ACCUMULATED COPPER AND ZINC IN LETTUCE PLANTATION IN SOIL AMENDED WITH DIFFERENT BOTTOM ASH RATIOS

PRAT INTRARASAKSIT

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Thesis
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ABSTRACT

This study was conducted to determine copper and zinc accumulation in lettuce planted in sida soil amended with lignite bottom ash. The copper and zinc accumulation in sida soil, bottom ash, sida soil added with bottom ash, lettuce root and lettuce leaf were investigated. Nitrogen, phosphorus and potassium in sida soil, in bottom ash and in sida soil added with bottom ash, and the lettuce yield were also investigated. Lettuce seed were cultured and grown in five ratios of sida soil added with bottom ash. Lettuce was planted and grown under laboratory conditions. The collected data were analyzed using one-way ANOVA and the mean comparison was conducted using LSD.

Results indicated that mixed sample ratio 0.6:0.4 had the highest copper and zinc accumulation (26.13±2.30 and 137.74±4.13 mg/kg). Copper and zinc accumulation in mixed sample ratio showed significant difference as bottom ash ratio increased ($p<0.05$). The highest copper and zinc accumulation was found at ratio of 0.6:0.4 in lettuce root (21.46±5.90 mg/kg and 113.47±4.13 mg/kg), followed by lettuce leaf (8.18±1.20 mg/kg and 32.94±7.34 mg/kg). The accumulation in root and leaf of lettuce was significantly different as bottom ash ratio increased ($p<0.05$). The highest lettuce yield was found at both ratio of 0.8:0.2 and 0.6:0.4 with 1.43±0.06 and 1.43±0.03 gm/plant, respectively. A significant difference in lettuce yield with the increase of bottom ash ratio was observed ($p<0.05$).

The results suggest that lettuce leaf at all ratios are not harmful for consumers. The lettuce leaf at all mixed sample ratios did not exceed the heavy metal contamination according to the Criteria of Food and Drug Administration. However, leachate generated, cultured lettuce in vegetable garden, and use of other crop types still requires additional study for future use.

KEY WORDS: LETTUCE / BOTTOM ASH / SIDA SOIL/
COPPER ACCUMULATION / ZINC ACCUMULATION

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การสะสมของทองแดงและสังกะสีในผักกาดหอมที่ปลูกในดินผสมด้วยเถ้าหนักลิกไนต์ในอัตราส่วนที่ต่างกัน
(ACCUMULATED COPPER AND ZINC IN LETTUCE PLANTATION IN SOIL AMENDED WITH DIFFERENT BOTTOM ASH RATIOS.)

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บทคัดย่อ

การวิจัยนี้ได้ศึกษาการสะสมของทองแดงและสังกะสีในผักกาดหอมที่ปลูกในดินสีดาที่ได้รับการผสมด้วยเถ้าหนักลิกไนต์ โดยทำการทดลองหาการสะสมของทองแดงและสังกะสีในดินสีดาในเถ้าหนักลิกไนต์ในดินสีดาที่ผสมด้วยเถ้าหนักลิกไนต์และในผักกาดหอมที่ปลูกในดินสีดาที่ผสมด้วยเถ้าหนักลิกไนต์ที่อัตราส่วนต่างๆกันและหาการสะสมของไนโตรเจนฟอสฟอรัส โพแทสเซียม และน้ำหนักของผักกาดหอม การศึกษานี้ดำเนินการภายใต้การทดลองในห้องปฏิบัติการและใช้สถิติการวิเคราะห์ความแปรปรวนทางเดียวและ LSD ในการคำนวณ

ผลการศึกษาพบว่าในดินผสมด้วยเถ้าหนักที่อัตราส่วน0.6:0.4มีทองแดงและสังกะสีสะสมสูงสุดคือ 26.13±2.30 และ137.74±4.13 มิลลิกรัมต่อกิโลกรัม ทองแดงและสังกะสีสะสมในดินที่ผสมด้วยเถ้ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่ออัตราส่วนของเถ้าเพิ่มขึ้น($p<0.05$) ทองแดงและสังกะสีที่รากและใบนั้นมีการสะสมสูงสุดที่อัตราส่วน0.6:0.4ที่รากพบทองแดงและสังกะสี 21.46±5.90และ113.47±4.13มิลลิกรัมต่อกิโลกรัมที่ใบพบทองแดงและสังกะสี8.18±1.20และ 32.94±7.34มิลลิกรัมต่อกิโลกรัม ปริมาณทองแดงและสังกะสีที่สะสมในรากและใบของผักกาดหอมมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเมื่ออัตราส่วนของเถ้าเพิ่มขึ้น($p<0.05$) ผักกาดหอม มีน้ำหนักสูงที่สุดที่2อัตราส่วน คือ0.8:0.2 และ 0.6:0.4มีน้ำหนัก 1.43±0.06และ1.43±0.03กรัมต่อต้น น้ำหนักของผักกาดหอมมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเมื่ออัตราส่วนของเถ้าเพิ่มขึ้น ($p<0.05$)

ใบผักกาดหอมที่ปลูกจากทุกอัตราส่วนนำมารับประทานได้ไม่เป็นอันตรายเพราะว่าปริมาณทองแดงและสังกะสีนั้นมีค่าไม่เกินมาตรฐานตามประกาศของคณะกรรมการอาหารและยาใน การศึกษาครั้งต่อไปควรศึกษาเกี่ยวกับน้ำที่เกิดขึ้นหลังจากการรดน้ำและนำผักกาดหอมไปปลูกในแปลงผักในสภาวะแวดล้อมจริงและควรใช้ผักชนิดอื่นมาทำการทดลองในครั้งต่อไป

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
BA	Bottom ash
Cu	Copper
°C	Degree Celcius
gm	Gram
kg	Kilogram
mg	Milligram
ml	Milliliter
Om	Organic matter
Zn	Zinc

CHAPTER I
INTRODUCTION

1.1 Statement of problem

Nowadays, the demand of energy in Thailand has rose sharply during recent years. Thailand has many sources of electricity generating such as hydroelectricity from dam, natural gas from the gulf of Thailand, crude oil and coal (1). The electricity generating from coal are very low capital. The biggest lignite coal mine in Thailand at Mae-moh district Lampang province, it has 630 million tons lignite coal. The process of electrical generator are burn lignite after that it has heat power and use heat power to boil de-ionnized water and use vapor to spin propeller adjoin motor generator. At Mae-Moh it use lignite coal about 40,000 tons per day at each process it make any pollutions such as SOx, NOx, fly ash and bottom ash.

Fly ash has been used to mix with cement for any construction like the use of Hungry Horse damn In Montana, United States. In Thailand, it has been used and mixed in any construction of Pak-Mul damn, wall of underground train, Pra-ram 8 bridge, and the supporting pillar of Suvarnabhumi Airport. Nowadays fly ash, are quite valuable materials (1).

However, bottom ash is different from the fly ash, it can not be used for construction. This is because when it falls down to bottom of kiln, bottom ash will submerge under the well and then it will be transported by machine belt. By this process, the characteristic of bottom ash will be damaged and not suitable for being used and mixed with cement. However, if to bottom ash is disposal. It will need to mix with sand or just purely dispose (2).

At present Mae-Moh power plant has produced bottom ash about 2,000 tons per day (3) and it is not suitable for any disposal. The chemical constituents of bottom ash can vary, depending on the coal type, source and plant operating parameters.

Major constituent include calcium(Ca), copper (Cu), aluminum(Al), iron(Fe), magnesium(Mg), potassium(K), silicone(Si), sodium(Na), and zinc (Zn)(4). Of these materials Ca, Cu, Fe, Mg, K and Si are essential for plant nutrients (2)(4).However chemical composition of bottom ash has hazard heavy metals such as Cu, Zn, Ni, Pb, Cd (5).

Heavy metals such as copper and zinc present in bottom ash can contaminate in soil, and their concentrations as pollutants or nutrients in soils and plants. The availability of copper and zinc must be determined because they are beneficial at low concentrations but can be harmful at higher concentrations.

Lettuce is a kind of crop most that Thai people like to eat and to use as food ornament. It is a vegetable that accumulates relatively high amount of heavy metals such as Cu, Zn and Pb. Therefore, it may be used as an indicator of metal contamination in soils. For soil amended with mine wastes it accumulated significantly more metals than other species such as bean and tomato (6).

Therefore, this study was focused on the copper and zinc in soils added with bottom ash and the uptake by lettuce in various parts (leaf and root). The optimum conditions to mix the variation of bottom ash and lettuce yield were also determined.

1.2 Objective of study

1.2.1 General objective

To investigate effect of bottom ash at various amendments with sida soil.

1.2.2 Specific objective

(1) To study physical and chemical characteristics of bottom ash and sida soil.

(2) To study the accumulation of copper and zinc in sida soil due to bottom ash application.

(3) To study the uptake of copper and zinc by lettuce (leaf and root) grown in different ratios of bottom ash.

(4) To study lettuce yield from sida soil mixed with different bottom ash ratios.

1.3 Hypotheses of study

1.3.1 The different ratios of bottom ash added with soil were different in copper and zinc accumulation in the part of lettuce (leaf and root).

1.3.2 The different ratios of bottom ash added with soil were different in copper and zinc accumulation in mixed sample.

1.3.3 The different ratios of bottom ash added with soil were different in lettuce yield

1.4 Variables of study

1.4.1 Independent variables

(1) Ratios of bottom ash.

1.4.2 Dependent variables

(1) Copper and zinc in parts of lettuce (leaf and root).

(2) Copper and zinc in each ratio of bottom ash mixed with sida soil.

(3) Lettuce yield.

1.4.3 Control variables

(1) Particle size of sida soil and bottom ash

(2) Water irrigations

(3) Soils weight

(4) An amount of lettuce in plastic pots.

1.5 Operation definition

1.5.1 Soil refers to trade mark of agricultural soil was obtained from Jatujak market.

1.5.2 Bottom ash refer to by-product of after burning finely ground coal on electricity generating Mae-Moh power plant, it was collected from the bottom of the furnace.

1.5.3 Bottom ash ratio is the ratio of soil adding with bottom ash, in this research was varied into 5 levels1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4 weight by weight.

1.5.4 Plant species refers to lettuce (*Lactuca sativa* L), seeds from Chia Tai Co., Ltd. will be used in this study.

1.5.5 Dry weight refers to the lettuce weight (leaf and root) after dry in hot air oven at 65 °C for 48 hours.

1.5.6 Plant parts in this experiment mean leaf and root. In this study separate part of plant by cut under the first leaf. All parts above the first leaf are leaf and all parts under the first leaf are root.

1.5.7 Uptake of heavy metals by leaf refers to the process, which leaf can accumulate copper and zinc.

1.5.8 Uptake of heavy metals by root refers to the process, which root can accumulate heavy metals copper and zinc.

1.5.9 Lettuce yield refers to lettuce weight include leaf and root after dry in hot air oven at 65 °C for 48 hours.

1.6 Scope of study

Sida soil was randomly selected from Jatujak market and the bottom ash used was the kind courtesy of Mae-Moh power plant from Amphoe Mae Moh, Lampang. The accumulation of heavy metals by lettuce was only tested under laboratory condition.

1.7 Expected outcome

The study of lettuce uptake heavy metals tasted with the bottom ash mixed with sida soils is the alternative for agriculturist to select a suitable ratio of mixed bottom ash with soil for the best lettuce yield and safe for human health.

1.8 Conceptual framework

Independent variables		Dependent variables
Ratio of Sida soil adding with bottom ash (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4)	→	- Copper and Zinc accumulation in mixed sample at each ratio - Uptake of copper and zinc by root and leaf - Lettuce yield

CHAPTER II
LITERATURE REVIEWS

2.1 Heavy metals

Heavy metal refers to metals with densities greater than 5 g cm^{-3}. Not including alkali and alkali earth, the atomic numbers of heavy metals are in the range of 23 to 92, and ranging from period 4 to 7. The definition often conflict and may include such elements as cadmium, copper, zinc, nickel, mercury, thallium, lead, arsenic, chromium, iron, manganese, zinc(7, 8).

2.2 Background of Copper and Zinc

2.2.1 Copper

Copper was the first metal used by humans and appears to have been discovered on the island of Cyprus around 2500 (9). Copper is a group IB of the periodic table that forms two series of compounds: copper (I) (cuprous) and copper (II) (cupric) compounds. Metallic copper is fairly resistant to corrosion and is not attacked by dry air, water, or non-oxidizing acid. Copper has the properties of malleability, ductility, and it is a good conductor of heat and electricity. The use of copper is in the production of wire and of its alloy, brass and bronze (8, 10, 11).

Table 2.1 General physical properties of copper (12, 13).

Item	**General physical properties**
Name, symbol, number	Copper, Cu, 29
Chemical series	Transition metal

Table 2.1 General physical properties of copper (continued) (12, 13).

Item	**General physical properties**
Electron configuration	$3d^{10} 4s^{1}$
Phase	Solids
Density	8.96 g·cm^{-3}
Melting point	1,357.77 K (1,084.62 °C, 1,984.32 °F)
Boling point	2,835 K (2,562 °C, 4,643 °F)

2.2.2 Zinc

Zinc is the twenty-fifth most abundant element. It is widely found in nature and makes up 0.02% by weight of the earth's crust (14). Zinc is a bluish-white lustrous when polished and soft metal placed in group IIB of the periodic table. In dry air, it is highly resistant to attack except at temperatures exceeding 225°C. Zinc is the most abundant heavy metal among the metals here discussed. It is major use is in metal coating and in alloys. Iron coated with zinc is known as galvanized iron and the mostly known alloy is brass. Zinc is also used in batteries, and zinc oxide is used as a pigment in paints and as filler in rubber (8, 15).

Table 2.2 General physical properties of zinc (12, 16).

Item	**General physical properties**
Name, symbol, number	zinc, Zn, 30
Chemical series	transition metals
Group, period, block	12, 4, d
Appearance	bluish pale gray
Standard atomic weight	65.409 g·mol^{-1}
Electron configuration	$3d^{10} 4s^{2}$
Phase	solid
Density	7.14 g·cm^{-3}
Melting point	692.68 K (419.53 °C, 787.15 °F)
Boling point	1,180 K (907 °C, 1,665 °F)

2.3 Source and Use of Copper and Zinc

2.3.1 Copper

Copper is found in wide variety of mineral salts and organic compounds, and can also be found naturally in the elemental or metallic form. Copper has a natural abundance of approximately 60 mg/kg in the earth's crust and 2.5×10^{-4} mg/liter in the sea (17). The world uses approximately 15×10^{6} tons of copper a year. Of this about one-third is derived from recycled metal, and the rest is supplied from the mining of ore bodies and refining of the extracted copper.

In 1994, 1.8 million metric tons were produced in the United States, primarily in Arizona, Utah and New Mexico. The United States produces 19% of the world's copper output, which exceeds 9.4 million metric tons annually. Mined ores of copper are concentrated by a flotation process and then are refined. Smelting consists of applying sufficient heat (1,100°C to 1,600°C) to concentrate the metal and fuse the remaining gangue (waste ore) into slag (18).

Some of the workers who encounter significant amounts of copper and copper compounds are asphalt makers, fungicide and insecticide workers, and welders (19).

Table 2.3 Copper compounds and uses (10).

Copper compounds	**Their uses**
Cupric oxide (blacks copper oxide)	Catalyst, batteries, electrodes, desulfurizing oils, paints, insecticides
Cupric oxide (red copper oxide)	Fungicide, antifouling paint, photoelectric cells.
Cupric borate	Pigment, fireproofing, wood preservative.
Cupric chloride	Catalyst, mordant, petroleum desulfurizing and deodorizing agent, inks.
Cupric chromate (VI)	Fungicide, seed protectant, wood preserving.
Cupric nitrate	Photocopying, colorant, mordant, fungicide.

2.3.2 Zinc

Zinc is a chalcophilic element like copper and lead, and a trace constituent in most rocks. Zinc rarely occurs naturally in it is metallic state, but many minerals contain zinc as a major component from which the metal may be economically recovered. Zinc has been used as an alloy with copper and tin since ancient times but probably was not recognized as a separate entity until the fifteenth century. Commercial production of zinc began in the eighteenth century (20). As of which the United States contributed 5%. Chile, Canada, Australia, and Russia are the principal producers. After 1991, zinc was no longer considered a strategic metal, and the sale of the national defense stockpiles was authorized (21). Metallic zinc is used principally in galvanizing iron and steel to prevent corrosion and oxidation. Zinc metal also is die-cast for automotive components, electrical equipment, tools, hardware, toys, and fancy goods.

Table 2.4 Zinc compounds and their uses (10).

Zinc Compounds	**Their uses**
Zinc acetate	Wood preserving, mordant, glazes
Zinc carbonate	Pigment, feed additives, rubber
Zinc chloride	Wood preservative, paper, glues dye
Zinc cyanide	Electroplating
Zinc silicate	Television screens, neon lights
Zinc phosphide	Rodenticide

Zinc ore (smithsonite) has been used for the production of brass since 1400. In Europe, the production of elemental zinc started in 1743 (22). World mine production of zinc was 10,148,000tons in 2005 and 10,463,000tons in 2006.

Table 2.5 Total zinc production and consumption in 2005 and 2006 (23).

Geographical area	Zinc 2005 production (tons)	Zinc 2005 consumption tons	Zinc 2006 production tons	Zinc 2006 consumption tons
Europe	2,559,000	2,686,000	2,515000	2,793,000
Africa	274,000	204,000	260,000	199,000
America	1,883,000	190,4000	1,849,000	1,999,000
Asia	5,057,000	5,572,000	5,602,000	5,775,000
Oceania	457,000	253000	466,000	269,000
World total	10,229,000	10,617,000	10,691,000	11,035,000

2.4 Transport and distribution between media

2.4.1 Copper

The concentrations of copper in air depend on the proximity of the site to major sources such as smelters, power plants, and incinerators. Median total copper concentrations in uncontaminated soil were reported to be 30 mg/kg (range 2-250 mg/kg) (24). Copper can accumulate in soils from the long-term application of fertilizers or fungicides.

2.4.2 Zinc

Zinc in the atmosphere is primarily in the oxidized form in aerosols (25). Zinc is found on a particles of various sizes, the size being determined by the source of zinc emission. Waste incinerators release small zinc-containing particles to the atmosphere, whereas wear of vehicle tyres produces large particles (26).

The major sources of zinc in soils are the zinc sulfide minerals, such as sphalerite and wurtzite, and to a lesser extent minerals such as smithsonites ($ZnCo_3$), zincite (ZnO), zinkosite ($Znso_4$) and franklinite ($ZnFe_2O_4$).

2.5 Effect of Copper and Zinc on the human health

2.5.1 Copper

1) Route of exposure

Illness occurs when diet is deficient or intake is excessive. The principal route of exposure is through ingestion, but inhalation of copper dust and fumes occurs in industrial settings. Toxicity has resulted from treatment of burns using topical copper compounds (25), and copper azide impregnation of the skin after an explosion (28).

2) Absorption

Adults ingest 1.2 to 5 mg of copper per day, approximately one-half of which is absorbed (28). After ingestion, maximum absorption of copper occurs in the stomach and jejunum. Absorption through the intestinal wall is facilitated by active transport, although the exact mechanism is unknown. Copper is bound initially in the serum to albumin and transcuprein, the later is bound more firmly to ceruloplasmin, which binds more than 75% of circulating copper (29). In acute poisoning, copper is bound also to metallothionein in the liver and kidney (30). Absorption is increased in copper deficiency and is impaired in small-bowel disease. Copper is distributed throughout the body but is stored primarily in liver, muscle, and bone. Normal serum levels are approximately 1 mg per mL. In all mammal, copper is an essential trace element involved in fundamental cellular respiration, free radical defense, connective tissue synthesis, iron metabolism, and neurotransmission.

3) Metabolism

Absorbed copper initially is bound to albumin and is transported from gastrointestinal tract to the liver. There it is transferred to ceruloplasmin, which is the primary transport vehicle for incorporating copper into the copper-dependent enzymes. Urinary excretion is enhanced by increased molybdenum intake, cirrhosis, and biliary obstruction (31).

4) Elimination

Copper is eliminated principally through the feces after excretion into the bile, which is copper's primary excretory route. Biliary copper is absorbed poorly (32). . Healthy adults have urinary concentrations of less than 100µg per 24 hours. A daily intake of 2 mg of copper results in a urinary concentration of between 11 and 48 µg per 24 hours (33).

5) Acute toxicity

Copper is an essential element, toxicity is uncommon, as with all essential elements. Most reports of acute toxicity are from suicidal ingestion of copper sulfate. However, death is rare, owing to copper sulfate's emetic properties. Mild forms of poisoning produce only nausea, vomiting, diarrhea, and malaise and have been described in patients poisoned by eating or drinking from copper-containing vessels (34) or from a soft-drink dispenser. Symptoms associated with severe poisoning usually follow the order of metallic taste, nausea, vomiting, hematemesis, diarrhea, melena, hypotension, com and death (35).

6) Chronic toxicity and long-term effects

Acquired chronic copper toxicity, with the exception of that in vineyard sprayer's lung, has not been established firmly. Chronic disease from excessive copper storage is epitomized by Wilson's disease, an inherited, autosomal recessive error in copper metabolism. The disease is characterized by excess copper deposition in most organs, especially the liver, kidneys, brain, and eyes. Wilson's disease also is termed hepatolenticular degeneration, owing to the prominent effects of the liver (cirrhosis) and eye (kayser-Fleischer rings) (28).

2.5.2 Zinc

1) Route of exposure

The most common route of exposure to zinc is that of diet. Inhalation of zinc fumes and dust occurs in some of the aforementioned industrial settings. Absorption occurs across broken epithelium when zinc oxide is applied to treat burns or wounds (20).

2) Absorption

Absorption of zinc occurs throughout the intestine but mainly in the jejunum (36). The mechanism of passage through the gastrointestinal mucosa is not understood completely but involves metallothionein binding or other zinc-protein complexes in luminal cells. Absorption ranges from 25% to 90% after Zn oral administration in humans and is influenced by dietary factors. Zinc absorption is decreased when consumed with some vegetable proteins, calcium, and phosphorus but is increased when consumed with animal proteins (20). Prasad noted that, after oral administration of Zn, measurable zinc levels were found in the blood within 15 to 20 minutes, with peak levels in 2 to 4 hours. Plasma and serum levels were higher than in whole blood (37).

3) Elimination

Zinc is biological half-life exceed 300 days. A total of 70% - 80% of ingested zinc is excreted in the feces via bile and pancreatic secretions, which are enhanced by dietary protein of plant origin. Urinary and sweat excretion together account for roughly 15% but, in hot climates 25% can be excreted by sweating alone. Breast milk also contains significant concentrations of zinc.

4) Acute toxicity

Acute symptoms of oral zinc poisoning are primarily gastrointestinal. Symptoms include nausea, vomiting, abdominal pain, diarrhea, and hematemesis. Fever also is reported. With supportive care, zinc toxicity usually is self-limited, and resolution of symptoms occurs in a matter of hours or days.

5) Chronic toxicity and long-term effects

Other than producing corneal and lens opacities after ocular zinc salt injury and anemia from zinc-induced copper deficiency, zinc toxicity does not result in any known chronic effects.

Table 2.6 Common uses and principal toxic effects of selected metals.

Metals	Common industrial uses	Principal toxic effect
Copper	Electrical wiring, water pipes, sheet metal, alloys	Environmental exposures are relatively non toxic
Zinc	Batteries, alloys, galvanizing, dyes, pharmaceuticals	Gastrointestinal effects, anemia

2.6 Copper and Zinc for plant and soil

2.6.1 Copper

Copper (Cu) is an essential nutrient for plant growth because this element is constituent of a number of plant enzymes (38), but because only a small amount is needed, it is classified as a micronutrient. Copper is required in small amounts: 5-20 mg/kg in plant tissue is adequate for normal growth less than 4 mg/kg is considered deficient and more than 20 mg/kg is considered toxic (39). However, depending on the plant species, plant organ, developmental stage, and nitrogen supply, these ranges can be larger (40).

Copper is an important component of proteins found in the enzymes that regulate the rate of many biochemical reactions in plants. Plants would not grow without the presence of these specific enzymes. Copper is absorbed by plant roots as Cu(II) ions and translocated to the shoots predominantly in anionic form and possibly some as the shoots predominantly in anionic form and possibly some as the free ion. Copper is an essential constituent of a group of enzymes known as oxidases in which molecular oxygen is used directly in the oxidation of substrate; these include cytochrome oxidase, phenol oxidase, laccase, ascorbic acid oxidase, and amine oxidase. Copper also plays a role in photosynthesis as an essential constituent of plastocyanin. Crops vary in their response to low supplies of copper. Cereals and fruit trees can be seriously affected with substantial losses in yield while, for example, sugar beet shows few visible symptoms of deficiency appear during tillering when the leaves become twisted or rolled and their tips turn grey or white; ear emergence and grain filling are seriously affected. In fruit trees the leaves of the terminal shoots becomes dark green and curled and may then develop brown or necrotic areas. This is usually followed by withering of the leaves, defoliation and death, or 'die-back', of the shoot (41).

Deficiency of copper in crops has been reported from many countries (42). The total copper content of soil commonly ranges from 2.5 to 60 µg Cu g^{-1} but values up to several hundred µg g^{-1} are found in contaminated soils. Copper deficiencies in crops may occur if the content of total soil copper is very low, for example when well-developed podzols are brought into agricultural production, or in soils over inherently low copper parent materials such as granites, sandstones or sandy glacial deposits.

Copper deficiency in crops can be severe when peats are drained especially if their surface becomes dry. It occurs on thin organic soils over chalk and on many strongly weathered soils in Australia (42).

Table 2.7 Amount of metal per hectare calculated for a soil depth of 15 cm and a bulk density of 1.3 g cm^{-3}(43).

Metals	**Earth's crust (µg g^{-1})**	**Soils (µg g^{-1})**	**Soils [a] (µg g^{-1})**	**Rocks with highest concentration**
Cu	14	2-300	4-600	Granite
Zn	75	10-300	20-600	Shales and clays

2.6.2 Copper exposure in food

The actual concentration of copper in food and beverages from various countries varies widely depending upon the food product, the growing conditions (soil, use of fertilizers high in copper, water, use of copper fungicides) and the type of processing used; in particular, pH levels and the use of copper vessels (44).

Copper levels in common foodstuffs and beverages have been determined in many countries, copper levels in representative foodstuffs given in table 2.8.

Table 2.8 Levels of copper in foodstuffs (45).

Foodstuffs	Mean (mg/kg wet weight)	Minimum (mg/kg wet weight)	Maximum (mg/kg wet weight)
Beef	1.1	0.74	1.6
Pork	1.4	0.44	7.22
Liver beef	39	8.8	87
Liver pork	9.0	0.9	29
Apples	0.25	0.21	0.31
Lettuce	0.72	0.20	1.4
Tomato	0.55	0.29	1.1
Cow milk	0.06	Trace	0.14

2.6.3 Zinc

Zinc is absorbed as Zn(II) and translocated to the shoots primarily as the free ion; it is concentration in plant dry matter is at least three- or four-fold greater than copper. Many factors affect the bioavailability of zinc in soils, including total zinc content, ph, organic matter, adsorption site, microbial activity and moisture content. Zinc is known to be an essential constituent of only three plant enzymes, namely carbonic anhydrase, alcohol dehydrogenase and superoxide dismutase (46).

The major sources of zinc in soils are the zinc sulfide minerals, such as sphalerite and wurtzite, and to a lesser extent minerals such as smithsonites ($ZnCo_3$), zincite (ZnO), zinkosite ($Znso_4$) and franklinite ($ZnFe_2O_4$).

Zinc in soil is distributed between the following fractions dissolved in soil water, exchangeably bound to soil particles, bound to organic ligands, occulded in secondary clay minerals and metal oxides/hydroxides, present in primary minerals. Only those fractions of zinc that are soluble or may be solubilized are available to plant. Under most conditions, the amount of zinc present in adsorbed soil fractions is much higher than the soluble fraction that remains in the pore waters or soil solution. The change in any of the above factors will result in a change in the overall equilibrium of the soil, with zinc transformed to different forms until a new equilibrium is reached. Such equilibrium displacements may occur as a result of plant uptake, losses by leaching, zinc input changes in soils moisture content, changes in pH, mineralization of organic matter, and changes in the redox status of the soil. The selective adsorption of zinc and the occurrence of an adsorption/desorption hysteresis effect is controlled by the following parameter

- Number of pH-dependent adsorption sites
- Interactions with amorphous hydroxides
- Affinity for the formation of organomineral complexes, and their stability
- Formation of hydroxyl complexed
- Steric factors
- Properties of zinc including: ionic radius, polarizability, thickness of the hydration sheet, equivalent conductance, hydration enthalpy and entropy.

There are numerous reports that zinc deficiency is induced by the application of phosphate fertilizers, the best known example being with citrus trees. This effect is unlikely to be due to an interaction between phosphorus and zinc in the soil or to a dilution of zinc in the plant tissues owing to the growth response to phosphorus. There is, however, evidence that phosphate may interfere with the translocation of zinc or with it is utilization, but no mechanisms have been suggested for either effect (47). With potato it has been found that phosphate does not affect the translocation of zinc, but that a ratio of phosphorus to zinc in the leaves in excess of 400 to 1 is associated with zinc deficiency (48).

Total zinc concentrations are found in soils but values from 50 to 300 μg $^{g-1}$ are most common in agricultural soils. The characteristic symptom of zinc deficiency is a failure of leaves to expand and stems to elongate, giving a terminal rosette effects. These effects are believed to be associated with a disturbance of auxin metabolism. Fruit trees, particularly citrus, are frequently affected by zinc deficiency and mize, tomato and cotton are also especially sensitive. In fruit trees such as citrus, apple and peach, the mature leaves may show the first symptoms as pale green to yellow interveinal mottling, while more severe effects on growth and resetting often occur in the terminal leaflets of young shoots. The older leaves of maize may have purple tints while yellow or white interveinal chlorotic stripes develop in the younger leaves. In tomato and cotton the symptoms appear as irregular areas of interveinal chlorosis which become necrotic.

2.6.4 Zinc exposure in food

Zinc is an ubiquitous and essential element. Zinc content of some foods is show in table 9 Zinc levels of 10-15 mg/kg of fresh edible portion are found in vegetable and meat, eggs and dairy products contain more zinc than plants; liver is a particularly rich zinc source, with average values of 44-84 mg/kg of edible portion (49).

Table 2.9 Zinc concentrations in some foodstuffs (50).

Foodstuffs	Zinc concentrations (mg/kg of edible portion)
Meat	31.7
Beef	31
Pork	19
Liver	44(sheep)-84(calf)
Kidney	3.7(pig)-28(sheep)
Chicken	8.5
Chicken eggs	8-20
Shrimps	23.1
Cheese	11-106
Apple	1.2
Banana	2.2
Tomato	2.4
Lettuce, cabbage	2.2

2.7 Standards value for heavy metals contamination levels allowed in food

In Thailand, The Ministry of Public Health (1986) set permissible limits for the standard heavy metals contamination levels in vegetables (51). As being shown in table 2.10

Table 2.10 Standard value for heavy metals contamination levels (51).

Heavy metals	Standard value (mg/kg)
Copper	Not exceed 20
Zinc	Not exceed 100

2.8 Bottom ash

Bottom ash refers to the non combustible constituents of coal with traces of combustibles embedded in forming clinkers and sticking to hot side walls of furnace during the furnace working. The clinkers fall by themselves into the water or sometimes by poking manually, and get cooled. The clinker lumps get crushed to small sizes by clinker grinders mounted under water and fall down into a trough from where a water ejector takes them out to a sump. From there it is pumped out by suitable rotary pumps to dumping yard far away. In another arrangement a continuous link chain scrapes out the clinkers from under water and feeds them to clinker grinders outside the bottom ash hopper.

2.8.1 Ash quantity

Mae Moh power plant is the largest lignite thermal power plant in Thailand, situated Amphoe Mae Moh, and Lumpang province, northern of Thailand. The Lignite is mined from Mae Moh coalfield, which situated the same area of the power plant. Total capacity of electricity production from this power plant in the year 2005 consume lignite up to 40,000 tons per day. The Mae Moh lignite contains approximately 30 percent ash , which after burning, 80 percent of the total ash formed during lignite combustion is fly ash. The remainder is bottom ash or slag, which is collected at the bottom or the wall of the boiler (52).

The production of ashes exceeds 10,000 tons per day at Mae Moh thermal power plant. The ash disposal poses a serious problem in terms of storage, space, cost and environmental impact. Considering these factors, effective utilized of this beneficial. Proper utilization of this industrial waste would also mitigate adverse environmental effects of open dumps. Generally, large quantities of ashes are disposed in the allocated areas in from of stockpiles near the power plant and sold as the reinforce material for blending with the cement in construction (53).

2.8.2 Ash characteristics

1) Chemical characteristics

The chemical composition of ash is a function of several variables such as the coal source, degree of coal pulverization, actual firing process, furnace type and firing mechanism and handling and storing methods.

Table 2.11 Typical chemical compositions of bottom ash from power plant (52).

Chemical compositions	Average value in percent weight (% wt)
SiO_2	51.91
Al_2O_3	22.14
Fe_2O_3	7.12
CaO	4.44
MgO	1.54
Na_2O	0.41
K_2O	1.27

2) Physical characteristics

Bottom ash is pozzolanic materials. A pozzolan is a material that can react with lime in the presence of water to produce a cementitious material. The reaction of aluminosilicious material, lime and water results in the formation of concrete-like product termed pozzolanic concrete. The resulting pozzolanic structures are termed amorphous aluminosilicates. fly ash and bottom ash is the most commonly employed pozzolans. In addition, the amount of unburned carbon may vary from source to source (53).

Physical characteristics of Mae Moh plant bottom ash from power plant are shown in Table 2.12.

Table 2.12 Physical and chemical properties of bottom ash from power (52).

Physical characteristics	**Average value**	
	Year 1992	**Year 1993**
Specific gravity	1.99	2.51
Specific surface, m^2 /kg	270.60	295.70
Loss of Ignition (%)	0.64	0.69
Bulk density kg/m^3	1,261.20	1,268.10

2.8.3 Heavy metals content in bottom ash

Metals contained in bottom ash from Mae Moh power plant are presented in Table 2.13.

Table 2.13 Heavy metals in bottom ash from Mae Moh power plant (54).

Parameter	**Bottom ash**
	Total heavy metals (mg/kg)
Cu	19.80
Zn	63.54
Pb	<D.L.
Ni	18.92
Cd	<D.L.
Total aluminum (mg/kg)	2996.38
pH	8.74
Moisture content (%)	19.86

Remark: <D.L = Less than detection limit

Size of ash particle

The ash samples collected from bottom ash conveyer in Mae Moh power plant appeared as a brick-color light friable lumps, or dark gray to black, hard lump plant (52).

Table 2.14 Typical grading of bottom ahs from coal burned in a pulverized fuel boiler plant (52)

Sieve sizes(mm)	Percent passing of bottom ash
9.50	97.20
4.75	93.70
2.36	85.90
1.18	72.70
0.60	58.80
0.30	44.80
0.15	24.90

2.8.5 Ratio of lignite coal production and consumption in Thailand

Since 1986 to 2006 production and consumption of lignite coal in Thailand has rose sharply has show in table 15.

Table 2.15 Lignite production and consumption in Thailand (55).

Years	Mae-Moh		Whole Kingdom	
	Production (tons)	Consumption (tons)	Production (tons)	Consumption (tons)
1992	11,844,352	12,129,274	15,388,561	15,563,652
1993	11,221,088	11,239,942	15,537,927	15,712,753
1994	11,906,553	11,908,173	17,094,871	17,053,592
1995	13,191,880	13,374,488	18,420,798	18,506,630
1996	16,381,301	16,405,340	21,690,394	21,093,807
1997	17,692,415	18,010,806	23,438,556	23,246,465
1998	14,419,327	15,388,095	19,996,195	20,732,418
1999	12,021,265	13,893,584	18,261,308	18,978,543
2000	13,621,615	14,120,569	17,785,747	17,550,581
2001	15,314,436	15,744,116	19,606,995	19,934,560
2002	14,994,739	15,035,329	19,571,985	19,540,868
2003	15,591,841	15,406,532	18,830,469	17,948,028
2004	16,561,572	16,536,694	20,038,376	20,461,638
2005	16,534,142	16,571,091	21,429,019	21,015,290
2006	15,763,798	15,815,374	19,070,603	18,867,082

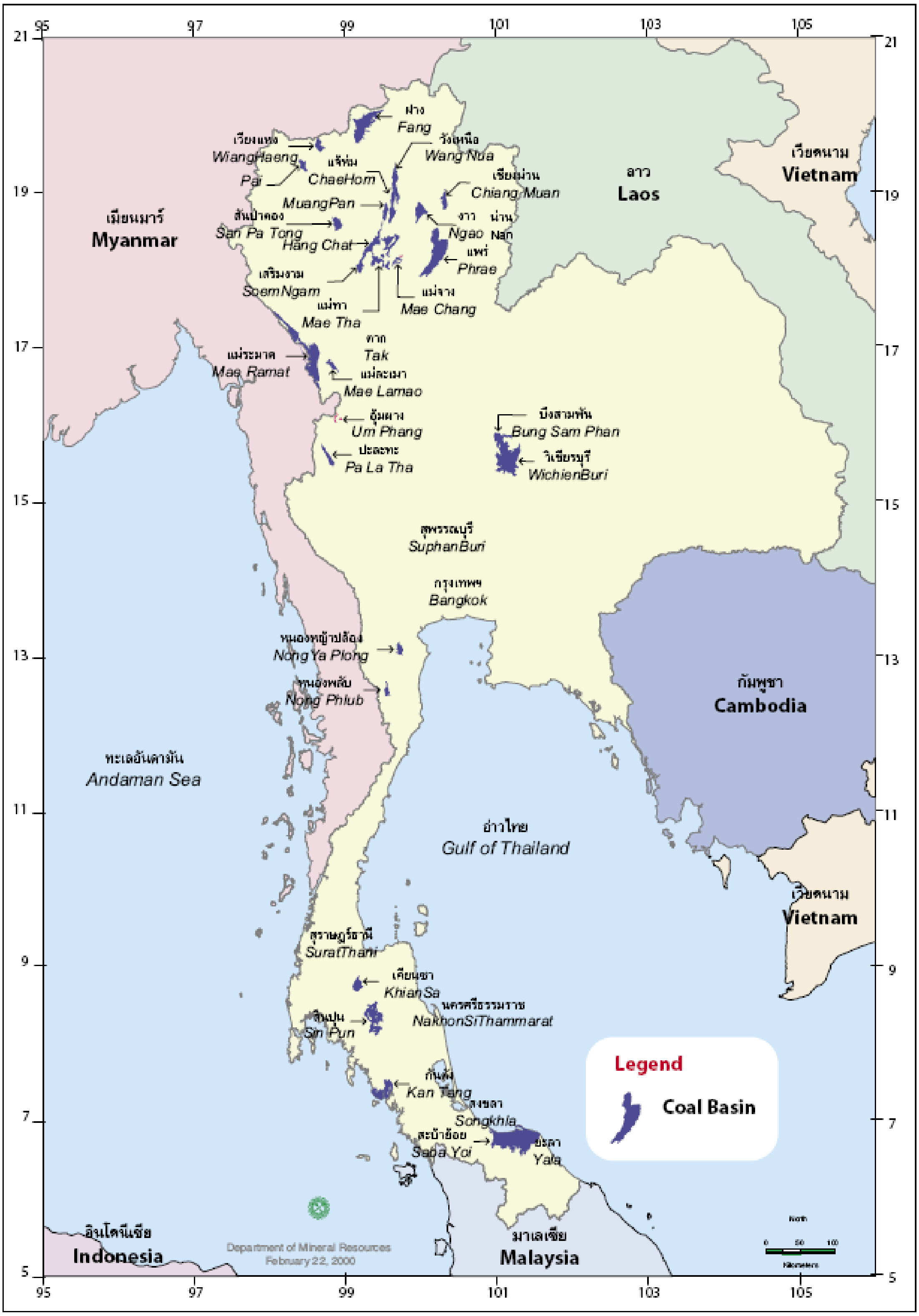


Figure 2.1 Map of coals reserve in Thailand (55).

2.9 Sida soil

Sida soil refer to trade mark of agricultural soil was obtained from Jatujak market. Sunthong(56) investigated chemical characteristics, nutrients content and heavy metal accumulation of sida soil. The data are shown in Table 2.16 Table 2.17 and Table 2.18.

Table 2.16 Average (±SD) of pH, moisture content and organic matter in sida soil (56).

Parameter	**Average (±SD)**
pH	6.66±0.27
Moisture content (%)	25.59±2.69
Organic matter (%)	12.92±1.92

Table 2.17 Average (±SD) of nitrogen, phosphorus and potassium in sida soil (56).

Parameter	**Average (±SD)**
Total nitrogen (%)	0.31±0.06
Available phosphorus (%)	0.20±0.01
Exchangeable potassium (%)	0.08±0.02

Table 2.18 Average (±SD) of total heavy metal in sida soil (56).

Parameter	**Average (±SD)**
Copper (mg/kg)	13.13±0.93
Zinc (mg/kg)	24.38±1.04
Nickel (mg/kg)	Trace
Lead (mg/kg)	Trace
Cadmium (mg/kg)	Trace
Mercury (mg/kg)	Trace

2.10 Crop species

Lettuce (*Lactuca sativa* L)

Figure 2.2 *Lactuca sativa* L

Scientific classification

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Asterales
Family:	Asteraceae
Genus:	Lactuca
Species:	sativa

Lettuce is a temperate annual or biennial plant most often grown as a leaf vegetable. In some countries, it is typically eaten cold and raw, in salads, hamburgers, tacos, and many other dishes. In some places, including China, lettuce is typically eaten cooked and use of the stem is as important as use of the leaf.

The Lettuce plant has a short stem initially (a rosette growth habit), but when it blooms the stem lengthens and branches, and it produces many flower heads that look like those of dandelions, but smaller. This is called bolting. When grown to eat, lettuce is harvested before it bolts.

Lettuce is known to be sensitive to phytotoxic substances, which could be present in the raw water or released by roots and microorganisms, and some reports have been published regarding lettuce growth in relation to the autotoxic potentials and the identification of phytotoxic root exudates (57).

2.11 The related Literatures

Juntaramitree (54) determine the optimum conditions of ashes mixed sludge both fly ash and bottom ash were applied to anaerobic digested and dewatered sewage sludge from Huay Kwang wastewater treatment plant in ratio 1:1, 1:5 and 1:10 by weight, respectively. The experiment showed that 1:5 fly ash-sludge mixture and 1:10 bottom ash-sludge mixture were the ratios that minimized toxic elements and provided sufficient nutrients. As a result of seed germination test, the seed germination percentages increased when soils were applied with sludge and ash-sludge mixtures. The increased seed germination percentages were high as incubation time increased.

Sirisukhodom (58) Investigated sewage sludge, which contaminated with heavy metals, to agricultural area for consideration about suitable vegetable species and heavy metal contaminated in soil. Effect of two sewage sludge application rates (1,600 and 3,200 kg/rai) on growth and heavy metals (Pb, Cd, Ni, Cu, Mn, and Fe) accumulation in four vegetables (chinese kale, lettuce, edible rape and Kang-kong). The result of lettuce showed that fertilizer gave lettuce products higher than applied sewage sludge 1,600 kg/rai. Sewage sludge application at both rate had no effect on accumulation of heavy metals in Chinese kale and edible rape difference among treatments but there were resulted in increased Cu and Fe accumulation in the root system of lettuce. Applied sewage sludge 3,200 kg/rai enabled the increasing of Zn accumulation in the shoot system of lettuce and in the root system of kang-kong.

C.P. Jordao et al (6). Studied cattle manure verimcompost enriched with Cu, Ni, and Zn to increase plant yield. An oxisol amended with the metal-enriched vermicompost at the doses 0, 25, 50, 65 and 80 t ha^{-1} was used to grown lettuce (Lettuca sativa L.). The distribution of the metals in the plants was determined and the metal compared with the levels commonly found in plants, as well as with the range of the critical levels of toxicity to plants. Dose of 50, 65 and 80 t ha^{-1} of metal-enriched vermicomposts applied these was a decrease in lettuce yield as compared to the correspondent 50, 65 and 80 t ha^{-1} doses of natural vermicompost applied. The Cu concentrations in the lettuce leaf from the pots with vermin compost enriched with this element were in the range commonly found in plant (from 5.9 – 13.9 $mg.kg^{-1}$), although in the roots they were relatively high (from 76.25 to 244.56 $mg.kg^{-1}$). The Ni concentrations in the leaves from the pots with the vermicompost contaminated with this elements with this elements were with in the range of critical levels of toxicity to plants, i.e., from 10 to 50 mg kg^{-1}. In the case of Zn, it is concentrations in the leaves from the pots with vermicompost enriched with element were, in general, above the range of critical levels of toxicity to plants, i.e., from 200 to 500 mg kg^{-1}.

Guang wen et al (59). Investigated the effect of irradiation and compositing on Copper (Cu) availability in sludge and manure using a yield control approach. They use four organic wastes digested and dewatered: DSS, digested and irradiated: DISS, composted: DICSS, sewage sludge and composted: CLM. Were applied four rates (10, 20, 30 and 40 t solid ha^{-1} year) with supplemented N and k fertilizers. A control treatment (CT) received n and K fertilizers only. Beans, lettuce and petunias were grown in first and lettuce were harvest twice in second year. Bean appeared to have a strong ability to absorb Cu compared with other test crops. Lettuce were accumulate increase when quantity of compose fertilizer increase. Copper applied in digest and dewaterd sludge and digested and irradiated sludge increase lettuce Cu concentration to greather than Cu in composted sewage sludge. Copper concentration in first cut of lettuce was higher at CLM 40 t ha^{-1} rate of applications than other rate.

Marlon et al (60) used lettuce (*Lactuca Sativa* L.) to bioassay they used four soil samples with different element concentrations were used. Two from soils under natural conditions, and two were from soils under human influence. They use seeds germination to bioassay and root elongation, germination rate. They found the concentrations of soluble elements in the solutions, exerted a stronger effects on root elongation than on seed germination in lettuce. The most sensitive variables in the bioassay with these solutions are germination rate and root necrosis, in these cases the solution causes a deduction of 44% and 67% respectively, in relation to control (distilled water). The test using soil-water solutions is sensitive and reproducible to determine phytotoxicity in lettuce caused by potentially pollutant elements is soils.

Henry & Harrison (61) studied the uptake of metals by turfgrass, tomatoes, lettuce and carrots grown in different soils (control soil, soil amended with NPK fertilizer, compost, and a 1:1 soil-compost mixture). The loading rates of zinc in the loading rates of zinc in the control soil, compost mixture and compost were 232, 239 and 245 kg/ha, respectively. The order of uptake by plants was in the order lettuce > grass > carrots > tomatoes. Uptake slopes for lettuce, grass and carrots grown in compost were higher for than those for plants grass grown in compost and the compost-soil mixture than in plants grown in either the control or fertilized soils. The zinc concentrations in the tomatoes showed no variation.

Maria greger et al (62) was to determine the edible parts of crop plants grown in various macroalgae composts contain elevated concentrations of heavy metals. The vegetables produced were compared with ones cultivated in composted horse manure and in soil interms of transfer of cadmium and in some case of cadmium and in some case of copper, lead and mercury from the different substrates to the edible parts of the plants. Concentrations of copper, mercury and lead were not elevated in either of the composts or in the crop plants compared with limit values for cultivated plants and soil. However the concentration of cadmium in the composts and crop plants was greater than the limit values for arable soil and cultivated plants, respectively. The cadmium concentrations in lettuce and oat cultivated in the seaweed composts exceeded official EU limit values, while the concentrations in root vegetables and leguminous plants were lower than the limit values.

C.P. jordao et al (63) used glass column and loaded with cattle manure vermicompost and effluents were passed through it. The experiments on adding effluent aliquots into the columns were continued until the metal concentrations in the elutant reached the maximum values established for effluent discharges in water courses by the Brazilian quality criteria. Vermicompost residues obtained from this process were used for lettuce cultivation. The Cu concentrations in lettuce leaves from the treatment with vermicompost enriched with this metal were below the range of critical toxicity level to plants. The estimated Cu concentrations in the roots from the treatment with vermicompost enriched with Cu were much larger than that of the treatment with the natural vermicompost, reaching 246.3 mg L^{-1}. The Ni and Zn concentrations in lettuce were above the range of critical toxicity levels to plants. A greater absorption of Cu and Ni by roots was found in treatments with vermicompost enriched with these elements, whereas Zn was found preferentially in the leaves.

Speir et al (64) was investigated the effects, on plant growth and element uptake, of soil amendment with Cu- Cr- and As-(CCA) treated, or boric-treated sawdust. Three indicator plants (beetroot, white clover, lettuce) were chosen and comparisons were made with an untreated sawdust amendment and with a non-sawdust control. Amendment with 10% treated-sawdust (v/v) increased soil concentrations of Cu, Cr, As and B. These treatments had no important effects on the uptake of major and minor nutrient elements by the plants.

CHAPTER III
MATERIALS AND METHODS

3.1 Research design

This study was designed as experimental research. The purpose of this study was investigated characteristics of sida soil and bottom ash. The effects of heavy metal in sida soil mix with bottom ash uptake by lettuce and lettuce yield also study. The concentration of copper and zinc in sida soil, bottom ash and lettuce was measure by using Atomic Absorption Spectrophotometer (AAS).

3.2 Experiment laboratory

3.2.1 Analysis of copper and zinc concentration by Atomic Absorption Spectrophotometer (AAS) done at Faculty of Science, Mahidol University.

3.2.2 Analysis of physical and nutrient of sida soil and bottom ash was done at Faculty of Public Health, Mahidol University.

3.3 Plant materials

The following plant species would be used in this experiment as lettuce *(Lactuca sativa L.).* These plants would be cultivated and grown in plastic pots.

3.4 Experiment conditions

The lettuce was cultivated and grown in plastic pots has sida soils mix with bottom ash (1:0, 0.9;0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) under nature light and temperature.

3.5 Materials, equipment and reagents

3.5.1 Materials

(1) Sida soils

(2) Bottom ash

3.5.2 Equipment

(1) pH meter

(2) Hot air oven

(3) Atomic Absorption Spectrophotometer (AAS)

(4) Hood

(5) Desiccator

(6) Furnace

(7) Crusible

(8) Sieve No.20

(9) Filter paper No.5 and No.42

(10) Plastic bag

(11) Digestion blocks

(12) Distillation unit

(13) Spectophotometer

(14) Light meter

(15) Plastic pots

3.5.3 Chemical reagent.

(1) Nitric acid (HNO_3), Merck, Germany

(2) Hydrochloric (HCl), Merck, Germany

(3) Sodiumhydroxide (NaOH), Merck, Germany

(4) Boric acid (H_3BO_3), Merck, Germany

(5) Hydrofluoric, Merck, Germany

(6) Sulfuric acid, Merck, Germany

(7) Perchloric acid, Merck, Germany

(8) Ethyl alcohol

3.6 Glassware

(1) Volumetric flask size 25, 50, 100, 500 and 1000 ml.

(2) Beaker size 50, 100, 500 and 1000 ml.

(3) Cylinder size 50, 100, 500 and 1000 ml.

(4) Pipette size 1, 5, 10, 50 and 100 ml.

(5) Dropper

(6) Erlenmayer flask size 50, 100 and 250 ml.

(7) Burette size 50 ml.

(8) Funnel

(9) Digestion tubes

3.7 Experimental procedure

3.7.1 Sample collection.

The sida soils bought from Jatujak maket were use in this study. The bottom ash samples obtained from Mae Moh power plant in Lampang province operated by the electricity Generation Authority of Thailand (EGAT) were also used for the study.

3.7.2 Preparation of samples.

Soil samples were prepared for use in the experiment as follows.

1. Soils samples was spread out on a flat surface, no more than 2 to 3 cm deep. Them allow them to air dry for 7 day (do not oven dry)

2. After the sample has air-dried, it was passed through a 2 mm screen sieve. Large aggregates will be crushed, without grinding, using a clean mortar and a rubber-tipped pestle.

3. Mix the sieved material until the sample is homogeneous. Use a riffle splitter, or the other unbiased splitting procedure, to obtain sub-samples of appropriate size.

Bottom ash samples were prepared for use in the experiment as follows.

The bottom ash was air-dried in a room free from dust and excessive air currents. Stirring at intervals will lessen air-drying time. After that, reduce the gross or divided sample to a top size of No.20 sieve before use in experiment.

The sample of sida soils added with bottom ash was prepared as follows.

Weigh the sida soils and bottom ash follow the ratios 1:0 (soils 600 grams: bottom ash 0 grams), 0.9:0.1 (soils 540 grams: bottom ash 60 grams), 0.8:0.2 (soils 480grams: bottom ash 120 grams), 0.7:0.3 (soils 420 grams: bottom ash 180 grams) and 0.6:0.4 (soils 360 grams: bottom ash 240 grams) prepared samples by weight.

3.7.3 Physical and chemical properties analysis.

The physical and chemical properties of sida soils samples was analyzed by the methods and instrument as following Table 3.1

Table 3.1 Parameter and test methods for sida soil

Parameter	Method/Equipment
Physical properties	
Moisture content	Gravimetric method
Chemical properties	
pH	1:10 water/soil, measurement by pH meter
% Organic matter	Walkey & Black method
Nitrogen	Kjedahl method
Phosphorus	Bray II
Potassium	NH_4OAc 1N pH7
Heavy metals (Cu, Zn)	HNO_3-HCl digestion and measurement by Atomic absorption spectrophotometer

In addition, the physical and chemical properties of bottom ash sample were analyzed by the methods and instrument as following Table 3.2

Table 3.2 Parameter and test methods for bottom ash

Parameter	Method/Equipment
Physical properties	
Moisture content	Gravimetric method
Chemical properties	
pH	1:10 water/soil, measurement by pH meter
% Organic matter	Walkey & Black method
Nitrogen	Kjedahl method
Phosphorus	Bray II
Potassium	NH_4OAc 1N pH7
Heavy metals (Cu, Zn)	HNO_3-HCl digestion and measurement by Atomic absorption spectrophotometer

The physical and chemical properties of sida soil adding with bottom ash were analyzed by the methods and instrument as following Table 3.3

Table 3.3 Parameter and test method for sida soil adding with bottom ash

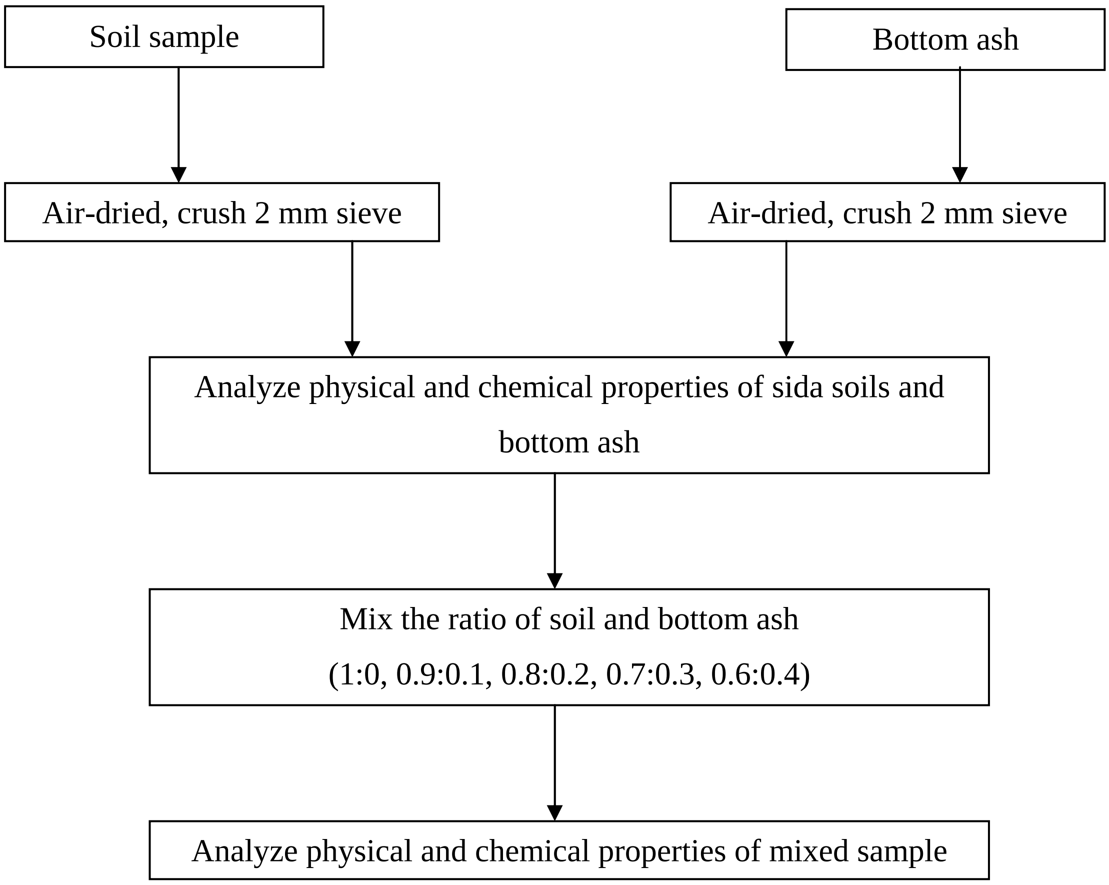
Parameter	Method/Equipment
Physical properties	
Moisture content	Gravimetric method
Chemical properties	
pH	1:10 water/soil, measurement by pH meter
% Organic matter	Walkey & Black method
Nitrogen	Kjedahl method
Phosphorus	Bray II
Potassium	NH_4OAc 1N pH7
Heavy metals (Cu, Zn)	HNO_3-HCl digestion and measurement by Atomic absorption spectrophotometer

3.7.4 Determining lettuce grown in 5 ratios of sida soil mix with bottom ash.

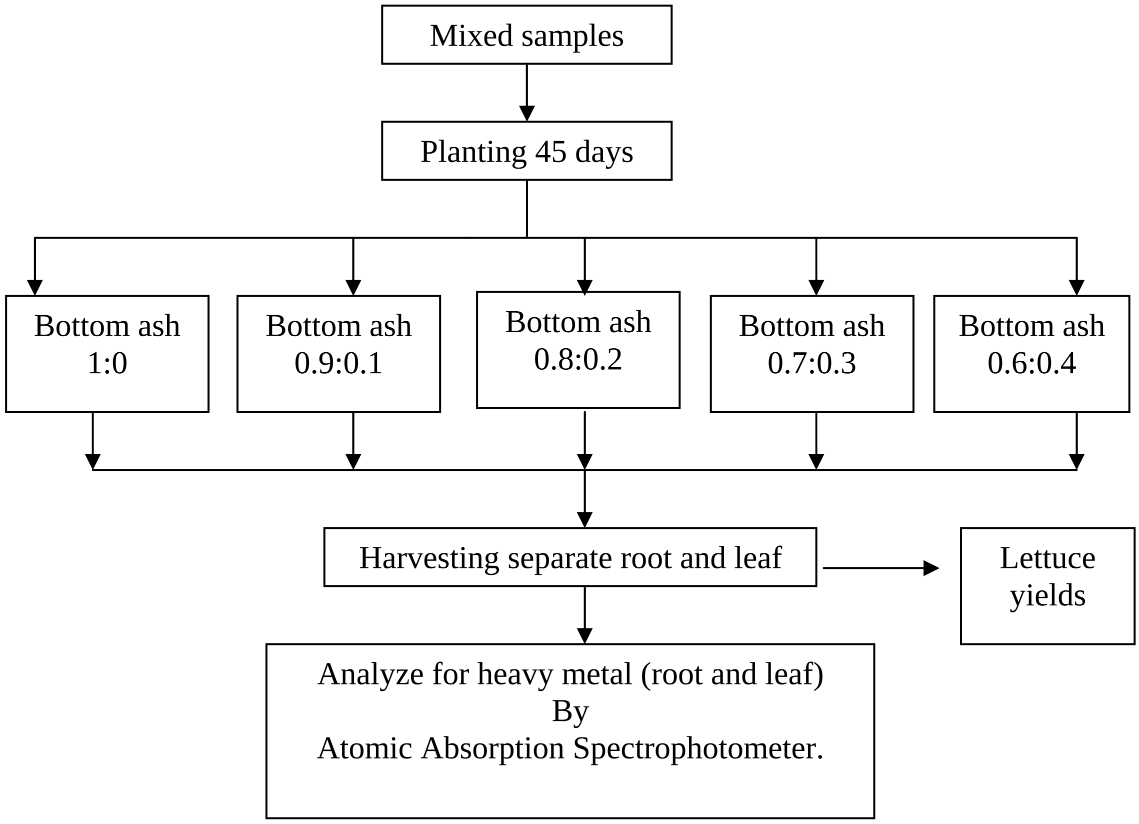
This study for the ratio of sida soil adding bottom ash for plant lettuce by using vary ratio (1:0, 0.9;0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) from prepare sample (3.7.2) and following this step.

1. Place the sample 500 g. of each ratio of sida soil adding with bottom ash in plastic pots.
2. About 20 seeds were sow in each plastic posts. Tap water will be applied about 200 ml/day in each plastic pots.
3. Third strong seeding were left to grown when they were 20 days old and will harvest when 45 days old. All experiment will be done 4 replicates.
4. Lettuce root and leaf part were separated and dry at 65 °C in the oven for 48 hours. Dried plant will be weight, ground with agate mortar, and kept in plastic bags until use.
5. Copper and zinc in plants were digested with HNO_3-HCl and measurement by Atomic Absorption Spectrophotometer.

3.8 Research diagram



3.8 Research diagram (continued)



3.9 Data analysis

Descriptive analysis

Copper and zinc accumulation and crop yield were determined as mean and standard deviation. Every treatment has four independent replicates. This data were presented as mean ±SD of four independent experiments.

Inferential statistics

The data were analyzed using the one-way ANOVA. The different lettuce effect to copper and zinc accumulation and growth in different lettuce parts (leaf and root) at different rations of sida soils mixed with bottom ash was determined using LSD at α level of 0.05.

CHAPTER IV
RESULTS

4.1 Characteristics of sida soil bottom ash and mixed soil.

The characteristics of sida soil, bottom ash and mixed samples at all ratios were analyzed. Results are shown in Table 4.1, 4.2 and 4.3 and Figure 4.1, 4.2 and 4.3.

4.1.1 pH, moisture content and organic matter in the sida soil bottom ash and mixed samples.

This experiment was carried out in order to determine the pH, moisture content and organic matter. The average pH value of sida soil was 6.64±0.78 whereas average pH value of bottom ash was 8.79±0.1. There were significant differences in pH of mixed soil ratios ($p<0.05$) (Table 4.1, 4.2 and 4.3). According to the experiment, the increase of pH values was found as the ratio increased. The greatest pH value was observed when the ratio was 0.6:0.4.

The average moisture content value of sida soil was 10.55±0.33% whereas average moisture content of bottom ash was 79.94±0.29%. There were significant differences in moisture content of mixed soil ratios ($p<0.05$) (Table 4.1, 4.2 and 4.3). According to the experiment, the increase of moisture content values was found as the ratio increased. The greatest moisture value was observed when the ratio was 0.6:0.4 at 14.22±0.24%.

The average organic matter value of sida soil was 5.04±0.38% whereas average organic matter of bottom ash was 1.64±0.61%. There were significant differences in organic matter of mixed soil ratios ($p<0.05$) (Table 4.1, 4.2 and 4.3). According to the experiment, the decrease of organic matter values was found as the ratio increased. The greatest organic matter value was observed when the ratio was 1:0 at 5.04±0.38%.

Table 4.1 Average (±SD) of pH, moisture content and organic matter in mixed soil at various ratios.

Ratios of sida soil added with bottom ash	Average (±SD)		
	pH	**Moisture content (%)**	**Organic matter (%)**
0.9:0.1	6.71 ± 0.57^{a}	11.07 ± 0.51^{a}	4.85 ± 0.74^{a}
0.8:0.2	6.78 ± 0.22^{a}	12.22 ± 0.59^{a}	4.24 ± 0.36^{a}
0.7:0.3	6.85 ± 0.18^{a}	12.85 ± 0.54^{a}	2.45 ± 0.18^{a}
0.6:0.4	6.89 ± 0.98^{b}	14.22 ± 0.24^{b}	1.86 ± 0.85^{a}

Remark : The same letter in the column was not significantly different at the α level of 0.05.

Table 4.2 Average (±SD) of pH, moisture content and organic matter in bottom ash.

Parameter	**Average (±SD)**
pH	8.79±0.01
Moisture content (%)	79.94±0.29
Organic matter (%)	1.64±0.61

Table 4.3 Average (±SD) of pH, moisture content and organic matter in soil.

Parameter	**Average (±SD)**
pH	6.64±0.78
Moisture content (%)	10.55±0.33
Organic matter (%)	5.04±0.38

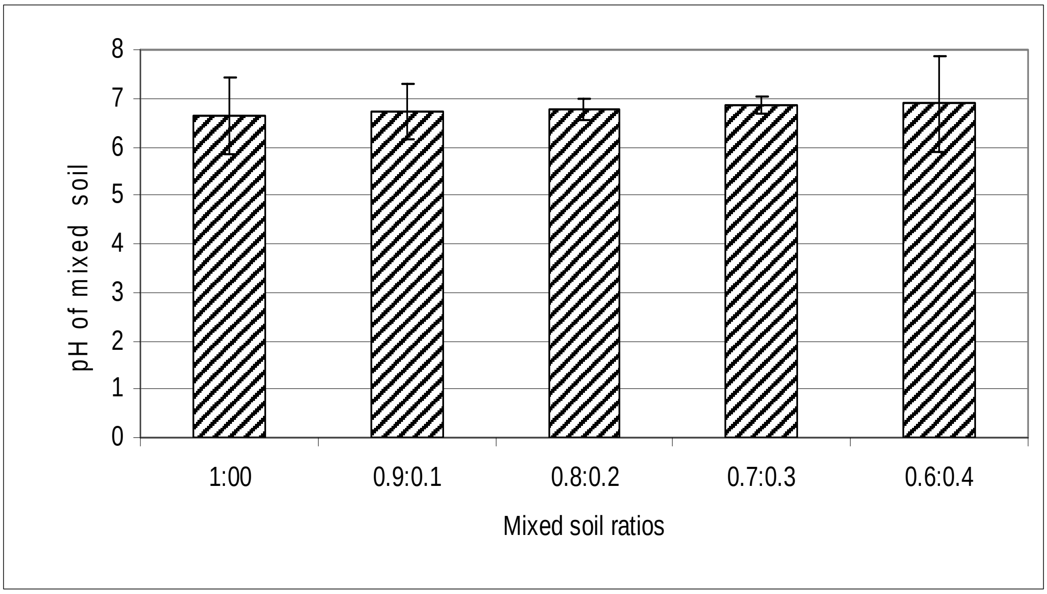


Figure 4.1 pH of sida soil at various ratios.

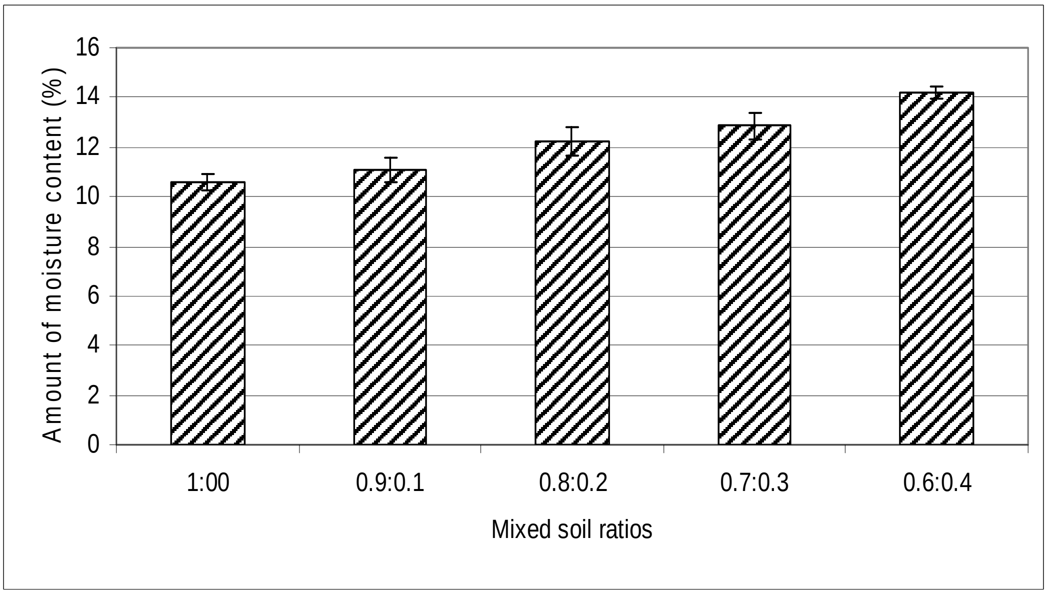


Figure 4.2 Amount of moisture content of sida soil at various ratios.

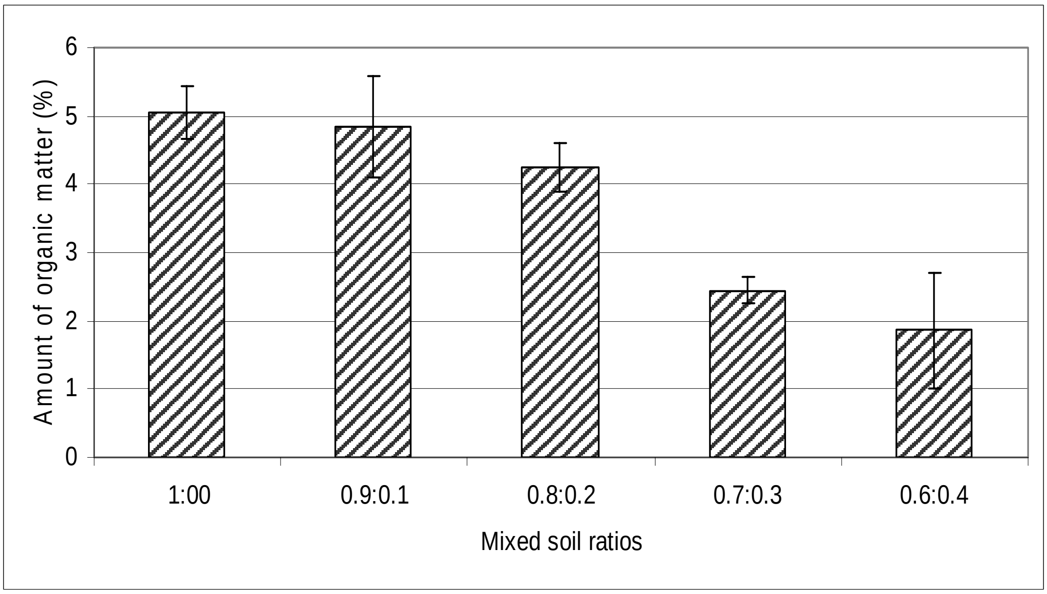


Figure 4.3 Amount of organic matter in sida soil at various ratios.

4.1.2 Nitrogen, phosphorus and potassium.

The average amount of nitrogen in sida soil was 0.35±0.04% whereas those of bottom ash were 0.15±0.03%. %. It was found that there was a significant difference of nitrogen amount among the ratios of mixed soil ($p<0.05$). According to the experiment, the average maximum nitrogen was found at the ratio of 0.9:0.1 at 0.40±0.02.

The average amount of phosphorus in sida soil was 44.99±1.35 ppm whereas those of bottom ash were 25.35±0.02 ppm. It was found that there was a significant difference of phosphorus amount among the ratios of mixed soil ($p<0.05$). According to the experiment, the average maximum phosphorus was found at the ratio of 0.9:0.1 at 66.35±2.94 ppm.

The average amount of potassium in sida soil was 0.93±0.02% whereas those of bottom ash were 0.03±0.005%. It was found that there was no significant difference of potassium amount among the ratios of mixed soil ($p>0.05$). According to the experiment, the average maximum potassium was found at the ratio of 0.8:0.2 at 0.96±0.01%. All results are showed in Table 4.4 and 4.5 and Figure 4.4, 4.5 and 4.6.

Table 4.4 Average (±SD) of nitrogen, phosphorus and potassium in mixed soil at various ratios.

Ratios of sida soil added with bottom ash	**Average (±SD)**		
	Nitrogen (%)	**Phosphorus (ppm)**	**Potassium (%)**
0.9:0.1	0.40±0.02[a]	66.35±2.94[a]	0.94±0.03[a]
0.8:0.2	0.39±0.05[a]	56.91±1.11[b]	0.96±0.01[b]
0.7:0.3	0.35±0.03[a]	52.46±4.41[b]	0.93±0.02[a]
0.6:0.4	0.31±0.03[a]	50.82±2.64[b]	0.92±0.01[a]

Remark : The same letter in the column was not significantly different at the α level of 0.05.

Table 4.5 Average (±SD) of nitrogen, phosphorus and potassium in bottom ash.

Parameter	**Average (±SD)**
Nitrogen, %	0.15±0.03
Phosphorus, ppm	25.35±0.02
Potassium, %	0.03±0.005

Table 4.6 Average (±SD) of nitrogen, phosphorus and potassium in sida soil.

Parameter	**Average (±SD)**
Nitrogen, %	0.35±0.04
Phosphorus, ppm	44.99±1.35
Potassium, %	0.93±0.02

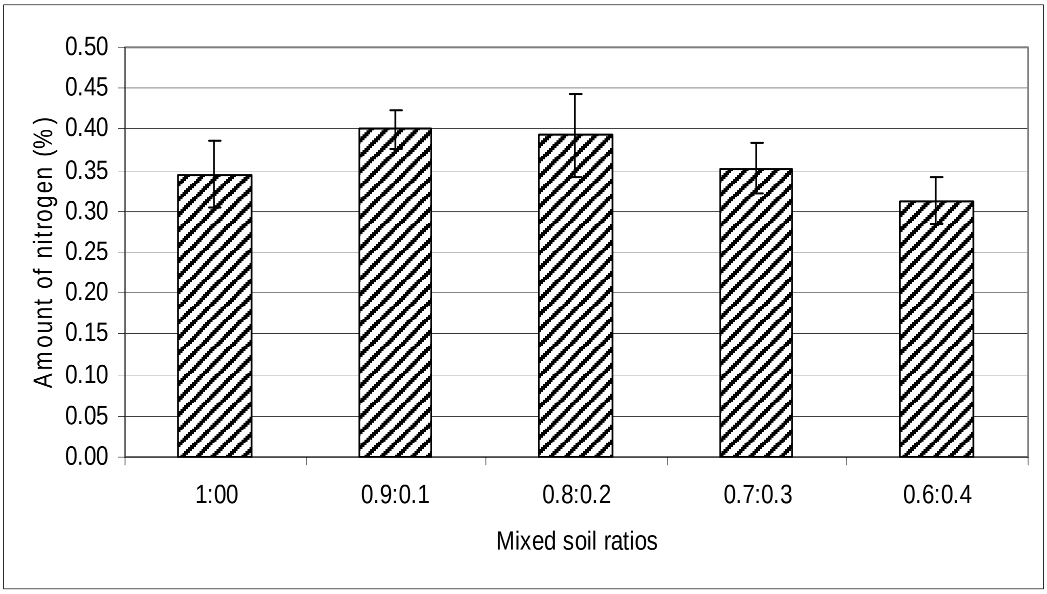


Figure 4.4 Amount of nitrogen in sida soil at various ratios.

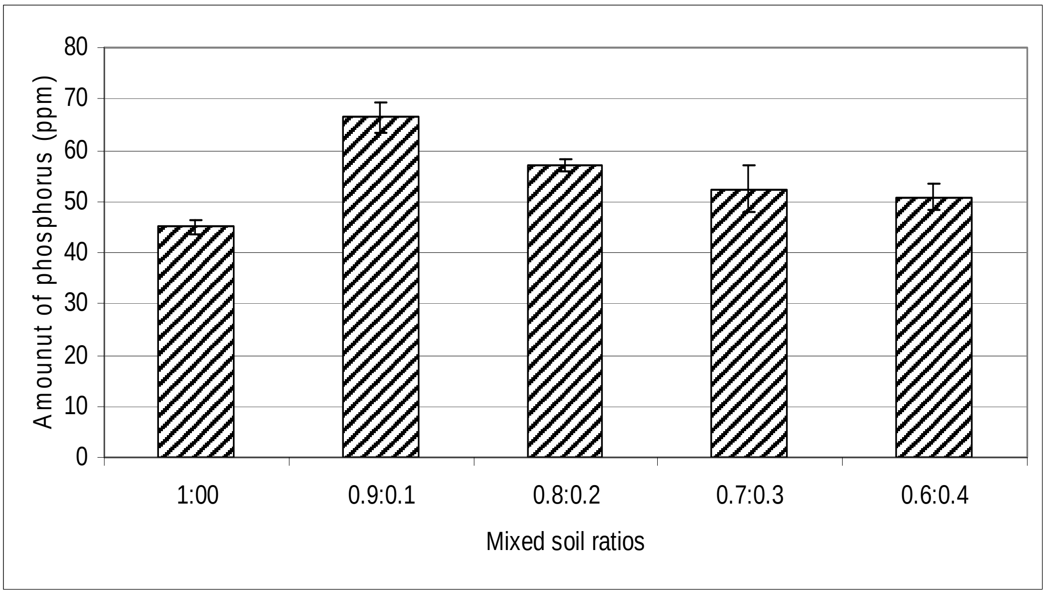


Figure 4.5 Amount of phosphorus in sida soil at various ratios.

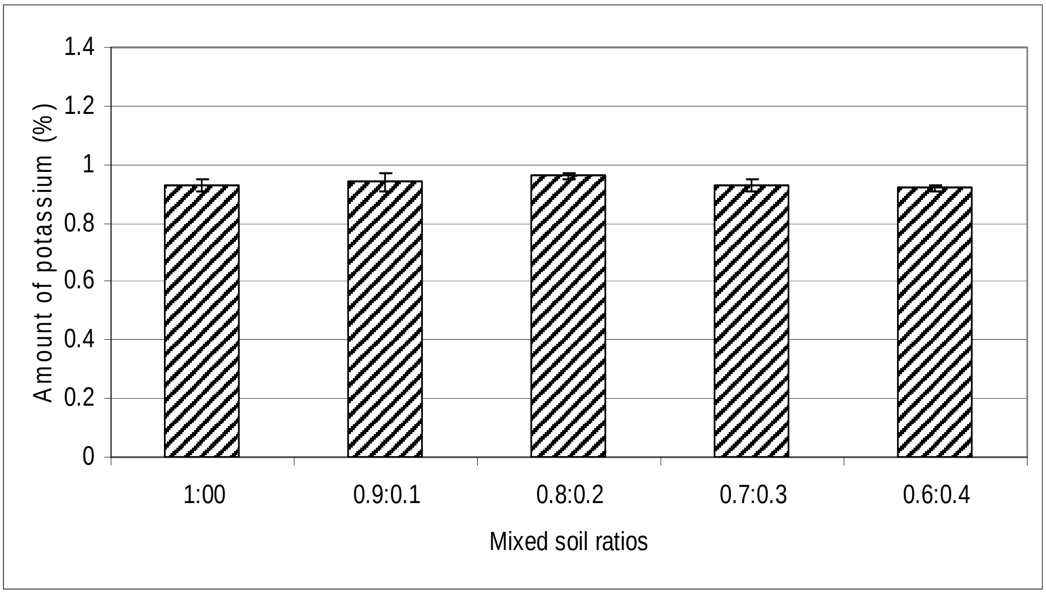


Figure 4.6 Amount of potassium in sida soil at various ratios.

4.2 Amount of copper and zinc in sida soil due to bottom ash application.

This experiment was carried out in order to determine the heavy metals accumulation in sida soil due to bottom ash application. Four mixed samples (0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) of bottom ash had been mixed with sida soil. Effect of mixed sample and bottom ash was shown in Table 4.7, 4.8 and 4.9 and Figure 4.7.

The amount of copper accumulated in sida soil and bottom ash was 13.12±1.71 mg/kg and 20.12±0.61 mg/kg, respectively. It was found that there was a significant difference of copper accumulation amount among the ratios of mixed soil ($p<0.05$). According to the experiment, the average maximum amount of copper was found at the ratio of 0.6:0.4 at 26.13±2.30 mg/kg. The average minimum amount of copper was found at the ratio of 1:0 at 13.12±1.71 mg/kg.

For zinc, the amount of zinc accumulated in sida soil and bottom ash was 66.84±5.84 and 57.67±1.32 mg/kg, respectively. It was found that, there was a significant difference of zinc accumulation amount among the ratios of mixed soil ($p<0.05$). According to the experiment, the average maximum amount of zinc was found at the ratio of 0.6:0.4 at 137.74±4.13 mg/kg. The average minimum amount of zinc was found at the ratio of 1:0 at 66.84±5.84 mg/kg.

Table 4.7 Average (±SD) of copper and zinc content in mixed soil at various ratios and bottom ash.

Ratios of sida soil added with bottom ash	Average (±SD)	
	Copper (mg/kg)	Zinc (mg/kg)
0.9:0.1	17.15±1.88[a]	87.27±5.46[a]
0.8:0.2	17.77±1.12[a]	106.04±12.38[b]
0.7:0.3	19.23±2.80[a]	121.63±8.17[b]
0.6:0.4	26.13±2.30[b]	137.74±4.13[bc]

Remark : The same letter in the column was not significantly different at the α level of 0.05.

Table 4.8 Average (±SD) of copper and zinc in bottom ash.

Parameter	Average (±SD)
Copper (mg/kg)	20.12±0.61
Zinc (mg/kg)	57.67±1.32

Table 4.9 Average (±SD) of copper and zinc in sida soil.

Parameter	Average (±SD)
Copper (mg/kg)	13.12±1.71
Zinc (mg/kg)	66.84±5.84

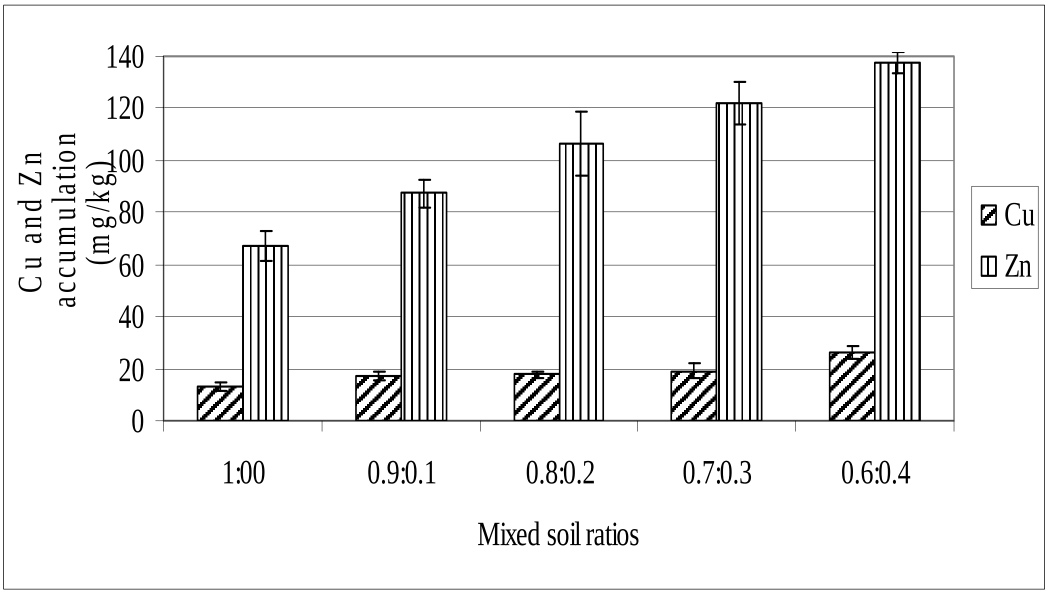


Figure 4.7 Copper and zinc accumulated in mixed soil at various ratios.

4.3 Amount of copper and zinc up-taken by root and leaf lettuce grown in different ratios of bottom ash.

This experiment was carried out in order to determine the heavy metal accumulated in parts of lettuce (root and leaf). Four mixed ratios (0.9:0.1, 0.8:0.2, 0.7:0.3 and 0.6:0.4) and sida soil (1:0) were used for culture lettuce for 45 days. All experiments were done four replicates. The results of copper and zinc accumulated in root and leaf at all experiment were shown in Table 4.7 and Figure 4.8 and 4.9.

The amount of copper accumulation in root at all ratio (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) were 9.85±1.35, 14.57±1.85, 14.86±1.12, 16.54±3.76 and 21.46±5.90 mg/kg, respectively. The average maximum accumulation in root was found at the ratio of 0.6:0.4 whereas the average minimum accumulation in root was found at the ratio of 1:0. It was found that there was significant difference of copper accumulation in root amount among the ratios of mixed soil ($p<0.05$).

The amount of copper accumulated in lettuce leaf at ratio (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) were 2.92±1.05, 4.65±1.14, 5.53±1.08, 6.34±0.23 and 8.18±1.20 mg/kg, respectively. The average maximum accumulation in leaf was found at the ratio of 0.6:0.4 whereas the average minimum accumulation in leaf was found at the ratio of 1:0. It was found that there was significant difference of copper accumulation in leaf amount among the ratios of mixed soil ($p<0.05$).

The amount of zinc accumulation in root at all ratio (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) were 42.30±4.90, 58.07±8.06, 83.01±6.33, 105.47±4.77 and 113.47±4.13 mg/kg, respectively. The average maximum accumulation in root was found at the ratio of 0.6:0.4 whereas the average minimum accumulation in root was found at the ratio of 1:0. It was found that there was significant difference of zinc accumulation in root amount among the ratios of mixed soil ($p<0.05$).

The amount of zinc accumulated in lettuce leaf at ratio (1:0, 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4) were 19.18±3.59, 21.12±1.77, 25.15±1.65, 30.564.54 and 32.94±7.34 mg/kg, respectively. The average maximum accumulation in leaf was found at the ratio of 0.6:0.4 whereas the minimum accumulation in leaf was found at the ratio of 1:0. It was found that there was significant difference of zinc accumulation in leaf amount among the ratios of mixed soil ($p<0.05$).

Table 4.10 Average (±SD) of copper and zinc accumulation in root and leaf at various ratios and bottom ash.

Ratios of sida soil added with bottom ash	**Average of copper (mg/kg)**		**Average of zinc (mg/kg)**	
	Root	**Leaf**	**Root**	**Leaf**
1:0	9.85±1.35[a]	2.92±1.05[a]	42.30±4.90[a]	19.18±3.59[a]
0.9:0.1	14.57±1.85[a]	4.65±1.14[a]	58.07±8.06[a]	21.12±1.77[a]
0.8:0.2	14.86±1.12[a]	5.53±1.08[a]	83.01±6.33[a]	25.15±1.65[a]
0.7:0.3	16.54±3.76[a]	6.34±0.23[a]	105.47±4.77[a]	30.56±4.94[a]
0.6:0.4	21.46±5.90[a]	8.18±1.20[a]	113.47±4.13[a]	32.94±7.34[a]

Remark : The same letter in the column was not significantly different at the α level of 0.05.

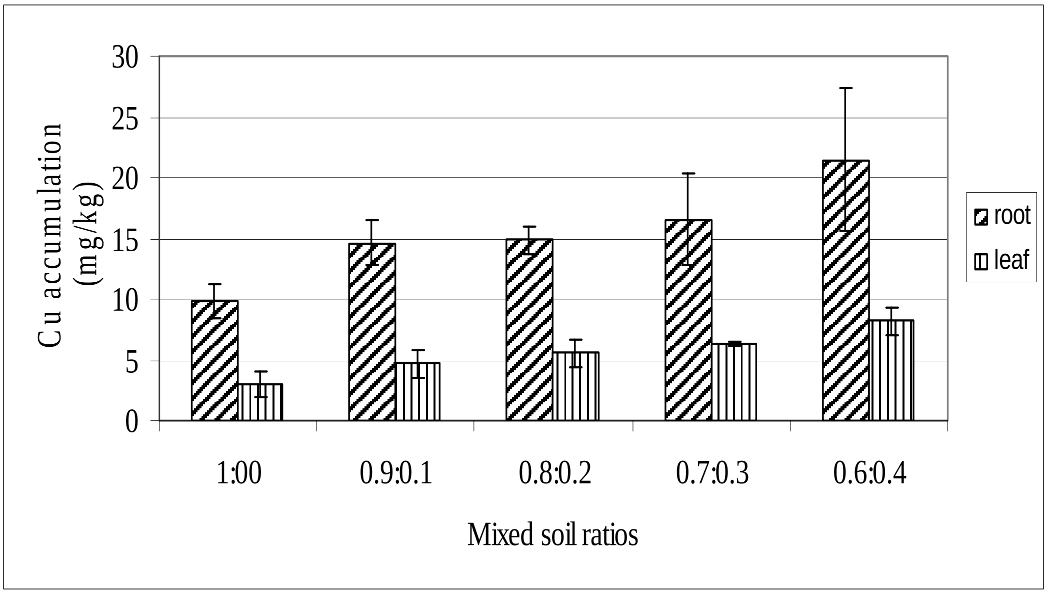


Figure 4.8 Copper accumulated in root and leaf at various ratios of mixed soil.

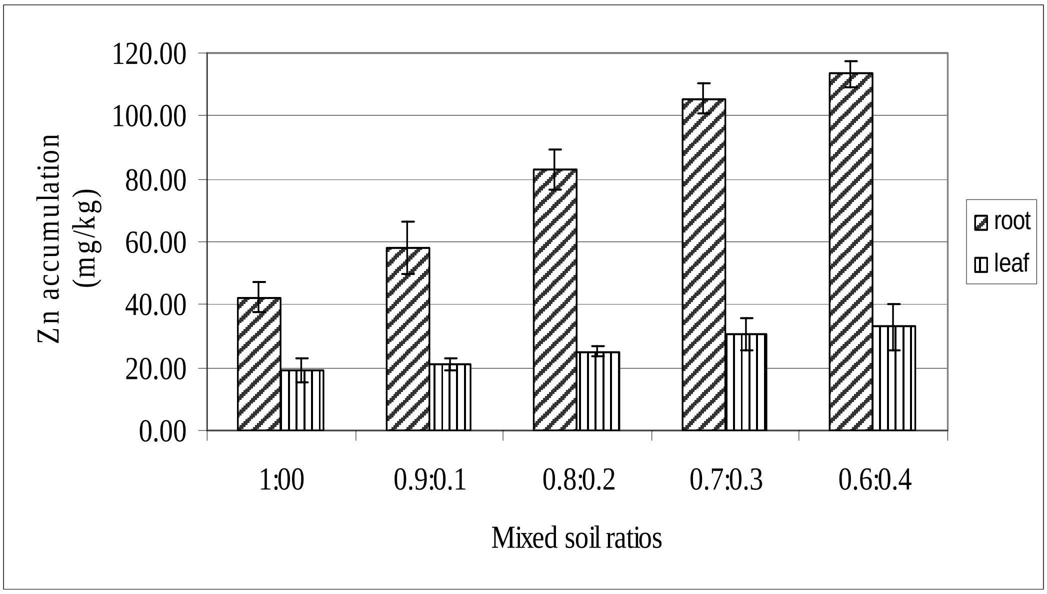


Figure 4.9 Zinc accumulated in root and leaf at various ratios of mixed soil.

4.4 Yield of lettuce grown in mixed soils.

This experiment was carried out in order to determine the lettuce yield. Dry weights (lettuce yield) of four mixed ratio and control ratio are presented in Table 4.8 and Figure 4.10

The average amount of lettuce yield in sida soil was 1.31±0.26 gm/plant. According to the experiment, the average maximum lettuce yield was found at the ratio of 0.6:0.4 and the ratio of 0.8:0.2 at 1.43±0.06 and 1.43±0.03 gm/plant, respectively. The average minimum lettuce yield was found at the ratio of 1:0 at 1.31±0.26 gm/plant. It was found that there was a significant difference of lettuce yield amount among the ratios of mixed soil ($p<0.05$).

Table 4.11 Average (±SD) of lettuce yield at various soil ratios

Ratios of sida soil added with bottom ash	Lettuce yield (gm/plant)
1:0	1.31±0.03[a]
0.9:0.1	1.32±0.01[b]
0.8:0.2	1.43±0.06[b]
0.7:0.3	1.36±0.08[b]
0.6:0.4	1.43±0.03[b]

Remark : The same letter in the column was not significantly different at the α level of 0.05.

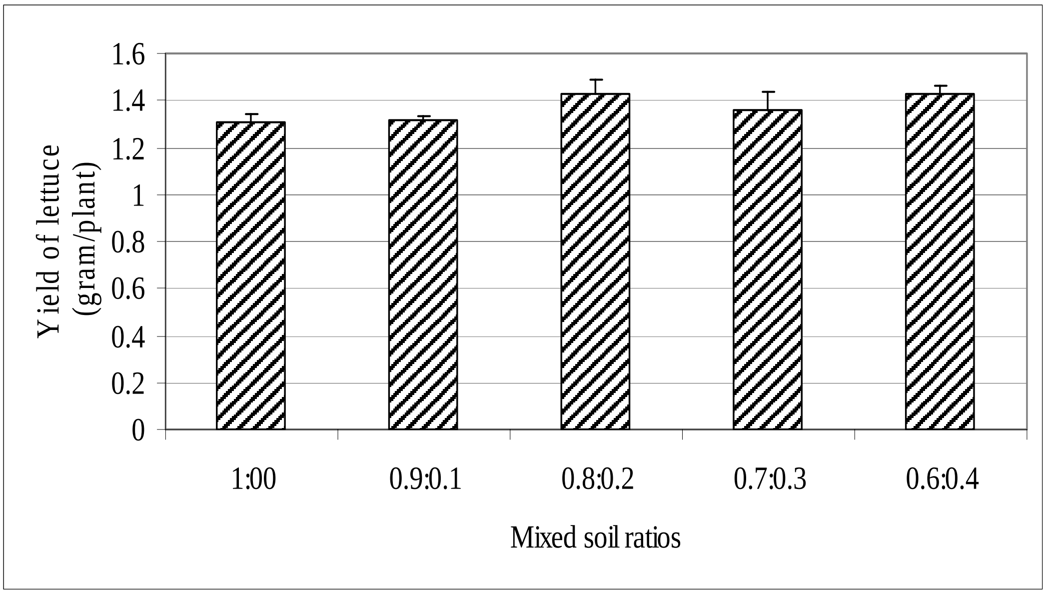


Figure 4.10 Yield of lettuce grown at various ratios of mixed soil.

CHAPTER V
DISCUSSIONS

5.1 Effect of macronutrients from added bottom ash in sida soil at various ratios.

The plant nutrients may be divided two categories as macronutrients and micronutrients. Macronutrient are found and needed in plants in relatively high concentrations. The following elements may be defined as macronutrients. Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, Sulfur, Potassium, Calcium and Magnesium. Macronutrients are not important to a plant's metabolism more than micronutrients, but since they are needed in large amounts, deficiencies are more common with the macronutrients. From the result of experiment, application of bottom ash from Mae Moh power plant in sida soil at all ratios showed that value of nitrogen, phosphorus and potassium are different.

For nitrogen, bottom ash had effect to tendency decrease nitrogen value while increased bottom ash ratios. Because sida soil is produce from soil mixed with other materials and when mixed it with bottom ash it may be not homogeneous such as liquid solution. When sampling mixed soil for nitrogen analysis it may be obtain other materials not soil and bottom ash such as coconut flake or sand.

Phosphorus values at all ratios decreased as ratios of bottom ash increased. Sungthong (56) suggested that pH is not only major factors of plant growth, in other words pH can indicate phosphorus solubility and include other micronutrients such as zinc and manganese too. Phosphorus was very well solubility at pH about 6-7. At pH below 6, phosphorus can be fixed by Fe, Mn and Mg, on the other hand calcium and magnesium can fixed phosphorus at pH over 7. Krutkul (65) also found that concentration of phosphorus was decreased as value of pH increased. According to the experiment, the average maximum phosphorus was found at the ratio of 0.9:0.1, might be due to pH at this ratio has suitable to release maximum phosphorus.

At all mixed sample ratios except 0.6:0.4, the potassium value is higher than control ratio (1:0). This may indicate that bottom ash has minor potassium content and influence to change potassium level. The result found that different of mixed sample ratio had no significant difference of potassium content ratio ($p>0.05$).

5.2 Effect of copper and zinc on the lettuce parts.

5.2.1 Copper and zinc accumulation.

Copper and zinc are consider as micronutrients for crop because crop needed for small amount (38). On the other hand, it cause deleterious to consumer especially in human if crop accumulated copper exceed 20 mg/kg and zinc exceed 100 mg/kg (52). In this experiment copper and zinc were increased with increasing ratios of bottom ash.

Copper accumulated in the root part higher than in leaf part at all ratios. From the results, at all ratios of mixed samples, copper accumulated in root and leaf were significantly increased ($p<0.05$) as ratios of bottom ash was increased. It has been reported that concentration of Cu at 20-100 mg/kg can be toxic to plant (66). The observed 2.92 to 8.1 mg/kg Cu content in level was found to be in a normal range compared to other studies as found that Cu content in dry mater in lettuce is between 3.19 – 13.90 mg g^{-1}(67)(68). However, in the root of lettuce grown in the all ratios of mixed samples except 0.6:0.4 ratio the Cu concentrations were close limit for Cu toxicity in plants.

Similar to copper accumulation of zinc it the root part is higher than in leaf. Moreover accumulation of zinc in the root and leaf was found to have significant increase at to the increase of bottom ash and soil ratio ($p<0.05$).

About heavy metals uptake by plant, it has two main structural component: (1) the root system, which anchors the plant in the soil, and is the site for absorption of materials from it, and (2) the shoot system consisted of the stem and leaves. The stem supports the plant above the ground, and is the part through which mineral and water absorbed from roots are conveyed to leaves and the carbohydrates produced in leaves are transferred to roots.

The movement of elements into roots occurs either by passive diffusion through the cell membrane, or by the more common process of active transfer against concentration and/or electrochemical potential gradients (69).

The foliar uptake is another route of entry of metals into plant cells. The entry of metals into plant cells through leaves is of particular significance from the pollution point of view because of aerosol deposit (69).

From the experiment of Prasad (69) in soil, some metals are more mobile than others, e.g. Cd and Zn, while, e.g., Cu and Pb are immobile and easily form organic complexes with fulvic acid

5.2.2 Factors influencing heavy metal uptake.

Many factors influence the uptake of metals, and include the growing environment, such as temperature, soil pH, soil aeration, Eh condition (particular of aquatic environment) and fertilization, competition between the plant species, the type of plant, its size, the root system, the availability of the elements in the soil or foliar deposits, the type of leaves, soil moisture and the plant energy supply to the roots and leaves (69).

Prasad (69) and Siriratpiriya found that, the increasing of soil pH caused the decreasing of metals plant uptake due to the solubility of exchangeable metal ions reduced when soil pH arise. According to the experiment, although pH increase as the ratio increased but it is not has influence to decrease lettuce uptake copper and zinc similar Siriratpiriya and Prasad. Because bottom ash has a few influence to increase pH in soil, that not enough able to decrease copper and zinc uptake by lettuce.

Metal accumulation depends on both uptake into the tissue and leakage into the surrounding medium. Metals are first taken into the apoplast of the roots. Then some of the total amount of the metal is transported further into the cells, some is transported further in the apoplast, and some becomes bound to cell wall substances. (69)

The movement of metals from the external solution into the cell walls is a non-metabolic, passive process, driven by diffusion or massflow (70).

Part of metal that has been taken into the apoplast is further transported through the plasma membrane into the cytoplasm. Metals are taken up in cationic forms, except Mo, which is taken up as molybdate anion (69).

The Cu net uptake could be composed by a low and high affinity biphasic Cu uptake system based on Michaelis-Menten kinetics and an ATP- depended efflux of Cu (71) (72).

Zn transporters, with a higher abundance in zinc accumulators species than in non accumulator species (73). Zinc is also shown to be actively transported as a free ion across the tomoplast, and depending on the tolerance of the tolerance of the plant there are two or more parallel pathways (74).

5.2.3 Heavy metal translocation and distribution.

Heavy metals are largely transported apoplastically in plant tissue. To be able to reach the xylem vessels of the roots, the metals have to cross the endodermis and the suberinized. Casparian strips, which is difficult. Consequently, most of the metal uptake is performed by the younger parts of the root where the casparian strips are not yet folly developed. How the metals are thereafter transported into the xylem vessels is still unknown (75). In the xylem, zinc may be translocated chelated to organic acid, copper in complex with amino acid (76). Distribution, during their transportation through the plant, metals get bound largely on the cell wall, which explains why most of the metal taken up is commonly found in the roots (about 75-90%) and smaller amounts are distributed in the shoots. According to the experiment, copper and zinc accumulated in lettuce root more than lettuce leaf. That are close conformity with the previous research.

5.3 Effect of copper and zinc on lettuce yields.

Lettuce yields are calculated from dry weights. Lettuce was harvest after 45 days grown clean lettuce by tap water afterwards, take it to hot air oven 65 C° for 48 hours and weight.

From the results, all lettuce yields grown from all ratios of mixed sample had weight more than lettuce from control experiment. Because bottom ash has high moisture content and when mix with sida soil bottom ash make soil moisture increase and influence to soil aggregation.

Soil aggregation has effect as (1) can help root spread (2) increase soil aerator (3) increase water efficiency and (4) induce microorganism activity for release nutrients (77).

Moreover bottom ash has copper and zinc that are micronutrients for plant such as John J. Hassett and Wayne L. Banwart (78) suggest that copper occurs primarily in organic complexes in the plant, whereas zinc occurs primarily as inorganic species. A deficiency of copper interferes with protein synthesis and causes a buildup of soluble nitrogen compounds in the plant.

According to data mentioned above, it can be concluded that soil aggregation and copper and zinc accumulation they are factors for lettuce cultivated and growth in soil added with bottom ash are has yields (dry weight) more than lettuce yield (dry weight) from control ratio.

CHAPTER VI
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The aim of this research was to investigate effect of bottom ash at various amendments with sida soil. The physical and chemical of sida soil and bottom ash were determined.

Based on results obtained from this study, the following conclusions can be made:

6.1.1 Heavy metals (copper and zinc) contamination were acceptable in sida soil when compared with criteria of heavy metal contamination for agricultural purpose. (Appendix C)

6.1.2 Heavy metal (copper and zinc) contaminations in bottom ash from this study were similar to the experimental results carried out by Juntaramitree (54).

6.1.3 From the results of this study, copper and zinc in mixed sample at all ratios were significant increased ($p<0.05$) when ratios of bottom ash increased.

6.1.4 At all ratios of mixed sample, copper and zinc accumulated in lettuce parts (leaf and root) were significant increased ($p<0.05$) when ratios of bottom ash increased.

6.1.5 Lettuce leaf (edible part) from cultivated and growth at all mixed ratios, copper and zinc accumulated were acceptable when compared with standard value of heavy metals contamination issued by Food and Drug Administration.

6.1.6 The maximum dry weight of lettuce yield at 0.8:0.2 and 0.6:0.4 was 1.43 gm/plant, whereas, the minimum dry weight at 1:0 was 1.31 gm/plant.

6.2 Recommendation

6.2.1 Recommendation for further study

6.2.1.1 After the application of tap water, heavy metal in the leachate generation should be investigated.

6.2.1.2 Bottom ash ratio should be used until 100 percent.

6.2.1.3 In this experiment, lettuce was planted and growth in plastic pots, it should be carried out in the real situation for further study. In addition, the unit of mixing ration should be in kg/rai. instead of weight/weight.

6.2.1.4 Lettuces are one type of crops in this study, for next experiment, other crops should be used.

6.2.2 Recommendations for applications.

This study aimed to use bottom ash for alternative way for agriculturist to select a suitable ratio of mixed bottom ash with soil for best of lettuce yield and protect human health from hazard of heavy metals.

All mixing ratios in this study can be employed for the real applications since the heavy metal contamination in lettuce leaf was acceptable when compared with the criteria of Food and Drug Administration (52).

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APPENDIX

APPENDIX A
RESULTS

Table A-1 Mean, S.D and value of nitrogen, phosphorus and potassium at all mixed soil ratios.

Characteristics of mixed soil ratio	Ratios of mixed soil				
	1:0	**0.9:0.1**	**0.8:0.2**	**0.7:0.3**	**0.6:0.4**
Nitrogen (%)					
Rep 1	0.31	0.42	0.35	0.31	0.28
Rep 2	0.38	0.42	0.42	0.35	0.31
Rep 3	0.31	0.38	0.35	0.38	0.31
Rep 4	0.38	0.38	0.45	0.37	0.35
S.D	0.04	0.02	0.05	0.03	0.03
Mean	0.35	0.40	0.39	0.35	0.31
Phosphorus (ppm)					
Rep 1	42.99	61.98	55.39	58.21	48.86
Rep 2	45.88	68.35	56.8	48.55	48.28
Rep 3	45.36	67.51	57.51	49.51	52.57
Rep 4	45.71	67.57	57.93	53.55	53.58
S.D	1.35	2.94	1.11	4.41	2.64
Mean	44.99	66.35	56.91	52.46	50.82
Potassium (%)					
Rep 1	0.94	0.97	0.97	0.90	0.92
Rep 2	0.90	0.92	0.96	0.93	0.92
Rep 3	0.95	0.91	0.97	0.96	0.90
Rep 4	0.94	0.94	0.95	0.93	0.92
S.D	0.02	0.26	0.01	0.02	0.01
Mean	0.93	0.94	0.96	0.93	0.92

Table A-2 Mean, S.D and value of pH, moisture content and organic matter at all mixed soil ratios.

Characteristics of mixed soil ratio	Ratios of mixed soil				
	1:0	**0.9:0.1**	**0.8:0.2**	**0.7:0.3**	**0.6:0.4**
pH					
Rep 1	6.75	6.66	6.79	6.84	6.81
Rep 2	6.60	6.79	6.75	6.87	6.80
Rep 3	6.61	6.69	6.78	6.83	6.97
Rep 4	6.58	6.69	6.80	6.86	6.98
S.D	0.78	0.57	0.22	0.18	0.98
Mean	6.64	6.71	6.78	6.85	6.89
Moisture content (%)					
Rep 1	10.10	10.80	12.90	12.70	14.20
Rep 2	10.90	10.50	11.50	12.80	13.90
Rep 3	10.60	11.40	12.10	12.30	14.40
Rep 4	10.60	11.60	12.40	13.60	14.40
S.D	0.33	0.51	0.59	0.54	0.24
Mean	10.55	11.07	11.07	12.85	14.22
Organic matter (%)					
Rep 1	5.40	4.67	4.09	2.48	2.34
Rep 2	4.96	5.69	3.80	2.48	0.88
Rep 3	4.53	5.11	4.53	2.19	2.77
Rep 4	5.26	3.94	4.53	2.63	1.46
S.D	0.38	0.74	0.36	0.18	0.85
Mean	5.04	4.85	4.24	2.45	1.86

Table A-3 Mean, S.D and value of copper accumulated in mixed soil, lettuce root and lettuce root at all ratios.

Characteristics of sample	**Ratios of mixed sample**				
	1:0	**0.9:0.1**	**0.8:0.2**	**0.7:0.3**	**0.6:0.4**
Mixed soil ratio (mg/kg)					
Rep 1	11.43	17.40	17.66	20.40	29.33
Rep 2	15.23	15.50	19.22	21.23	25.30
Rep 3	12.09	16.00	17.7	15.08	23.90
Rep 4	13.73	19.70	16.5	20.21	26.00
S.D	1.71	1.88	1.12	2.80	2.30
Mean	13.12	17.15	17.77	19.23	26.13
Root (mg/kg)					
Rep 1	8.18	15.81	15.12	14.29	29.33
Rep 2	11.34	12.43	16.32	20.39	21.23
Rep 3	10.40	13.66	14.10	12.48	15.08
Rep 4	9.47	16.38	13.88	18.99	20.21
S.D	1.35	1.85	1.12	3.76	5.90
Mean	9.85	14.57	14.86	16.54	21.46
Leaf (mg/kg)					
Rep 1	3.13	4.96	4.21	6.5	9.16
Rep 2	2.02	4.60	5.08	5.99	8.95
Rep 3	2.20	3.14	6.49	6.45	8.08
Rep 4	4.32	5.88	6.32	6.41	6.52
S.D	1.05	1.14	1.08	0.23	1.20
Mean	2.92	4.65	5.53	6.34	8.18

Table A-4 Mean, S.D and value of zinc accumulated in mixed soil, lettuce root and lettuce root at all ratios.

Characteristics of sample	**Ratios of mixed sample**				
	1:0	**0.9:0.1**	**0.8:0.2**	**0.7:0.3**	**0.6:0.4**
Mixed soil ratio (mg/kg)					
Rep 1	71.15	94.29	108.07	115.24	139.09
Rep 2	58.24	84.72	122.30	127.94	132.35
Rep 3	69.42	88.46	93.45	129.41	142.22
Rep 4	68.53	81.59	100.34	113.94	137.29
S.D	5.84	5.46	12.38	8.17	4.13
Mean	66.84	87.27	106.04	121.63	137.74
Root (mg/kg)					
Rep 1	41.60	67.31	76.52	103.79	112.65
Rep 2	43.60	53.43	79.19	100.87	108.21
Rep 3	47.90	61.98	90.45	112.12	117.98
Rep 4	36.10	49.54	85.86	105.09	115.03
S.D	4.90	8.06	6.33	4.77	4.13
Mean	42.30	58.07	83.01	105.47	113.47
Leaf (mg/kg)					
Rep 1	23.59	23.23	24.46	26.14	42.46
Rep 2	15.62	19.87	23.97	37.64	24.64
Rep 3	17.00	19.46	24.57	29.08	33.16
Rep 4	20.52	21.91	27.59	29.37	31.53
S.D	3.59	1.77	1.65	4.94	7.34
Mean	19.18	21.12	25.15	30.56	32.94

Table A-5 Mean, S.D and value of lettuce yield at all ratios.

Crop yield (grams/plant)	**Ratios of mixed sample**				
	1:0	**0.9:0.1**	**0.8:0.2**	**0.7:0.3**	**0.6:0.4**
Rep 1	1.27	1.32	1.36	1.37	1.40
Rep 2	1.33	1.33	1.47	1.26	1.42
Rep 3	1.30	1.31	1.41	1.34	1.47
Rep 4	1.32	1.33	1.48	1.45	1.43
S.D	0.03	0.01	0.06	0.08	0.03
Mean	1.31	1.32	1.43	1.36	1.43

APPENDIX B
STATISTICAL ANALYSIS

1. Analysis of variance between different ratios of mixed soil and nitrogen.

Table B-1.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Nitrogen	1:0	.3450	.04041	4
	0.9:0.1	.4000	.02309	4
	0.8:0.2	.3925	.05058	4
	0.7:0.3	.3525	.03096	4
	0.6:0.4	.3125	.02872	4

Table B-1.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.021	4	.005	3.989	.021
Within Groups	.020	15	.001		
Total	.024	19			

Table B-1.3 Multiple comparisons between different ratios of mixed soil and nitrogen.

Dependent Variable: Nitrogen accumulation

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-.05500(*)	.02551	.048	-.1094	-.0006
	0.8:0.2	-.04750	.02551	.082	-.1019	.0069
	0.7:0.3	-.00750	.02551	.773	-.0619	.0469
	0.6:0.4	.03250	.02551	.222	-.0219	.0869
0.9:0.1	1:0	.05500(*)	.02551	.048	.0006	.1094
	0.8:0.2	.00750	.02551	.773	-.0469	.0619
	0.7:0.3	.04750	.02551	.082	-.0069	.1019
	0.6:0.4	.08750(*)	.02551	.004	.0331	.1419
0.8:0.2	1:0	.04750	.02551	.082	-.0069	.1019
	0.9:0.1	-.00750	.02551	.773	-.0619	.0469
	0.7:0.3	.04000	.02551	.138	-.0144	.0944
	0.6:0.4	.08000(*)	.02551	.007	.0256	.1344
0.7:0.3	1:0	.00750	.02551	.773	-.0469	.0619
	0.9:0.1	-.04750	.02551	.082	-.1019	.0069
	0.8:0.2	-.04000	.02551	.138	-.0944	.0144
	0.6:0.4	.04000	.02551	.138	-.0144	.0944
0.6:0.4	1:0	-.03250	.02551	.222	-.0869	.0219
	0.9:0.1	-.08750(*)	.02551	.004	-.1419	-.0331
	0.8:0.2	-.08000(*)	.02551	.007	-.1344	-.0256
	0.7:0.3	-.04000	.02551	.138	-.0944	.0144

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

2. Analysis of variance between different ratios of mixed soil and phosphorus.

Table B-2.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Phosphorus	1:0	44.9850	1.34750	4
	0.9:0.1	66.3525	2.94000	4
	0.8:0.2	56.9075	1.34750	4
	0.7:0.3	52.4550	4.40610	4
	0.6:0.4	44.9850	2.64407	4

Table B-2.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1017.312	4	254.328	33.372	.000
Within Groups	114.315	15	7.621		
Total	1131.627	19			

Table B-1.3 Multiple comparisons between different ratios of mixed soil and phosphorus.

Dependent Variable: Phosphorus accumulation

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-21.36750(*)	1.95205	.000	-25.5282	-17.2068
	0.8:0.2	-11.92250(*)	1.95205	.000	-16.0832	-7.7618
	0.7:0.3	-7.47000(*)	1.95205	.002	-11.6307	-3.3093
	0.6:0.4	-5.83750(*)	1.95205	.009	-9.9982	-1.6768
0.9:0.1	1:0	21.36750(*)	1.95205	.000	17.2068	25.5282
	0.8:0.2	9.44500(*)	1.95205	.000	5.2843	13.6057
	0.7:0.3	13.89750(*)	1.95205	.000	9.7368	18.0582
	0.6:0.4	15.53000(*)	1.95205	.000	11.3693	19.6907
0.8:0.2	1:0	11.92250(*)	1.95205	.000	7.7618	16.0832
	0.9:0.1	-9.44500(*)	1.95205	.000	-13.6057	-5.2843
	0.7:0.3	4.45250(*)	1.95205	.038	.2918	8.6132
	0.6:0.4	6.08500(*)	1.95205	.007	1.9243	10.2457
0.7:0.3	1:0	7.47000(*)	1.95205	.002	3.3093	11.6307
	0.9:0.1	-13.89750(*)	1.95205	.000	-18.0582	-9.7368
	0.8:0.2	-4.45250(*)	1.95205	.038	-8.6132	-.2918
	0.6:0.4	1.63250	1.95205	.416	-2.5282	5.7932
0.6:0.4	1:0	5.83750(*)	1.95205	.009	1.6768	9.9982
	0.9:0.1	-15.53000(*)	1.95205	.000	-19.6907	-11.3693
	0.8:0.2	-6.08500(*)	1.95205	.007	-10.2457	-1.9243
	0.7:0.3	-1.63250	1.95205	.416	-5.7932	2.5282

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

3. Analysis of variance between different ratios of mixed soil and potassium.

Table B-3.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
potassium	1:0	0.93	0.02	4
	0.9:0.1	0.94	0.03	4
	0.8:0.2	0.96	0.01	4
	0.7:0.3	0.93	0.02	4
	0.6:0.4	0.92	0.01	4

Table B-3.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.005	4	.001	2.994	.053
Within Groups	.006	15	.000		
Total	.011	19			

Table B-3.3 Multiple comparisons between different ratios of mixed soil and potassium.

Dependent Variable: Potassium accumulation

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-.00250	.01408	.861	-.0325	.0275
	0.8:0.2	-.03000	.01408	.050	-.0600	.0000
	0.7:0.3	.00250	.01408	.861	-.0275	.0325
	0.6:0.4	.01750	.01408	.233	-.0125	.0475
0.9:0.1	1:0	.00250	.01408	.861	-.0275	.0325
	0.8:0.2	-.02750	.01408	.070	-.0575	.0025
	0.7:0.3	.00500	.01408	.728	-.0250	.0350
	0.6:0.4	.02000	.01408	.176	-.0100	.0500
0.8:0.2	1:0	.03000	.01408	.050	.0000	.0600
	0.9:0.1	.02750	.01408	.070	-.0025	.0575
	0.7:0.3	.03250(*)	.01408	.036	.0025	.0625
	0.6:0.4	.04750(*)	.01408	.004	.0175	.0775
0.7:0.3	1:0	-.00250	.01408	.861	-.0325	.0275
	0.9:0.1	-.00500	.01408	.728	-.0350	.0250
	0.8:0.2	-.03250(*)	.01408	.036	-.0625	-.0025
	0.6:0.4	.01500	.01408	.304	-.0150	.0450
0.6:0.4	1:0	-.01750	.01408	.233	-.0475	.0125
	0.9:0.1	-.02000	.01408	.176	-.0500	.0100
	0.8:0.2	-.04750(*)	.01408	.004	-.0775	-.0175
	0.7:0.3	-.01500	.01408	.304	-.0450	.0150

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

4. Analysis of variance between different ratios of mixed soil and organic matter.

Table B-4.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Organic matter	1:0	5.0375	.38491	4
	0.9:0.1	4.8525	.73794	4
	0.8:0.2	4.2375	.35790	4
	0.7:0.3	2.4450	.18412	4
	0.6:0.4	1.8625	.85223	4

Table B-4.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.427	4	8.357	26.428	.000
Within Groups	4.743	15	.316		
Total	38.170	19			

Table B-4.3 Multiple comparisons between different ratios of mixed soil and organic matter.

Dependent Variable: Organic matter

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	.18500	.39762	.648	-.6625	1.0325
	0.8:0.2	.80000	.39762	.063	-.0475	1.6475
	0.7:0.3	2.59250(*)	.39762	.000	1.7450	3.4400
	0.6:0.4	3.17500(*)	.39762	.000	2.3275	4.0225
0.9:0.1	1:0	-.18500	.39762	.648	-1.0325	.6625
	0.8:0.2	.61500	.39762	.143	-.2325	1.4625
	0.7:0.3	2.40750(*)	.39762	.000	1.5600	3.2550
	0.6:0.4	2.99000(*)	.39762	.000	2.1425	3.8375
0.8:0.2	1:0	-.80000	.39762	.063	-1.6475	.0475
	0.9:0.1	-.61500	.39762	.143	-1.4625	.2325
	0.7:0.3	1.79250(*)	.39762	.000	.9450	2.6400
	0.6:0.4	2.37500(*)	.39762	.000	1.5275	3.2225
0.7:0.3	1:0	-2.59250(*)	.39762	.000	-3.4400	-1.7450
	0.9:0.1	-2.40750(*)	.39762	.000	-3.2550	-1.5600
	0.8:0.2	-1.79250(*)	.39762	.000	-2.6400	-.9450
	0.6:0.4	.58250	.39762	.164	-.2650	1.4300
0.6:0.4	1:0	-3.17500(*)	.39762	.000	-4.0225	-2.3275
	0.9:0.1	-2.99000(*)	.39762	.000	-3.8375	-2.1425
	0.8:0.2	-2.37500(*)	.39762	.000	-3.2225	-1.5275
	0.7:0.3	-.58250	.39762	.164	-1.4300	.2650

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

5. Analysis of variance between different ratios of mixed soil and pH.

Table B-5.1 Descriptive statistics

Items	**Mixed soil ratios**	**Mean**	**Std.Deviation**	**N**
pH	1:0	6.6350	.07767	4
	0.9:0.1	6.7075	.05679	4
	0.8:0.2	6.7800	.02160	4
	0.7:0.3	6.8500	.01826	4
	0.6:0.4	6.8900	.09832	4

Table B-5.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.172	4	.043	10.900	.000
Within Groups	.059	15	.004		
Total	.231	19			

Table B-5.3 Multiple comparisons between different ratios of mixed soil and pH.

Dependent Variable: pH

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-.07250	.04441	.123	-.1672	.0222
	0.8:0.2	-.14500(*)	.04441	.005	-.2397	-.0503
	0.7:0.3	-.21500(*)	.04441	.000	-.3097	-.1203
	0.6:0.4	-.25500(*)	.04441	.000	-.3497	-.1603
0.9:0.1	1:0	.07250	.04441	.123	-.0222	.1672
	0.8:0.2	-.07250	.04441	.123	-.1672	.0222
	0.7:0.3	-.14250(*)	.04441	.006	-.2372	-.0478
	0.6:0.4	-.18250(*)	.04441	.001	-.2772	-.0878
0.8:0.2	1:0	.14500(*)	.04441	.005	.0503	.2397
	0.9:0.1	.07250	.04441	.123	-.0222	.1672
	0.7:0.3	-.07000	.04441	.136	-.1647	.0247
	0.6:0.4	-.11000(*)	.04441	.026	-.2047	-.0153
0.7:0.3	1:0	.21500(*)	.04441	.000	.1203	.3097
	0.9:0.1	.14250(*)	.04441	.006	.0478	.2372
	0.8:0.2	.07000	.04441	.136	-.0247	.1647
	0.6:0.4	-.04000	.04441	.382	-.1347	.0547
0.6:0.4	1:0	.25500(*)	.04441	.000	.1603	.3497
	0.9:0.1	.18250(*)	.04441	.001	.0878	.2772
	0.8:0.2	.11000(*)	.04441	.026	.0153	.2047
	0.7:0.3	.04000	.04441	.382	-.0547	.1347

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

6. Analysis of variance between different ratios of mixed soil and moisture content.

Table B-6.1 Descriptive statistics

Items	**Mixed soil ratios**	**Mean**	**Std.Deviation**	**N**
Moisture content	1:0	10.5500	.33166	4
	0.9:0.1	11.0750	.51235	4
	0.8:0.2	12.2250	.58523	4
	0.7:0.3	12.8500	.54467	4
	0.6:0.4	14.2250	.23629	4

Table B-6.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	34.043	4	8.511	39.863	.000
Within Groups	3.203	15	.214		
Total	37.245	19			

Table B-6.3 Multiple comparisons between different ratios of mixed soil and moisture content.

Dependent Variable: moisture content

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-.52500	.32673	.129	-1.2214	.1714
	0.8:0.2	-1.67500(*)	.32673	.000	-2.3714	-.9786
	0.7:0.3	-2.30000(*)	.32673	.000	-2.9964	-1.6036
	0.6:0.4	-3.67500(*)	.32673	.000	-4.3714	-2.9786
0.9:0.1	1:0	.52500	.32673	.129	-.1714	1.2214
	0.8:0.2	-1.15000(*)	.32673	.003	-1.8464	-.4536
	0.7:0.3	-1.77500(*)	.32673	.000	-2.4714	-1.0786
	0.6:0.4	-3.15000(*)	.32673	.000	-3.8464	-2.4536
0.8:0.2	1:0	1.67500(*)	.32673	.000	.9786	2.3714
	0.9:0.1	1.15000(*)	.32673	.003	.4536	1.8464
	0.7:0.3	-.62500	.32673	.075	-1.3214	.0714
	0.6:0.4	-2.00000(*)	.32673	.000	-2.6964	-1.3036
0.7:0.3	1:0	2.30000(*)	.32673	.000	1.6036	2.9964
	0.9:0.1	1.77500(*)	.32673	.000	1.0786	2.4714
	0.8:0.2	.62500	.32673	.075	-.0714	1.3214
	0.6:0.4	-1.37500(*)	.32673	.001	-2.0714	-.6786
0.6:0.4	1:0	3.67500(*)	.32673	.000	2.9786	4.3714
	0.9:0.1	3.15000(*)	.32673	.000	2.4536	3.8464
	0.8:0.2	2.00000(*)	.32673	.000	1.3036	2.6964
	0.7:0.3	1.37500(*)	.32673	.001	.6786	2.0714

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

7. Analysis of variance between different ratios of mixed soil and lettuce yield.

Table B-7.1 Descriptive statistics

Items	**Mixed soil ratios**	**Mean**	**Std.Deviation**	**N**
Lettuce yield	1:0	1.3050	.02646	4
	0.9:0.1	1.3225	.00957	4
	0.8:0.2	1.4300	.05598	4
	0.7:0.3	1.3550	.07853	4
	0.6:0.4	1.4300	.02944	4

Table B-7.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.056	4	.014	6.340	.003
Within Groups	.033	15	.002		
Total	.088	19			

Table B-7.3 Multiple comparisons between different ratios of mixed soil and lettuce yield.

Dependent Variable: lettuce yield

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-.01750	.03310	.605	-.0881	.0531
	0.8:0.2	-.12500(*)	.03310	.002	-.1956	-.0544
	0.7:0.3	-.05000	.03310	.152	-.1206	.0206
	0.6:0.4	-.12500(*)	.03310	.002	-.1956	-.0544
0.9:0.1	1:0	.01750	.03310	.605	-.0531	.0881
	0.8:0.2	-.10750(*)	.03310	.005	-.1781	-.0369
	0.7:0.3	-.03250	.03310	.342	-.1031	.0381
	0.6:0.4	-.10750(*)	.03310	.005	-.1781	-.0369
0.8:0.2	1:0	.12500(*)	.03310	.002	.0544	.1956
	0.9:0.1	.10750(*)	.03310	.005	.0369	.1781
	0.7:0.3	.07500(*)	.03310	.039	.0044	.1456
	0.6:0.4	.00000	.03310	1.000	-.0706	.0706
0.7:0.3	1:0	.05000	.03310	.152	-.0206	.1206
	0.9:0.1	.03250	.03310	.342	-.0381	.1031
	0.8:0.2	-.07500(*)	.03310	.039	-.1456	-.0044
	0.6:0.4	-.07500(*)	.03310	.039	-.1456	-.0044
0.6:0.4	1:0	.12500(*)	.03310	.002	.0544	.1956
	0.9:0.1	.10750(*)	.03310	.005	.0369	.1781
	0.8:0.2	.00000	.03310	1.000	-.0706	.0706
	0.7:0.3	.07500(*)	.03310	.039	.0044	.1456

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

8. Analysis of variance between different ratios of mixed soil and copper accumulation in mixed soil.

Table B-8.1 Descriptive statistics

Items	**Mixed soil ratios**	**Mean**	**Std.Deviation**	**N**
Copper	1:0	13.1200	1.70697	4
	0.9:0.1	17.1500	1.88060	4
	0.8:0.2	17.7700	1.11541	4
	0.7:0.3	19.2300	2.80189	4
	0.6:0.4	26.1325	2.30352	4

Table B-8.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	359.699	4	89.925	21.563	.000
Within Groups	62.554	15	4.170		
Total	422.253	19			

Table B-8.3 Multiple comparisons between different ratios of mixed soil and copper accumulation in mixed soil.

Dependent Variable: copper

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-4.03000(*)	1.44400	.014	-7.1078	-.9522
	0.8:0.2	-4.65000(*)	1.44400	.006	-7.7278	-1.5722
	0.7:0.3	-6.11000(*)	1.44400	.001	-9.1878	-3.0322
	0.6:0.4	-13.01250(*)	1.44400	.000	-16.0903	-9.9347
0.9:0.1	1:0	4.03000(*)	1.44400	.014	.9522	7.1078
	0.8:0.2	-.62000	1.44400	.674	-3.6978	2.4578
	0.7:0.3	-2.08000	1.44400	.170	-5.1578	.9978
	0.6:0.4	-8.98250(*)	1.44400	.000	-12.0603	-5.9047
0.8:0.2	1:0	4.65000(*)	1.44400	.006	1.5722	7.7278
	0.9:0.1	.62000	1.44400	.674	-2.4578	3.6978
	0.7:0.3	-1.46000	1.44400	.328	-4.5378	1.6178
	0.6:0.4	-8.36250(*)	1.44400	.000	-11.4403	-5.2847
0.7:0.3	1:0	6.11000(*)	1.44400	.001	3.0322	9.1878
	0.9:0.1	2.08000	1.44400	.170	-.9978	5.1578
	0.8:0.2	1.46000	1.44400	.328	-1.6178	4.5378
	0.6:0.4	-6.90250(*)	1.44400	.000	-9.9803	-3.8247
0.6:0.4	1:0	13.01250(*)	1.44400	.000	9.9347	16.0903
	0.9:0.1	8.98250(*)	1.44400	.000	5.9047	12.0603
	0.8:0.2	8.36250(*)	1.44400	.000	5.2847	11.4403
	0.7:0.3	6.90250(*)	1.44400	.000	3.8247	9.9803

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

9. Analysis of variance between different ratios of mixed soil and zinc accumulation in mixed soil.

Table B-9.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Zinc	1:0	66.8352	5.83561	4
	0.9:0.1	87.2657	5.45756	4
	0.8:0.2	106.0413	12.37653	4
	0.7:0.3	121.6347	8.17038	4
	0.6:0.4	137.7372	4.12804	4

Table B-9.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12458.266	4	3114.566	51.769	.000
Within Groups	902.441	15	60.163		
Total	13360.707	19			

Table B-9.3 Multiple comparisons between different ratios of mixed soil and zinc accumulation in mixed soil.

Dependent Variable: zinc

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-20.43057(*)	5.48465	.002	-32.1208	-8.7403
	0.8:0.2	-39.20616(*)	5.48465	.000	-50.8964	-27.5159
	0.7:0.3	-54.79959(*)	5.48465	.000	-66.4898	-43.1093
	0.6:0.4	-70.90207(*)	5.48465	.000	-82.5923	-59.2118
0.9:0.1	1:0	20.43057(*)	5.48465	.002	8.7403	32.1208
	0.8:0.2	-18.77560(*)	5.48465	.004	-30.4658	-7.0853
	0.7:0.3	-34.36902(*)	5.48465	.000	-46.0593	-22.6788
	0.6:0.4	-50.47151(*)	5.48465	.000	-62.1618	-38.7813
0.8:0.2	1:0	39.20616(*)	5.48465	.000	27.5159	50.8964
	0.9:0.1	18.77560(*)	5.48465	.004	7.0853	30.4658
	0.7:0.3	-15.59343(*)	5.48465	.012	-27.2837	-3.9032
	0.6:0.4	-31.69591(*)	5.48465	.000	-43.3862	-20.0057
0.7:0.3	1:0	54.79959(*)	5.48465	.000	43.1093	66.4898
	0.9:0.1	34.36902(*)	5.48465	.000	22.6788	46.0593
	0.8:0.2	15.59343(*)	5.48465	.012	3.9032	27.2837
	0.6:0.4	-16.10248(*)	5.48465	.010	-27.7927	-4.4122
0.6:0.4	1:0	70.90207(*)	5.48465	.000	59.2118	82.5923
	0.9:0.1	50.47151(*)	5.48465	.000	38.7813	62.1618
	0.8:0.2	31.69591(*)	5.48465	.000	20.0057	43.3862
	0.7:0.3	16.10248(*)	5.48465	.010	4.4122	27.7927

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

10. Analysis of variance between different ratios of mixed soil and copper accumulation in root.

Table B-10.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Copper	1:0	9.8475	1.34856	4
	0.9:0.1	14.5700	1.84584	4
	0.8:0.2	14.8550	1.11611	4
	0.7:0.3	16.5375	3.75815	4
	0.6:0.4	21.4625	5.89510	4

Table B-10.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	279.397	4	69.849	6.310	.003
Within Groups	166.042	15	11.069		
Total	445.439	19			

Table B-10.3 Multiple comparisons between different ratios of mixed soil and copper accumulation in root.

Dependent Variable: copper

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-4.72250	2.35260	.063	-9.7370	.2920
	0.8:0.2	-5.00750	2.35260	.050	-10.0220	.0070
	0.7:0.3	-6.69000(*)	2.35260	.012	-11.7045	-1.6755
	0.6:0.4	-11.61500(*)	2.35260	.000	-16.6295	-6.6005
0.9:0.1	1:0	4.72250	2.35260	.063	-.2920	9.7370
	0.8:0.2	-.28500	2.35260	.905	-5.2995	4.7295
	0.7:0.3	-1.96750	2.35260	.416	-6.9820	3.0470
	0.6:0.4	-6.89250(*)	2.35260	.010	-11.9070	-1.8780
0.8:0.2	1:0	5.00750	2.35260	.050	-.0070	10.0220
	0.9:0.1	.28500	2.35260	.905	-4.7295	5.2995
	0.7:0.3	-1.68250	2.35260	.485	-6.6970	3.3320
	0.6:0.4	-6.60750(*)	2.35260	.013	-11.6220	-1.5930
0.7:0.3	1:0	6.69000(*)	2.35260	.012	1.6755	11.7045
	0.9:0.1	1.96750	2.35260	.416	-3.0470	6.9820
	0.8:0.2	1.68250	2.35260	.485	-3.3320	6.6970
	0.6:0.4	-4.92500	2.35260	.054	-9.9395	.0895
0.6:0.4	1:0	11.61500(*)	2.35260	.000	6.6005	16.6295
	0.9:0.1	6.89250(*)	2.35260	.010	1.8780	11.9070
	0.8:0.2	6.60750(*)	2.35260	.013	1.5930	11.6220
	0.7:0.3	4.92500	2.35260	.054	-.0895	9.9395

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

11. Analysis of variance between different ratios of mixed soil and zinc accumulation in root.

Table B-11.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Zinc	1:0	42.3000	4.89830	4
	0.9:0.1	58.0650	8.06135	4
	0.8:0.2	83.0050	6.32952	4
	0.7:0.3	105.4675	4.77316	4
	0.6:0.4	113.4675	4.12770	4
	Total	80.4610		20

Table B-11.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14716.275	4	3679.069	108.937	.000
Within Groups	506.588	15	33.773		
Total	15222.863	19			

Table B-11.3 Multiple comparisons between different ratios of mixed soil and zinc accumulation in root.

Dependent Variable: zinc

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-15.76500(*)	4.10929	.002	-24.5237	-7.0063
	0.8:0.2	-40.70500(*)	4.10929	.000	-49.4637	-31.9463
	0.7:0.3	-63.16750(*)	4.10929	.000	-71.9262	-54.4088
	0.6:0.4	-71.16750(*)	4.10929	.000	-79.9262	-62.4088
0.9:0.1	1:0	15.76500(*)	4.10929	.002	7.0063	24.5237
	0.8:0.2	-24.94000(*)	4.10929	.000	-33.6987	-16.1813
	0.7:0.3	-47.40250(*)	4.10929	.000	-56.1612	-38.6438
	0.6:0.4	-55.40250(*)	4.10929	.000	-64.1612	-46.6438
0.8:0.2	1:0	40.70500(*)	4.10929	.000	31.9463	49.4637
	0.9:0.1	24.94000(*)	4.10929	.000	16.1813	33.6987
	0.7:0.3	-22.46250(*)	4.10929	.000	-31.2212	-13.7038
	0.6:0.4	-30.46250(*)	4.10929	.000	-39.2212	-21.7038
0.7:0.3	1:0	63.16750(*)	4.10929	.000	54.4088	71.9262
	0.9:0.1	47.40250(*)	4.10929	.000	38.6438	56.1612
	0.8:0.2	22.46250(*)	4.10929	.000	13.7038	31.2212
	0.6:0.4	-8.00000	4.10929	.071	-16.7587	.7587
0.6:0.4	1:0	71.16750(*)	4.10929	.000	62.4088	79.9262
	0.9:0.1	55.40250(*)	4.10929	.000	46.6438	64.1612
	0.8:0.2	30.46250(*)	4.10929	.000	21.7038	39.2212
	0.7:0.3	8.00000	4.10929	.071	-.7587	16.7587

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

12. Analysis of variance between different ratios of mixed soil and copper accumulation in leaf.

Table B-12.1 Descriptive statistics

Items	**Mixed soil ratios**	**Mean**	**Std.Deviation**	**N**
Copper	1:0	2.9175	1.05396	4
	0.9:0.1	4.6450	1.13893	4
	0.8:0.2	5.5250	1.07866	4
	0.7:0.3	6.3375	.23457	4
	0.6:0.4	8.1775	1.19984	4

Table B-12.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	61.077	4	15.269	15.070	.000
Within Groups	15.198	15	1.013		
Total	76.275	19			

Table B-12.3 Multiple comparisons between different ratios of mixed soil and copper accumulation in leaf.

Dependent Variable: copper

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-1.72750(*)	.71177	.028	-3.2446	-.2104
	0.8:0.2	-2.60750(*)	.71177	.002	-4.1246	-1.0904
	0.7:0.3	-3.42000(*)	.71177	.000	-4.9371	-1.9029
	0.6:0.4	-5.26000(*)	.71177	.000	-6.7771	-3.7429
0.9:0.1	1:0	1.72750(*)	.71177	.028	.2104	3.2446
	0.8:0.2	-.88000	.71177	.235	-2.3971	.6371
	0.7:0.3	-1.69250(*)	.71177	.031	-3.2096	-.1754
	0.6:0.4	-3.53250(*)	.71177	.000	-5.0496	-2.0154
0.8:0.2	1:0	2.60750(*)	.71177	.002	1.0904	4.1246
	0.9:0.1	.88000	.71177	.235	-.6371	2.3971
	0.7:0.3	-.81250	.71177	.272	-2.3296	.7046
	0.6:0.4	-2.65250(*)	.71177	.002	-4.1696	-1.1354
0.7:0.3	1:0	3.42000(*)	.71177	.000	1.9029	4.9371
	0.9:0.1	1.69250(*)	.71177	.031	.1754	3.2096
	0.8:0.2	.81250	.71177	.272	-.7046	2.3296
	0.6:0.4	-1.84000(*)	.71177	.021	-3.3571	-.3229
0.6:0.4	1:0	5.26000(*)	.71177	.000	3.7429	6.7771
	0.9:0.1	3.53250(*)	.71177	.000	2.0154	5.0496
	0.8:0.2	2.65250(*)	.71177	.002	1.1354	4.1696
	0.7:0.3	1.84000(*)	.71177	.021	.3229	3.3571

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

13. Analysis of variance between different ratios of mixed soil and zinc accumulation in leaf.

Table B-13.1 Descriptive statistics

Items	Mixed soil ratios	Mean	Std.Deviation	N
Zinc	1:0	19.1839	3.59332	4
	0.9:0.1	21.1181	1.77111	4
	0.8:0.2	25.1456	1.65011	4
	0.7:0.3	30.5589	4.94382	4
	0.6:0.4	32.9448	7.33777	4
	Total	25.7902		20

Table B-13.2 One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	559.262	4	139.816	7.203	.002
Within Groups	291.167	15	19.411		
Total	850.430	19			

Table B-13.3 Multiple comparisons between different ratios of mixed soil and zinc accumulation in leaf.

Dependent Variable: zinc

LSD

(I) name	(J) name	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1:0	0.9:0.1	-1.93419	3.11538	.544	-8.5745	4.7061
	0.8:0.2	-5.96169	3.11538	.075	-12.6020	.6786
	0.7:0.3	-11.37498(*)	3.11538	.002	-18.0153	-4.7347
	0.6:0.4	-13.76087(*)	3.11538	.000	-20.4011	-7.1206
0.9:0.1	1:0	1.93419	3.11538	.544	-4.7061	8.5745
	0.8:0.2	-4.02750	3.11538	.216	-10.6678	2.6128
	0.7:0.3	-9.44079(*)	3.11538	.008	-16.0811	-2.8005
	0.6:0.4	-11.82668(*)	3.11538	.002	-18.4670	-5.1864
0.8:0.2	1:0	5.96169	3.11538	.075	-.6786	12.6020
	0.9:0.1	4.02750	3.11538	.216	-2.6128	10.6678
	0.7:0.3	-5.41329	3.11538	.103	-12.0536	1.2270
	0.6:0.4	-7.79918(*)	3.11538	.024	-14.4395	-1.1589
0.7:0.3	1:0	11.37498(*)	3.11538	.002	4.7347	18.0153
	0.9:0.1	9.44079(*)	3.11538	.008	2.8005	16.0811
	0.8:0.2	5.41329	3.11538	.103	-1.2270	12.0536
	0.6:0.4	-2.38589	3.11538	.456	-9.0262	4.2544
0.6:0.4	1:0	13.76087(*)	3.11538	.000	7.1206	20.4011
	0.9:0.1	11.82668(*)	3.11538	.002	5.1864	18.4670
	0.8:0.2	7.79918(*)	3.11538	.024	1.1589	14.4395
	0.7:0.3	2.38589	3.11538	.456	-4.2544	9.0262

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

APPENDIX C
SOIL QUALITY STANDARDS FOR HABITAT AND AGRICULTURE

Parameter	Standard value (mg/kg)
1. Volatile organic compounds	
1) Benzene	Not exceed 6.5
2) Carboon Tetrachloride	Not exceed 2.5
3) 1,2 – Dichloroethane	Not exceed 3.5
4) 1,1 -Dicholroehtylene	Not exceed 0.5
5) cis-1,2- Dichloroethylene	Not exceed 43
6) trans-1,2- Dichloroethylene	Not exceed 63
7) Dichloromethane	Not exceed 89
8) Ethylbenzene	Not exceed 230
9) Styrene	Not exceed 1,700
10) Tetrachloroethylene	Not exceed 57
11) Toluene	Not exceed 520
12) Trichloroetyhlene	Not exceed 28
13) 1,1,1 –Trichloroethane	Not exceed 630
14) 1,1,2 Trichloroethane	Not exceed 8.4
15) Total Xylene	Not exceed 210
2. Heavy metal	
1) Arsenic	Not exceed 3.9
2) Cadmium and compounds	Not exceed 37
3) Hexavalent Chromium	Not exceed 300
4) Lead	Not exceed 400

SOIL QUALITY STANDARDS FOR HABITAT AND AGRICULTURE (Continued)

Parameter	Standard value (mg/kg)
3. Pesticides	
1) Aitrazine	Not exceed 22
2) Chlordane	Not exceed 16
3) 2,4 –D	Not exceed 690
4) DDT	Not exceed 17
5) Dieldrin	Not exceed 0.3
6) Heptachlor	Not exceed 1.1
7) Heptachlor Epoxide	Not exceed 0.5
8) Lindane	Not exceed 4.4
9) Pentachlorophenol	Not exceed 30
4. Others	
1) Benzo (a) pyrene	Not exceed 0.6
2) Cyanide and compounds	Not exceed 11
3) PCBs	Not exceed 2.2
4) Vinyl Chloride	Not exceed 1.5

Source: Notification of National Environmental Board No. 25, B.E. 2004

APPENDIX D
FOOD CONTAMINATED STANDARDS FOR CONSUMERS

Parameter	Standard value (mg/kg)
Arsenic	Not exceed 2
Copper	Not exceed 20
Lead	Not exceed 1
Mercury	Not exceed 0.5
Tin	Not exceed 250
Zinc	Not exceed 100

Source: Food and Drug Administration, B.E. 1987

APPENDIX E
SOIL QUALITY STANDARDS FOR HABITAT AND AGRICULTURE (Europe region)

Country	**Heavy metal (mg/kg)**	
	Zn	**Cu**
England	280	140
German	300	100
France	300	100
Holland	100	50

Source: Daoroong Sungthong, B.E. 1996

APPENDIX F
PICTURES

Figure F-1 Sida soil from Jatujak market

Figure F-2 Bottom ash from Mae-Moh Mine

Figure F-3 Lettuce shelf

Figure F-4 Rinse lettuce by tap water

Figure F- 5 Pack lettuce in plastic bag and bring it to hot air oven

Figure F-6 Root and leaf in plastic bag after dry in hot air oven at 65 °C for 48 hours

Figure F-7 Digester blocks

Figure F-8 Atomic Absorption Spectrophotometer

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