{-1}$). Similar to vetiver grass, lead was found to accumulate more in roots ($10,720 \text{ mg kg}^{-1}$) than in shoots (370 mg kg^{-1}). In the present study, *T. maxima* showed better growth performance than vetiver grass. In addition, in the field, *T. maxima* has a very fast growth rate, is very large in size and possesses a very deep root system, and can tolerate extremely high lead concentrations. The only drawback is they commonly grow well at high elevation from about 2500–5000 feet (Rhind, 1945). Nevertheless, *T. maxima* can be another alternative for phytostabilization of lead mine soil. In mine lands, there are some major constraints to plant growth and the success of restoration is dependent upon overcoming these problems (Bradshaw, 1997). The present study indicated that Bo Ngam lead mine soil contained high levels of total and DTPA-extractable lead and low levels of organic matter and major nutrients (N, P, K). Therefore, the application

of pig manure or inorganic fertilizer should improve these soil properties. The application of pig manure to lead mine soils resulted in a significant increase in EC value because pig manure contained large amount of soluble salts such as Fe and K ions. However, it reduced the total and DTPA-extractable lead in the soil. This may be due to chelation, complexation, and adsorption between metals in soils and the organic matter that is contained in organic amendments and also the dilution effect when they are mixed with soils (Friedland, 1989; Lozano-cerezo et al., 1999; Chiu et al., 2006). Similar results were obtained when applying lime, manure compost and sewage sludge to Pb/Zn mine tailings (Ye et al., 1999; Chiu et al., 2006). In contrast, the application of fertilizer increased the total and DTPA-extractable lead in our lead mine soils. The present study demonstrated that the application dosage of 20% pig manure was the most effective in improving the biomass of vetiver grown in lead mine soils. However, the application of inorganic fertilizer did not effectively improve vetiver growth. These results suggested that pig manure amendment could provide enough nutrients for the growth of vetiver in lead mine soils and may improve their physical conditions. Analysis of pig manure in this study showed 100 times more organic matter, N and P, and 14 times more K than in the lead mine soil. These could enhance growth in vetiver. Similar results were reported by Ye et al. (1999); adding inorganic fertilizer alone did not significantly improve the growth of *Agropyron elongatum* or *Trifolium repens*. On the contrary, *T. maxima* had high biomass when grown in lead mine soils amended with 150 mg kg⁻¹ inorganic fertilizer and very poor when amended with pig manure. This may be due to the increase in EC value by pig manure amendment (from 0.23 to 3.96 dS m⁻¹). In general, most plant species survive in the EC range of 0–2 dS m⁻¹ and sensitive species are affected by EC of 4–8 dS m⁻¹, while only tolerant species can achieve satisfactory growth when the EC is greater than 8 dS m⁻¹ (Shu et al., 2001). The results indicated that vetiver is more salt tolerant than *T. maxima*.

The root/shoot ratio is a highly representative indicator of environmental stress encountered by plants (Chiu et al., 2006). Plants have been documented to increase root/shoot ratio when stressed by harsh environments (Xia, 2004). In the present study, *T. maxima* and vetiver grown in high lead concentrations (10000 mg kg⁻¹) exhibited the highest root/shoot ratios. The results from the amendment study

correlated well with the growth performance. Vetiver grass grown in lead mine soil amended with pig manure showed lower root/shoot ratios and better growth performance than those grown in lead mine soil alone or amended with fertilizer. On the other hand, *T. maxima* showed the greatest stress (with highest value of root/shoot ratio) when grown in lead mine soil amended with 20% pig manure. This again suggested that *T. maxima* cannot tolerate soil with high soluble salts. The addition of pig manure to the lead mine soil reduced the total and extractable lead while inorganic fertilizer amendment resulted in the increase of lead in the soil. Hence, both *T. maxima* and *V. zizanioides* showed a decrease in lead uptake when grown in soil amended with pig manure. On the other hand, higher applications of inorganic fertilizer (150 mg kg^{-1}) caused an increase in lead uptake in these grass species, while lower additions (75 mg kg^{-1}) did not have any significant effect on lead uptake. Several other studies have reported that refuse, pig manure and CaCO_3 , cow manure, manure compost and sewage sludge effectively reduce lead availability to plants leading to lower lead uptake (Scialdone et al., 1980; Wong and Lau, 1985; Ye et al., 1999; Chiu et al., 2006). It was suggested that soil had a relatively large capacity for the immobilization of ionic lead and that the precipitation of lead was primarily through the fixation by organic matter (Alloway, 1995). Hence, the high organic carbon content of amended soil caused the lower uptake of lead by these grass species.

3. Lead tolerance and accumulation in the grass *Vetiveria zizanioides* and the effects of lead on vetiver oil production

The results of this research demonstrate the expected effect that elevated concentrations of lead in solution decrease the length of shoots and roots of plants due to lead toxicity. However, vetiver grown in highly contaminated soils showed no apparent phytotoxicity symptoms. A possible explanation for this lack of phytotoxicity in plants grown in the pot study would be the high organic matter content of the growth medium which has a high affinity for the metal (Zheljazkov et al., 2006). According to the results of Kabata-Pendias and Pendias (1992), lead concentrations in mature leaf tissues greater than 30 ppm (DW) are considered excessive or toxic to plants. However, in this study, lead concentrations in the shoots

and roots of vetiver plants grown in hydroponic were up to 144 and 19,530 mg kg⁻¹ DW, and those grown in soil were 38 and 629 mg kg⁻¹ DW, respectively. The results of our study show that vetiver can tolerate high lead concentrations in soil as suggested in earlier studies by Chen et al. (2004) and Chantachon et al. (2004). Vetiver grass has been cultivated widely for use in the prevention of soil erosion and stabilization of heavy metals in contaminated land. Rotkittikhun et al. (2007) showed that vetiver is a good choice for phytostabilization of lead mine soil in Thailand. It has a high tolerance to lead and can accumulate much more lead in roots than in shoots.

Previous studies have suggested that the oil yield and composition in vetiver could be influenced by several factors such as geographical origin, cultivar, cultivation methods, the presence of microorganisms, and environmental parameters, such as temperature. Champagnat et al. (2006) reported different oil composition of vetiver from 9 countries (different geographical origins) but no meaningful differences were observed. Variations in percentage oil yield and oil composition were observed in different vetiver DNA-fingerprinted cultivars (Adams et al., 2003). Pripdeevech et al. (2006) reported different oil yields and constituents of vetiver oil in vetiver grown under different cultivation conditions. A higher oil yield was obtained by cultivation in normal soil with added microbes, while vetiver oil from semi-hydroponic cultivation, which contained no fertilizer and no effective microbes, gave the lowest yield (Pripdeevech et al., 2006). Similarly, Adams et al. (2004) also showed that tissue cultured (cleansed) vetiver plants grown in sterilized soil produced lower oil yield than normal vetiver grown in unsterilized soil. They produced only trace amounts of oil and a strikingly different oil compositions compared to the oils from normal vetiver plants. Furthermore, it has been shown that soil fertilization increased oil yields dramatically without changing the composition (Dethier et al., 1997; Adams et al., 2003). In addition, vetiver oil production is closely related to plant metabolism that is affected by temperature (Massardo et al., 2006). Low temperature (such as in winter) causes a decrease in plant metabolic activities and hence a decrease in oil production (Massardo et al., 2006).

The present study demonstrated that heavy metals such as lead clearly had an effect on vetiver oil production and composition. Lead stimulated the oil production and constituents. Lavania (2003) reported that vetiver roots may give a yield of about

0.3-2.0% essential oil on a fresh weight basis. From this study, the oil yield ranged from 0.42-1.29%. The highest oil yields were found in plants grown in nutrient solution with 100 mg Pb L⁻¹ for 5 weeks (1.29%) and 7 weeks (1.22%). The total constituents of vetiver oil also varied due to the presence of lead in the growth medium. They were in the range of 47-143 compounds. The highest number of total constituents was found in plants grown in soil spiked with 1000 mg Pb kg⁻¹ (143 compounds), followed by those grown in solution with 100 mg Pb L⁻¹ (129 compounds). Massardo et al. (2006) explained the increase in oil yield as the result of warm temperature was due to the symbiotic intracellular bacteria associated with oil-producing gland cells in vetiver roots. These bacteria were thought to be involved in essential oil metabolism in vetiver (Bertea and Camusso, 2002; Adams et al., 2004; Massardo et al., 2006; Pripdeevech et al., 2006). We did not observe the vetiver root gland cells for the presence of intracellular bacteria but we assumed that since we used normal vetiver plants and unsterilized solution and soil, these bacteria should be present in the root gland cells. These symbiotic bacteria may be affected by lead in the medium and lead could have stimulated essential oil metabolism by these bacteria.

A typical analysis of vetiver oil indicates three major fractions: the lower boiling or sesquiterpene hydrocarbon fraction, the intermediate fraction containing the bulk of oxygenated derivatives, and the third fraction represented by khusimol fraction, which contains the main alcohol component in the vetiver oil (Lemberg and Hale, 1978; Massardo et al., 2006). Furthermore, these major constituents are responsible for the base note of vetiver oil (Weyerstahl et al., 2000). From our results, the largest component was khusimol (10.7-18.1%) followed by (*E*)-isovalencenol (10.3-15.6%). The smaller compounds were vetiselinol (2.7-9.3%), α -vetivone (1.6-4.9%), and β -vetivone (1.7-4.0%). These main components of vetiver oil in the present study are somewhat similar to the previous studies. Adams et al. (2003) reported that different cultivars of vetiver had khusimol (14-31%), (*E*)-isovalencenol (10-16%), β -vetivone (2-6%) and α -vetivone (3-6%) as the main components. The characteristic components of vetivers from different geographical origins were β -vetiseprene (1.6-4.5%), khusimol (3.4-13.7%), vetiselinol (1.3-7.8%) and α -vetivone (2.5-6.3%). Massardo et al. (2006) reported (*E*)-isovalencenol (19.5-25%) as the main component of vetiver, followed by khusimol (11.1-12.9%), and α -vetivone

as small component. The quality of vetiver or volatile oil usually depends on the amounts of alcohols (mainly khusimol) which are mainly responsible for the desired woody odour of vetiver oil (Arctander, 1960).



CHAPTER VII

CONCLUSION

Our results indicated that the lead mine area was a harsh habitat for vegetation development, with soil lead content as high as 14-15%. However, 48 species of plants (mostly herbs, grasses and undershrubs) grew in this area. There were a total of 11 species of lead hyperaccumulators. They were able to accumulate >0.1% of lead in their shoots and had a shoot/root quotient >1. The highest lead content (2.8%) was found in *Sp. mauritiana*. Interestingly, three grass species (*M. ciliatum*, *D. aegyptium* and *Pe. polystachyon*) were able to accumulate 0.6-1.2% of lead in their shoots. These accumulators, when confirmed under laboratory and field trial conditions, could be useful in practical phytoremediation approaches and reduction of the risk from metals to human health.

T. maxima and *V. zizanioides* (Surat Thani and Songkhla) are good choices for phytostabilization of lead mine soil. They showed very high tolerance to lead and could accumulate much more lead in roots than in shoots. The applications of pig manure and inorganic fertilizer to the lead mine soil could increase nutrients for plant growth. *T. maxima* showed the best growth when grown in lead mine soil amended with 150 mg kg⁻¹ inorganic fertilizer while the amendment with 20% pig manure resulted in the best growth of *V. zizanioides*. In addition, the use of pig manure also resulted in decreased pH and lead concentrations, and increases in EC and organic matter.

The results demonstrate that lead could increase the oil production of vetiver. Due to its high lead tolerance, planting vetiver grass in a lead mine area could serve the dual purpose of stabilizing the mine site and at the same time producing oil with a high commercial value. More research is needed to investigate the soil lead concentration and methods of soil amendment that will promote the highest yield of vetiver oil by further pot and field trial experiments.

BIBLIOGRAPHY

- Adams, R.P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*. Allured Publishing Corp, Carol Stream, IL.
- Adams, R.P., Pandey, R.N., Dafforn, M.R., & James, S.A. (2003). Vetiver DNA-Fingerprinted cultivars: Effects of environment on growth, oil yields and composition. *Journal of Essential Oil Research*, 15, 363-371.
- Adams, R.P., Habte, M., Park, S., & Dafforn, M.R. (2004). Preliminary comparison of vetiver root essential oils from cleansed (bacteria-and fungus-free) versus non-cleansed (normal) vetiver plants. *Biochemical Systematics and Ecology*, 32, 1137-1144.
- Allen, S.E., Grimshaw, H.M., Parkinson, H.M., & Quarmby, J.A. (1974). *Chemical Analysis of Ecological Materials*. Oxford: Blackwell.
- Alloway, B.J. (1995). Soil process and the behavior of metals. In B.J. Alloway (Ed.), *Heavy Metals in Soils* (pp. 11-37). New York: John Wiley.
- Anderson, N.H. (1970). Biogenetic implications of the antipodal sesquiterpenes of vetiver soil. *Phytochemistry*, 9, 145-151.
- Angle, J.S., Baker, A.J.M., Whiting, S.N., & Chaney, R.L. (2003). Soil moisture effects on uptake of metals by *Thlaspi*, *Alyssum*, and *Berkheya*. *Plant and Soil*, 256, 325-332.
- APHA, AWWA, & WEF. (1998). *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association.
- Arctander, S. (1960). *Perfume and Flavor Materials of Natural Origin*. New Jersey: Arctander
- Ayerst, J.M. (1978). The effect of compaction on the revegetation of spoil heaps. *Reclamation & Revegetation Research*, 1, 27-30.
- Baker, A.J.M. (1981). Accumulators and excluders- strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, 3, 643-654.

- Baker, A.J.M., & Brooks, R.R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology, and phytochemistry. *Biorecovery*, 1, 81-126.
- Baker, A.J.M., McGrath, S.P., Sidoli, C.M.D., & Reeves, R.D. (1994). The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resources, Conservation and Recycling*, 11, 41-49.
- Baker, A.J.M., Reeves, R.D., & Hajar, A.S.M. (1994). Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J.&C. Presl (Brassicaceae). *New Phytologist*, 127, 61-68.
- Baker, A.J.M., McGrath, S.P., Reeves, R.D., & Smith, J.A.C. (2000). Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In N. Terry, & G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soil and Water* (pp. 359-376). Florida: Lewis.
- Baker, A.J.M., & Whiting, S.N. (2002). In search of the Holy Grail-a further step in understanding metal hyperaccumulation? *New Phytologist*, 155, 1-7.
- Barceló, J., & Poschenrieder, Ch. (1999). Structural and ultrastructural changes in heavy metal exposed plants. In M.N.V. Prasad, & J. Hagemeyer (Eds.), *Heavy Metal Stress in Plants: From Molecules to Ecosystems* (pp. 183-206). Heidelberg: Springer.
- Barceló, J., & Poschenrieder, Ch. (2003). Phytoremediation: principles and perspectives. *Contributions to Science*, 2, 333-344.
- Barry, S.A.S., & Clark, S.C. (1978). Problems of interpreting the relationship between the amounts of lead and zinc in plants and soil on metalliferous wastes. *New Phytologist*, 81, 773-783.
- Bazzar, F.A., Rolfe, G.L., & Windle, P. (1974). Differing sensitivity of corn and soybean photosynthesis and transpiration to lead contamination. *Journal of Environmental Quality*, 3, 156-158.
- Bertea, C., & Camusso, W. (2002). Anatomy, biochemistry and physiology. In M. Maffei. (Ed.), *Vetiveria. The Genus Vetiveria* (pp. 19-43). London: Taylor & Francis.
- Berti, W.R., & Cunningham, S.D. (2000). Phytostabilization of metal. In L. Raskin,

- B.D. Ensley (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* (pp. 71-88). New York: Wiley.
- Black, G.R. (1965). *Bulk Density: Method of Soil Analysis*. Monograph No.9 Part I. Wisconsin: American Society of Agronomy Inc.
- Blaylock, M.J., & Huang, J.W. (2000). Phytoextraction of metals. In I. Raskin, B.D. Ensley (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* (pp. 53-70). New York: Wiley.
- Bleeker, P.M., Assunção, A.G.L., Teiga, P.M., de Koe, T., & Verkleij, J.A.C. (2002). Revegetation of the acidic, As contaminated Jales mine spoil tips using a combination of spoil amendments and tolerant grasses. *Science of The Total Environment*, 300, 1–13.
- Bradshaw, A.D. (1997). Restoration of mined lands-using natural processes. *Ecological Engineering*, 8, 255-269.
- Bradshaw, A.D. (2000). The use of natural processes in reclamation-advantages and difficulties. *Landscape and Urban Planning*, 51, 89-100.
- Bradshaw, A.D., & Chadwick, M.J. (1980). *The Restoration of Land*. Oxford: Blackwell.
- Bray, R.H., & Kurtz, L.T. (1945). Determination of total, organic and available forms of phosphorus in soil. *Soil Science*, 59, 39-45.
- Brown, S.L., Chaney, R.L., Angle, S.J., & Baker, A.J.M. (1994). Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soil. *Journal of Environmental Quality*, 23, 1151-1157.
- Champagnat, P., Figueredo, G., & Chalchat, J.C. (2006). A study on the composition of commercial *Vetiveria zizanioides* oils from different geographical origins. *Journal of Essential Oil Research*, 18, 416-422.
- Chaney, R.L., Brown, S.L., Li, J.M., Angle, S.J., & Gree, C. (1995). Potential use of heavy metal accumulators. *Mining Environmental Management*, 3, 9-11.
- Chantachon, S., Kruatrachue, M., Pokethitiyook, P., Upatham, E.S., Tantanasarit, S., & Soonthornsarathool, V. (2004). Phytoextraction and accumulation of lead from contaminated soil by vetiver grass: Laboratory and simulated field study. *Water Air and Soil Pollution*, 154, 37-55.
- Chen, H.M., Zheng, C.R., Tu, C., & Shen, Z.G. (2000). Chemical methods and

- phytoremediation of soil contaminated with heavy metals. *Chemosphere*, 41, 229-234.
- Chen, Y., Shen, Z., & Li, X. (2004). The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. *Applied Geochemistry*, 19, 1553-1565.
- Chiu, K.K., Ye, Z.H., & Wong, M.H. (2006). Growth of *Vetiveria zizanioides* and *Phragmites australis* on Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge: A greenhouse study. *Bioresource Technology*, 97, 158-170.
- Chomchalow, N. (2000). *Manual of the International Training Course on the Vetiver System*. Bangkok: Office of Royal Development Project Board.
- Chomchalow, N. (2001). *The Utilization of Vetiver as Medicinal and Aromatic Plants with Special Reference to Thailand*. (Technical Bulletin No. 2001/1). Bangkok: Office of the Royal Development Projects Board.
- Clemens, S., Palmgren, M.G., & Krämer, U. (2002). A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science*, 7, 309-315.
- Conesa, H.M., Faz, Á., & Arnaldos, R. (2007). Initial studies for the phytostabilization of a mine tailing from the Cartagena-La Union Mining District (SE Spain). *Chemosphere*, 66, 38-44.
- Coppin, N.J., & Bradshaw, A.D. (1982). *Quarry Reclamation*. London: Mining Journal Books.
- Crowley, D.E., Wang, Y.C., Reid, C.P.P., & Azanislo, P.J. (1991). Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant and Soil*, 130, 179-198.
- Cunningham, S.D., & Berti, W.R. (1993). Remediation of contaminated soils with green plants: An overview. *In Vitro Cellular and Developmental Biology*, 20, 207-212.
- Cunningham, S.D., Berti, W.R., & Huang J.W. (1995). Phytoremediation of contaminated soils. *Trends in Biotechnology*, 13, 393-397.
- Cunningham, S.D., & Ow, D.W. (1996). Promises and prospects of phytoremediation. *Plant Physiology*, 110, 715-719.

- Cunningham, S.D., Shann, J.R., Crowley, D.E., & Anderson, T.A. (1997). Phytoremediation of contaminated water and soil. In E.L. Kruger, T.A. Anderson, & J.R. Coats (Eds.), *Phytoremediation of Soil and Water Contaminants* (pp. 2-19). Washington, DC: American Chemical Society.
- Cunningham, S.D., & Berti, W.R. (2000). Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue. In N. Terry, & G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soil and Water* (pp. 359-376). Florida: Lewis.
- Dancer, W.S., Handley, J.F., & Bradshaw, A.D. (1977). Nitrogen accumulation in kaolin mining wastes in Cornwall II forage legumes. *Plant and Soil*, 48, 303-314.
- Demole, E.P., Holzner, G.W., & Youssefi, M.J. (1995). Malodor formation in alcoholic perfumes containing vetiveryl acetate and vetiver oil. *Perfume Flavor*, 20, 35-40.
- Department of Primary Industries and Mines, (1998). *Proposed Lead Potential Area Delineated from the on-going Declared Lum Klong Ngu National Park*. Bangkok: Ministry of Industry.
- Deram, A., & Petit, D. (1997). Ecology of bioaccumulation in *Arrhenatherum elatius* L. (Poaceae) populations-applications of phytoremediation of zinc, lead and cadmium contaminated soils. *Journal of Experimental Botany*, 48, 98.
- Dethier, M., Sakubu, S., Ciza, A., Cordier, Y., Menut, C., & Lamaty, G. (1997). Aromatic plants of tropical central Africa. XXVIII. Influence of cultural treatment and harvest time on vetiver oil quality in Burundi. *Journal of Essential Oil Research*, 9, 447-451.
- Ernst, W.H.O. (1996). Bioavailability of heavy metals and decontamination of soil by plants. *Applied Geochemistry*, 11, 163-167.
- Ernst, W.H.O. (2005). Phytoextraction of mine wastes-options and impossibilities. *Chemie der Erde*, 65, 29-42.
- Ewers, U., & Schlipkötter, H.W. (1991). Lead. In E. Merian (Ed.), *Metals and their Compounds in the Environment: Occurrence, Analysis, and Biological Relevance* (pp. 971-1014). Weinheim: VCH Verlagsgesellschaft.
- Förstner, U., & Wittmann, G.T.W. (1979). *Metal Pollution in the Aquatic Environment*. Heidelberg: Springer.

- Freitas, H., Prasad, M.N.V., & Pratas, J. (2004). Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications. *Environment International*, 30, 65–72.
- Frieland, A. J. (1989). The movement of metals through soils and ecosystem. In A. J. Shaw (Ed.), *Heavy Metal Tolerance in Plants: Evolutionary Aspects* (pp. 7-19). Boca Raton: CRC Press.
- Garbisu, C. & Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology*, 77, 229-236.
- Godbold, D.L., Horst, W.J., Thurmann, D.A., & Marschner, H. (1984). Accumulation of zinc and organic acids in roots of zinc-tolerant and non-tolerant ecotypes of *Deschampsia caepitosa*. *Journal of Plant Physiology*, 116, 59.
- Greger, M. (1999). Metal availability and bioconcentration in plants. In M.N.V. Prasad, & J. Hagemeyer (Eds.), *Heavy Metal Stress in Plants: From Molecules to Ecosystems* (pp. 1-28). Heidelberg: Springer.
- Guenther, E. (1950). *The Essential Oils*. Canada: Nostrand Company Inc.
- Gunn, J.M. (1995). *Restoration and Recovery of an Industrial Region*. New York: Springer.
- Harris, J.A., Birch, P., & Palmer, J.P. (1996). *Land Restoration and Reclamation: Principles and Practice*. London: Longman.
- Huang, J.W. (1992). Aluminium effects on calcium fluxes at the root apex of aluminium-tolerant and aluminium-sensitive wheat cultivars. *Plant Physiology*, 98, 230-237.
- Huang, J.W., & Cunningham, S.D. (1996). Lead phytoextraction-species variation in lead uptake and translocation. *New Phytologist*, 134, 75-84.
- International Center for Agricultural Research in the Dry Areas (ICARDA). (2001). *Soil and Plant Analysis Laboratory Manual*. Syria: ICARDA and NARC.
- Irmer, U., Wachholz, I., Schäfer, H., & Lorch, D.W. (1986). Influence of lead on *Chlamydomonas reinhardtii* Danegard (Volvocales, Chlorophyta): accumulation, toxicity and ultrastructural changes. *Environmental and Experimental Botany*, 26, 97-105.
- Kabata-Pendias, A., & Pendias, H. (1984). *Trace Metals in Soils and Plants*. Florida:

CRC Press

Kabata-Pendias, A., & Pendias, H. (2000). *Trace Elements in Soils and Plants*.

Florida: CRC Press.

Kartusch, R., & Kartusch, B. (1978). Nachweis and Lokalisierung der wurzel von

Vetiveria zizanioides (L.). *Mikroskopie*, 34, 195-201.

Kinnersely, A.M. (1993). The role of phytochelates in plant growth and productivity.

Plant Growth Regulation, 12, 207-217.

Kochian, L.V. (1993). Zinc absorption from hydroponic solutions by plants roots. In

A.D. Robson (Ed.), *Zinc in Soils and Plants* (pp. 45-57). Boston: Kluwer Academic Publishers.

Krämer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, A.J.M., & Smith, J.A.

(1996). Free histidine as a metal chelator in plants that accumulate nickel.

Nature, 379, 635-637.

Kumar, P.B.A.N., Dushenkov, V., Motto, H., & Raskin, I. (1995). Phytoextraction:

The use of plants to remove heavy metals from soils. *Environmental Science & Technology*, 27, 1232-1237.

Kuo, S., Jellum, E.J., & Baker, A.J. (1985). The effect of pH on metal uptake.

American Society of Soil Science, 139, 122.

Lasat, M.M. (2002). Phytoextraction of toxic metals: a review of biological

mechanisms. *Journal of Environmental Quality*, 31, 109-120.

Lavana, U.C. (2003). *Other Uses, and Utilization of Vetiver: Vetiver Oil*.

Guangzhou: Proceedings of the Third International Conference on Vetiver.

Lozano-cerezo, M.L., Fernández-marcos, M.L., & Álvarez-rodriguez, E. (1999).

Heavy metals in mine soils amended with sewage sludge. *Land Degradation & Development*, 10, 555-564.

Lemberg, S., & Hale, R.B. (1978). Vetiver oils of different geographical origins.

Perfume Flavor, 3, 23-27.

Lindsay, W.L. (1979). *Chemical Equilibration in Soils*. New York: Wiley.

Ma, Q. (1993) Immobilization of soil Pb by apatite. *Environmental Science &*

Technology, 27, 1803-1810.

Maffei, M. (2002). Introduction to the genus *Vetiveria*. In M. Maffei (Ed.), *Vetiveria*.

The Genus Vetiveria (pp. 1-18). New York: Taylor & Francis.

- Marshall, J. (1994). Membrane potential-dependent calcium transport in right-side-out plasma membrane vesicles from *Zea mays* L. roots. *Plant Journal*, 5, 683-694.
- Marshall, J.A., & Anderson, N.H. (1967). The structure of α -vetivone (isonootkatone). *Tetrahedron Letters*, 17, 1611-1615.
- Marshall, J.A., & Johnson, P.C. (1968). The total synthesis of β -vetivone. *Chemical Communication*, 391.
- Marshall, J.A., & Johnson, P.C. (1970). Structure and synthesis of β -vetivone. *Journal of Organic Chemistry*, 35, 192-196.
- Martinez, J., Rosa, P.T.V., Menut, C., Leydet, A., Brat, P., Pallet, D., & Meireles, M.A.A. (2004). Valorization of Brazilian vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) oil. *Journal of Agricultural and Food Chemistry*, 52, 6578-6584.
- Massardo, D., Senatore, F., Alifano, P., Giudice, L.D., & Pontieri, P. (2006). Vetiver oil production correlates with early root growth. *Biochemical Systematics and Ecology*, 34, 376-382.
- Nigam, I.C., & Komae, H. (1968). Essential oils and their constituents XXXVIII, isolation and structure of khusenol, a new sesquiterpenic primary alcohol from oil of vetiver. *Journal of Pharmaceutical Sciences*, 57, 1029-1030.
- Nriagu, J.O. (1989). *The History of Leaded Gasoline*. Geneva: Proceedings of the Seventh International Conference on Heavy Metal in the Environment.
- Palmer, J.P., & Iverson, L.R. (1983). Factors affecting nitrogen fixation by white clover (*Trifolium repens*) on colliery spoil. *Journal of Applied Ecology*, 20, 287-301.
- Pang, J., Chan, G.S.Y., Zhang, J., Liang, J., & Wong, M.H. (2003). Physiological aspects of vetiver grass for rehabilitation in abandoned metalliferous mine wastes. *Chemosphere*, 52, 1559-1570.
- Parker, D.R., Norvell, W.A., & Chaney, R.L. (1993). GEOCHEM-PC a chemical speciation program for IBM and compatible personal computers. In R.H. Loeppert, A.P. Schwab, & S. Goldberg (Eds.), *Soil Chemical Equilibrium and Reaction Models* (pp. 253-269). Wisconsin: Soil Science Society of America.
- Peyron, L. (1989). Vetiver in perfumery. *Quintessenza* 13, 4-14.
- Pichtel, J., Kuroiwa, K., & Sawyer, H.T. (2000). Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. *Environmental Pollution*, 110, 171-

178.

- Prasad, M.N.V. (2006). Stabilization, remediation, and integrated management of metal-contaminated ecosystems by grasses (Poaceae). In M.N.V. Prasad, K.S. Sajwan, & R. Naidu (Eds.), *Trace Elements in the Environment; Biogeochemistry, Biotechnology and Bioremediation* (pp. 405-424). Florida: Taylor&Francis.
- Prasad, M.N.V., & Strzalka, K. (1999). Impact of heavy metals on photosynthesis. In M.N.V. Prasad, & J. Hagemeyer (Eds.), *Heavy Metal Stress in Plants: From Molecules to Ecosystems* (pp. 117-138). Heidelberg: Springer.
- Prezemeck, E., & Haase, N.U. (1991). The bonding of manganese, copper and cadmium to peptides of the xylem sap of plant roots. *Water Air and Soil Pollution*, 57-58, 569-577.
- Pripdeevech, P., Wongpornchai, S., & Promsiri, A. (2006). Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions. *Molecules*, 11, 817-826.
- Pulford, I.D., & Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees-a review. *Environment International*, 29, 529-540.
- Purves, D. (1985). *Trace-element Contamination of the Environment*. New York: Elsevier.
- Radloff, B., Walsh, K., & Melzer, A. (1995). *Direct Revegetation of Coal Tailings at BHP. Saraji Mine*. Darwin: Australian Mining Council Environment.
- Raskin, I., Kumar, P.B.A.N., Dushenkov, S., & Salt, D. (1994). Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 5, 285-290.
- Raskin, I., Smith, R.D., & Salt, D.E. (1997). Phytoremediation of metals: using plants to remove pollutants from the environment. *Current opinion in Biotechnology*, 8, 221-226.
- Reeves, R.D., & Brooks, R.R. (1983). Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. *Environmental Pollution Series A*, 31, 277-285.
- Rhind, D. (1945). *The Grasses of Burma*. Calcutta: The Baptist Mission Press.
- Rizzi, L., Petruzzelli, G., Poggio, G., & Vigna Guidi, G. (2004). Soil physical changes and plant availability of Zn and Pb in a treatability test of phytostabilization.

- Chemosphere*, 57, 1039–1046.
- Roongtanakiat, N., & Chairroj, P. (2002). *Vetiver Grass for Remedying soil Contaminated with Heavy Metals*. Bangkok: Proceedings of the 17th World Congress on Soil Science.
- Rotkittikhun, P., Kruatrachue, M., Chaiyarat, R., Ngernsarsaruay, C., Pokethitiyook, P., Paijitprapaporn, A., et al. (2006). Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand. *Environmental Pollution*, 144, 681-688.
- Rotkittikhun, P., Chaiyarat, R., Kruatrachue, M., Pokethitiyook, P., & Baker, A.J.M. (2007). Growth and lead accumulation by the grasses *Vetiveria zizanioides* and *Thysanolaena maxima* in lead-contaminated soil amended with pig manure and fertilizer: A glasshouse study. *Chemosphere*, 66, 45-53.
- Saager, R. (1984). *Encyclopedia of Metallic Raw Materials*. Zurich: Bank von Tobel.
- Salomons, W. (1995). Environmental impact of metals derived from mining activities: processes, predictions, prevention. *Journal of Geochemical Exploration*, 52, 5-23.
- Salt, D.E., Blaylock, M., Kumar, P.B.A.N., Dushenkov, V., Ensley, B.D., Chet, L., & Raskin, L. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology*, 13, 468-474.
- Saxena, P.K., KrishnaRaj, S., Dan, T., Perras, M.R., & Vettakkorumakankav, N.N. (1999). Phytoremediation of heavy metal contaminated and polluted soils. In M.N.V. Prasad, & J. Hagemeyer (Eds.), *Heavy Metal Stress in Plants: From Molecules to Ecosystems* (pp. 305-330). Heidelberg: Springer.
- Scialdone, D., Scogamiglio, D., & Ramunni, A.U. (1980). The short and medium term effects of organic amendments on lead availability. *Water Air and Soil Pollution*, 13, 267–274.
- Shen, Z.G., & Liu, Y.L. (1998). Progress in the study on the plants that hyperaccumulate heavy metal. *Plant Physiology Community*, 34, 133-139.
- Shimwell, D.W., & Laurie, A.E. (1972). Lead and zinc contamination of vegetation in the Southern Pennines. *Environmental Pollution*, 3, 291-301.
- Shiowatana, J., Tantidanai, N., Nookabkaew, S., & Nacapricha, D. (2001). A flow system for the determination of metal speciation in soil by sequential extraction.

- Environment International*, 26, 381-387.
- Shu, W.S. (2003). Exploring the potential utilization of vetiver in treating acid mine drainage (AMD). Guangzhou: Proceedings of the Third International Conference on Vetiver.
- Shu, W.S., Ye, Z.H., Lan, C.Y., Zhang, Z.Q., & Wong, M.H. (2001). Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. *Environment International*, 26, 389-394.
- Smithinand, T. (1980). *Thai Plant Names*. Bangkok: Funny Publishing Limited Partnership.
- Taylor, M.D., & Percival, H.J. (2001). Cadmium in soil solutions from a transect of soils away from a fertilizer bin. *Environmental Pollution*, 113, 35–40.
- Temmerman, L.O., Hoenig, M., & Scokart, P.O. (1984). Determination of “normal” levels and upper limit values of trace elements in soils. *Z Pflanzenernähr Bodenkd*, 147, 687-694.
- Tomsig, J.L., & Suszkiw, J.B. (1991). Permeation of Pb^{2+} through calcium channels: fura-2 measurements of voltage- and dihydropyridine-sensitive Pb^{2+} entry in isolated bovine chromaffin cells. *Biochimica ET Biophysica ACTA*, 1069, 197-200.
- Tordoff, G.M., Baker, A.J.M., & Willis, A.J. (2000). Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere*, 41, 219–228.
- Truong, P., & Baker, D. (1998). *Vetiver Grass System for Environmental Protection*. (Technical Bulletin No. 1998/1). Bangkok: Office of the Royal Development Projects Board.
- Truong, P. (1999). *Vetiver Grass Technology for Mine Rehabilitation*. (Technical Bulletin No. 1999/2). Bangkok: Office of the Royal Development Projects Board.
- Truong, P. (2000). *Vetiver Grass Technology for Environmental Protection*. Cha Am: Proceedings of the Second International Conference on Vetiver.
- United States Environmental Protection Agency. (1998). *A Citizen's Guide to Phytoremediation* (EPA 542-F-98-011). Washington, DC: Office of Solid Waste and Emergency Response.

- Vassil, A.D., Kapulnik, Y., Raskin, I., & Salt, D.E. (1998). The role of EDTA in lead transport accumulation by Indian mustard. *Plant Physiology*, 117, 447-453.
- Viona, J., Gaydou, E., & Smadja, J. (1991). Sur la presence de bacteries intracellulaires dans les raciness du *Vetiveria zizanioides* (L.) Staph. *Rev. Cytol. Biol. Végét-Bot.*, 14, 65-70.
- Visoottiviseth, P., Francesconi, K., & Sridokchan, W. (2002). The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environmental Pollution*, 118, 453-461.
- Walkley, A., & Black, C.A. (1934). An examination of degradation method for determining soil organic matter: a proposed modification of the chromic acid titration method. *Soil Science*, 37, 29-35.
- Weyerstahl, P., Marschall, H., & Splittgerber, U. (1996). New sesquiterpenes ethers from vetiver oil. *Liebigs Annalen*, 7, 1195-1199.
- Weyerstahl, P., Marschall, H., Splittgerber, U., Wolf, D., & Surburg, H. (2000). Constituents of Haitian vetiver oil. *Flavour and Fragrance Journal*, 15, 391-394.
- Williams, S.T., McNeilly, T., & Wellington, E.M.H. (1977). The decomposition of vegetation growing on metal mine waste. *Soil Biology & Biochemistry*, 9, 271-275.
- Wong, M.H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, 50, 775-780.
- Wong, M.H., & Lau, W.M. (1985). The effects of applications of phosphate, lime, EDTA, refuse compost and pig manure on the Pb contents of crops. *Agricultural Wastes*, 12, 61-75.
- World Health Organization Geneva. (1989). *Environmental Health Criteria: Lead-Environmental Aspects*. Finland.
- World Metal Statistics. (1983). *World Bureau of Metal Statistics*. London.
- Woźny, A., Schneider, J., & Gwóźdź, E.A. (1995). The effects of lead and kinetin on greening barley leaves. *Biology Plantarum*, 35, 541-552.
- Xia, H.P. (2001). *Development of the Vetiver System in Guangdong, China*. (Technical Bulletin No. 2001/3). Bangkok: Office of the Royal Development Projects Board.
- Xia, H.P. (2004). Ecological rehabilitation and phytoremediation with four grasses in

- oil shale mined land. *Chemosphere*, 54, 345-353.
- Yang, B., Shu, W.S., Ye, Z.H., Lan, C.Y., & Wong, M.H. (2003). Growth and metal accumulation in vetiver and two *Sesbania* species on lead/zinc mine tailings. *Chemosphere*, 52, 1593-1600.
- Ye, Z.H., Wong, J.W.C., Wong, M.H., Lan, C.Y., & Baker, A.J.M. (1999). Lime and pig manure as ameliorants for revegetating lead/zinc mine tailings: a greenhouse study. *Bioresource Technology*, 69, 35-43.
- Zhang, P., Ryan, J.A., & Bryndzia, L.T. (1997). Pyromorphite formation from goethite adsorbed lead. *Environmental Science & Technology*, 31, 2673-2678.
- Zheljazkov, V.D., Craker, L.E., & Xing, B. (2006). Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. *Environmental and Experimental Botany*, 58, 9-16.
- Zhu, B.C.R., Henderson, G., Chen, F., Fei, H.X., & Laine, R.A. (2001). Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. *Journal of Chemical Ecology*, 27, 1617-1625.



APPENDIX

1. Lead speciation in the hydroponic solution, 10 mg L⁻¹ Pb treatment

K	NO₃
99.94 % as a free metal	99.94 % as a free ligand
.06 % complexed with SO ₄	.06 % complexed with Pb
Ca	NH₃
98.75 % as a free metal	99.99 % complexed with H ⁺
.02 % complexed with PO ₄	PO₄
1.18 % complexed with SO ₄	.97 % complexed with Ca
.04 % complexed with MES	.04 % complexed with Mg
Mg	.16 % complexed with Pb
99.00 % as a free metal	98.83 % complexed with H ⁺
.94 % complexed with SO ₄	SO₄
.05 % complexed with MES	92.83 % as a free ligand
Zn	.25 % complexed with K
98.31 % as a free metal	4.62 % complexed with Ca
.29 % complexed with NO ₃	.92 % complexed with Mg
.04 % complexed with PO ₄	.02 % complexed with Mn ⁺²
1.18 % complexed with SO ₄	.20 % complexed with Na
.17 % complexed with OH ⁻	1.07 % complexed with Pb
Mn⁺²	.08 % complexed with H ⁺
98.76 % as a free metal	B(OH)₄
1.18 % complexed with SO ₄	.04 % complexed with Pb
.04 % complexed with MES	99.95 % complexed with H ⁺
.01 % complexed with Cl	MoO₄
Cu⁺²	1.72 % as a free ligand
97.87 % as a free metal	97.99 % in solid form with Pb
.37 % complexed with NO ₃	.29 % complexed with H ⁺
.02 % complexed with NH ₃	MES
.04 % complexed with PO ₄	4.05 % as a free ligand

1.47 % complexed with SO_4
 .05 % complexed with HBED
 .18 % complexed with OH^-

Fe⁺³

100.00 % complexed with HBED

Na

99.96 % as a free metal

.04 % complexed with SO_4

Pb

95.20 % as a free metal

1.80 % complexed with NO_3

.03 % complexed with PO_4

2.28 % complexed with SO_4

.41 % in solid form with MoO_4

.12 % complexed with Cl

.16 % complexed with OH^-

.02 % complexed with Ca

95.93 % complexed with H^+

Cl

99.79 % as a free ligand

.05 % complexed with Na

.15 % complexed with Pb

HBED

100.00 % complexed with Fe^{+3}

2. Lead speciation in the hydroponic solution, 50 mg L⁻¹ Pb treatment

K

99.94 % as a free metal

.06 % complexed with SO_4

Ca

98.86 % as a free metal

1.09 % complexed with SO_4

.04 % complexed with MES

Mg

99.08 % as a free metal

.87 % complexed with SO_4

.05 % complexed with MES

Zn

98.37 % as a free metal

NO₃

99.72 % as a free ligand

.28 % complexed with Pb

NH₃

99.99 % complexed with H^+

PO₄

.33 % complexed with Ca

.01 % complexed with Mg

.26 % complexed with Pb

64.67 % in solid form with Pb

34.72 % complexed with H^+

SO₄

89.55 % as a free ligand

.36 % complexed with NO₃

.02 % complexed with PO₄

1.08 % complexed with SO₄

.16 % complexed with OH⁻

Mn⁺²

98.86 % as a free metal

1.09 % complexed with SO₄

.04 % complexed with MES

.01 % complexed with Cl

Cu⁺²

97.93 % as a free metal

.45 % complexed with NO₃

.02 % complexed with NH₃

.01 % complexed with PO₄

1.36 % complexed with SO₄

.04 % complexed with HBED

.18 % complexed with OH⁻

Fe⁺³

100.00 % complexed with HBED

Na

99.96 % as a free metal

.04 % complexed with SO₄

Pb

92.76 % as a free metal

2.15 % complexed with NO₃

.01 % complexed with PO₄

2.68 % in solid form with PO₄

2.04 % complexed with SO₄

.08 % in solid form with MoO₄

.12 % complexed with Cl

.16 % complexed with OH⁻

.23 % complexed with K

4.26 % complexed with Ca

.85 % complexed with Mg

.02 % complexed with Mn⁺²

.19 % complexed with Na

4.82 % complexed with Pb

.07 % complexed with H⁺

B(OH)₄

.18 % complexed with Pb

99.81 % complexed with H⁺

MoO₄

.37 % as a free ligand

99.57 % in solid form with Pb

.06 % complexed with H⁺

MES

4.03 % as a free ligand

.02 % complexed with Ca

95.95 % complexed with H⁺

Cl

99.24 % as a free ligand

.05 % complexed with Na

.69 % complexed with Pb

HBED

100.00 % complexed with Fe⁺³

3. Lead speciation in the hydroponic solution, 100 mg L⁻¹ Pb treatment

K	NO₃
99.96 % as a free metal	99.49 % as a free ligand
.04 % complexed with SO ₄	.51 % complexed with Pb
Ca	NH₃
99.22 % as a free metal	99.99 % complexed with H ⁺
.74 % complexed with SO ₄	PO₄
.04 % complexed with MES	.18 % complexed with Ca
Mg	.26 % complexed with Pb
99.36 % as a free metal	80.77 % in solid form with Pb
.59 % complexed with SO ₄	18.78 % complexed with H ⁺
.05 % complexed with MES	SO₄
Zn	61.69 % as a free ligand
98.65 % as a free metal	.16 % complexed with K
.44 % complexed with NO ₃	2.88 % complexed with Ca
.73 % complexed with SO ₄	.57 % complexed with Mg
.16 % complexed with OH ⁻	.01 % complexed with Mn ⁺²
Mn⁺²	.13 % complexed with Na
99.21 % as a free metal	6.17 % complexed with Pb
.74 % complexed with SO ₄	28.34 % in solid form with Pb
.04 % complexed with MES	.05 % complexed with H ⁺
.01 % complexed with Cl	B(OH)₄
Cu⁺²	.35 % complexed with Pb
98.28 % as a free metal	99.65 % complexed with H ⁺
.56 % complexed with NO ₃	MoO₄
.02 % complexed with NH ₃	.20 % as a free ligand
.92 % complexed with SO ₄	99.77 % in solid form with Pb
.04 % complexed with HBED	.03 % complexed with H ⁺
.17 % complexed with OH ⁻	MES
Fe⁺³	4.10 % as a free ligand
100.00 % complexed with HBED	.02 % complexed with Ca

Na 95.88 % complexed with H^+

99.97 % as a free metal

.02 % complexed with SO_4

Pb

88.20 % as a free metal

2.50 % complexed with NO_3

1.67 % in solid form with PO_4

1.31 % complexed with SO_4

6.01 % in solid form with SO_4

.04 % in solid form with MoO_4

.11 % complexed with Cl

.15 % complexed with OH^-

Cl

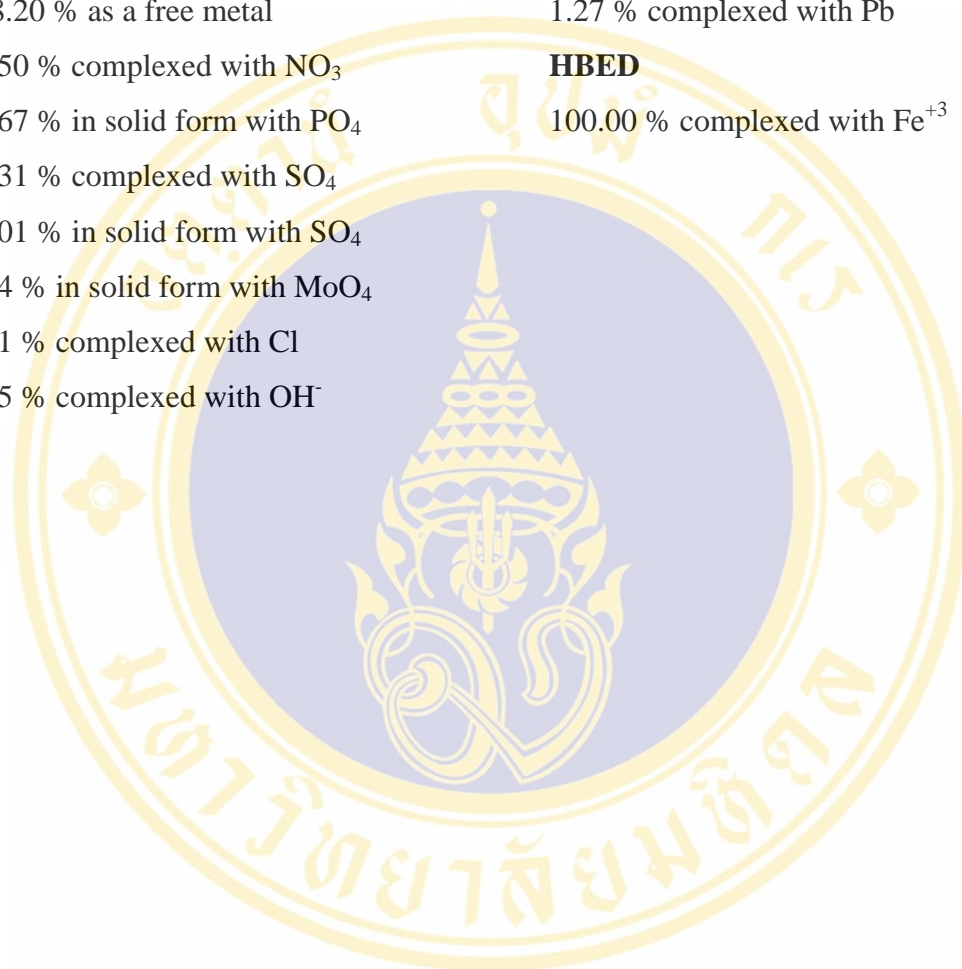
98.66 % as a free ligand

.05 % complexed with Na

1.27 % complexed with Pb

HBED

100.00 % complexed with Fe^{+3}



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