



รายงานวิจัยฉบับสมบูรณ์

การศึกษาการรักษาสมดุลโลหะของพืชท้องถิ่นที่สะสมสารตะกั่วภายใต้สภาวะควบคุม

In vitro Studies of Metal Homeostasis in Local Lead Hyperaccumulator Plant Species

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31 พฤษภาคม 2550

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา และ สำนักงานกองทุนสนับสนุนการวิจัย
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บทคัดย่อ - การปนเปื้อนของสารตะกั่วในธรรมชาติทำให้เกิดความเสี่ยงต่อการได้รับสารพิษของมนุษย์ งานวิจัยนี้มุ่งเน้นการศึกษาความแตกต่างของระดับของการสะสมสารตะกั่วในพืชผักท้องถิ่นจำนวนสิบชนิด และความเป็นพิษของสารตะกั่วในพืชที่ได้รับสารตะกั่วที่ความเข้มข้น 5, 15 และ 30 มิลลิกรัมต่อกิโลกรัมในอาหารภายใต้สภาวะควบคุม ผลการวิเคราะห์ปริมาณตะกั่วที่สะสมในน้ำหนักแห้งของพืชตัวอย่าง จัดแบ่งได้ 3 กลุ่มคือ กลุ่มที่สะสมสารตะกั่วในปริมาณที่สูงคือ ถั่วเขียว (*Vigna radiata* (L.) R. Wilczek) และ ผักบุ้ง (*Ipomoea reptans* Poir.) ซึ่งสะสมในปริมาณ 145.2 และ 74.7 มิลลิกรัมต่อกิโลกรัม ตามลำดับ โดยแตงกวา (*Cucumis sativus* L.), คะน้า (*Brassica alboglabra* L.H. Bailey), กะหล่ำปลี (*Brassica oleracea* L. var. *capitata* L.), และ แมงลัก (*Ocimum basilicum* L.) สะสมตะกั่วในระดับปานกลางที่ 40.1, 38.1, 33.7 และ 29.1 มิลลิกรัมต่อกิโลกรัม ตามลำดับ นอกจากนี้ ผักกาดหอม (*Lactuca sativa* L.), กระเพรา (*Ocimum tenuiflorum* L.), มะเขือเทศ (*Lycopersicon esculentum* Mill.), และพริกชี้ฟ้า (*Capsicum annuum* L.) สะสมสารตะกั่วในระดับต่ำที่ 20.2, 11.7, 11.9 และ 5.1 มิลลิกรัมต่อกิโลกรัม ตามลำดับ จากการวิเคราะห์ปริมาณสะสมของสารตะกั่วในพืชตัวอย่างพบว่าเมื่อปริมาณความเข้มข้นของสารตะกั่วในอาหารเพิ่มขึ้น พืชตัวอย่างมีการสะสมสารตะกั่วในปริมาณที่สูงขึ้นเช่นกัน และจากการเปรียบเทียบกับมาตรฐานความปลอดภัยพบว่าคะน้า กะหล่ำปลี แมงลัก และผักกาดหอม อาจเป็นพืชตัวอย่างที่มีศักยภาพในการนำไปปลูกในพื้นที่ที่มีสารตะกั่วปนเปื้อนได้ ซึ่งพืชเหล่านี้แสดงพิษจากสารตะกั่วในระดับต่ำ และสะสมปริมาณสารตะกั่วในเนื้อเยื่อต่ำกว่าพืชตัวอย่างที่ศึกษาอย่างมีนัยสำคัญ นอกจากนี้ คณะผู้ศึกษายังได้ทดสอบอิทธิพลของสารตะกั่วที่ความเข้มข้นต่างๆ ต่อพืชท้องถิ่นที่สะสมสารตะกั่ว จากแหล่งที่มีการปนเปื้อนและจากแหล่งที่ไม่มีการปนเปื้อน พบว่าสาบแร้งสาบกา (*Ageratum conyzoides*) จากแหล่งที่ไม่มีการปนเปื้อนสามารถสะสมสารตะกั่วในปริมาณที่สูงกว่าพืชจากแหล่งที่มีการปนเปื้อน แต่สาบเสือ *Chromolaena odorata* จากแหล่งที่มีการปนเปื้อนสามารถสะสมสารตะกั่วได้ในปริมาณที่สูงกว่าพืชจากแหล่งที่ไม่มีการปนเปื้อน

***In vitro* Studies of Metal Homeostasis in Local Lead Hyperaccumulator Plant Species**

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Abstract - Lead contamination in environment poses intoxication risk to human. This study focused on differences in level of lead accumulation in ten common vegetable and on lead phytotoxicity when studied plants treated at 5, 15 and 30 mg kg⁻¹ *in vitro*. Treated plants could be categorized into three groups according to their lead accumulation level in dried weight determined with atomic absorption spectrophotometers after four weeks post-exposure. Mung bean (*Vigna radiata* (L.) R. Wilczek) and morning glory (*Ipomoea reptans* Poir.) accumulated high lead contents, at 145.2 and 74.7 mg kg⁻¹, respectively. Cucumber (*Cucumis sativus* L.), collard (*Brassica alboglabra* L.H. Bailey), cabbage (*Brassica oleracea* L. var. *capitata* L.), and sweet basil (*Ocimum basilicum* L.) accumulated moderate lead contents, at 40.1, 38.1, 33.7 and 29.1 mg kg⁻¹, respectively. Lettuce (*Lactuca sativa* L.), holy basil (*Ocimum tenuiflorum* L.), tomato (*Lycopersicon esculentum* Mill.), and bird pepper (*Capsicum annum* L.) accumulated low lead contents, at 20.2, 11.7, and 5.1 mg kg⁻¹, respectively. The accumulated lead contents increased in most plants when they were exposed to higher lead concentrations. According to safety standard, collard, cabbage, lettuce and sweet basil seemed to be good candidates that might grow better, be less affected by lead contamination, yet posing lower health hazard on human consumption. Besides, lead phytotoxicity was assessed on the development of different ecotypes of lead hyperaccumulators *Ageratum conyzoides* and *Chromolaena odorata*. Although *Ageratum conyzoides* collected from a non-contaminated site accumulated significantly higher lead contents than those from a contaminated site, we observed the opposite on *Chromolaena odorata*.

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Executive Summary

This study determined the effects of lead compounds on development of ten common vegetable in Thailand. Based on the results, seed germination of the studied plants was not affected when exposed to lead compounds in the culture medium at the lead concentrations of 5, 15, and 30 mg kg⁻¹. During their development, mung bean, cucumber, morning glory, sweet basil, lettuce, kale, tomato, cabbage, holy basil, and bird pepper manifested different response to lead concentrations.

Mung bean showed high sensitivity to lead concentrations in the media. Growth retardation was observed from as early as one week after exposure to lead. In tomato, growth retardation was observed after exposure to lead of two week. Holy basil seemed to be less sensitive to lead concentration than mung bean and tomato. However, its growth retardation was observed after exposure to lead of three week. Bird pepper seemed to be less sensitivity to lead concentration than mung bean, tomato and holy basil. Growth retardation of bird pepper was observed after four week exposure to lead. Morning glory, cucumber, lettuce, sweet basil, kale, and cabbage did not seem to be affected by lead concentrations during the four week exposure, growth retardation of these plants was not observed. Interestingly, holy basil and sweet basil are close as they belong to the same genus. Nevertheless, from this study, it cannot be generalized to have similar response to lead concentrations.

In addition, development of the root system seemed to be negatively affected by the presence of lead, even as low as 5 mg kg⁻¹ in all plant species in this study. Even though, average growth, measured by the length from shoots to root, did not show any effects, formation of lateral roots was impaired in all treatments.

Therefore, based on the results, it can be concluded that lettuce, holy basil, cabbage, and collard might be suitable for cultivation in lead contaminated sites as they tolerated lead toxication; yet, accumulating low lead contents.

In considering the feasibility of phytoextraction, it is unfortunate that some of the best hyperaccumulators are relatively small in size and grows very slowly, making it difficult to harvest them mechanically, and limiting the metal extraction that can be achieved. This study showed that the ecotype of plants could influence lead accumulation potential.

For further study, longer exposure period maybe required to define plant parts of the suggested species whether their lead accumulated contents remain safe for human consumption when they are cultivated in lead contaminated area.

Introduction

1.1 Rationale

Human activity has led to high levels of heavy metals being accumulated from the metal related industries, the premises of old mines, and also rural areas where the soil along highways and roads is polluted by automotive exhausts and in fields contaminated with fertilizers containing heavy metal ingredients (Antosiewicz, 1992). Lead is the most dangerous heavy metal because of its elevated level in the environment in certain areas. These areas include urban regions polluted by wastes that are beginning to reach thresholds able to evoke the first signs of toxicity in humans. Plants are an important link in the pathway by which excessive amounts of heavy metals are channelled into the food chain and biological cycles (Todd et al., 1996). This is because plants are able to accumulate lead in their tissues. Lead is toxic to many organ systems of human body, such as the central and peripheral nervous system, the red blood cells, the kidneys, the cardiovascular systems, and the male and female reproductive organs. Lead can decrease sperm counts and increase prevalence of morphologically abnormal sperm in male, and increase risk of miscarriage in female (Mengel et al., 1980).

The levels of lead in soils that are toxic to plant are not easy to evaluate. However, it is generally agreed that soil lead concentration ranging from 100 to 500 mg kg⁻¹ are considered to be excessive (Pendias et al., 1984). The toxic symptoms of lead in plant are not very specific. There is much evidence that lead toxicity resulted in retardation of plant growth.

The inhibitory effects may be due to interference with enzymes essential for normal metabolic and development, photosynthetic processes, water and mineral nutrients absorption, and changes in cell ultrastructure (Van et al., 1990).

Plants are an important link in the pathway by which excessive amounts of heavy metals are channeled into the food chain and biological cycles (Todd et al., 1996). This is because plants are able to accumulate lead in their tissue. Lead from the soil enters plants through their root system, while lead from dusts and automotive exhaust aerosols deposits directly on their overground parts (Zimdahl, 1976). The localization of lead in root cells and tissues effects on cell division, only a small part of the lead taken up by the roots from the soil is transported via the xylem to the above-ground parts of the plant (Jones et al., 1973). There still remains the problem, however, of the degree to which exogenous lead, as that from direct atmospheric pollution or soil solutions in contact with seeds, is able to pass through the seed coat into the seed and consequently affect germination (Magorzata et al., 1998).

Plants are the crucial link in the pathway of food chain and biological cycle with contaminated excessive amount of heavy metals (Todd et al., 1996). Compounds are adsorbed by the roots of plants and moved upward to the shoots by the xylem vessel through the transpiration stream. While transported in the xylem, heavy metals may have some influences on the plants metabolic processes. When plants can develop mechanisms against this kind of toxicity, the highest risk of human health is occurred (A. Kabata-Pendias, 1984). Xylem will contain low-molecular weight organic acids, inorganic anions (0.01%-0.05% w/w) such as nitrite, sulfate, phosphate and a limited number of amino acid, amides and other solutes (D. Prima-Putra, 1998).

The nitric acid digestion technique is commonly used in preparation of sample vegetal materials. Determination of the lead present in concentration is in ranges of mg kg^{-1} in various samples of biological origin. Two most common techniques used for analyze accumulation of lead concentration are flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectroscopy (GF-AAS) due to their sensitivities and small amount of samples required.

In this study, we conducted our experiment mainly in a plant tissue culture laboratory. The objectives of this project were to study effects of lead at different concentrations on germination of common vegetables and to identify level of tolerance to lead in common vegetables and to study effects of lead at different concentration on physiological changes during plant development. We also investigated variation in accumulation of lead by cultivars of thirteen common vegetables and herbs from a medium containing various concentrations of lead compounds, and studied the effects of ecotype on the accumulation of common vegetables and herbs and to identify the level of accumulation of common vegetables and herbs in different lead concentration. This work may help to understand possible mechanisms of plant physiological processes when lead is involved.

1.3 Problem statement

Contamination of lead may impair growth and development of plant in agriculture. Lead is one of the prevalent heavy metals present naturally in the soil. Contamination of lead in soil can be from the natural event itself, e.g. volcanic activities, mineral decomposition, and from human activities such as mining and related activities. For the natural source, the indigenous plant species may have well adapted to the concentration and be able to regulate heavy metal to provide protection from phytotoxicity. Nevertheless, introduced species may suffer from lead at different levels. These species often include plants and vegetables of economic importance.

Thailand and Thai people rely heavily on agriculture for their everyday life. The fundamental problems to be tackled in this research could provide us a tool to plan and decide which plant species would grow better and be less affected by lead contamination in agricultural. The data obtained from this study may elucidate the tolerance of common vegetables to lead concentrations.

1.4 Objectives

1. To study effects of lead at different concentrations on germination of common vegetables.
2. To identify level of tolerance of common vegetables to different lead concentrations.
3. To study effects of lead at different concentrations on physiological changes during plant development
4. To investigate variation in accumulation of lead by cultivars of thirteen common vegetables and herbs from a medium containing various concentration of lead compounds.
5. To study the ecotype effects the accumulation of common vegetables and herbs.
6. To identify the accumulation level of common vegetables and herbs in different lead concentration.

1.5 Scope

In this study, we conducted our experiment mainly in a plant tissue culture laboratory. Plant tissue culture techniques have become important to both research and development field and agriculture industry. It allows us to understand metal regulation and strategies that plants use to survive under stress from the heavy metal. This experiment was to find out the effects of lead at various concentrations on germination and development of common vegetables in Thailand. For lead accumulated content analysis, flame atomic absorption spectrometry-(FAAS) and graphite furnace atomic absorption spectrometry (GFAAS) were used to investigate and understand lead accumulation in plants and strategies of plant used to survive under stress from heavy metal. This research was to analyze the lead accumulation in local vegetable plant species exposed to various concentrations of lead compounds and study the ecotypes effect on lead accumulation.

1.6 Hypothesis

We hypothesized that each species or variety of common vegetables would carry different tolerance to lead; thus, enabling us to characterize vegetables of economic importance of which inherent tolerance to lead will be challenged for their limits. Also, each species or variety of

common vegetables and herbs would carry different accumulation level of lead; thus, enabling us to characterize vegetables of economic importance for their inherent tolerance of lead and their limits within the health hazard.

1.7 Expected outcomes

This experiment was to find out the effects of lead at various concentrations on germination and development of common vegetables in Thailand. At the end of the experiment we will know that which of the ten common vegetables are affected by lead at different concentrations on germination and development of common vegetables. We can also identify level of tolerance of common vegetables to lead concentrations

Literature Review

2.1 *In vitro* study of plant physiology

Plants require elements and minerals to maintain proper growth and development. However, nutrient in the planting substrate at different location may naturally contain those elements and minerals in different forms and concentration. Study on plant physiology can be conducted either *in vivo* or *in vitro*, which attempts to provide answers to different questions. Experiment in natural setting could also provide answers to a real world situation. Nevertheless, due to its complexity and numerous uncontrollable parameters, *in vitro* study often gives us preliminary information and hints to look at the problem in a clearer direction.

2.2 Phytoremediation, plant-based strategies for cleaning up contaminated soils

The use of such plants to cleanup soils and water contaminated with pollutants, a technique known as phytoremediation, is emerging as a new tool for *in situ* remediation. Phytoremediation takes advantage of the fact that a living plant acts as a solar-driven pump, which can extract and concentrate certain heavy metals from the environment (Raskin et al., 1997). Plants that take up heavy metals (Figure 1) from the soil offer an alternative and less expensive method to strip heavy metals directly from the soil. Plants have constitutive and adaptive mechanisms for accumulating or tolerating high contaminant concentrations. This remediation method maintains the biological properties and physical structure of the soil. The technique is environmentally friendly, potentially cheap, and offers the possibility of bio-recovery of the heavy metals.

Phytoremediation strategies can offer suitable approaches for decontaminating polluted soil, water, and air by trace metals as well as organic substances (Table 1).

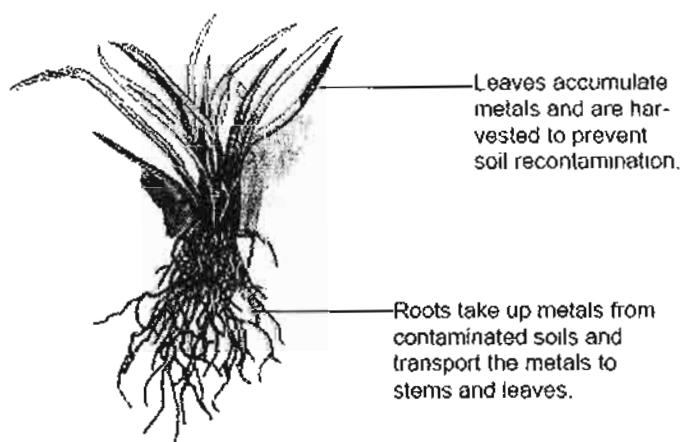


Figure 1: Phytoremediation: Using plants to clean up soil (Leon, 2000)

Table 1: Lists of phytoremediation strategies (Xiaoe et al., 2005)

Phytoremediation Techniques	Action mechanism	Medium treated
Phytoextraction	Direct accumulation of contaminants into plant shoots with subsequent removal of the plant shoots	Soil
Rhizofiltration (phytofiltration)	Absorb and adsorb pollutants in plant Roots	Surface water and water pumped through roots
Phytostabilization	Root exudates cause metals to precipitate and biomass becomes less bioavailable	Groundwater, soil, mine tailings
Phytovolatilization	Plants evaporate certain metal ions and volatile organics	Soil, groundwater
Phytodegradation (plant-assisted bioremediation)	Microbial degradation in the rhizosphere region	Groundwater within the rhizosphere and soil
Phytotransformation	Plant uptake of organic contaminants and degradation	Surface- and groundwater

Phytoextraction is a specific type of phytoremediation that refers to the uptake of metal contaminants by plant roots in plant stems and leaves. In mechanisms (Figure 2) that require translocation of metals through plant tissues, there may be steps involving such as: (a) transport of metals across the plasma membrane of root cells; (b) xylem loading and translocation; and (c) detoxification and sequestration of metals at the cellular and the whole plant levels (Rupali et al., 2004).

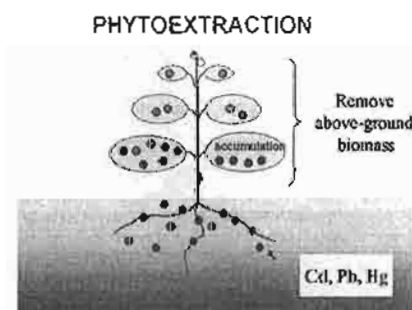


Figure 2: Mechanisms of phytoextraction

2.3 Hyperaccumulation of heavy metals by plants

Hyperaccumulation of heavy metals by higher plants is a complex phenomenon. These plants are called hyperaccumulators, absorbing high levels of contaminants concentrated either in their roots, shoots, and/or leaves. Plants show different levels of tolerance and accumulation to different metals. The first characterized hyperaccumulators were members of Family Brassicaceae and Family Fabaceae. More than 400 plant species have been reported so far that hyperaccumulate metals (McIntyre, 2003). The accumulation ability of a given metal is determined by the uptake capacity and intracellular transportation of plant. The major processes that are assumed to be influencing metal accumulation rates in plant are illustrated in Figure 3.

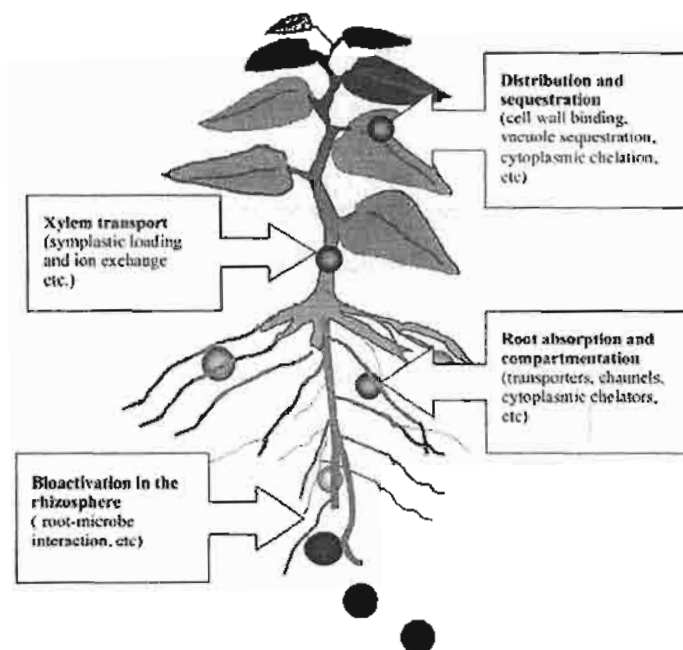


Figure 3: Major processes proposed to be involved in heavy metal hyperaccumulation (Xiaoe et al., 2005)

2.4 Type of phytoextraction

2.4.1 Natural phytoextraction

Natural phytoextraction is the removal of metal depends on the natural ability of the plant to remediate contamination. At least 45 families have been identified to have hyperaccumulate plants; some of the families are Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae, and Scrophulariaceae (Dushenkov, 2003; Salt et al., 1998). The best-known hyperaccumulators is *Brassica juncea*, has been found to have a good ability to transport lead from the roots to the shoots. Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed), and *Azolla pinnata* (water velvet) have been

investigated for use in rhizofiltration, phytodegradation, and phytoextraction (Salt et al., 1997). Recently, a fern *Pteris vittata* has been shown to accumulate as much as 14,500 mg kg⁻¹ arsenic in fronds without showing symptoms of toxicity (Ma et al., 2001).

2.4.2 Induced phytoextraction

Induced phytoextraction or chelate assisted phytoextraction is the method in which artificial chelates are added to increase the mobility and uptake of metal contamination. Chelators have been isolated from plants that are strongly involved in the uptake of heavy metals and their detoxification. Chelating agents like ethylenediaminetetraacetic acid (EDTA) are applied to lead-contaminated soils that increase the amount of bioavailability lead in the soil and a greater accumulation in plants is observed (Huang et al., 1997).

2.5 Limitations of phytoextraction

Plants express an incomplete set of remediating features. For example, most of the metal hyperaccumulators are small and slow growing (Mitch, 2002). Phytoextraction and plant-assisted bioremediation is most effective if soil contamination is limited to within 3 feet of the surface, and if groundwater is within 10 feet of the surface (Raskin et al., 1994). It is applicable to sites with low to moderate soil contamination over large areas, and to sites with large volumes of groundwater with low levels of contamination that have to be cleaned to low (strict) standards (Salt et. al., 1995). Since chemical chelators have additional toxicity to plants, thus they may increase the uptake of metals but decrease plant growth thus proving to be of limited benefit.

Enhanced root-to-shoot transport is another key component of metal/metalloid hyperaccumulation. This may be achieved by a reduced sequestration of the metal in the root vacuoles or by enhanced xylem loading, although there has been little progress in research on this aspect (Steve et al., 2003).

2.6 Effects of lead on plant

Although lead is not an essential element for plants, it gets easily absorbed and accumulated in different plant parts. Excess lead causes a number of toxicity symptoms in plants e.g. stunted growth, chlorosis and blackening of root system. Lead inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability (Pallavi et al., 2005). The uptake, transport, and accumulation of lead by plants are

strongly governed by soil and plant factors, and they differ significantly with plant species (Eltrop et al., 1991).

2.7 Role of EDTA in lead transport and accumulation

The synthetic chelate EDTA forms a soluble complex with many metals, including lead and can solubilize lead. Recently, application of EDTA to lead contaminated soils has been shown to induce the uptake of lead by plants (Huang et al., 1997). The synthetic chelates including EDTA destroy the physiological barrier(s) in roots that normally function to control uptake and translocation of solutes. The plasma membrane surrounding root cells is thought to play a major role in forming this barrier. Both Zn^{2+} and Ca^{2+} are involved in stabilizing plasma membranes. Therefore, synthetic chelates may induce metal-chelate uptake and accumulation by removal of stabilizing Zn^{2+} and Ca^{2+} from the plasma membrane. Lead is known to be effective at displacing various cationic metals from roots, suggesting that lead may also play a role in destabilizing the physiological barrier to solute movement in roots (Andrew et al., 1998).

2.8 Frequently asked questions about lead contamination

2.8.1 How much lead is there in our soil?

The natural background level of lead in soil is less than 100 parts per million (mg kg⁻¹). Lead in surface soil in residential communities is commonly higher than 200 mg kg⁻¹. In older, urban residential areas lead in soil on some properties may range from 500 to 1000 mg kg⁻¹, even when there is no local industrial source.

2.8.2 Can I eat vegetables from the garden?

Lead enters and is stored in vegetables grown in lead-contaminated garden soils. The amount of lead taken up and stored in these vegetables will vary depending on the type of vegetable, the type of soil, your gardening practices and the amount of lead in the soil. Although lead normally increases in plants as they age, it is taken up and stored differently in roots and in plant leaves. Therefore, it is not always safe to assume that root vegetables will contain more lead than leafy vegetables. Fruit crops such as tomatoes, berries, apples and cucumbers, present a much lower risk because they take up and store very little lead.

2.8.3 Is lead in soil harmful?

Children take in an average of 80 milligrams of soil and dust (equal to the size of a grain of rice) each day while they play. Depending on the concentration of lead in the soil, they may develop elevated levels of lead in their blood. Soil and dust are considered a major route of exposure for children. The Ministry of the Environment advises that there is minimal risk from exposure to soil with lead levels below 200 mg kg⁻¹.

There is minimal risk in consuming homegrown vegetables grown in soil containing less than 200 mg kg⁻¹ of lead. However, this is only a guide and it should be remembered that eating vegetables grown in soil contaminated with lead will always increase your exposure to lead and the risk to your health, especially for infants and young children if they are used in baby food recipes. You should not eat any vegetables out of your garden if lead levels are above 1000 mg kg⁻¹.

2.9 Names of common vegetables in Thailand

Ten species of common vegetable seeds were chosen (Table 2). Name of each species was classified in term of family, species, common name and Thai name.

Table 2: Names of common vegetables used in this study

Family	Species	Common name	Thai name
Fabaceae	<i>Vigna radiata</i> (L.) R.wilczek	Mung bean	ถั่วเขียว
Cucurbitaceae	<i>Cucumis sativus</i> L.	Cucumber	แตงกวา
Convolvulaceae	<i>Ipomoea reptans</i> Poir.	Morning glory	ผักบุ้ง
Asteraceae	<i>Lactuca sativa</i> L.	Lettuce	ผักกาดหอม
Lamiaceae	<i>Ocimum basilicum</i> L.	Sweet basil	โหระพา
	<i>Ocimum tenuiflorum</i> L.	Holy basil	กระเพรา
Brassicaceae	<i>Brassica alboglabra</i> L.H. Bailey	Chinese kale	คะน้า
	<i>Brassica oleracea</i> L.var. <i>capitata</i> L.	Cabbage	กะหล่ำปลี
Solanaceae	<i>Lycopersicon esculentum</i> Mill.	Tomato	มะเขือเทศ
	<i>Capsicum annuum</i> L.	Bird pepper	พริกชี้ฟ้า

Materials and Methods

3.1 Experimental locations

3.1.1 Plant culture and experimental design were performed at Department of Biology, Faculty of Science, Mahidol University.

3.1.2 Plant samples were bought from seed providers.

3.1.3 Lead hyperaccumulators were collected from two locations

- Lead contaminated sites in Kanchanaburi province
- Non-contaminated sites in Petchaboon province

3.2 Chemical reagents and equipments

3.2.1 Chemical reagents

- 1) Modified White (1963) medium-see appendix A
- 2) Lead standard solution --Pb (NO₃)₂ in HNO₃ 0.5 mol/l, 1000mg/l Pb-CertiPUR
- 3) Agarose, BactoTMAgar-Becton, Dickinson and company
- 4) Sucrose
- 5) Distilled water
- 6) Sodium hypochlorite 8 %
- 7) Detergents 0.5%
- 8) EDTA 0.5 M
- 9) NaOH
- 10) HCl

3.2.2 Equipments

Analytical balance- Mettler PJ300

pH meter- Suntex and Stirrer-Ikamag@Rce-G

Laminar air-flow cabinet- Issco Model

Autoclave

Pipette and glasswares

Flame Atomic Absorption Spectrophotometer (FAAS-SpectrAA 55B)-Variance

Graphite Furnace Atomic Absorption Spectroscopy (GFAAS-GBC UltraZ)-GBC

GFAAS sample load cup

Graphite tube

Argon (Ar) Gas

3.3 Vegetable seeds

The common vegetable seeds were bought from The Mall Departmentstore, Bangkok, Thailand. Nine species of the studied plants were purchased from Chia Tai Company, Thailand. Mung bean was purchased from Thai-Ha Company.

Table 3: Sources of vegetable seeds

Common name	Sources
1. Mung bean	Thai-Ha, Thailand
2. Cucumber	Chia Tai, Thailand
3. Morning glory	Chia Tai, Thailand
4. Lettuce	Chia Tai, Thailand
5. Sweet basil	Chia Tai, Thailand
6. Holy basil	Chia Tai, Thailand
7. Chinese kale	Chia Tai, Thailand
8. Cabbage	Chia Tai, Thailand
9. Tomato	Chia Tai, Thailand
10. Bird pepper	Chia Tai, Thailand

3.4 Experimental procedure for physiological study

This experimental procedure was divided into four steps; the first step was media preparation where both the control and the media treated with lead were prepared. The second steps were surface sterilization of vegetable seeds. The third steps were plant culture. The fourth steps were data collection and analysis.

3.4.1 Preparation of media

The culture medium was modified from White (1963) and was designed for lead experiment. The solutions of modified White's medium were prepared from stocks ranging from 200-600 times the final concentrations. A series of solutions was prepared as Appendix A.

Place a volume of deionized water, equal to approximately half the total volume of media to be prepared, in a beaker. Stock solutions numbers 1-6 were added into the beaker and the volume was adjusted to the final desired amount with deionized water.

Table 4: Preparation of media with lead

Media concentration (mg kg ⁻¹)	Nutrient solution (ml)	Lead standard solution (ml)	Final volume (ml)
0	1000	0	1000
5	995	5	1000
15	985	15	1000
30	970	30	1000

The media with lead were prepared as the following. Media with lead were prepared in 0 mg kg⁻¹ (control), 5 mg kg⁻¹, 15 mg kg⁻¹ and 30 mg kg⁻¹ of lead standard solution –Pb (NO₃)₂ in HNO₃ 0.5 mol/l, 1000mg/l. The media with lead were prepared as in Table 4. The pH was adjusted to 5.5-5.7 with HCl, NaOH and 0.5 M EDTA, as known, nitrates are soluble in water. However, adding Pb (NO₃)₂ into the stock solutions will cause precipitation. This is due to the reactions between Na₂SO₄, KCl, and KI. The results of white precipitates were not desirable for agar solution. 0.5 M of EDTA, a chelating agent was applied to completely dissolve Pb (NO₃)₂ in the stock solution. One ml of 0.5 M EDTA was used in four treatments. The amount of EDTA used was determined by dissolving the highest concentration (30 mg kg⁻¹) of Pb (NO₃)₂ in the stock solution and was applied to other treatments, 0 mg kg⁻¹ (control), 5 mg kg⁻¹ and 15 mg kg⁻¹. 8 g of agar was added in each concentration. Placing into the oven until the agar is dissolved. 20 g of sucrose was added into the media and let dissolved by stirring. Melted media were poured into the bottles and bottles were covered. Culture media were autoclaved sterilization for 15 min at 121°C. After sterilization the media were kept in the tissue culture room.

3.4.2 Surface sterilization of vegetable seeds

Two hundreds seeds of each vegetable were counted and put into 250 ml of Erlenmeyer flask. The surface sterilization of vegetable seeds was performed as following sequence: 8% bleach and 0.5 % detergents, shaken at 150 rpm. for 10 min, washed with sterilized water for 10 min, shaken at 150 rpm. for 10 min, repeated washing again.

3.4.3 Plant culture

Four treatments at concentrations of 0 mg kg⁻¹ (control), 5 mg kg⁻¹, 15 mg kg⁻¹ and 30 mg kg⁻¹ of lead, supplied in the form of Pb (NO₃)₂ were set up. 32 bottles were used for each plant species and 8 bottles were used for each treatment. After the seeds were sterilized, seeds were planted into the media with each medium containing 5 seeds as replication. The plants were cultured under 12-12 lights & dark at 25°C.

3.4.4 Data collection and analysis

Each replicate was assigned to a specific treatment. When assigning replicates to treatments, it was important to make assignments in a manner such that all have an equal chance of receiving a given treatment. This is called randomization. The data were collected weekly by each bottle of each treatment was randomized collecting.

The seedlings were harvested and the germination rate, length were recorded. The germination rate was determined in percentages; seeds were scored as germinated when the breakage of seed coat was visible. Dry weight was measured after drying at 60°C for 2 days. The data were analyzed to determine the effects of the treatments, and least significant difference (LSD) tests were performed to determine the statistical significance of differences between means of treatments.

A completely randomized design (CRD) was used to analyze the results. A majority of plant cell and tissues culture studied employ a CRD because cell cultures are generally grown in environmental chambers that accurately control light, temperature and humidity. In the CRD, treatments are assigned to experimental units at random. The numbers of treatments and replicates per treatment that can be tested are not limited by CRD.

3.5 Experimental procedure for accumulation analysis

For lead accumulated content analysis, the procedure was divided into four steps; the first step was sample preparation where both control and lead contaminated samples. The second steps were nitric acid digestion of samples. The third were determined the lead accumulation in samples by using Flame Atomic Absorption Spectrophotometer (FAAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). The fourth steps were data collection and analysis.

3.5.1 Sample preparation

Dry sample plants were ground with a mortar and pestle. The powder samples were measured the weight by using analytical balance.

3.5.2 Nitric acid digestion

Dry weight of plant samples, 0.5 g or all amount of plant was used in case that weight of plant was less than 0.5g, was digested with nitric acid (1g dry-weight: 1ml nitric acid (69% HNO_3 ; BDH) (APHA, 1998).

3.5.3 Heavy metal (Lead) analysis

After digestion, Pb concentrations in plant samples were measured by flame atomic absorption spectrophotometer (Variance SpectrAA 55B) and by graphite furnace atomic absorption spectroscopy (GBC UltraZ).

3.5.4 Data collection

After obtained the absorbance values from FAAS and GFAAS, The three replicate data from each sample were calculated with standard curve of lead concentration in order to get real lead concentration in plant samples in the unit of mg.kg^{-1} dried weight.

3.5.5 Data analysis

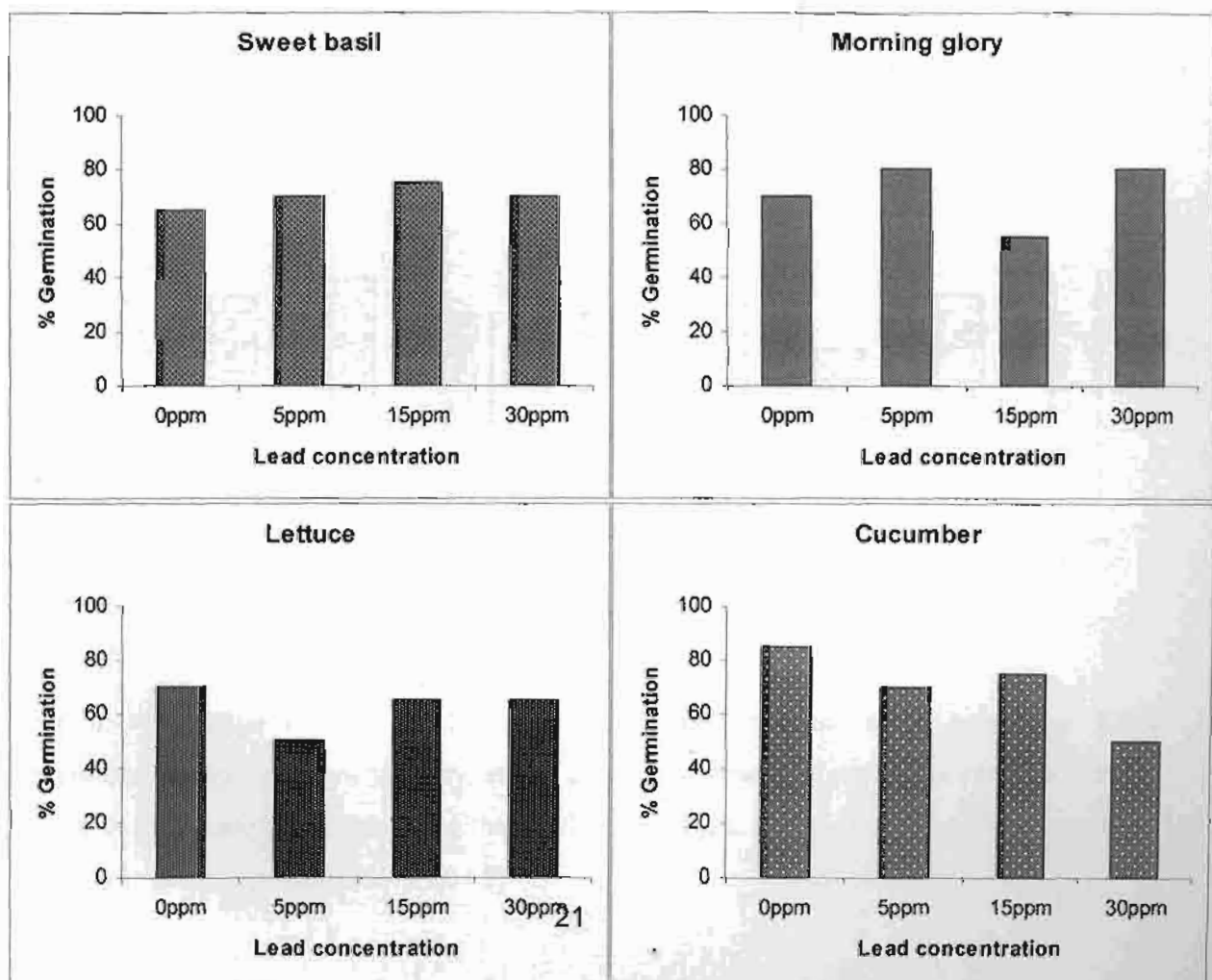
The relative growth rate (RGR) was calculated according to Hunt (1982). $\text{RGR} = [\ln(W_2) - \ln(W_1)] / (t_2 - t_1)$; W_1 and W_2 are plant dry weight (g) at time t_1 and t_2 .

The bioaccumulation coefficient (BC), or phytoextraction rate, was described as the heavy metal concentration in plant divided by heavy metal concentration in the solution (Nanda-Kumar et al., 1995).

Results and Discussion

4.1 Percentage of seed germination

In this study required observation of germination in each bottle. The number of germination seeds was counted in four week cultures. Seeds were considered to germinate when the breakage of seed coat was visible. Figure 4, the different lead concentrations (0 mg kg⁻¹, 5 mg kg⁻¹, 15 mg kg⁻¹ and 30 mg kg⁻¹) did not affect seed on germination in the ten species of common vegetables. Mung bean (100%) was germination at 0 to 30 mg kg⁻¹ of lead concentrations. It showed that lead did not affect mung bean on germination. Cucumber, morning glory, sweet basil, lettuce, kale, tomato, cabbage, holy basil, and bird pepper were germination less than 100 percentages. However, there were no significant difference in percent germination ($P>0.05$), see Appendix B. All the ten species of common vegetables have seed coat. Presence of the seed coats plays a role in the selective penetration of different lead concentration into the seeds. In this study, only very low amounts of lead may be able to penetrate through even with a high lead concentration. Hence, no significant effect was observed in the seed germination.



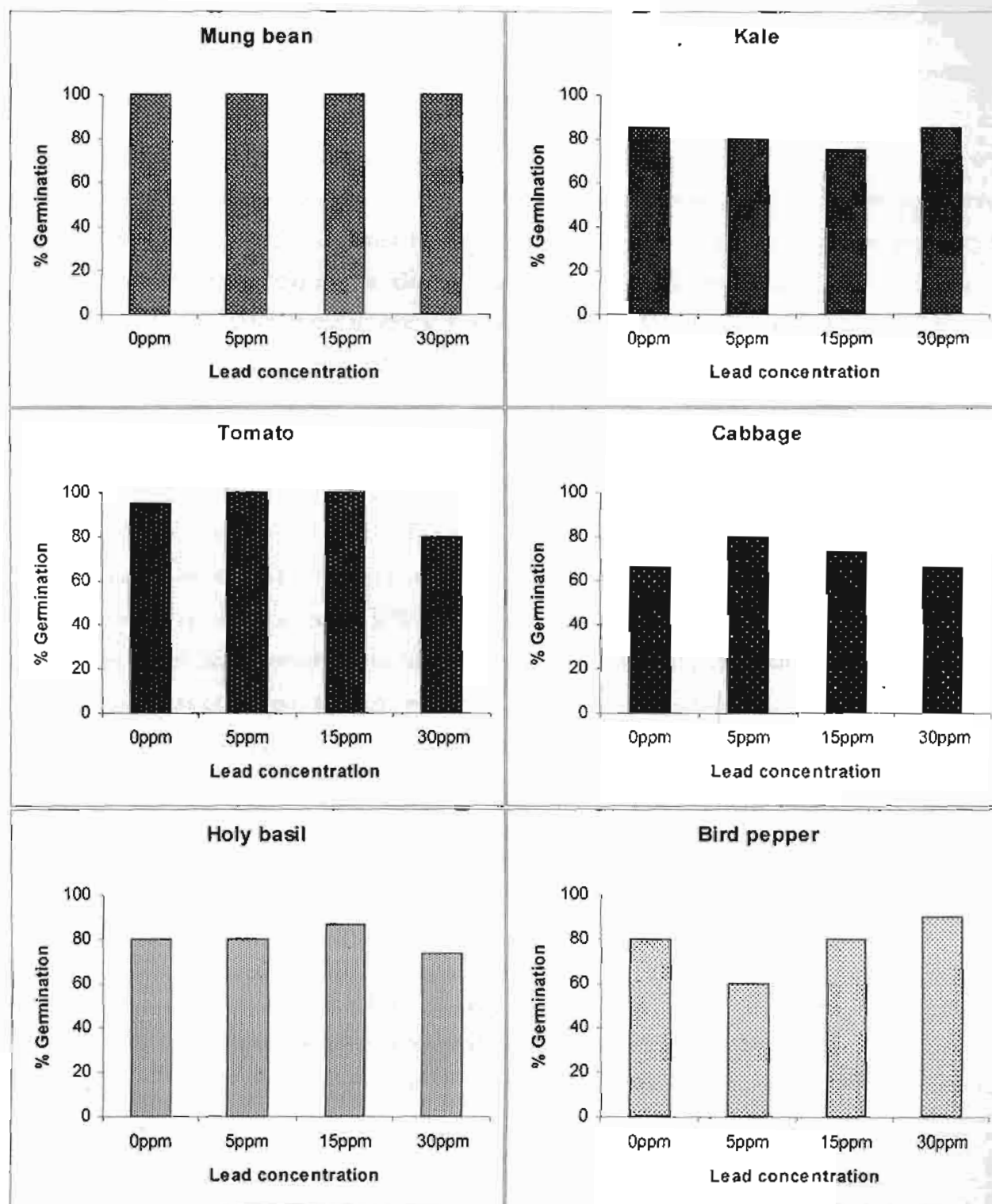


Figure 4: Percentage of seed germination in four week cultures. Mung bean had 100% germination. Cucumber, morning glory, sweet basil, lettuce, kale, tomato, cabbage, holy basil, and bird pepper were germination less than 100 percentages. However, there had no significant difference in percent germination ($P>0.05$).

4.2 Length measurement from root to shoot

Seedlings were grown in different concentrations of lead under same conditions. Seedlings were harvested weekly. Length of seedling was measured from root to shoot.

Average length of mung bean (Figure 5) had significant difference ($P<0.05$) after one week exposure to lead any concentration. Differences in the average length were observed when the seedlings were exposed for a longer period. Mung bean showed high sensitivity to lead concentrations in the media. Growth retardation was observed from as early as one week after exposure to lead concentrations. Average length of tomato (figure 6) had no significant difference ($P>0.05$) after one week exposure to lead any concentration, but it showed significant difference ($P<0.05$) after two week exposure to lead. Differences in the average length were observed. Growth retardation of tomato was observed after exposure to lead of two week onward.

Average length of holy basil (Figure 7) had no significant difference ($P>0.05$) after two week exposure to lead any concentration, but it showed significant difference ($P<0.05$) after three week exposure to lead. Differences in the average length were observed. Holy basil seemed to be less sensitivity to lead concentrations than mung bean and tomato, growth retardation was observed after exposure to lead of three week onward.

Average length of bird pepper (Figure 8) had no significant difference ($P>0.05$) after three week exposure to lead any concentration. However, it showed significant difference ($P<0.05$) after four week exposure to lead. Differences in the average length were observed. Bird pepper seemed to be very less sensitivity to lead concentrations than mung bean, tomato and holy basil. Growth retardation of bird pepper was observed after exposure to lead of four week.

Figure 9, average length of morning glory, cucumber, lettuce, sweet basil, kale, and cabbage showed no significant difference ($P>0.05$) after one to four week exposure to lead any concentration. Growth retardation of six species can not be observed, all six species did not seem to be affected by lead concentrations during the four week exposure.

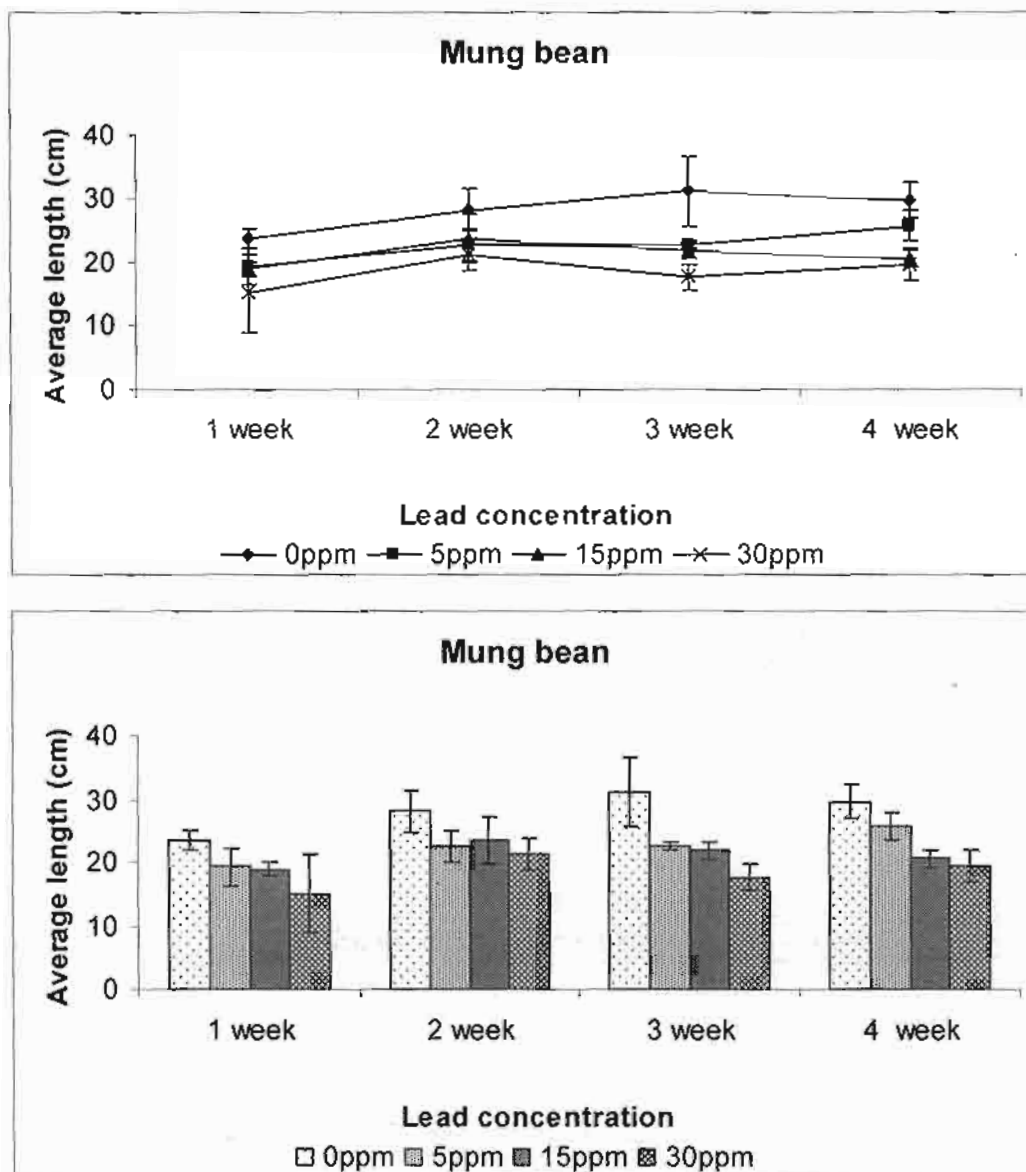


Figure 5: Column and line chart types for average length of mung bean had a significant difference ($P < 0.05$) after one week exposure to lead onward. Values represent the mean $\pm$ SD of five replicate samples.

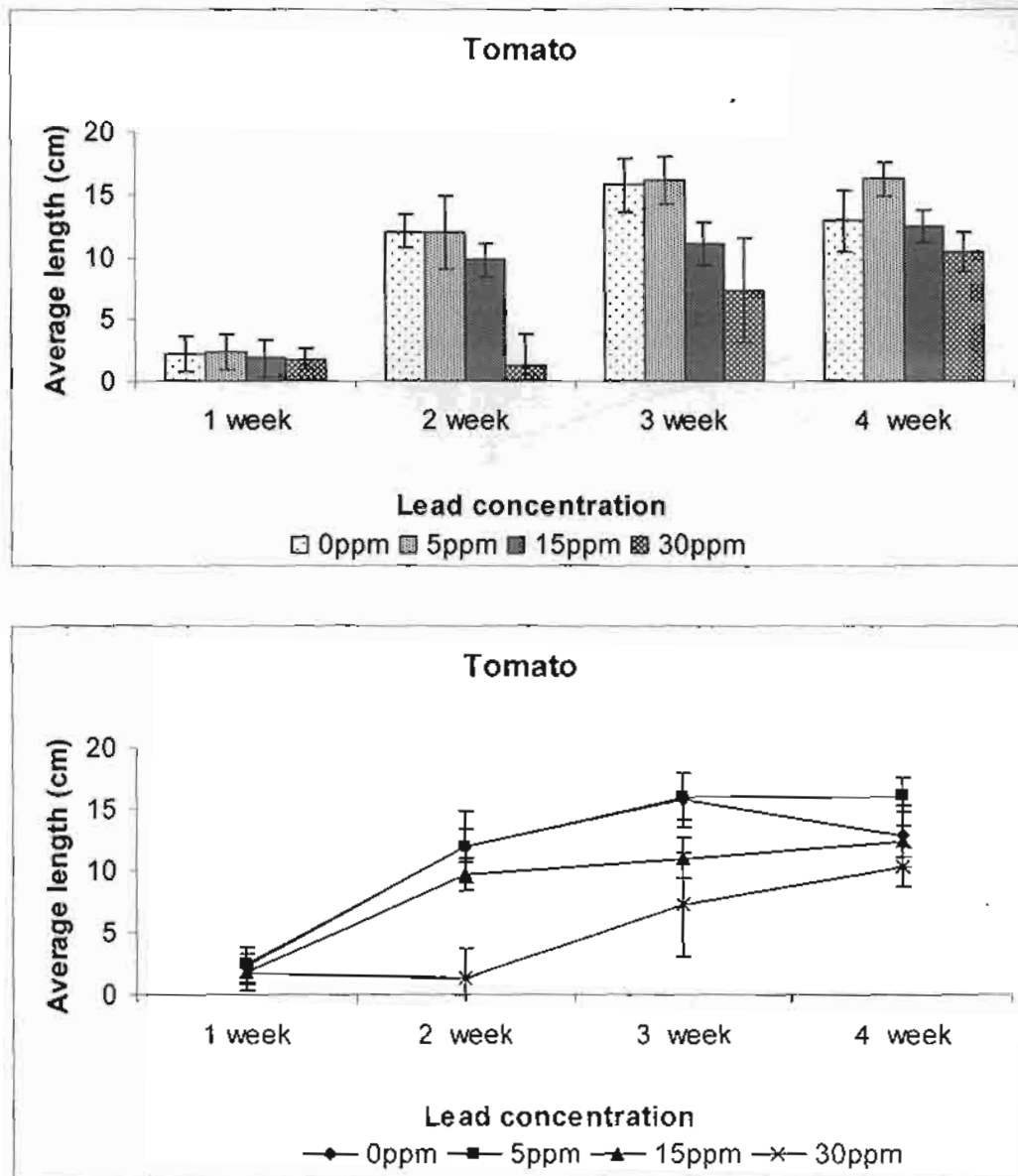


Figure 6: Column and line chart types for average length of tomato showed no significant difference ($P>0.05$) after one week exposure to lead. However, average length showed significant difference ($P<0.05$) after exposure to lead of two week onward. Values represent the mean $\pm$ SD of five replicate samples.

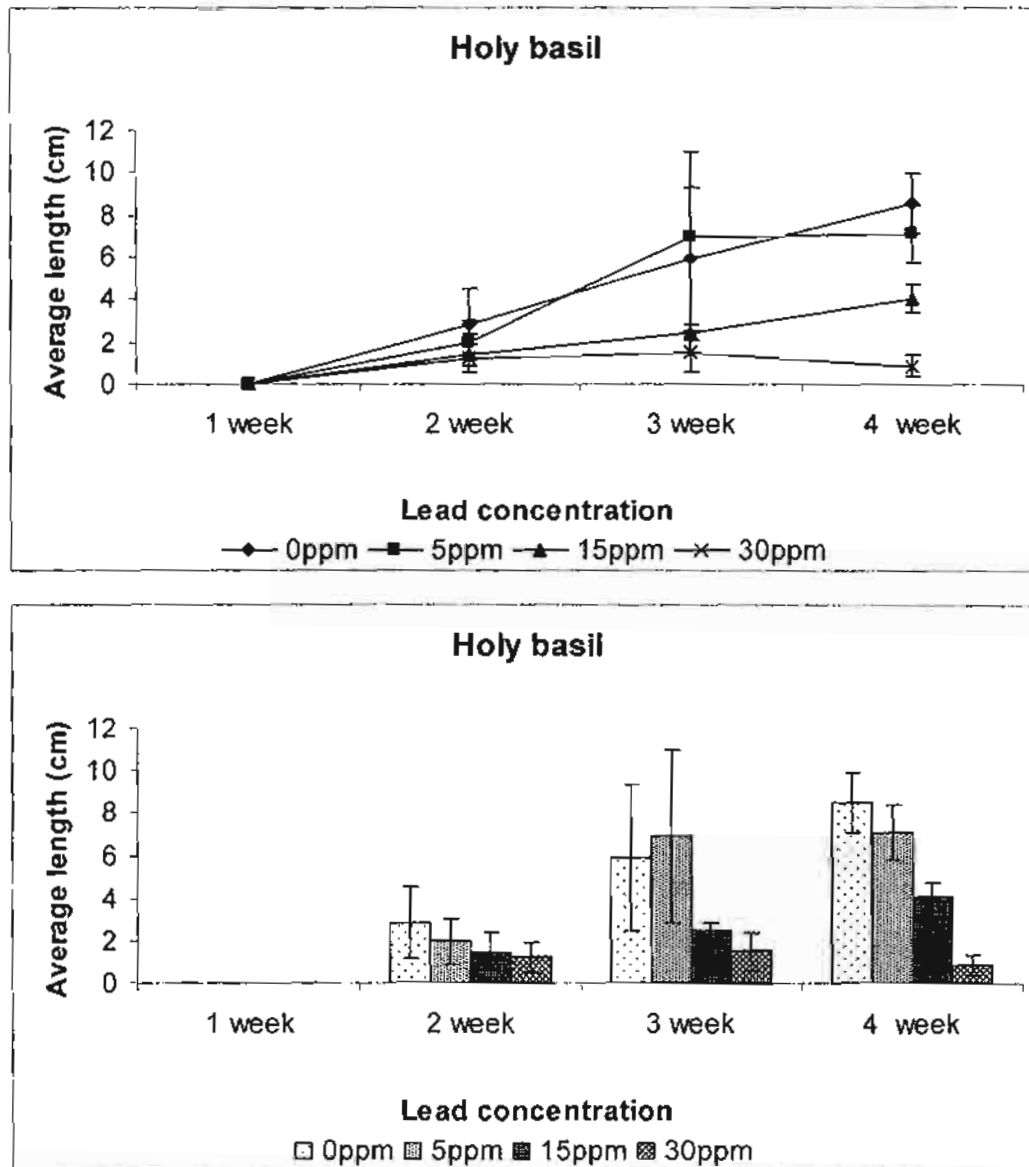


Figure 7: Column and line chart types for average length of holy basil showed no significant difference ($P>0.05$) after one week exposure to lead. However, there was a significant difference ($P<0.05$) after exposure to lead of three week onward. Values represent the mean $\pm$ SD of five replicate samples.

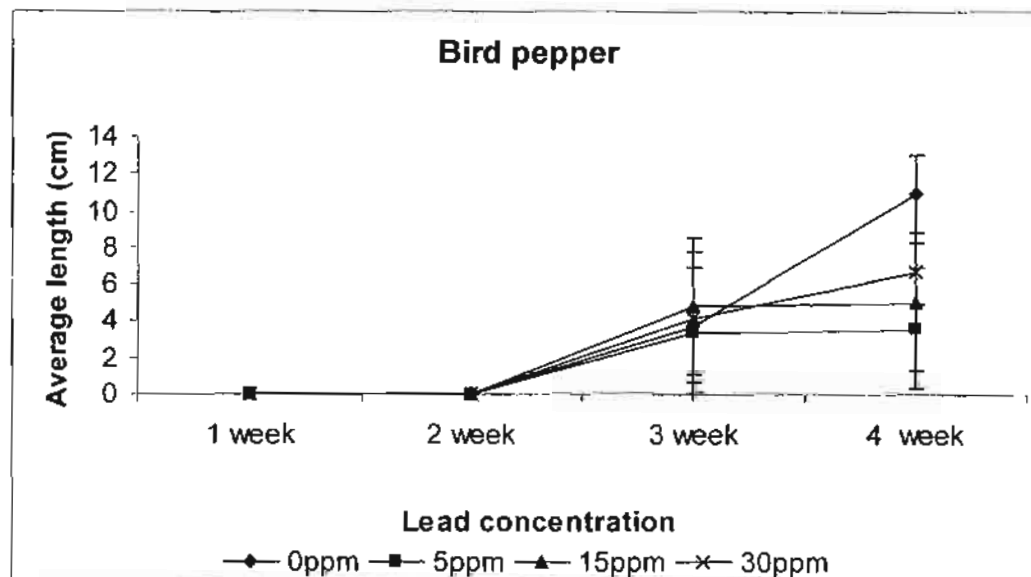
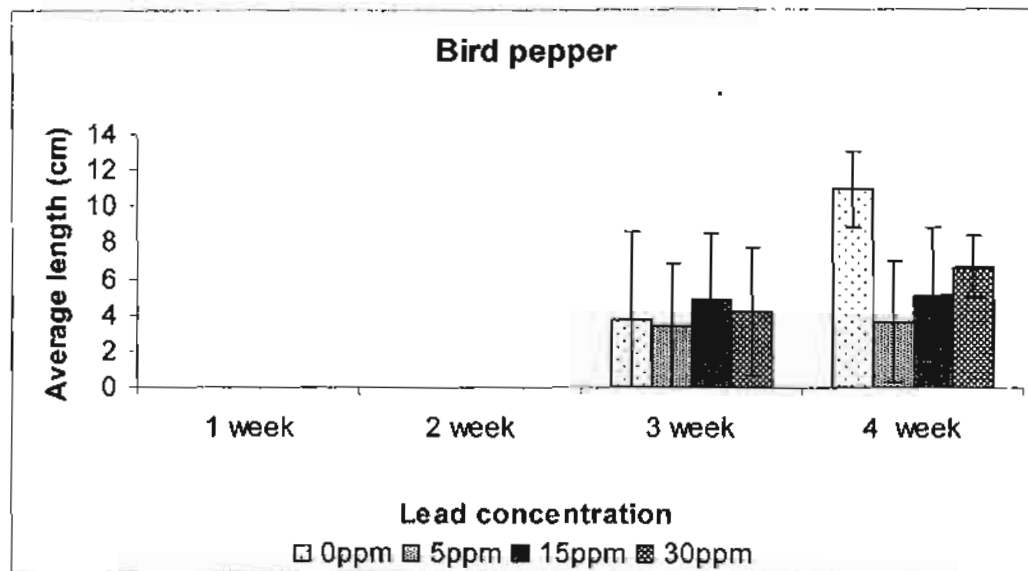


Figure 8: Column and line chart types for average length of bird pepper showed no significant difference ($P>0.05$) after three week exposure to lead. However, there was a significant difference ($P<0.05$) after four week exposure to lead. Values represent the mean $\pm$ SD of five replicate samples.

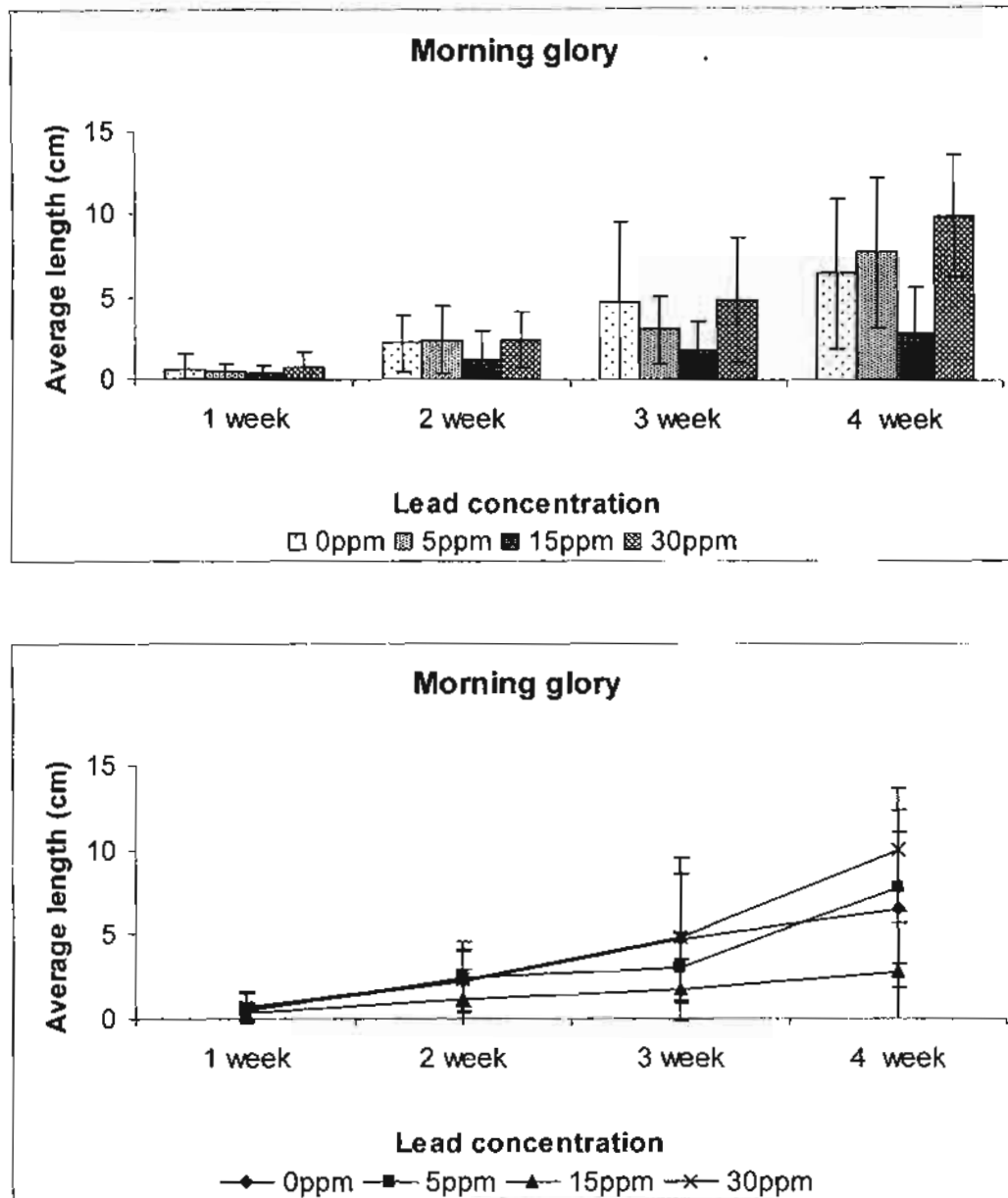


Figure 9: Column and line chart types for average length of morning glory showed no significant difference ($P>0.05$) after four week exposure to lead any concentration. Values represent the mean $\pm$ SD of five replicate samples.

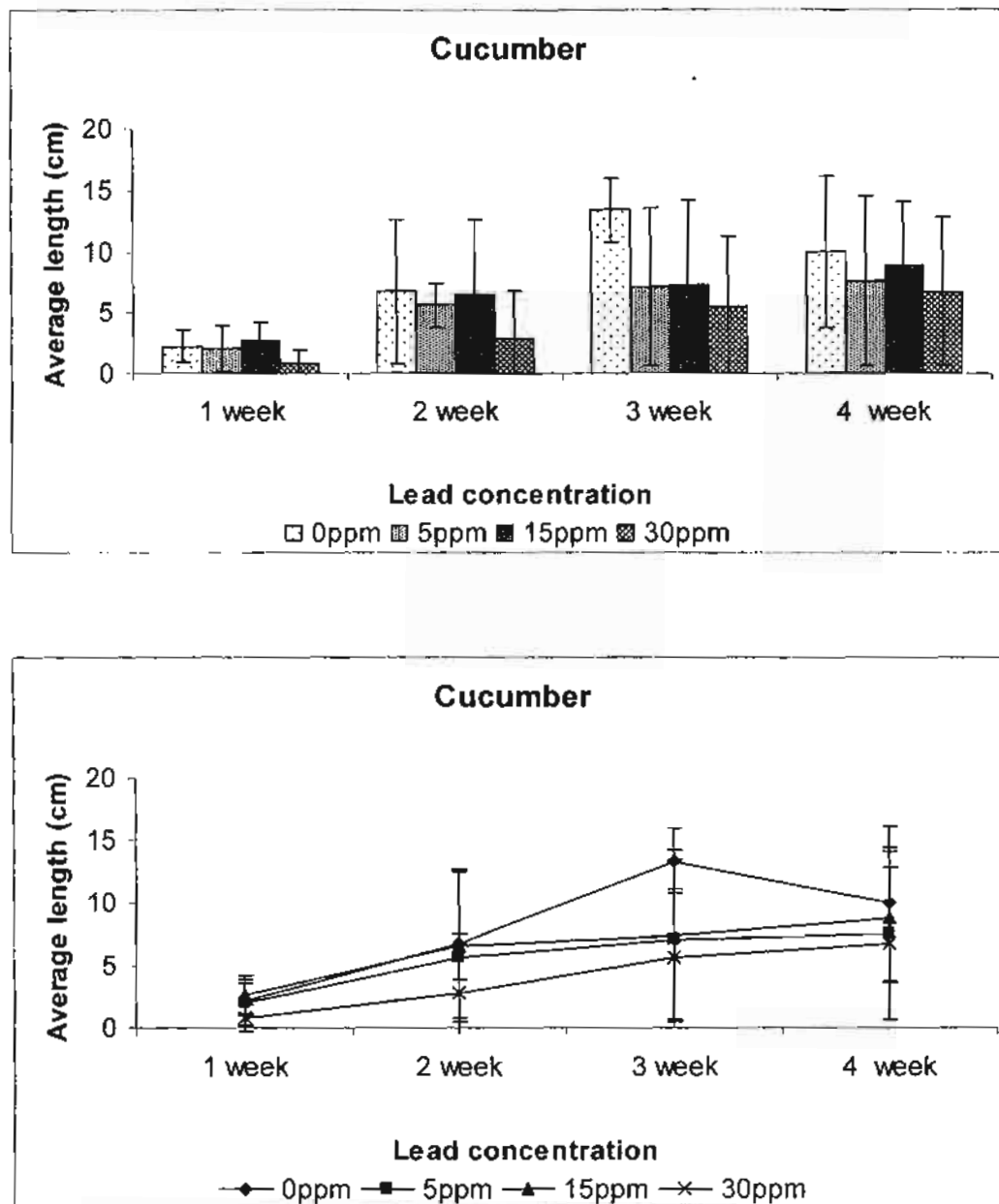


Figure 10: Column and line chart types for average length of cucumber showed no significant difference ($P>0.05$) after four week exposure to lead any concentration. Values represent the mean $\pm$ SD of five replicate samples.

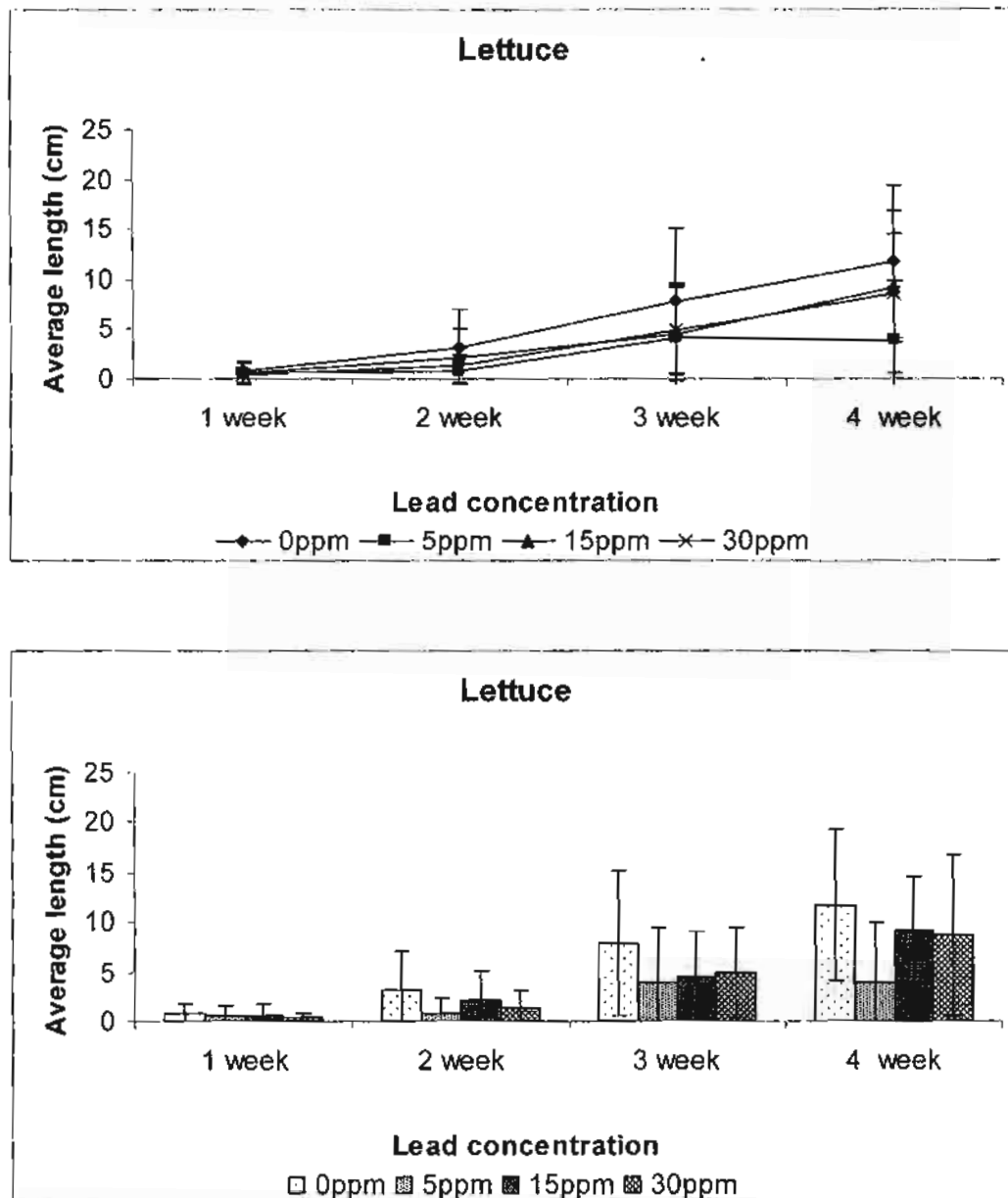


Figure 11: Column and line chart types for average length of lettuce showed no significant difference ($P>0.05$) after four week exposure to lead any concentration. Values represent the mean $\pm$ SD of five replicate samples.

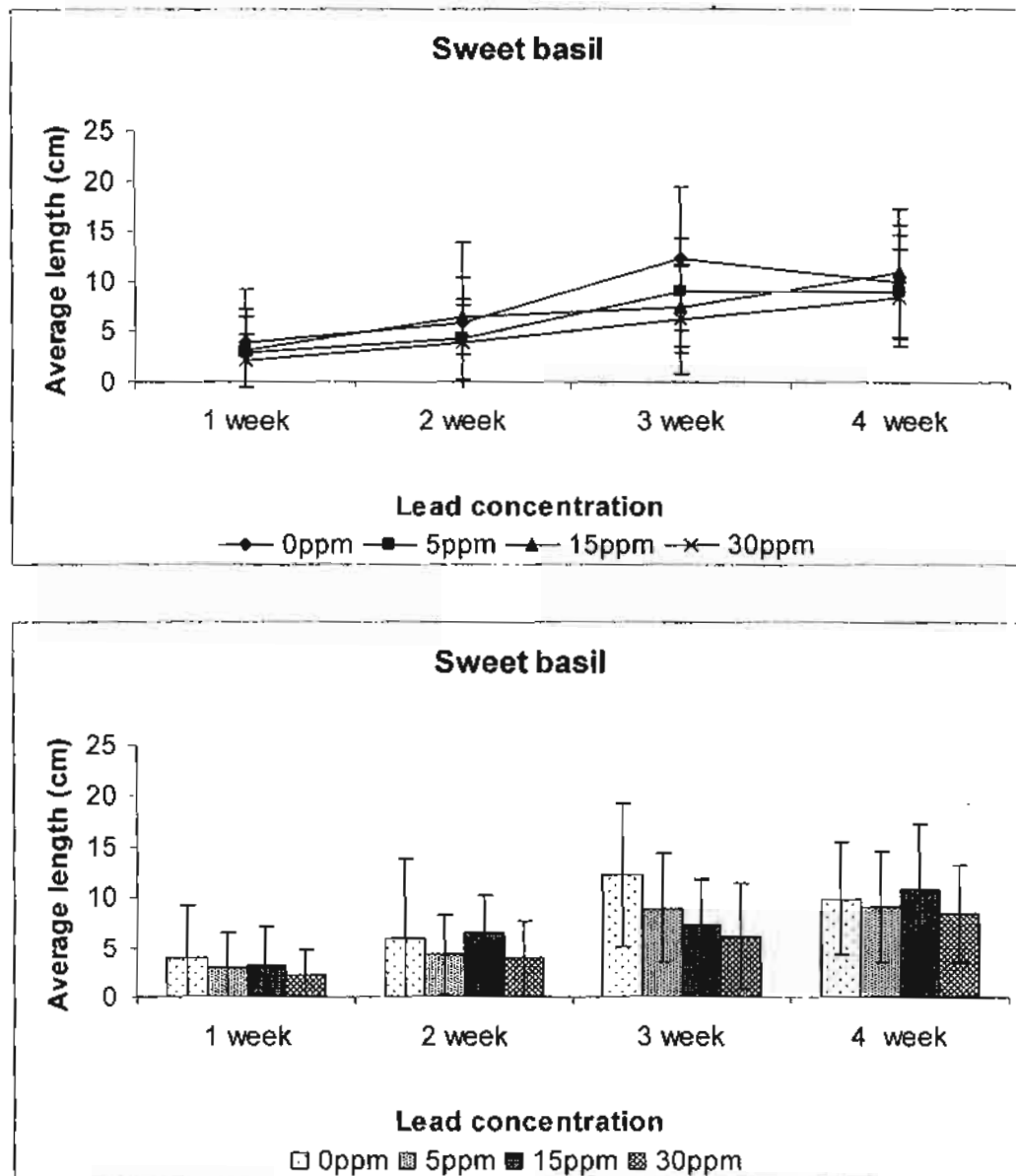


Figure 12: Column and line chart types for average length of sweet basil showed no significant difference ($P>0.05$) after four week exposure to lead any concentration. Values represent the mean $\pm$ SD of five replicate samples.

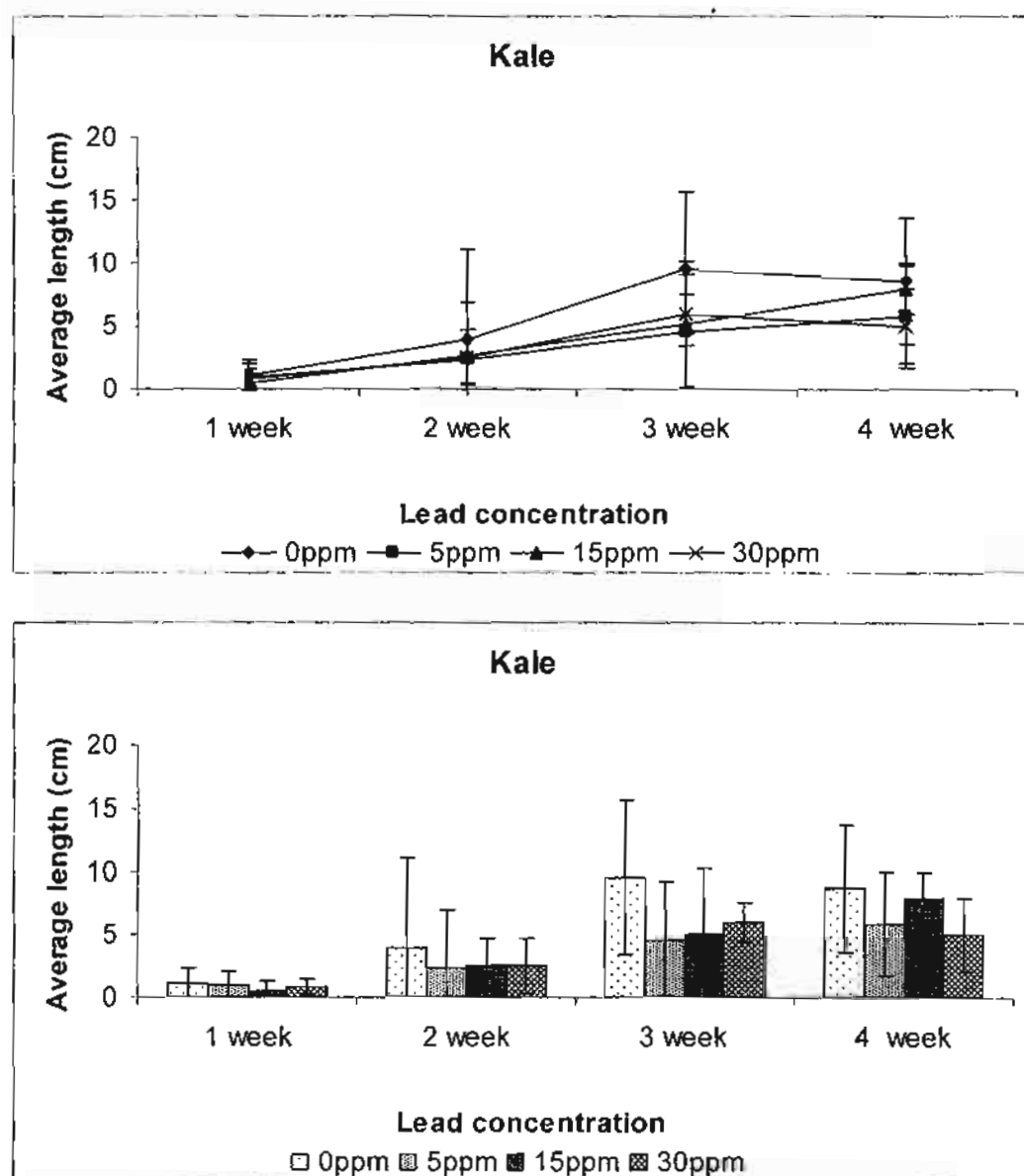


Figure 13: Column and line chart types for average length of kale showed no significant difference ($P>0.05$) after four week exposure to lead any concentration. Values represent the mean $\pm$ SD of five replicate samples.

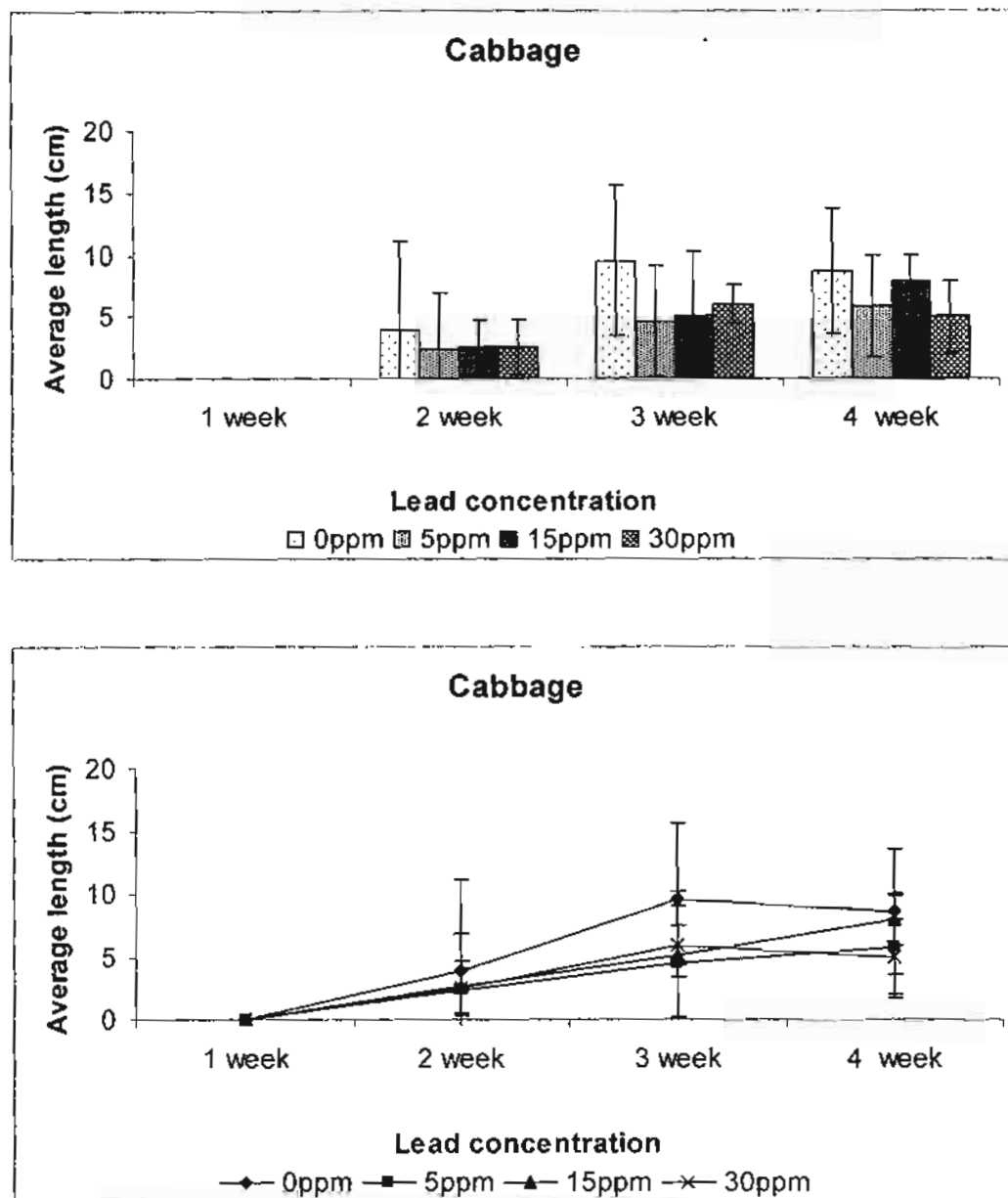


Figure 14: Column and line chart types for average length of cabbage showed no significant difference ($P>0.05$) after four week exposure to lead any concentration. Values represent the mean $\pm$ SD of five replicate samples.

4.3 Plant physiological changes

The seedling still grew in the presence of high concentrations of lead. However, the subsequent seedlings growth (after the breakage of seed coat) was severely inhibited at much lower concentrations of lead.

Mung bean, cucumber, morning glory, sweet basil, lettuce, kale, tomato, cabbage, holy basil, and bird pepper are dicotyledonous plants. In many dicots the primary roots continues to elongate and forms the taproot. Many smaller branch roots may grow from the taproot (Martin and Rene, 2006). The effect of lead on root growth was observed as a decrease in the growth of vegetables during taproot elongation, with increasing lead concentration (from 5 mg kg⁻¹ to 30 mg kg⁻¹). The taproot growth was decreased after exposure to lead at 5 mg kg⁻¹, 15 mg kg⁻¹ and 30 mg kg⁻¹ of lead concentrations as compared to the control.

EDTA was added to completely dissolve the lead nitrate solution. EDTA also caused easier and higher rate of translocation of lead to the shoot as compared to other parts of the plants as research done by Andrew D. Vassil and Co. in Indian mustard. EDTA destroys the physiological barrier(s) in roots by removal of stabilizing Zn²⁺ and Ca²⁺ from the plasma membrane. The primary effect of lead toxicity in plants is a rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip (Lee Y, 2000). So in this study Pb and EDTA may play an importance role in decreasing a taproots elongation.

This result indicated that lead had negatively effects on root elongation of mung bean, cucumber, morning glory, sweet basil, lettuce, kale, tomato, cabbage, holy basil, and bird pepper. The vegetables were not tolerant to lead toxicity even at low (5 mg kg⁻¹) concentrations.

Afterward, each plant samples were analysed for its lead accumulated content by using either flame atomic absorption spectrophotometer or graphite furnace atomic absorption spectrophotometer, depending on the amount of lead present in the samples. Plants which were treated with lead concentrations of 5, 15 and 30 mg kg⁻¹ *in vitro* for four weeks of exposure, lead concentration was determined. Results were focused on uptake and accumulation of lead in plants, Relative Growth Rate (RGR) and Bioaccumulation coefficient (BC) of the plants.

4.1 Study of uptake and accumulation of lead in plants

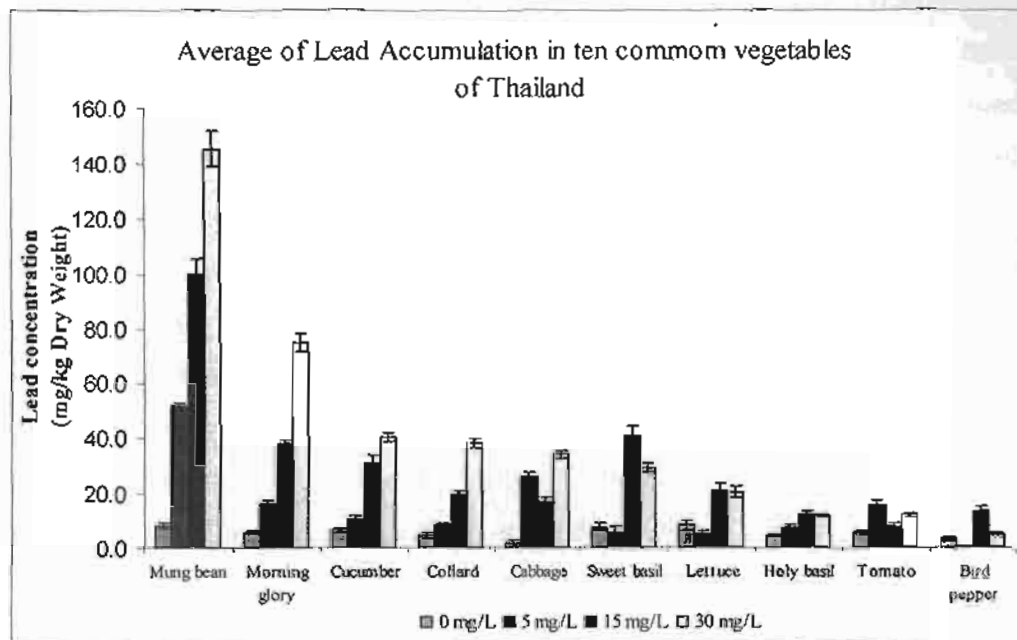


Figure 15: Average of lead accumulation in ten common vegetables of Thailand. Values represent the mean $\pm$ SD of three replicate samples at week 4.

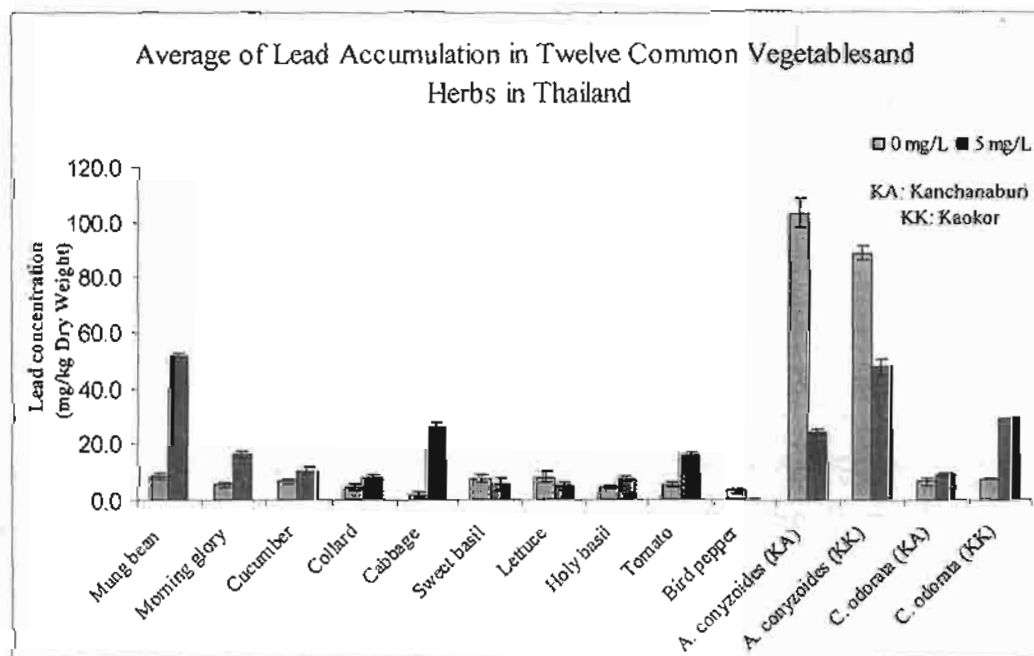


Figure 16: Average of lead accumulation in twelve common vegetables and herbs of Thailand. Values represent the mean $\pm$ SD of three replicate samples at week 4. (KK: Kaokor, KA: Kanchanaburi)

In this study, we investigated the lead accumulation in each cultivar of twelve common vegetables and herbs from a common medium which contained various concentrations of lead compounds. Concentration of lead which plants accumulated was determined by flame atomic absorption spectrometry (FAAS) technique and graphite furnace atomic absorption spectrometry (GFAAS) technique. Due to the limit detection of FAAS, some plants that had lead content lower than 0.02 mg kg⁻¹ used in this study were determined by GFAAS that can be used for detection the lead content in plant samples with lead content higher than 1 µg kg⁻¹. Lead was accumulated in plant by transfer the lead metal from root to plant shoot. Most of the plant samples were accumulated lead rise from lower concentration to higher concentration of lead compounds. The plants also increasing accumulated lead compounds when the exposure day was rising. Lead had effect on seed germination of *Sonchus arvensis* due to their low biomass production. The heavy metal analysis of *S. arvensis* could not perform the test. The highest lead accumulation was found in the forth week of lead exposed. Most of the plants showed highest accumulation at 30 mg kg⁻¹ of lead concentration in the media. From the result at week 4 (Figure 15), they can be categorized into three groups of plants according to level of lead accumulations: high, moderate and low lead. Mung bean (145.2 mg kg⁻¹ DW) and morning glory (74.7 mg kg⁻¹ DW) demonstrated significantly in high lead content among ten common vegetables. The moderate lead accumulations are cucumber (40.1 mg kg⁻¹ DW), collard (38.1 mg kg⁻¹ DW), cabbage (33.7 mg kg⁻¹ DW) and sweet basil (29.1 mg kg⁻¹ DW). The low lead accumulations were lettuce (20.2 mg kg⁻¹ DW), holy basil (11.7 mg kg⁻¹ DW), tomato (11.9 mg kg⁻¹ DW) and bird pepper (5.1 mg kg⁻¹ DW). Lead metal accumulation by *A. conyzoides* and *C. odorata* were summarized in Figure 16 compared to ten common vegetables.

In Figure 16, both populations of *A. conyzoides* at week 4, the accumulation of lead was higher in control than to 5 mg kg⁻¹ of lead treated and non contaminated site (Kaokor) had higher lead accumulated than the contaminated site (Kanchanaburi). Where as the both population of *C. odorata* at week 4, demonstrated the high level of lead accumulation of lead in contaminated site as compared to non contaminated site with 5 mg kg⁻¹ treated lead compound was higher accumulated than control.

In this study, we found that relative growth rate (RGR) of plant at week 4 was inverse proportion to the concentration of lead contamination. Lead concentration was not significantly affected to the growth of plants (Figure 17). Some plants can be developed themselves to tolerate the toxic of lead and capable to growth with increasing their biomass. However, from the results interpreted that all treated plants continued to develop new leaves and roots. At week 4, *A. conyzoides* had developed their sensitivity to lead exposure in contaminated site.

RGR of *A. conyzoides* at contaminated site (Kanchanaburi) was higher than those in non contaminated site (Kaokor). In *C. odorata*, the non contaminated site (Kaokor) was shown higher relative growth rate than the contaminated site (Kanchanaburi) (Figure 18).

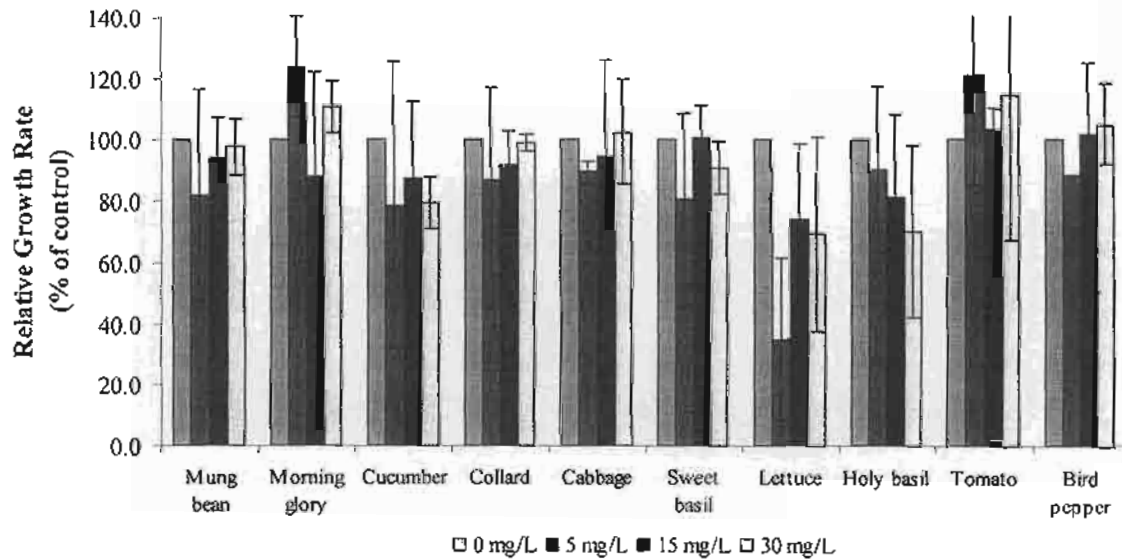


Figure 17: Effect of lead on average of relative growth rate (RGR) in ten common vegetables of Thailand. Values represent the mean $\pm$ SD of three replicate samples.

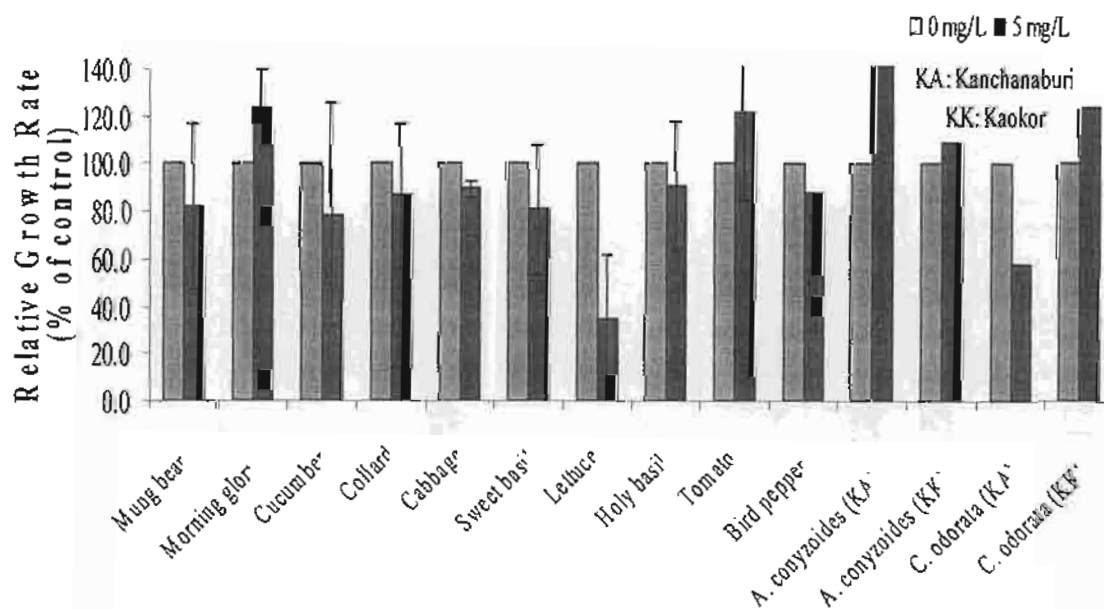


Figure 18: Effect of lead on average of relative growth rate (RGR) in twelve common vegetables and herbs of Thailand. Values represent the mean $\pm$ SD of three replicate samples. (KK: Kaokor, KA: Kanchanaburi)

4.3 Bioaccumulation coefficient (BC)

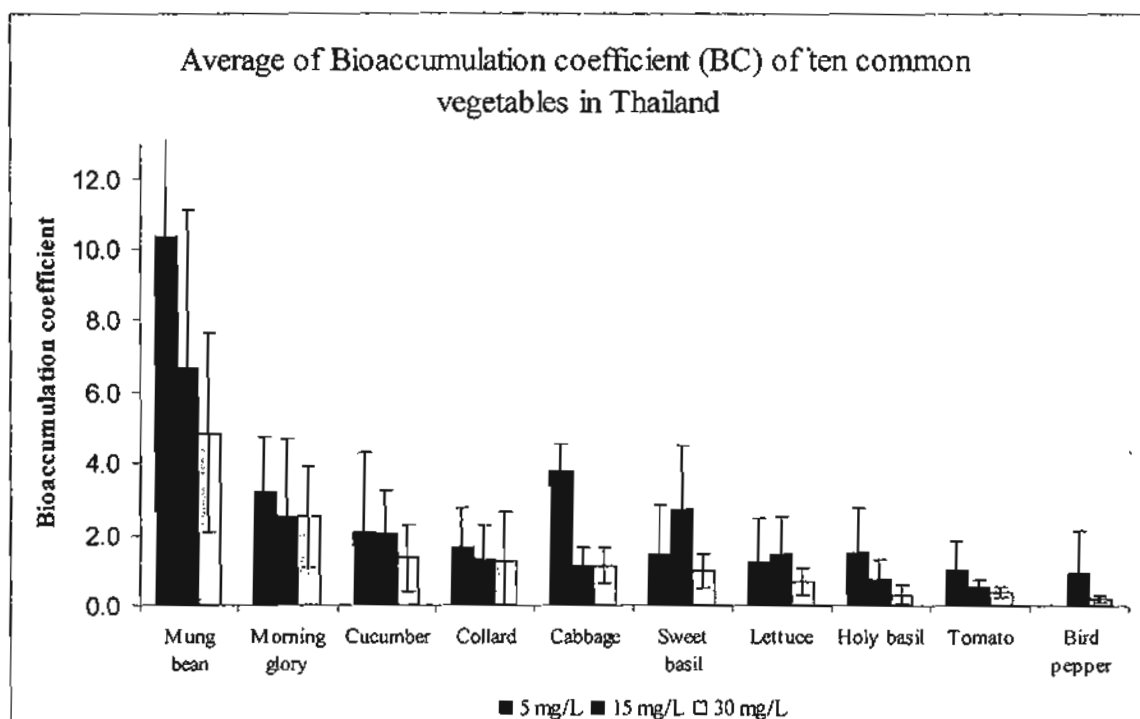


Figure 19: Average of bioaccumulation coefficient (BC) of ten common vegetables of Thailand. Values represent the mean $\pm$ SD of three replicate samples at week 4.

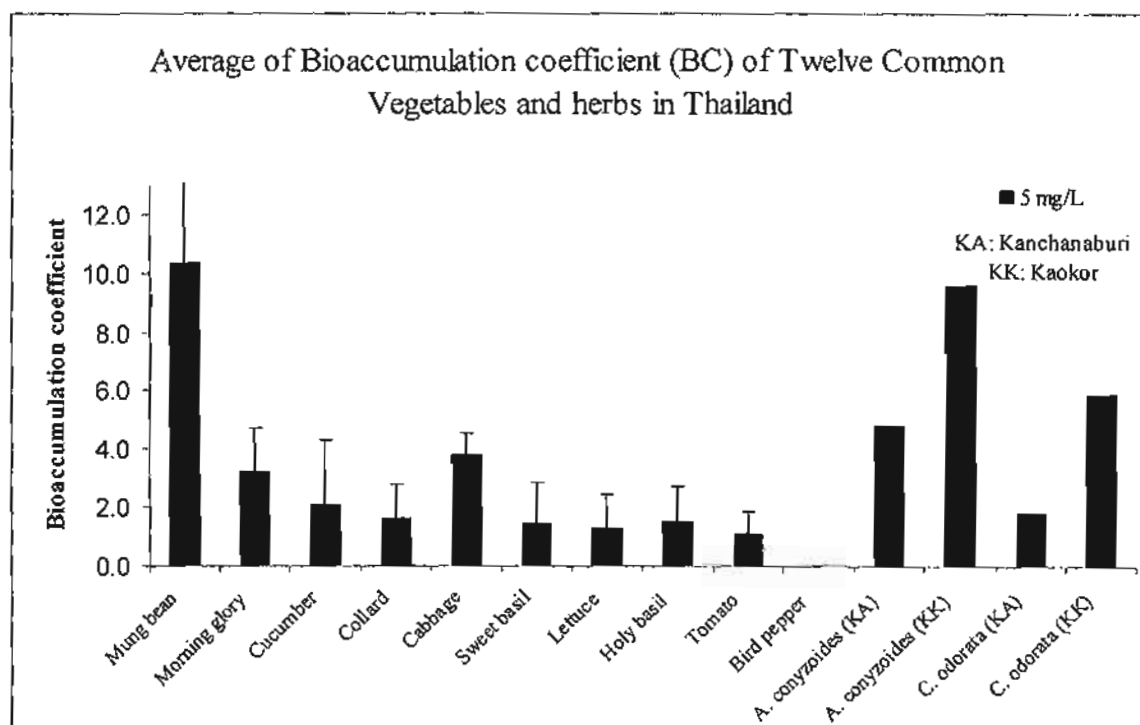


Figure 20: Average of bioaccumulation coefficient (BC) of twelve common vegetables and herbs of Thailand. Values represent the mean $\pm$ SD of three replicate samples at week 4. (KK: Kaokor, KA: Kanchanaburi)

The BC at week 4 in each vegetable had same trend decreasing from low concentration of lead accumulation to high lead accumulation. The highest BC in each vegetable increased from mung bean > morning glory > cucumber > collard > cabbage > sweet basil > lettuce > holy basil > tomato > bird pepper (Figure 20). The highest BC for lead in each vegetable was 10.3, 3.2, 2.1, 1.6, 3.8, 2.7, 1.5, 1.0, 1.5, and 0.9 respectively. At 5 mg kg⁻¹ of lead treated in week 4 of *A. conyzoides* and *C. odorata* in contaminated site (Kanchanaburi) were shown lower than the noncontaminated site (Kaokor) (Figure 20).

Markert (1994) gave the values of metal concentration of normal plant with which the uptakes in a species could be compared, and showed that the normal compositions of lead in plant are 1 mg kg⁻¹ DW. Lead is a non-essential element and can be toxic to photosynthesis (Skórzyńska-Polit and Baszyński, 1997), chlorophyll synthesis (Stobart et al., 1985) and antioxidase enzyme (Somashekaraiah et al., 1992). In this study, all vegetables and herbs showed abnormal lead concentration in their tissue. In general, the mean level of lead in plants collected increased with the increased concentration of lead in media. Numerous studies have demonstrated that heavy metal concentration in plants is a function of heavy metal content in the environment (Xiong, 1998).

There are three indicators to define a lead hyperaccumulator: (1) the concentration of lead in plant shoots > 1000 mg kg⁻¹ (Barker and Brooks, 1989); (2) the concentration of lead in shoots is 10-500 times more than that in plants from non-polluted areas (Pb 5 mg kg⁻¹) (Shen and Liu, 1998); (3) the TF or shoot:root ratio >1 (Barker and Brooks, 1989, Baker et al., 1994). In this study, although the translocation factor (TF) or shoot:root ratio was not determined but with two above indicators can evaluated that all ten common vegetables could not be considered as hyperaccumulator. The highest lead accumulation was mung bean (145.2 mg kg⁻¹ DW) or 0.014% of lead in total weight of plant.

In the soil, the bioavailability of lead is quite low due to low solubility of most lead compounds, and the readily precipitation of lead by sulfate and phosphate at the root system (Baker et al., 2000). Arvik and Zimdahl (1974) indicated that Pb uptake did not require any energetic exposure. Lead can be taken up from the surrounding solution against concentration gradients and deposited in large amounts in the roots (Wierzbicka, 1987). Heavy metals are transported from roots to shoots in terrestrial plants to different parts. Different metals are different motile and within a plant, lead is less motile than extends other Cu, Zn, and Cd (Greger, 2004).

Most of the shoot accumulation was found in stems but not pass through leaves. Lead deposition in the cell membrane and cell wall (Sahi et al., 2002). There was no report of plants

with ability to solubilize lead from the soil matrix which lead in nature is insoluble form (Blaylock and Huang, 2000). These knowledge can be explained that lead will not pass through leaves. The vegetables that consumed only leaves and fruits (tomato, bird pepper) can be consumed. However, lead may present in fruit vegetables such as tomato but in very less amount. Even though the seed of mung bean that people consumed as sweet seed mung bean soup, the lead may pass through the seed in very little amount because the lead was heavy molecule and mostly accumulate in root (Hussein, Obuid-Allah et al. 2006)

The results in sweet basil confirm the understanding that high heavy metal concentrations in the growth medium may increase metal accumulation in plant tissue, but not in the essential oil, which is the final marketable product (Zheljazkov and Warman 2003). It can imply that sweet-basil could be grown as an essential oil crop in contaminated soil without a risk of contamination of the end product, the essential oil.

Lead was not significantly effect on relative growth rate in all ten common vegetables. But lead showed significantly effect on shoot and root length extension in bird pepper, tomato, mung bean and holy basil (Piemyoo S., 2005). The genotypic differences in accumulation between cultivars are important. In this study, all crops were grown at the same location so it is expected that the deposition would have been relatively similar. In the view of the relatively high accumulation of lead shown by mung bean and morning glory, these species would not be suitable for edible vegetables that cultivar in contaminated area.

For health consequences of lead in edibles, after lead is ingested, it can only adversely affect health if it is absorbed. Adults absorb approximately 11% of ingested lead (USFDA, 1998), and excrete approximately 50-60% of that ingested over the short term (at a half-life of approximately 20 days) and an additional 25% over many months. The residual lead accumulates in mineralizing tissues (i.e. bones and teeth). Children can absorb lead from 30-75% of ingested lead (USFDA, 1998) and infant can excrete only approximately $5\mu\text{g kg}^{-1} \text{ day}^{-1}$ (Ziegler et al., 1978). Accumulation of lead in women of child bearing age is problematic, as transfer of lead to the fetus can occur, and lead stored to bone is mobilized during pregnancy (hence, made available to transfer to the fetus) (Gomaa et al., 2002). Diets laden with urban-grown herbs may substantially contribute to a person's lead burden. For example, if a person were to consume as little as 1 tablespoon of dried cilantro (weighing approximately 1.75 g), with a lead concentration of 49 mg of lead per gram dry weight of sample, they would be ingesting 85.75 mg of lead. As a result, this value would contribute to their total body burden of lead, for it exceeds the US FDA's recommended Provisional Total Tolerable Intake Levels (PTTIL) for all age groups, which are defined at 6 mg lead/day for children up to 6 years of age, 15 mg

leady/day for children 7 years and older, 25 mg lead/day for pregnant woman and 75 mg lead/day for other adults (US FDA, 1993). In 1991, the Centre for Disease Control and Prevention, Atlanta, United States of America provided guideline of lead poisoning in blood lead levels is equal or more than 10 microgram per deciliter (US EPA, 2001).

However, the level of safe lead concentration in edible vegetable is not clearly identified. It is because of many factors influences such as amount of vegetables that people consumed and times that people consumed in each species of plants. However, United States Food and Drug Administration defined the range of lead concentration in vegetable that can be safe for consumption. It is ranging from 15-40 ppm of lead that accumulated in plant (US FDA, 1993).

These results indicate that the vegetables that lead had less effect on growth and still low accumulation are lettuce, sweet basil, cabbage and collard respectively.

The contrast pattern of Bioaccumulation coefficient and lead accumulation pattern, Kim et al. (2003) suggested such discrepancies arise due to variation in heavy metal concentration, form of metal present and plant species.

The ecotype differences in *A. conyzoides* and *C. odorata* were showed significantly different in lead accumulation in plant in both contaminated site (Karnjanburi) and non contaminated site (Kaokor). *A. conyzoides* from (Kaokor) developed themselves to accumulate lead more than the species from contaminated site (Karnjanaburi). P. Tanhan (2007) indicate that *C. odorata* was that hyperaccumulator but in the study, the genotypic different effect the accumulation of the plant. However, *C. odorata* has certain detoxification mechanisms within the tissue, which allow plants to accumulate such high amount of lead (Greger, 2004). It may be recognized that the medium lead treated instead of soil field, probably gave rise to enhanced uptake (Alexander P.D., 2006).

Because urban gardening is a wide spread activity with potential health impacts. The following lists recommendations urban gardeners may elect to follow so to lower risks associated with gardening.

Recommendations for urban gardeners (M.E. Finster et al., 2003)

- Survey the property to determine the potential lead hazards, extent of the contamination and location of high-risk areas.
 - Plan to locate fruit and vegetable gardens away from buildings, especially if peeling paint is evident and sites where sludge with heavy metals was applied.
 - Do not grow food crops in a soil that is contaminated to levels greater than 400 ppm.
-

-
- Analyze lead concentration in soil samples from areas where vegetable gardens exist or are planned.
 - Instead, use either containers or construct raised beds, with a semi-permeable barrier between the clean and contaminated soil.
 - Where container or raised bed gardening is not possible, fruiting crops should be grown.
 - Root vegetables, leafy greens and herbs should not be planted in contaminated soils.
 - Test new topsoil before using it and annually retest the garden soil to monitor for recontamination.
 - Do not use plants grown in contaminated soils for compost.
 - Use mulch or a weed tarp in garden beds to reduce the potential for aerial soil dust deposition or soil splash up on crops.
-

The risk of gardening in lead contaminated soil is both from the lead contamination of the edibles and the practices that might promote ingestion of lead contaminated soil (e.g. oral behaviors, soil track-in to the home). While there are no federal standards or guidelines for soil lead concentration for home gardening, it is recommended that all food crops should be grown in a soil in which the lead concentration is less than 400 ppm, the current US regulatory soil hazard standard that is considered safe for child play (USEPA, 2001). However, the gardener should recognize that any regulatory cutoff point does not ensure safety and keep in mind that background soil lead contamination levels are less than one-tenth this suggested 400 ppm soil hazard level (Shacklette and Boerngen, 1984).

Moreover, it is important that plants grown in contaminated soils are not used for compost, for this would result in lead recycling within a garden since most plants were shown to accumulate lead to some extent, particularly within their roots. Due to concern about directly ingesting lead from soil adhered to the leaves, fruits or roots of crops, it is important to remove outer leaves of leafy greens, peel vegetables when possible, and thoroughly wash all items with a detergent before consumption. Finally, when consumed the vegetables, it is recommended that wash the vegetables with 1% vinegar solution (2.5 tablespoons per gallon) for 15 min to dissolve lead contaminated on the surface skin of vegetables.

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Appendix A

Media formulation

The compositions of White (1963) used in tissue culture medium to study effects of lead on germination and development of common vegetables.

Table A-1: Compositions of nutrient solution in the media
(Modified White 1963)

Stock No.	Compounds	Amount (g/50ml)	Stock	Used (ml/l)
1	KNO ₃	0.8	200x	5
	Ca (NO ₃) ₂	2		
2	MgSO ₄ .7H ₂ O	7.2	200x	5
3	MnSO ₄ .4 H ₂ O	0.053	200x	5
	ZnSO ₄ .7 H ₂ O	0.030		
	Fe (SO ₄) ₃	0.035		
	Na ₂ SO ₄	2		
	KCl	1.3		
4	KI	0.015	400x	2.5
	H ₃ BO ₃	0.030		
5	NaH ₂ .PO ₄ .H ₂ O	0.186	200x	5
6	Glycine	0.009	600x	1.66
	Nicotinic acid	0.015		
	Vitamin B ₁	0.003		
	Vitamin B ₆	0.030		

Appendix B

Percentage of seed germination

Table B-1: Percentage of seed germinations in four week cultures

Species	Lead concentration			
	0mg	5mg kg-	15mg kg-	30mg kg-
	kg-1	1	1	1
Mung bean	100	100	100	100
Cucumber	85	70	75	50
Morning glory	70	80	55	80
Sweet basil	65	70	75	70
Lettuce	70	50	65	65
Kale	85	80	75	85
Tomato	95	100	100	80
Cabbage	66.67	80	73.33	66.67
Holy basil	80	80	86.67	73.33
Bird pepper	80	60	80	90

Table B-2: Statistic analysis of percent mung bean germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	4
Con.	2	5ppm	4
	3	15ppm	4
	4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.000 ^a	3	.000	.	.
Intercept	400.000	1	400.000	.	.
TRT	.000	3	.000	.	.
Error	.000	12	.000		
Total	400.000	16			
Corrected Total	.000	15			

a. R Squared = . (Adjusted R Squared = .)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound
LSD	0ppm	5ppm	.00	1.000	.a	.
		15ppm	.00	1.000	.a	.
		30ppm	.00	1.000	.a	.
	5ppm	0ppm	.00	1.000	.a	.
		15ppm	.00	1.000	.a	.
		30ppm	.00	1.000	.a	.
	15ppm	0ppm	.00	1.000	.a	.
		5ppm	.00	1.000	.a	.
		30ppm	.00	1.000	.a	.
	30ppm	0ppm	.00	1.000	.a	.
		5ppm	.00	1.000	.a	.
		15ppm	.00	1.000	.a	.

Based on observed means.

a. Range values cannot be computed.

Table B-3: Statistic analysis of percent cucumber germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	4
Con.	2	5ppm	4
	3	15ppm	4
	4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.500 ^a	3	2.167	3.467	.051
Intercept	196.000	1	196.000	313.600	.000
TRT	6.500	3	2.167	3.467	.051
Error	7.500	12	.625		
Total	210.000	16			
Corrected Total	14.000	15			

a. R Squared = .464 (Adjusted R Squared = .330)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound	
LSD	0ppm	5ppm	.75	.56	.205	-.47	1.97
		15ppm	.50	.56	.389	-.72	1.72
		30ppm	1.75*	.56	.009	.53	2.97
	5ppm	0ppm	-.75	.56	.205	-1.97	.47
		15ppm	-.25	.56	.663	-1.47	.97
		30ppm	1.00	.56	.099	-.22	2.22
	15ppm	0ppm	-.50	.56	.389	-1.72	.72
		5ppm	.25	.56	.663	-.97	1.47
		30ppm	1.25*	.56	.045	3.20E-02	2.47
	30ppm	0ppm	-1.75*	.56	.009	-2.97	-.53
		5ppm	-1.00	.56	.099	-2.22	.22
		15ppm	-1.25*	.56	.045	-2.47	-3.20E-02

Based on observed means.

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

Table B-4: Statistic analysis of percent morning glory germination in four week cultures

Between-Subjects Factors

	Value Label	N
Pb 1	0ppm	4
Con. 2	5ppm	4
3	15ppm	4
4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.188 ^a	3	1.396	2.913	.078
Intercept	203.063	1	203.063	423.783	.000
TRT	4.188	3	1.396	2.913	.078
Error	5.750	12	.479		
Total	213.000	16			
Corrected Total	9.938	15			

a. R Squared = .421 (Adjusted R Squared = .277)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

	(I) Pb Con.	(J) Pb Con.	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	0ppm	5ppm	-.50	.49	.327	-1.57	.57
		15ppm	.75	.49	.151	-.32	1.82
		30ppm	-.50	.49	.327	-1.57	.57
	5ppm	0ppm	.50	.49	.327	-.57	1.57
		15ppm	1.25*	.49	.025	.18	2.32
		30ppm	.00	.49	1.000	-1.07	1.07
	15ppm	0ppm	-.75	.49	.151	-1.82	.32
		5ppm	-1.25*	.49	.025	-2.32	-.18
		30ppm	-1.25*	.49	.025	-2.32	-.18
	30ppm	0ppm	.50	.49	.327	-.57	1.57
		5ppm	.00	.49	1.000	-1.07	1.07
		15ppm	1.25*	.49	.025	.18	2.32

Based on observed means.

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

Table B-5: Statistic analysis of percent sweet basil germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	4
Con.	2	5ppm	4
	3	15ppm	4
	4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.500 ^a	3	.167	.364	.780
Intercept	196.000	1	196.000	427.636	.000
TRT	.500	3	.167	.364	.780
Error	5.500	12	.458		
Total	202.000	16			
Corrected Total	6.000	15			

a. R Squared = .083 (Adjusted R Squared = -.146)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound	
LSD	0ppm	5ppm	-.25	.48	.611	-1.29	.79
		15ppm	-.50	.48	.317	-1.54	.54
		30ppm	-.25	.48	.611	-1.29	.79
	5ppm	0ppm	.25	.48	.611	-.79	1.29
		15ppm	-.25	.48	.611	-1.29	.79
		30ppm	.00	.48	1.000	-1.04	1.04
	15ppm	0ppm	.50	.48	.317	-.54	1.54
		5ppm	.25	.48	.611	-.79	1.29
		30ppm	.25	.48	.611	-.79	1.29
	30ppm	0ppm	.25	.48	.611	-.79	1.29
		5ppm	.00	.48	1.000	-1.04	1.04
		15ppm	-.25	.48	.611	-1.29	.79

Based on observed means.

Table B-6: Statistic analysis of percent lettuce germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	4
Con.	2	5ppm	4
	3	15ppm	4
	4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.250 ^a	3	.750	1.636	.233
Intercept	156.250	1	156.250	340.909	.000
TRT	2.250	3	.750	1.636	.233
Error	5.500	12	.458		
Total	164.000	16			
Corrected Total	7.750	15			

a. R Squared = .290 (Adjusted R Squared = .113)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound	
LSD	0ppm	5ppm	1.00	.48	.059	-4.30E-02	2.04
		15ppm	.25	.48	.611	-.79	1.29
		30ppm	.25	.48	.611	-.79	1.29
	5ppm	0ppm	-1.00	.48	.059	-2.04	4.30E-02
		15ppm	-.75	.48	.143	-1.79	.29
		30ppm	-.75	.48	.143	-1.79	.29
	15ppm	0ppm	-.25	.48	.611	-1.29	.79
		5ppm	.75	.48	.143	-.29	1.79
		30ppm	.00	.48	1.000	-1.04	1.04
	30ppm	0ppm	-.25	.48	.611	-1.29	.79
		5ppm	.75	.48	.143	-.29	1.79
		15ppm	.00	.48	1.000	-1.04	1.04

Based on observed means.

Table B-7: Statistic analysis of percent kale germination in four week cultures

Between-Subjects Factors

	Value Label	N
Pb 1	0ppm	4
Con. 2	5ppm	4
3	15ppm	4
4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.687 ^a	3	.229	.440	.729
Intercept	264.063	1	264.063	507.000	.000
TRT	.688	3	.229	.440	.729
Error	6.250	12	.521		
Total	271.000	16			
Corrected Total	6.937	15			

a. R Squared = .099 (Adjusted R Squared = -.126)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

	(I) Pb Con.	(J) Pb Con.	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	0ppm	5ppm	.25	.51	.633	-.86	1.36
		15ppm	.50	.51	.347	-.61	1.61
		30ppm	.00	.51	1.000	-1.11	1.11
	5ppm	0ppm	-.25	.51	.633	-1.36	.86
		15ppm	.25	.51	.633	-.86	1.36
		30ppm	-.25	.51	.633	-1.36	.86
	15ppm	0ppm	-.50	.51	.347	-1.61	.61
		5ppm	-.25	.51	.633	-1.36	.86
		30ppm	-.50	.51	.347	-1.61	.61
	30ppm	0ppm	.00	.51	1.000	-1.11	1.11
		5ppm	.25	.51	.633	-.86	1.36
		15ppm	.50	.51	.347	-.61	1.61

Based on observed means.

Table B-8: Statistic analysis of percent tomato germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	4
Con.	2	5ppm	4
	3	15ppm	4
	4	30ppm	4

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.250 ^a	3	.750	1.200	.352
Intercept	342.250	1	342.250	547.600	.000
TRT	2.250	3	.750	1.200	.352
Error	7.500	12	.625		
Total	352.000	16			
Corrected Total	9.750	15			

a. R Squared = .231 (Adjusted R Squared = .038)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

	(I) Pb Con.	(J) Pb Con.	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	0ppm	5ppm	-.25	.56	.663	-1.47	.97
		15ppm	.00	.56	1.000	-1.22	1.22
		30ppm	.75	.56	.205	-.47	1.97
	5ppm	0ppm	.25	.56	.663	-.97	1.47
		15ppm	.25	.56	.663	-.97	1.47
		30ppm	1.00	.56	.099	-.22	2.22
	15ppm	0ppm	.00	.56	1.000	-1.22	1.22
		5ppm	-.25	.56	.663	-1.47	.97
		30ppm	.75	.56	.205	-.47	1.97
	30ppm	0ppm	-.75	.56	.205	-1.97	.47
		5ppm	-1.00	.56	.099	-2.22	.22
		15ppm	-.75	.56	.205	-1.97	.47

Based on observed means.

Table B-9: Statistic analysis of percent cabbage germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	3
Con.	2	5ppm	3
	3	15ppm	3
	4	30ppm	3

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.917 ^a	3	.306	.244	.863
Intercept	154.083	1	154.083	123.267	.000
TRT	.917	3	.306	.244	.863
Error	10.000	8	1.250		
Total	165.000	12			
Corrected Total	10.917	11			

a. R Squared = .084 (Adjusted R Squared = -.260)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound	
LSD	0ppm	5ppm	-.67	.91	.486	-2.77	1.44
		15ppm	-.33	.91	.724	-2.44	1.77
		30ppm	.00	.91	1.000	-2.11	2.11
	5ppm	0ppm	.67	.91	.486	-1.44	2.77
		15ppm	.33	.91	.724	-1.77	2.44
		30ppm	.67	.91	.486	-1.44	2.77
	15ppm	0ppm	.33	.91	.724	-1.77	2.44
		5ppm	-.33	.91	.724	-2.44	1.77
		30ppm	.33	.91	.724	-1.77	2.44
	30ppm	0ppm	.00	.91	1.000	-2.11	2.11
		5ppm	-.67	.91	.486	-2.77	1.44
		15ppm	-.33	.91	.724	-2.44	1.77

Based on observed means.

Table B-10: Statistic analysis of percent holy basil germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	3
Con.	2	5ppm	3
	3	15ppm	3
	4	30ppm	3

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.667 ^a	3	.222	.242	.864
Intercept	192.000	1	192.000	209.455	.000
TRT	.667	3	.222	.242	.864
Error	7.333	8	.917		
Total	200.000	12			
Corrected Total	8.000	11			

a. R Squared = .083 (Adjusted R Squared = -.260)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound	
LSD	0ppm	5ppm	.00	.78	1.000	-1.80	1.80
		15ppm	-.33	.78	.681	-2.14	1.47
		30ppm	.33	.78	.681	-1.47	2.14
	5ppm	0ppm	.00	.78	1.000	-1.80	1.80
		15ppm	-.33	.78	.681	-2.14	1.47
		30ppm	.33	.78	.681	-1.47	2.14
	15ppm	0ppm	.33	.78	.681	-1.47	2.14
		5ppm	.33	.78	.681	-1.47	2.14
		30ppm	.67	.78	.419	-1.14	2.47
	30ppm	0ppm	-.33	.78	.681	-2.14	1.47
		5ppm	-.33	.78	.681	-2.14	1.47
		15ppm	-.67	.78	.419	-2.47	1.14

Based on observed means.

Table B-11: Statistic analysis of percent bird pepper germination in four week cultures

Between-Subjects Factors

		Value Label	N
Pb	1	0ppm	2
Con.	2	5ppm	2
	3	15ppm	2
	4	30ppm	2

Tests of Between-Subjects Effects

Dependent Variable: %Germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.375 ^a	3	.792	1.267	.398
Intercept	120.125	1	120.125	192.200	.000
TRT	2.375	3	.792	1.267	.398
Error	2.500	4	.625		
Total	125.000	8			
Corrected Total	4.875	7			

a. R Squared = .487 (Adjusted R Squared = .103)

Post Hoc Tests

Pb Con.

Multiple Comparisons

Dependent Variable: %Germination

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
(I) Pb Con.	(J) Pb Con.				Lower Bound	Upper Bound	
LSD	0ppm	5ppm	1.00	.79	.275	-1.19	3.19
		15ppm	.00	.79	1.000	-2.19	2.19
		30ppm	-.50	.79	.561	-2.69	1.69
	5ppm	0ppm	-1.00	.79	.275	-3.19	1.19
		15ppm	-1.00	.79	.275	-3.19	1.19
		30ppm	-1.50	.79	.131	-3.69	.69
	15ppm	0ppm	.00	.79	1.000	-2.19	2.19
		5ppm	1.00	.79	.275	-1.19	3.19
		30ppm	-.50	.79	.561	-2.69	1.69
	30ppm	0ppm	.50	.79	.561	-1.69	2.69
		5ppm	1.50	.79	.131	-.69	3.69
		15ppm	.50	.79	.561	-1.69	2.69

Based on observed means.

Appendix C

Experimental condition for lead determination with FAAS (Variance SpectrAA 55B)

Working condition	Lead (Pb)
Lamp current	5 mA
Fuel	Acetylene
Support	Air
Flame stoichiometry	Oxidizing
Wavelength	217.0 nm
Slit width	1.0 nm
Optimum working range	0.1-30 µg/ml

Experimental condition for lead determination with GFAAS (GBC UltraZ)

Working condition	Lead (Pb)
Lamp current	10.0 mA
Wavelength	217.0 nm
Slit width	1.0 nm
Gas flow	Argon

GBC UltraZ graphite furnace program

Step number	Final Temp. (C)	Ramp Time (s)	Hold Time (s)	Inert Gas	Aux. Gas	Read	Signal Graphics
Step 1	50	1	4	3	Off	Off	Off
Step 2	Inject Sample						
Step 3	90	10	15	3	Off	Off	Off
Step 4	120	15	10	3	Off	Off	Off
Step 5	800	10	5	3	Off	Off	Off
Step 6	800	0	1	Off	Off	Off	On
Step 7	2100	0.7	1.2	Off	Off	On	On
Step 8	2100	1	2	3	Off	Off	Off