POTENTIAL IMPACTS OF HEAVY METALS FROM THE PAPER SLUDGE – DERIVED FLOWERPOT

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POTENTIAL IMPACTS OF HEAVY METALS FROM THE PAPER SLUDGE – DERIVED FLOWERPOT

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ABSTRACT

The purpose of this research was to study potential impacts of Pb, Ni, and Cu from sludge-derived flowerpots (flowerpots made from paper mill sludge or PS-D flowerpot). Two types of the flowerpots were tested, without paraffin coating and coating with paraffin. The concentrations of leaching, percentage of seedling of Chinese kale, and total concentrations of the heavy metals accumulated in Chinese kale were determined. Planting materials used for growing Chinese kale included mixtures of various ratios of soil and crushed PS-D flowerpots.

Leaching levels of Pb and Cu in PS-D flowerpots without paraffin coating were higher than in those with paraffin coating. The leaching level of Ni from both types of flowerpot was lower than the detection limit of the instrument. However, leaching levels of all heavy metals were below the standard values for selected heavy metals based on leaching test. Therefore, leaching heavy metals from this PS-D flowerpot will not have an adverse effect in the environment.

Seedlings of Chinese kale (*Brassica oleracea var. alboglabra*) were assessed by cultivating 50 seeds in various mixtures of planting materials. The mixtures were 1) 100 % of soil; 2) 50 % of soil with 50 % of PS-D flowerpot without paraffin; 3) 100% of PS-D flowerpot without paraffin; 4) 50% of soil with 50% of PS-D flowerpot with paraffin; and 5) 100% of PS-D flowerpot with paraffin. The highest percentage of seedlings of Chinese kale, 89.3 %, was observed in the test using 100% crushed PS-D flowerpot without paraffin coating.

The analysis of accumulated heavy metals in the shoots and roots of Chinese kale showed that the levels of Pb exceeded the recommended maximum level of metal concentrations in leafy vegetable; while those of Ni and Cu did not. Thus, using PS-D flowerpots for growing edible plants should not be recommended.

KEY WORDS: PAPER MILL SLUDGE / PAPER SLUDGE-DERIVED FLOWERPOT / HEAVY METALS/ LEACHING / CHINESE KALE

102 pp.

ผลกระทบของโลหะหนักจากกระถางที่ผลิตจากกากตะกอนเยื่อกระดาษ
(POTENTIAL IMPACTS OF HEAVY METALS FROM THE PAPER SLUDGE-DERIVED FLOWERPOT)

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลกระทบของตะกั่ว นิกเกิล และทองแดง จากกระถางที่ผลิตจากกากตะกอนเยื่อกระดาษ ซึ่งมี 2 ชนิดคือกระถางชนิดที่ไม่ได้เคลือบพาราฟินและเคลือบด้วยพาราฟิน โดยศึกษาปริมาณโลหะหนักที่ถูกชะออกมา เปอร์เซ็นต์การงอกของเมล็ดคะน้าและปริมาณโลหะหนักที่สะสมในคะน้าที่ทำการปลูกในกระบะทดลองโดยมีส่วนผสมของวัสดุปลูกเป็นกระถางที่บดแล้วดังกล่าว

ปริมาณตะกั่วและทองแดงที่ถูกชะออกมาจากกระถางชนิดที่ไม่ได้เคลือบด้วยพาราฟินจะมีค่าสูงกว่าชนิดที่เคลือบด้วยพาราฟิน ส่วนปริมาณนิกเกิลที่ถูกชะออกมามีปริมาณน้อยมาก แต่ทั้งนี้ปริมาณโลหะหนักที่ถูกชะออกมาเมื่อเทียบกับค่ามาตรฐานโลหะหนักและสารพิษจากการทดสอบการชะแล้วพบว่ามีค่าไม่เกินเกณฑ์มาตรฐานที่กำหนด

ส่วนในด้านของผลกระทบต่อการงอกของเมล็ดคะน้า ศึกษาโดยการหว่านเมล็ดลงในกระบะทดลองที่มีการเตรียมวัสดุปลูก โดยมีอัตราส่วน (โดยน้ำหนัก) ดังนี้ 1.) ดิน 100 % 2.) ดิน 50 % ผสมกับกระถางชนิดที่ไม่เคลือบด้วยพาราฟิน 50% 3.) กระถางชนิดที่ไม่เคลือบด้วยพาราฟิน 100% 4.) ดิน50% ผสมกับกระถางชนิดที่เคลือบด้วยพาราฟิน 50% 5.) กระถางชนิดที่เคลือบด้วยพาราฟิน 100% ผลพบว่าเปอร์เซ็นต์การงอกของเมล็ดสูงสุดซึ่งคือ 89.3% เมื่อปลูกด้วยวัสดุปลูกคือกระถางชนิดที่ไม่ได้เคลือบด้วยพาราฟิน 100% ที่บดละเอียด

การศึกษาปริมาณตะกั่ว นิกเกิล และทองแดงที่สะสมในคะน้า ทำโดยการเก็บเกี่ยวคะน้าในกระบะทดลองแล้ววิเคราะห์หาปริมาณโลหะหนักในส่วนของลำต้นและราก พบว่าส่วนลำต้นมีปริมาณตะกั่วที่สูงกว่าค่าที่แนะนำให้มีได้ในพืชกินใบ ในขณะที่ปริมาณของนิกเกิลและทองแดงไม่เกินค่าที่แนะนำดังกล่าวข้างต้น ดังนั้นกระถางที่ผลิตจากกากตะกอนเยื่อกระดาษจึงไม่ควรนำมาใช้ปลูกพืชที่รับประทานได้

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LIST OF ABBREVIATIONS

AA = Atomic Absorption Spectrophotometry
Cu = Copper
mg = milligram
mg/l = milligram per liter
mg/kg = milligram per kilogram
Ni = Nickel
Pb = Lead
PS-D = Paper sludge derived

CHAPTER I

INTRODUCTION

1.1 Background

Nowadays, paper industry is important and related to many activities. For example, packaging (kraft paper) or education (write paper). One of the elements of material for making paper is fiber that comes from two sources. One is fiber of plant and the other comes from old paper. From the process of paper making, a lot of fiber will be discharged to wastewater treatment plant from which the excessive sludge will be dewatered and become sludge cake. Many of these sludge cake will dumped on landfills which may have some effects on landfills such as changing soil characteristics, due to heavy metals contained in the sludge cake striking onto soil layers. The contaminants will also be absorbed by the rain or groundwater; and some will be dispersed into the environment. Therefore, wastewater sludge should be properly managed to protect the environment.

The paper sludge–derived flowerpot (PS-D flowerpot) has come from this concept. Not only that it will decrease the sludge amount taken to landfills, but also make additional income to the industry. However, the sludge itself cannot be made into the flowerpot shape due to recycled fiber composition in it. It has to be mixed with clay with the optimum ratio of clay to sludge of 2:1 by weight. Although PS-D flowerpot can be digested in the ground, it is contaminated with toxic heavy metals such as lead, nickel and copper from the process of making paper. This has caused some concerns when using the flowerpot to grow plants due to some adverse effects these contaminants may have on human, animal, and plant. Therefore, it is important to assess the impact of these heavy metals contaminated in the flowerpot.

This study was carried out to determine the concentrations of heavy metals that may leach out from the flowerpot to affect seed cultivation, and to accumulate in plants.

1.2 Objectives of the study

1.2.1 Main objective

To assess the potential impact of heavy metals from the use of paper sludge-derived flowerpot (PS-D flowerpot).

1.2.2 Specific objectives

1. To determine concentrations of lead, nickel, and copper leaching from the PS-D flowerpot without paraffin coating and with paraffin coating.

2. To determine percentage of Chinese kale (*Brassica oleracea var. alboglabra*) seed cultivation under conditions of different mixture ratio of soil and crushed PS-D flowerpot without paraffin coating and with paraffin coating.

3. To determine concentrations of lead , nickel, and copper that accumulated in the shoot of Chinese kale (*Brassica oleracea var.alboglabra*) cultivated under conditions of different mixture ratio of soil and crushed PS-D flowerpot without paraffin coating and with paraffin coating.

4. To determine concentrations of lead, nickel, and copper that accumulated in the root of Chinese kale (*Brassica oleracea var.alboglabra*) cultivated under conditions of different mixture ratio of soil and crushed PS-D flowerpot without paraffin coating and with paraffin coating.

1.3 Hypothesis of the study

1. Concentrations of lead, nickel, and copper leaching from the flowerpot without coating paraffin will be higher than those from the PS-D flowerpot coating with paraffin.

2. Percentage of Chinese kale (*Brassica oleracea var.alboglabra*) seed cultivation will be decrease when the ratio of crushed PS-D flowerpot is increased in the mixture of cultivating condition.

3. Concentrations of lead, nickel, and copper that accumulated in the shoot of Chinese kale (*Brassica oleracea var. alboglabra*) will be higher when cultivated in higher ratio of crushed PS-D flowerpots.

4. Concentrations of lead, nickel, and copper that accumulated in the root of Chinese kale (*Brassica oleracea var. alboglabra*) will be higher when cultivated in higher ratio of crushed PS-D flowerpots.

1.4 Research Variables

1.4.1 Independent Variables

1. ratio of soil and crushed PS-D flowerpot
2. types of PS-D flowerpot (without coating paraffin and coating paraffin)

1.4.2 Dependent Variables

1. Seed cultivation percentage
2. Lead, nickel, and copper leaching concentrations
3. Concentrations of Lead, nickel, and copper in the shoot and root of Chinese kale (*Brassica oleracea var. alboglabra*)

1.4.3 Control Condition

1. Watering: 2 times (in morning and evening)
   volume : 200 ml each time
2. Protect sampling plot from the rain using clear plastic cover
3. Harvest: 45 days after cultivation.

1.5 Scope of the study

1. PS-D flowerpot was made from the mixture between clay and paper mill sludge cake with the ratio of 2:1.

2. Two types of PS-D flowerpot to be tested are PS-D flowerpot without coating and that coating with paraffin.

3. Toxic materials leached out from the PS-D flowerpot would be analyzed for lead, nickel, and copper only. Levels of lead, nickel and copper in PS-D flowerpot were selected to study as representative metals, due to high concentrations of three types of heavy metals (Pb, Ni and Cu) contaminated in materials of making paper (old fiber) such as old newspaper

4. Type of seed used in seed cultivation test is Chinese kale (*Brassica oleracea var. alboglabra*) from Chia Tai Co., Ltd.

5. Chuan Chom soil brand from Lopburi province was used to grow plant in this research. This type of soil is for commercial use.

1.6 Definition of Keywords

1. **Paper mill sludge cake:** excess sludge dewatered by screw press machine from paper industry.

2. **Paper sludge-derived flowerpots (PS-D Flowerpot):** flowerpot made from paper mill sludge and clay in ratio of clay : paper mill sludge 2 to 1. In this case, there are 2 types of flowerpot, without coating and coating with paraffin.

3. **Leaching Test**: method to determine heavy metals leached out from the PS-D flowerpot.

4. **Seed cultivation test:** test for the effect of heavy metals on seed cultivation. Fifty seeds were cultivated on crushed PS-D flowerpot for growing and collected number of seed that grow.

5. **Heavy metals accumulation in plant test:** method to determine total concentrations of heavy metals accumulated in plant**,** Pb, Ni and Cu in the shoot and root of plant.

6. **Shoot of plant:** combination of stalk and leaf of plant.

7. **Foliar absorption:** absorption of micronutrients into plant by leaf system such as cutical.

8. **Soil:** soil of Chuan Chom brand from Lopburi province.

1.7 Conceptual Framework

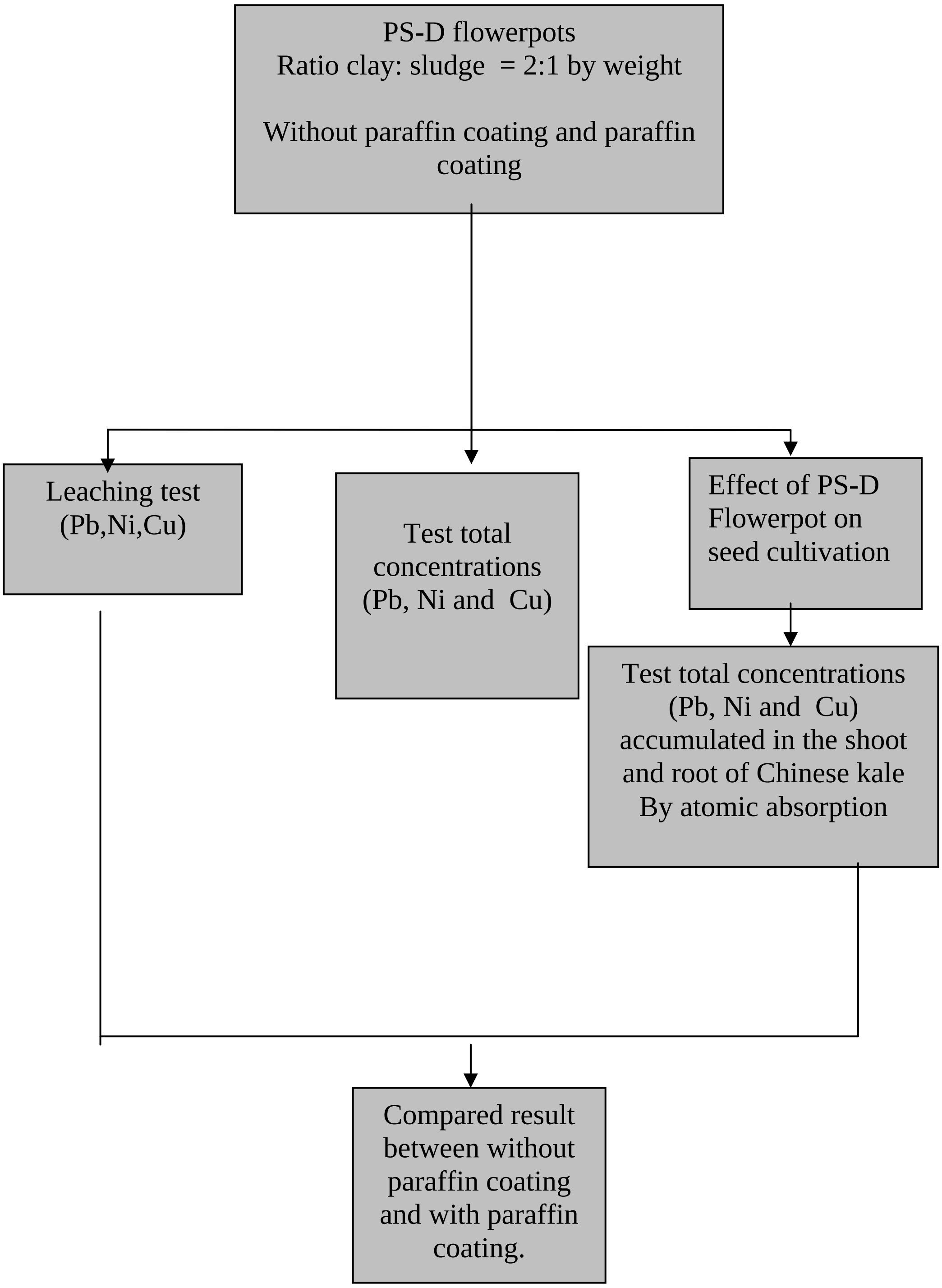


Figure 1.1 Conceptual Framework

CHAPTER II

LITERATURE REVIEW

2.1 Paper Mill Sludge Cakes

2.1.1 Pulp and Paper mill waste and treatment

In Thailand, most industries use a lot of water especially groundwater for production line due to lower price and better quality comparing to tap water and also available steady flow rate input. Industrial process usually produce a great amount of wastewater. Nowadays the industrial managers realize that wastewater would be able to damage river or water resources, if it is not properly treated before release into the receiving water body. So they construct wastewater treatment by using physical, chemical and biological treatment to comply with effluent standard before discharging effluent to the river. It looks quite good but wastewater treatment such as activated sludge and chemical precipitation only change or transfer major impurities into excess sludge sediment (Figure 2.1 & 2.2).

These excess sludge and precipitated toxic solids need further treatment. In general for industrial in Thailand, the processes consist of sludge thickening (by gravitational force), sludge conditioning such as put alum and ferric chloride into the sludge, sludge stabilization such as digester, sludge dewatering such as belt press and filter press, etc (Metcalf & Eddy, 2003). So excess sludge which sometimes include both organic matter and microorganisms and heavy metals (Lead, Copper, Zinc) will cause serious damage to water resources or agricultural area again if these sludge are not properly managed or disposed of.

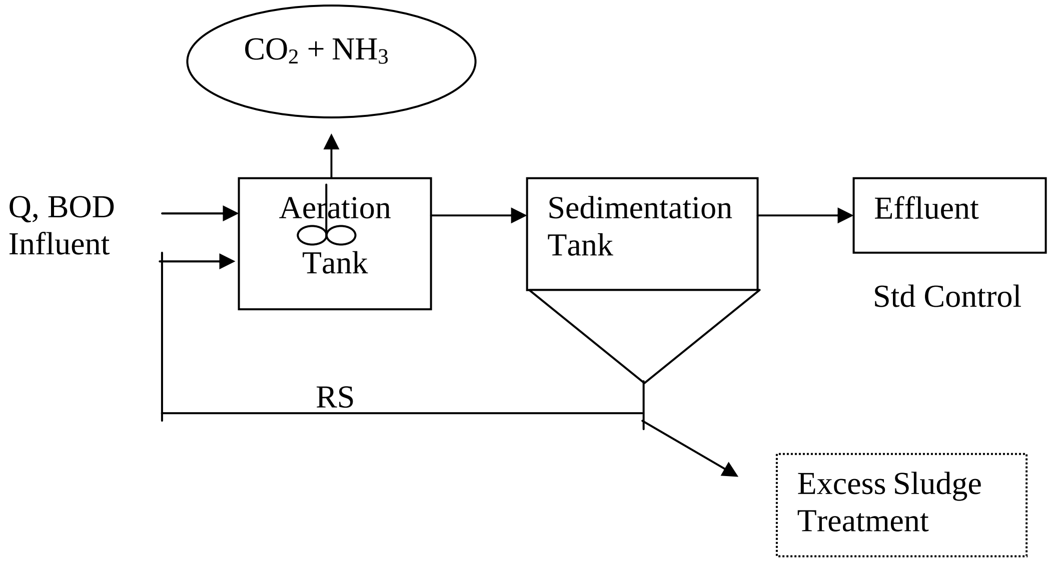


Figure 2.1 Flow diagram of activated sludge for wastewater treatment

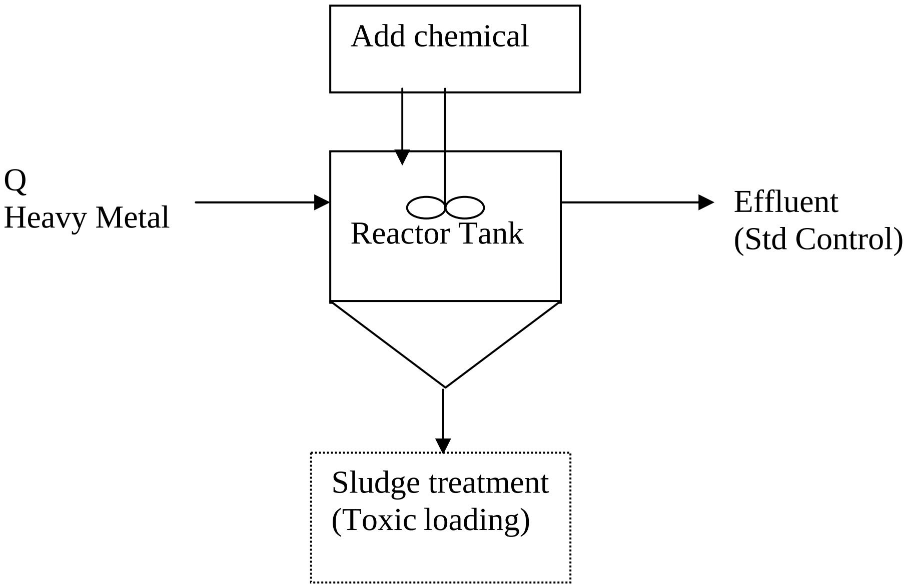


Figure 2.2 Batch scale (chemical precipitation) of wastewater treatment

The major portion of the pollution from paper making industry originates in the pulping processes. Raw materials are reduced to a fibrous pulp by either mechanical or chemical means. The bare is mechanically or hydraulically removed from wood before it is reduced to chips for cooking. Mechanically prepared (groundwood) pulp is made by grinding the wood on large emery or sandstone wheels and then carring it by water through screens. This type of pulp is low grade, usually highly colored, and contains relatively short fibers; it is mainly used to manufacture nondurable paper products such as newspaper. The screened bark effuent contains fine particles of bark and wood and some dissolved solids. Additional sources of waste from wood preparation are the pressing of rejects prior to burning and floor drainnings (Nelson, 1991).

The chief sources of waste at the pulp mills are the digester liquors and the chief sources at the paper mills are the beaters and paper machines. Fiber losses generally average 3 percent.

The current trends in waste treatment in the pulp and paper industry may be summarized in ten areas as follows:

1. In-plant changes have been successful in re-ducing both strength and quantity of waste. Extensive research is being undertaken on reuse and by-product recovery.
2. Disposal of spent liquor by deep well disposal or by conversion to salable products has been reported.
3. Sedimentation, flotation, and thickening: there has been considerable use of moving screens. In all of these processes, more and more attention is being paid to fiber recovery.
4. Chemical coagulation: contact flocculation, alum or ferrous salts, and activated silica have proved effective.
5. Solids handling: vacuum filtration, centrifugation, thickening, straining, pressing, incineration, wet-air oxidation, and landfill have been used.
6. Treatment of receiving waters: the paper industry, perhaps more than any other, has been emphasizing consideration of such techniques as: impoundment, intermittent storage at low flow, diffusion of effluent, mechanical aerators within the stream, etc.
7. Biological treatment: activated sludge (and all modification), trickling filtration, aerated lagoons, and anaerobic treatment are all being utilized, with the emphasis on activated sludge and aerated lagoons.

8. Irrigation disposal: excellent results have been reported on a number of crops.
9. Color removal: activated carbon adsorption.
10. Foam separation

2.2 Characteristics of paper mill sludge cakes

Sludge analysis which was conducted by The Office of Public Health and Environmental Technology Services, Faculty of Public Health, Mahidol University showed characteristics of sludge cake:

pH = 8.2
% Moisture = 40.5
% Ash = 23
Oil & Grease (mg/L) = 11.25
Pb (mg/L) = 0.556
Ni (mg/L) = 0.113
Cu (mg/L) = 1.006

The main feature is the percent sludge ash content, which has its average nearly 23%; it means that volatile solids is 77%. It also shows that the fiber left in the sludge cake is in the same amount. Almost all of such fibers should be short fiber and recycle fiber that pass through the wire screen in the production line at sheet forming part.

To test further, the sludge cake was analyzed using the electron microscope as shown in Figure 2.3. Many fibers were presented in the sludge cake as can be seen from this figure .It means that sludge cake can be brought back to make a flowerpot due to fibers can attach together and form a shape (Kongmuang U., 2003).

2.3 Utilization of paper mill sludge cakes

Disposal options for paper mill sludge can be one of the following five categories. These include the followings:

- Agricultural uses either as fertilizer or soil conditioner,
- Landfill disposal
- Land reclamation
- Incineration and
- Recycling and reuse options.

In this case, it is interesting to study about using sludge cake as soil conditioner, as the flowerpots will be added into soil; and to study about landfill disposal.

2.3.1 Use of sludge cake as soil conditioner

Amendments in agricultural production exemplify a strategy for converting wastes to resources including nutrient recycling. Paper mill sludge is one of the largest under utilized organic byproducts in Wisconsin . Use of paper mill sludge or paper mill sludge in annual crop production systems holds promise from a soil quality perspective, particularly in course textured sandy soils where increases in organic matter content can improve soil chemical, biological and physical properties. Land application of paper sludge can benefit the soil in many ways. It can add valuable nutrients to the soil such as nitrogen, phosphorus, potassium, and trace elements (Agri-Tech Inc, 2000).

John Peters from University of Wisconsin studied (1986 - present) the use of paper mill sludge from consolidated paper in a potato-sweet corn rotation supply either all or 50% of crop N needs. Results showed that approximately 25% of the total N content of Paper Sludge (PS) became available over the growing season from PS with a low C:N ratio (approximately 20:1). Bowen et al., (1985) studied using PS and PS compost in potato production showed that pulp sludge amended potato plots could produce yields equivalent to or higher than those receiving 200 lbs.N/ acre of

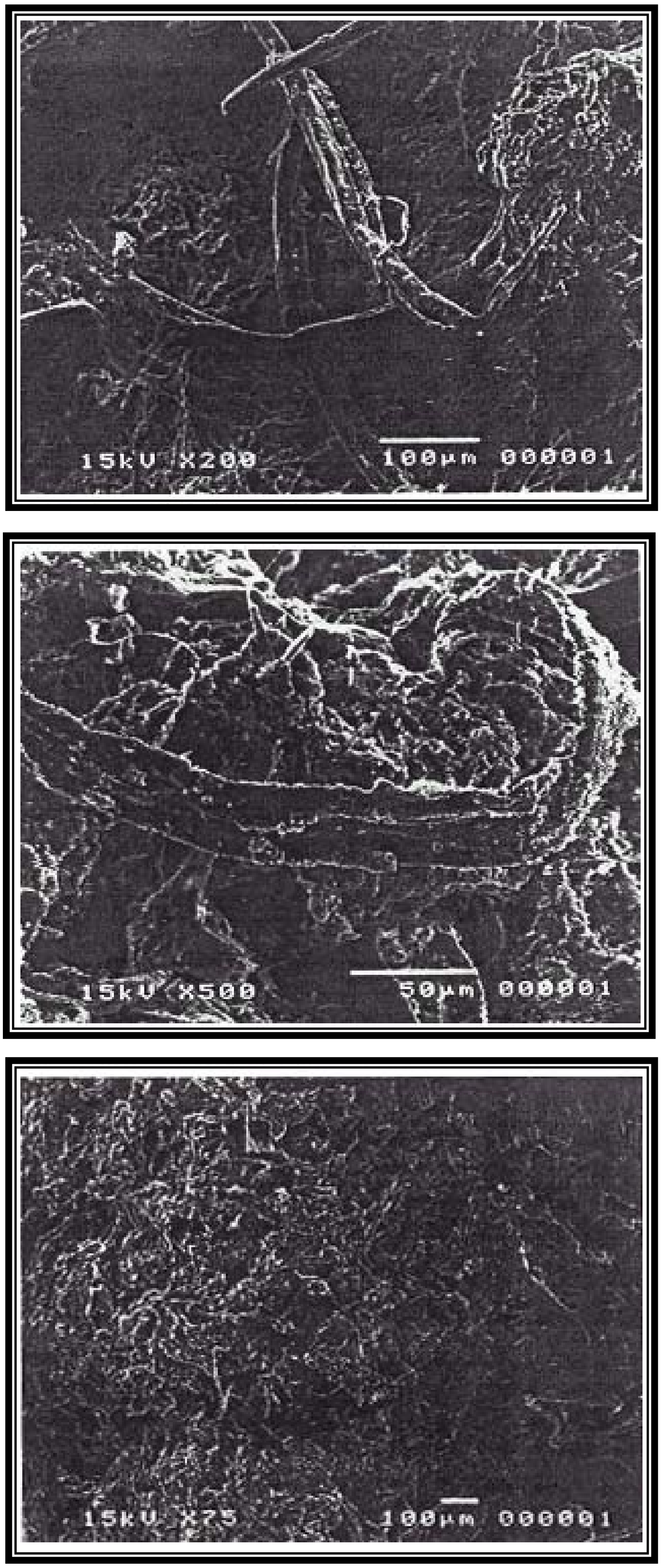


Figure 2.3 Fibers in sludge cake from scanning electron microscope

commercial fertilizer. It is quite clear from previous research did at field experiments that PS or PS compost could supply nitrogen to potato crops and PS itself was as good as commercial fertilizers when rate of application was high enough .

However, many vegetables processing plants will not accept crop growth with paper mill sludge because of growing concern for possible crop contamination due to organic chemical residues and trace metals remained. So PS has some restrictions in use only for non-vegetable field. Georgia’s Pulp and Paper Industry had continued project for year 1998 to recycling sludge to forestland. Recycling these materials to agricultural and forestland provides the potential for beneficial use of these materials (Energy Products, 1999).

The pulp and paper mill industry in Canada in the past had two methods to eliminate sludge cake by landfilling and incineration. At this time the most widely used methods of disposal, and in view of a growing trend in paper use, alternative methods of disposal are needed. Paper mill sludge contains significant amount of plant nutrients and are a widely available resource for composting, reducing landfilling and limiting greenhouse gas emission. Spectroscopic data revealed that the major components identified in the paper mill sludge were lipids, sterols, lignins, nitrogen compounds, and carbohydrates. Recycling of paper mill sludge by means of composting is becoming an acceptable practice for converting organic residues into useful soil amendments, while eliminating negative environmental impact (Smook G.A., 1982). Georgia’s Pulp and Paper Industry also had a new program for year 1998 proposed to develop and demonstrate the use of a controlled bioconversion process that can convert industrial solid wastes (sludge, wood yard debris, and ash) to environmentally sound value-added products for use in agriculture as a soil amendment (compost) and plant growth medium (Energy Products, 1999).

2.4. Paraffin and Toxicity

Paraffin is obtained from petroleum by distillation and is then purified by sweating or solvent refining. A waxy crystalline substance that in the pure form is white, more-or-less translucent, odorless, tasteless, waxy solid. It melts between 47°C and 65°C and is insoluble in water but soluble in ether, benzene, and certain esters

(Town and Laurence, 1951). Paraffin is unaffected by most common chemical reagents but burns readily in air. Obtained from petroleum during refining, it is used in candles, for coating paper, and for various other purposes. Chemically, paraffin is a mixture of high-molecular-weight alkanes , i.e., saturated hydrocarbons with the general formula $C_n H_{2n+2}$, where n is an integer between 22 and 27 (Johnson and Pauline, 1963).

2.4.1 Uses

Paraffin is usually used for the followings:

- Candlemaking
- Coatings for waxed paper or cloth.
- Coating for many kinds of hard cheese, like Edam cheese.
- Preparing specimens for histology.
- Solid propellant for hybrid rockets
- Sealing jars, cans, and bottles
- In dermatology, as an emollient (moisturiser)
- Surfing, for grip on surfboards as a component of surfwax.
- As a food additive

2.4.2 Toxicity

Petroleum – based (mineral) waxes normally will float on water. In stagnant or slow - flowing waterways, a wax layer can reduce the atmospheric oxygen exchange with the water system. If the wax layer is not removed, oxygen depletion can result in loss of marine life.

2.5. Heavy Metals and Their Impacts

Heavy metal is refered to an element which has atomic number higher than calcium (atomic number is 20) . These heavy metals have toxicity both in ions and complex forms. Different metals will be discharged from different kind of industries (Dean J.G., Bosqui F.L. and Laouette K.H.,1972).

2.5.1 Lead

Identity, physical and chemical properties

Lead is a bluish or silvery grey soft metal. Lead has 4 electrons in its balance shell, only 2 ionize readily. The usual Oxidation State of lead in inorganic compounds is, therefore, +2 rather than +4. The inorganic salts of lead (II), lead sulfide, and the oxides of lead are generally poorly soluble.

Source of human and environmental exposure

Mining, smelting and refining as well as the manufacture of lead containing compounds can give rise to lead emissions. The manufacture of electric storage batteries is responsible for the largest consumption of lead. This industry uses both metallic lead in the form of a lead – antimony alloy, and lead oxides in about equal proportions. About environmental transport and distribution of lead, sewage sludge is currently being considered for use as fertilizer. However, lead is not currently viewed a hazard in this case because sludge have a high phosphate content which tends to minimize the bio – availability of the lead for plants (Chaney, 1973).

Effects on humans and plants

Lead is capable of causing an adverse effect to humans and plants. Exposure to lead may arise through the following pathways :

1. Direct soil ingestion
2. Inhalation of suspended particulate matter
3. Dermal absorption from dust and dirt deposited to the skin
4. Ingestion of backyard garden produce grown in contaminated soil
5. Ingestion of contaminated groundwater where groundwater serves as a source of drinking water and where evidence demonstrates off-site migration of groundwater contamination from the INCO property and/or where surface-deposited lead contamination from the INCO stack percolates down to the water table.

The types of health and phytotoxicity impacts expected due to lead exposure are as follows :

Inhalation exposure

Hematological Effects : Lead inhibits the activity of enzymes ALAD and ferrochelatase which affects heme biosynthesis.

Oral exposure

Hematological Effects : Same as in inhalation exposure.

Renal Effects : Renal insufficiency, reflected by raised serum urea concentrations and hyperuricemia.

Occupational exposure

Colic is a well-recognized symptom of acute lead poisoning and is still reported in groups of lead-exposed industrial workers. Symptoms of colic include abdominal pain, constipation, cramps, nausea, vomiting, anorexia, weight loss and decreased appetite (Richardson M, 1992).

Exposure of children

Colic is seen in children and US EPA concluded that the lowest observed adverse effect level was in the range of 2.88-4.80 micromole/lit.

Lead exposure determinated by elevated blood Pb levels

Cardiovascular Effects : Increase in systolic and diastolic blood pressure, degenerative changes in myocardium, electrocardiogram abnormalities in children, ischemic electrocardiogram changes.

Hematological Effects : Enzyme effect: Increased ALAS and/or decrease ALAD, increased urinary or blood ALA, EP, ZPP, urinary coproporphyrin, decreased hemoglobin and resulting anaemia (Richardson M,1992).

Hepatic Effects : Decreased mixed function oxidase activity.

Renal Effects : Chronic nephropathy, gout.

Musculoskeletal Effects : Growth retardation in children.

Neurological Effects : Decreased performance on neurobehavioral tests, altered auditory evoked potential latency and decreased hearing acuity in children. Impaired postural balance. Peripheral neuropathy and reduced conduction velocity in children.

Developmental Effects : Reduced birth weight and/or reduced gestational age, and/or increased incidence of still birth and/or neonatal death. Impaired mental development in children, impaired motor development.

Reproductive Effects : Decreased fertility (low sperm count, decreased sperm mobility, abnormal sperm), increased incedence of miscarriages and stillbirths.

Phytotoxic effects

Relatively low toxicity, it mainly affects mitochondrial respiration and photosynthesis by disturbing electron transfer reactions. Corn root length reduces 48% in 500 ppm Pb. Wheat root weight reduces 22% in 1000 ppm.

A guideline for lead in soil of 200 mg/kg soil, to protect against potential non-cancer human health risks, has been established by the Ontario Ministry of Environment. Ontario has not established a guideline for lead to prevent phytotoxicity. However, a guideline for lead in soil of 400 mg/kg, to prevent phytotoxic effects, has been established by the Canadian Council of Ministers of Environment.

2.5.2 Nickel

Identity, physical, and chemical properties

Nickel is a metallic element belonging to group VIII B of the periodic table. It is resistant to alkalis, but generally dissolves in dilute oxidizing acids. Nickel carbonate, nickel sulfide, and nickel oxide are insoluble in water, whereas nickel chloride, nickel sulfate, and nickel nitrate are water-soluble.

Sources of human and environmental exposure

Nickel is a ubiquitous trace metal and occurs in soil, water air and in the biosphere. The average content in the earth's crust is about 0.008% (Mason, 1952). Farm soils contain between 3 and 1000 mg nickel/kg (Nas, 1975). Most of nickel is used for the production of stainless steel and other nickel alloys with high corrosion and temperature resistance. Nickel from various industrial processes and other sources finally reach wastewater. The electroplating industry was found to be the dominant source of nickel (62 %) in wastewater treatment plants (Klein et al.,1974). Chen et al.(1974) reported a removal efficiency of the secondary treatment process (activated sludge system) of 25 – 57%. The final effluent contained of 0.14 – 0.177 mg nickel / litres. Finally, residues from wastewater treatment are disposed of by deep – well injection, ocean dumping, land treatment or incineration.

Effects on humans and plants

Nickel is capable of causing an adverse effect to humans and plants. Exposure to nickel may arise through the following pathways:

1. Direct soil ingestion
2. Inhalation of suspended particulate matter
3. Dermal absorption from dust and dirt deposited to the skin
4. Ingestion of backyard garden produce grown in contaminated soil
5. Ingestion of contaminated groundwater where groundwater serves as a source of drinking water and where evidence demonstrates off-site migration of groundwater contamination from the INCO property and/or where surface-deposited nickel contamination from the INCO stack percolates down to the water table (Richardson M,1992).

The types of health and phytotoxicity impacts expected due to nickel exposure are as follows :

Inhalation exposure

Respiratory Effects : The respiratory system is the primary target of nickel toxicity following inhalation exposure. Increased incidence in deaths from respiratory disease has been found with exposure greater than 0.04 mg Ni/m^3 of nickel oxide or metallic nickel. Asthma occurs either from respiratory irritation, or as a result of allergic response.

Reproductive Effects : Increase incidence of spontaneous abortions may be associated with exposure to nickel compounds.

Carcinogenic Effects : Lung and nasal and throat cancers. Cancers were related primarily to exposure to less soluble compounds: oxidic and sulfidic. Classified as a Group 1 carcinogen by Health Canada (Richardson M, 1992).

Oral exposure

Dermal Effects : Contact dermatitis is the most prevalent effect of nickel in general population. Single oral dose of nickel can result in a flare-up in the dermatitis. Dose as low as 0.08 mg/kg, Ni will cause a reaction in nearly all cases.

Immunological and Lymphorticular Effects : Dermatitis resulting from nickel allergy (Richardson M,1992).

Dermal exposure

Dermal Effects : Allergy to nickel is the most frequent contact allergy in women.

Immunological and Lymphorticular Effects : Contact dermatitis resulting from nickel allergy (Richardson M, 1992).

Phytotoxic effects

Symptoms of Ni toxicity are generally Fe deficiency induced chlorosis and foliar necrosis. Excess nickel effects nutrient absorption by roots, root development, and metabolism, and it inhibits photosynthesis and transpiration. Nickel can replace Co and other heavy metals located at active sites in metallo-enzymes and disrupt their functioning.

Quantified outcomes of nickel toxicity (among others):
Ryegrass shoot weight – 180 ppm Ni – reduction by 66%.
Oats – 50 ppm Ni – 30% reduction in grain, 63% reduction in straw weight.
Corn – 294 ppm Ni – 21% reduction in plant weight.

General signs of nickel toxicity are reduced growth of roots and shoots, poor branching, deformation of various plant parts, decreased dry matter production, leaf spotting, abnormal flower shape, germination inhibition, and chlorosis that can lead to foliar necrosis. Reduced yields in celery, lettuce, radish, cabbage, and beets when exposed to 15 – 94 ppm Ni.

A guideline for nickel in soil of 310 mg/kg soil, to protect against potential non-cancer human health risks, has been established by the Ontario Ministry of Environment. A guideline of 200 mg/kg soil has also been established by the Ontario Ministry of Environment to protect against phytotoxicity (Richardson M,1992).

2.5.3 Copper

Identity, physical, and chemical properties.

Copper is a reddish – brown, ductile and malleable metal. It belongs to group IB of the periodic table. In compounds found in the environment, it usually has a valence of 2 but exist in the metallic, +1 and +3 valence states. Copper is found naturally in a wide variety of mineral salts and organic compounds, and in the metallic form. Copper posses high electrical and thermal conductivity and resists corrosion.

Sources of human and environmental exposure.

Average background concentrations of copper in uncontaminated sediments range is from 800 to 5,000 mg/kg (dry weight) (Forstner and wittmann, 1979). Copper level in marine sediment range is from 2 to 740 mg/kg (dry weight). Medium copper concentrations in uncontaminated soil were reported to average 30 mg cu/kg with 2 range of 2-250 mg/kg (Bowen, 1985).

Land treatment is increasingly being utilized as a method of waste disposal for sewage effluent and sludge. The intent is to soils, with safe land disposal of the large quantities of domestic sewage being generated (Juste and Mench, 1992). Copper concentrations in sewage sludge vary greatly. For example, Hedberg et al.(1996) quote copper concentrations from 0 to 16,000 mg/kg per day sludge for Finland, with a medium value of 214 mg Cu/kg. In 9 different sewage districts in Norway the levels in sludge varied from 100 to 500 mg Cu/kg.

In the great majority of sludge metal studies done to date, although copper is a constituent of the sludge, it is very rarely the element which imposes the limits for addition of sludges or sewage effluent to land.

Effects on humans and plants

Copper is capable of causing an adverse effect to humans and plants. Exposure to copper may arise through the following pathways :

1. Direct soil ingestion
2. Inhalation of suspended particulate matter
3. Dermal absorption from dust and dirt deposited to the skin
4. Ingestion of backyard garden produce grown in contaminated soil
5. Ingestion of contaminated groundwater where groundwater serves as a source of drinking water and where evidence demonstrates off-site migration of groundwater contamination from the INCO property and/or where surface-deposited copper contamination from the INCO stack percolates down to the water table (Richardson M,1992).

The types of health and phytotoxicity impacts expected due to copper exposure are as follows :

Inhalation exposure

Respiratory Effects : Copper is a respiratory irritant.

Dermal / Ocular Effects : Mucosal irritation of the eyes

Dermal exposure

Dermal / Ocular Effects : Eyes irritation after exposure to the copper dust.

Immunological Effects : In some cases pruritic dermatitis, allergic contact dermatitis.

Phytotoxic effects

The most common toxicity symptoms include reduced growth, poorly developed or malformed root system, and leaf chlorosis. The basic effect of Cu is related to the root system where it interferes with enzyme functioning. It also interferes with photosynthesis and fatty acid synthesis (Richardson M,1992).

Examples of damage resulting from exposure to copper: Bush beans – 200 ppm Cu – leaf weight reduced by 26%. Experiments in solution have revealed that shoot length and root mass were decreased by about 23% and 42% respectively at 0.64 ppm of copper.

A guideline for copper in soil of 1100 mg/kg soil, to protect against potential non-cancer human health risks, has been established by the Ontario Ministry of Environment. A guideline of 225 mg/kg soil has also been established by the Ontario Ministry of Environment to protect against phytotoxicity (Richardson M, 1992).

Case study, impact of heavy metals on forest trees from mining areas, by Peter Truby shows the investigation focused on uptake and distribution of heavy metals by forest trees from heavily contaminated sites. It was carried out on old mining areas from the Southern Black Forest and sites with recent mining activities in the Northern Eiffel Mountain, where air pollution even was extremely high. The aim was to trace the pathways of uptake, to find out the rules of internal distribution and to assess the impact of heavy metals on the state of health (Peter Truby, 1995).

On medieval ore mine spoils from the Black Forest Silver fir and Douglas fir have taken up relatively high amounts of heavy metals. Maximum Pb –contents in the stemwood of a 120-years old tree were 120 μg/g d.m.. However, there was no indication on negative effects regarding to growth and vitality.

The Eifel sites are characterized by a strong soil contamination (Pb 1.000-7.000 μg/g d.m.; Cu 100-2.000 μg/g d.m.) and a high input from atmosphere during several decades. The trees had individual heavy metal distribution patterns depending on soil properties, mainly. Even the high deposition rates heavy metals were taken up by the roots, predominantly. No significant uptake via leaves was observed.

Regarding to the potential toxidity of heavy metals there was no evidence on a specific damage. Norway spruce, Douglas fir, Scots pine, Silver fir, oak, and beech are able to grow normally without any symptoms even with high soil contamination and high atmospheric input.

2.6 Soil and plant relationships of heavy metals

Heavy metal contamination affects the biosphere in many places worldwide. Metal concentrations in soil range from less than 1 mg/kg (ppm) to high as 100,000 mg/kg, whether due to the geological origin of the soil or as a result of human activity. Excess concentrations of some heavy metals in soils such as Cd(II), Cr(VI), CuII), Ni(II), and Zn(II) have caused the disruption of natural aquatic and terrestrial ecosystems. Currently, cleanup processes of heavy metal pollution are expensive and environmentally destructive. Recently, scientists and engineers have started to generate cost-effective technologies that include the use of microorganisms, biomass, and live plants in the cleaning process of polluted areas. Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species. Researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds . Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on live plants. Most of these studies have been conducted using seedlings or adult plants. In a few studies, the seeds have been exposed to the contaminants.

Excess accumulation of heavy metals in soils is toxic to humans and other animals. Exposure to heavy metals is normally chronic (exposure over a longer period of time), due to food chain transfer. Acute (immediate) poisoning from heavy metals is rare though ingestion or dermal contact, but is possible.

Preventing heavy metals contamination is critical because cleaning contaminated soils is extremely expensive and difficult. Applicators of industrial waste or sludge must abide by the regulatory limits set by US Environmental Protection Agency (US EPA).

Soil and crop management can help preventing uptake of pollutants by plants. The soil becomes the sink to breaking (the soil-plant-animal or human cycle) through which its toxic effects are exerted (Brady and Weil, 1999). The following management practices, namely high temperature treatments, solidification and washing process, will not remove the heavy metal contaminants, but will help to immobilize them in the soil and reduce the potential adverse effects from the metals. Plant translocate larger quantities of metal to their leaves than to their fruit or seeds. The greater risk of food chain contamination is in leafy vegetables like lettuce or spinach. Another hazard is forage eaten by livestock. In Chernobyl, Ukraine sunflowers were used to remove radioactive contaminants from groundwater (US EPA, 1993).

Plant have been used to stabilize or remove metals from soil and water. The three mechanisms involved are phytoextraction, rhizofiltration, and phytostabilization. Rhizofiltration is the adsorption onto or absorption into plant roots of contaminants that are in solution surrounding the root zone (rhizosphere). Rhizofilltration is used to decontaminate groundwater. Phytostabilization is the use of perennial, non-harvested plants to stabilize or immobilize contaminants in the soil and groundwater. Metal-tolerant plants can be used to restore vegetation where natural vegetation is lacking, thus reducing the risk of water contamination, wind erosion and leaching. Phytoextraction is the process of growing plants in metal-contaminated soil. Plant roots translocate the metal into above ground portions of the plant. After plants have grown for some time, they are harvested and incenerated or composted.

2.6.1 Plant uptake of metals

2.6.1.1 Uptake of heavy metals from contaminated soils, water and air

The main causes of soil pollution from heavy metals (including lead, cadmium, chromium, zinc, copper, nickel, mercury, manganese, selenium, mercury and arsenic) are irrigation with water from streams and wastewater contaminated by industry, the application of contaminated solid wastes and the use of former industrial land contaminated by spilled oil and industrial wastes.

Important sources of heavy metals are smelters, refineries, manufacturing plants, vehicles, metalliferous mines, ceramic industry (lead and cadmium), leather tanneries (chromium salts), lignite-based power plants, aluminium industry, electronics industry, and metallurgical industry. Some heavy metals precipitate in sewage sludge, which can therefore contain rather high concentrations.

The heavy metals may accumulate in the edible parts of crops that are consumed by people or fed to animals. Plant uptake of heavy metals varies, which opens the possibility to adapt the choice of crops in relation to the degree and type of contamination. Generally, the highest amounts of heavy metals accumulate in the leaves, whereas the lowest contents are located in seeds. Beans, peas, melons, tomatoes and peppers show very low uptake figures. Plant uptake of heavy metals (especially of cadmium and lead) also varies with soil pH (Iretskaya and Chien, 1998).

Though heavy metal content in soils of most cities in developing countries are so high as to be able to cause acutely toxic symptoms, their increased concentration in the human food chain over a long period can provoke detectable damage to health (carcinogenic and mutagenic effects).

Puschenreiter et al. (1999) conclude that, after considering the several available pathways to reduce the transfer of heavy metals to the human food chain, urban soils with slight heavy metal contamination can be used safely for gardening and agriculture if proper precautions are followed. However, Birley and Lock (2000) argue that little is known of the chronic health effects of consuming tiny amounts of heavy metals over long periods of time, and that further research is needed.

Suggested prevention and control measures encountered in the literature, include the following :

- definition of norms regarding crop restrictions according to type and level of contamination of agricultural soils; testing of agricultural soils and irrigation water for heavy metals;
- a minimum distance is recommended between fields and main roads and/or boundary crops to be planted beside roads to reduce contamination of crops by lead and cadmium;
- soil treatment for immobilisation of heavy metals: application of lime increases pH and thus decreases the availability of metals, except for selenium; application of farmyard manure reduces the heavy metal content of nickel, zinc and copper (but may increase cadmium levels); iron oxides (like red mud) and zeolites are also known to absorb heavy metals like cadmium and arsenic;
- washing and processing of contaminated crops may effectively reduce heavy metal content: good results were obtained for lead (less so for cadmium) in green beans, spinach, potatoes, whereas peas virtually showed no change;
- use of plants like Indian grass (*Brassica juncea, L*) can be used for biological remediation of polluted soils or streams (when planted in hydroponic beds); and
- more research on chronic health impacts of heavy metals

The factors affecting the amounts of metal absorbed by a plant are those controlling:

1. The concentrations and speculation of the metal in the soil solution
2. The movement of the metal from the bulk soil to the root surface
3. The transport of the metal from the root surface into the root
4. Its translocation from the root to the shoot

Plant uptake of mobile ions present in the soil solution is largely determined by the total quantity of this ion in the soil but, in the case of strongly adsorbed ions, absorption is more dependent upon the amount of root produced. Absorption of heavy metals by plant roots can be by bout passive and active (metabolic) processes. Passive (non-metabolic) uptake involves diffusion of ions in the soil solution into the root endodermis. On the other hand, active uptakes takes place against a concentration gradient but requires metabolic energy and can therefore be inhibited by toxins. The mechanism appear to differ between metals. The absorption mechanisms can vary for different metal ions, but ions that are absorbed into the root by the same mechanisms are like to compete with each other (Alloway B.J., 1997).

The uptake of metal from soils is greater in plants grown in pots of soil in the greenhouse than from the same soil in the field. It is probably due to differences in mocroclimate and soil moisture, and to the roots of container-grown plants growing solely in contaminated soil, whereas those of field-grown plants may reach down to less contaminated soil. Relative differences in the uptake of metal ions between plant species and cultivates is genetically controlled and can be due to various factors, including surface area of the root, root CEC, root exudates and the rate of evapotranspiration. The transfer coefficient is the metal concentration in the soil. Although numerous soil and plants factors can affect the accumulation of metals in plants, the values given are intended as guides to the order of magnitude of the transfer coefficients and not precise values. Certain species of the plants have been found to accumulate very high concentrations of certain heavy metals and these are referred to as “hyperaccumulator” species.

2.6.1.2 Foliar absorption

In addition to root absorption, plants can also derive significant amounts of some elements through foliar absorption. This is exploited inagriculture as a means of supplying plants with micronutrients, such as Cd into the food chain. Foliar absorption of solutes depends on the plant species, its nutrientional status, the thickness of its cuticle, the age of the leaf, the presence of stomata guard cells, the humidity at the leaf surface and the nature of the solutes.

2.6.1.3 Translocation of metals within plants

Once the ions have been absorbed through the roots or leaves and have been transported to the xylem vessels, there is the possibility of movement throughout the whole plant. The rate and extent of movement within plants depends on the metal concerned, the plant organ and the age of the plant. In the leaves, metal ions may be incorporate into protiens or translocated around the plant in the phloem with phytosynthates. Following root absorption, the extent to which elements are translocated decreases in the order Cd>B>Zn>Cu>Pb.

2.7 Standard values

Standard values for selected heavy metals and other toxic elements based on leaching test according to The Notification of the Ministry of Industry Subject : Disposal of Wastes or Unusable Materials B.E 2548 (2005) are shown in Table 2.1. The values from the leaching test should not exceed the limits shown in the Table.

Table 2.1 Standard values for selected heavy metals and other toxic elements based on leaching test

Parameter	***Soluble Threshold Limit (mg/L)***
Arsenic and / or arsenic compounds	*5.00*
Cadmium and / or cadmium compounds	*1.00*
Chromium (VI) compounds	*5.00*
Chromium and / or chromium (III) compounds	*5.00*
Copper and / or copper compounds	*25.00*
Lead and / or lead compounds	*5.00*
Mercury and / or mercury compounds	*0.20*
Nickel and / or nickel compounds	*20.00*

Table 2.2 Soil Quality Standard for Habitat and Agriculture

Metal	*Amount (mg/kg)*
Arsenic	*3.9*
Cadmium and compounds	*37*
Hexavalent Chromium	*300*
Lead	*400*
Manganese and compounds	*1,800*
Mercury and compounds	*23*
Nickel soluble salt	*1,600*
Selenium	*390*

Source: Notification of National Environmental Board No. 25, B.E. 2547 (2004) under the Enhancement and Conservation of National Environmental Quality Act B.E. 2535 (1992)

Table 2.3 Standard levels of metal contaminants in food

Metal	***Amount (mg/kg)***
Cadmium	
Leafy vegetable	*0.1*
Liver of cattle, sheep and pig	*1.25*
Meat of cattle, sheep and pig (excluding offal)	*0.05*
Mollusca	*2*
Peanuts	*0.1*
Rice	*0.1*
Root and tuber vegetables	*0.1*
Wheat	*0.2*
Lead	
Brassicas	*0.3*
Fish	*0.5*
Fruit	*0.1*
Vegetables (except brassicas)	*0.1*

Source: Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

Table 2.4 Recommended maximum level of metal concentrations in leafy vegetables

Element	*Recommended max level in vegetables (mg/kg)*
Arsenic	*0.43*
Cadmium	*0.10*
Chromium	*2.30*
Copper	*73.30*
Iron	*425.50*
Manganese	*500.00*
Nickel	*67.90*
Lead	*0.30*
Zinc	*99.60*

Source: Australia Newzealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

2.8 Extraction and Leaching Test

Extraction and leaching test is generally to measure the performance of stabilized waste. Leaching test is to measure the potential of the stabilized waste to release contaminants to the environment. The standard extraction of leachate that would be produced by waste when placed in a landfill began since 1970. The extraction procedure for leaching test can be divided into two groups : batch and column tests.

In the batch test, the sample is continuously contacted with the same extraction in the reaction vessel while the column test , the fresh extract solution is continuous or intermittent introduced to contact with a packed column. Nowadays, the batch test is used to estimate the leachate quality that results from disposing of waste in the secure landfill (Rungsipanodorn C., 2002).

2.9 Relevant Research

Sirisukhodom S. (1992) studied effects of two sewage sludge application rates (1,600 and 3,200 kg/rai) on plant growth and heavy metals (Pb, Cd, Ni, Cu, Mn, and Fe) accumulation in four vegetables (Chinese Kale, Lettuce, Edible Rape and Kang-Kong); and studied heavy metals residual in the soil. Field experiment was carried out in an agricultural area at Pathum thani Province by using experimental design 2x4 factorial incompletely randomize.

The results showed that applying sewage sludge 1,600 and 3,200 kg/rai gave Chinese Kale and Kang-Kong products equal to adding fertilizer (25-7-7) 96 kg/rai. Only Edible Rape at the sewage sludge application rate 3,200 kg/rai gave production significantly higher than when adding fertilizer. Appling 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. All of heavy metals accumulated in four vegetables in this experiment were in the range that generally accumulated in plant tissue. The heavy metals content in the vegetables were within acceptable daily intake (ADI) of FAO/WHO.

Residue effect of heavy metals after appling sewage sludge were (1) non significant difference among treatments on Pb, Cd, Cu and Fe contents in the soil (2) increased both Ni content in the soil that grew Edible Rape and Zn content in the soil that grew four vegetables. Moreover, Zn residual in the soil was increased following the increasing rate of sewage sludge application. However, heavy metals residual in the soil were in the acceptable range for the agricultural soil.

Tansatit R (1989) compared the growth of lettuce (*Lactuca savita var. crispa*) and its heavy metal accumulation when planted in soil mixed with activated sludges from three different agroindustrial companies (brewery, mono sodium glutamate, and diary productions). Plant nutrients and heavy metals in sludge samples were analysed in the laboratory. The lettuce plants were grown in pots in a randomized complete block design which consist of three groups of soil treatments: (1) The control, with recommended rate of fertilizers (N,P,K 15-15-15 and urea) (2) With dry activated sludges which provided equal amount of N as in the recommended rate and (3) With inorganic fertilizers which provided equal amount of N,P and K as activated sludges used in group 2.

The results indicated that the growth of lettuce in the soil with sludge from the mono sodium glutamate industry was less than the control. No significant difference in growth was found between using the control and sludges from the other two companies. Concentration of heavy metals in leaf tissue and cultivated soil were very low and have no statistical significance ($p>0.05$).

Peralta J.R (2000) studied the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. Preliminary studies have shown that alfalfa plants (*Medicago sativa*) can grow in some heavy metal contaminated soils. Based on that, the study on individual effects of several doses of Cd(II), Cr(VI), Cu(II), Ni(II), and Zn(II) on the growth of live alfalfa plants using solid media was carried out. The doses used in this study were 0, 5, 10, 20, and 40 ppm. The seed germination and plant growth was significantly affected by Cd(II) and Cr(VI) at 10 ppm, as well as by Cu(II) and Ni(II) at 20 ppm and higher concentrations ($P<1\%$). Zn(II) did not affect seed germination. The roots of the plants exposed to 5 ppm-dose of Cd(II), and 5 and 10 ppm-dose of Cr(VI), Cu(II), Ni(II), and Zn(II), grew more than the roots of the control treatment by more than 30%. Exposures of 5 ppm of Cd(II) reduced the shoot size by 16% as compared to the control. While Cr(VI), Cu(II), Ni(II), and Zn(II) increased the shoot size by 14.0%, 60.0%, 36.0%, and 7.7%, respectively; only Zn(II) promoted the shoot growth at the doses of 20 and 40 ppm.

Jongbloed and Lennis (1997) reported that swine manure is mostly spread in the neighborhood and may lead to accumulation of mineral and metals such as phosphorus, copper and zinc. This may contribute, via leaching and runoff, to eutrophication of groundwater and freshwater sources. Eutrophication may cause excessive growth of algae and reduction of the biological diversity. De lange (2001) also emphasized that in areas with intensive swine facilities and the disposal of nutrient with pig manure. Nitrients that are of prime concern in regards to environmental pollution are copper, zinc, nitrogen and phosphorus. The values of copper and zinc are smaller in clean, healthy pigs. In some countries, such as the Netherlands,growth-promoting levels of copper and zinc are simply no longer allowed in finishing pig diet. These nutrients, if present in high concentrations, could stunt certain sensitive crops.

Lisk et al.(1982) studied the corn grown on municipal sewage sludge containing 115 mg Cd, 4,200 mg Zn and 538 mg Ni per kilogram of dry matter. This corn has higher ($p<0.01$) concentrations of Cd, Ni and Zn but when fed to the swine, no adversely effect was found.

Kachenko A. and Singh B. (2005) studied heavy metals contamination of home grown vegetables near metal smelters in NSW. The accumulation of Cd, Cu, Pb and Zn in soils and vegetables in the vicinity of 2 industrial regions, Port Kembla and Boolaroo, were investigated. Soil samples (n=37) were collected at depths of 0-30 and 60-90 cm, air dried and sieved to obtain <2mm fraction. Soil properties including pH, EC, organic carbon, total cation exchange capacity and total metal content were determined. Vegetable samples (n=40) predominantly leafy, included - lettuce, spinach, leek, mint and parsley. The plant samples were oven dried and analysed for total metal content.The high levels of soil contamination in the regions of Boolaroo and Port Kembla appear as a result of anthropogenic activities, with smelters in both regions are likely contributors. The degree of soil contamination related closely with the emissions released from the smelters during the duration of sampling and furthermore, all metals decreased in concentration between topsoil and subsoil layers. The topsoils sampled from Boolaroo were contaminated the most, containing the highest mean concentrations of Cd (5.5 mg kg -1), Pb (364 mg kg -1) and Zn (1061 mg kg -1). Similarly, topsoils sampled from Port Kembla were contaminated the most with Cu (83-1032 mg kg -1), and had high concentrations of Zn (192-1641 mg kg -1) and Cd (0.01-7.01 mg kg -1). Many of the topsoil samples from both regions exceeded Australian background levels and levels reported for the worlds soils.

This study highlights the potential danger of heavy metals accumulation, particularly Cd and Pb in vegetables grown in the vicinity of smelters. Vegetables from Boolaroo contained the highest levels of Cd (0.08-2.22 mg kg -1 DW) and Pb (0.69-57.5 mg kg -1 DW), and samples from Port Kembla had the highest level of Cu in all vegetable types. Almost all vegetables at Boolaroo exceeded Australian food standard guidelines for Cd and Pb.The site specific risk in growing vegetables in areas close to smelters and industry in general should be incorporated into the Australian

Food standard guidelines to highlight and minimise the potential health risks of ingesting vegetables containing high levels of heavy metals.

Homchan U. (1992) studied toxicity of heavy metals (iron, manganese, zinc, copper, nickel, lead and cadmium) from sewage sludge at the application rate of 20 tonnes sludge/ha. (50 gm./pot) on chinese kale (*Braddica oleracea L. Var. alboglabra Bailey*) and lettuce (*Lactuca sativeL.*). Agricultural soils from Tambon Banchang, Amphore Mueng, Changwat Pathumthani and sewage sludge from anaerobic digester of Havi Khavang treatment plant were example of case study. Pot experiment was conducted at a greenhouse. The experimental design was 2x4 factorial imcompletely randomize design with 3 replications. Kale and lettuce were planted on treated soils that had been applied with certain amount of heavy metal inorganic salts 4 level equal to heavy metal contents in the sludge (available form equal level l, total form equal level 4). Both heavy metal contents in soils and plants, and plant productivity were observed. The results showed that zinc contents in the soils increased significantly ($p<0.01$) by increasing the heavy metal content of inorganic salts. This behavior of zinc appeared in edible part of both kale and lettuce. However, this zinc contents in edible parts of lettuce planted in the soils applied with heavy metal inorganic salts equal to total content in the sludge was higher than the contents of various plants, but it was still lower than the toxic dose. Hence, Zinc will be chosen as an indicator to indicate the risk tendency of heavy metals from the sludge. Copper and cadmium contents in the soils for both kale and lettuce and only lead contents in the soil for kale followed the same pattern as already dircribed for zinc. However, there was no obvious tendency in plants. In addition, iron, manganese and nickel contents in both soils and plants including lead in the soils for lettuce showed no obvious tendency to follow the patern. Each heavy metal could be released from sludge differently after the first harvest in an order with the three fastest ones as follows: copper, zinc and cadmium respectively. When plant productivity was considered, the adverse effect of heavy metals was not observed.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Design

This research aims to study the effect of using PS-D flowerpot to grow plants. The concept of the experiment focuses on seed cultivation and the concentrations of heavy metals (lead, nickel, and copper) leached out of the PS-D flowerpot, and accumulated in the plants. The experiment was divided into four parts as follows:

Part 1 Total concentrations of heavy metals (Pb, Ni, and Cu)

This part involves the determination of total concentrations of Pb, Ni, and Cu in PS-D flowerpot and soil which will be used as background data for the next 2 parts (Test for the effect of PS-D flowerpot on seed cultivation and Test for heavy metal accumulation in plant).

Part 2 Leaching test

The test was performed to determine the concentrations of heavy metals (lead, nickel, and copper) leached out from the two types of PS-D flowerpot: (1) coating external surface with paraffin , (2) no coating external surface; and to determine the concentrations of the heavy metals (lead, nickel, and copper) leached out from soil. Then, compare the results with the standard value for selected heavy metals and other toxic elements based on leaching test.

Part 3 Test for the effect of PS-D flowerpot on seed cultivation

The following 5 groups of planting materials were used to determine the seed cultivation percentage of Chinese kale (*Brassica oleracea var. alboglabra*) :

1. Control group: using 1000 g of soil,
2. Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating ,
3. Using 1000 g of crushed PS-D flowerpots without paraffin coating,
4. Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating , and
5. Using 1000 g of crushed PS-D flowerpots with paraffin coating.

Group 3 and 5 should have the maximum adverse effect on seed cultivation for this study.

Observations were made on 5,10, 15, 20, 25 and 30 days with 3 replicates in each group.

Part 4 Test for heavy metal accumulation in plant

This test was conducted to determine the total concentrations of lead, nickel, and copper in 2 parts of plant: the shoot and root. Then, compare the total concentration in different mixtures of soil and crushed PS-D flowerpot without paraffin coating and with paraffin coating.

3.2 Test Method

3.2.1 Total concentration of heavy metals (Pb, Ni, and Cu)

The determination of total concentration of heavy metals in PS-D flowerpot and soil was performed as in the following step:

1. Five grams of crushed PS-D flowerpot without paraffin coating was digested with acid.

2. Then, analyze for lead, nickel, and copper by Atomic Absorption Spectrophotometer (AA)
3. Repeat each analysis for 3 times.
4. The analysis of PS-D flowerpot with paraffin coating and soil followed the same steps 1-3 as above.

3.2.2 Leaching test

Chemical reagent

1.Nitric acid
2.Sulfuric acid

Procedure

To determine heavy metals leached out from the flowerpot when rain or acid rain or infiltration intrude into the landfill and attack the flowerpot. Procedure of leaching test use USEPA SW846 which complied with the standard method of Notification of Ministry of Industry Subject: Disposal of Wastes or Unusable Materials B.E 2548 (2005);

1. Take the crushed samples for 100 g and transfer into an extractor bottle.
2. Prepare extraction leachant or synthetic acid rain extraction fluid that contain sulfuric acid and nitric acid in the ratio of 80: 20 by weight into distilled water until pH is 5 ± 0.2
3. Add 1000 cc of the extraction leachant (from 2) into extraction bottle.
4. Close the extractor bottle tightly, secure in rotary agitation device and rotate at 30 ± 2 rpm for 18 ± 2 hours at the temperature of 25°C
5. Acidify filtrate with nitric acid to pH < 2
6. Digest it until the volume remains half.
7. Separate the leachate with filter paper and preserve it to analyze.
8. Analyze lead, nickel, and copper by Atomic Absorption (AA)

9. Make 3 replicate in each sample.

3.2.3 Test for the effect of PS-D Flowerpot on seed cultivation

For this research, seed cultivation test is the test to determine adverse effect of heavy metals (Pb, Ni, and Cu) to plant growth.

Plant Test : use 50 seeds of Chinese kale (*Brassica oleracea var. alboglabra*) for growing

Materials requirement

1.Plastic tray size W x L x D = 12.5 x 28.5 x 6 cm^3. Four wall sides are rectangular screen which air and water can pass

2.Soil (Chuan Chom soil from Lopburi province)

3.Crushed PS-D flowerpots (2 types: without paraffin coating and with paraffin coating)

4.Seeds of Chinese kale (*Brassica oleracea var. alboglabra*)

Seed cultivation Procedure

The following materials were prepared for growing plant:

1. For group 1 (control group), fill 1000 g of soil in the plastic tray.
2. For group 2, fill 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.
3. For group 3, fill 1000 g of crushed PS-D flowerpots without paraffin coating.
4. For group 4, fill 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.
5. For group 5, fill 1000 g of crushed PS-D flowerpots with paraffin coating.
6. Fifty seeds were cultivated, for each replication, in the tray.
7. Water every replication 1 time in the morning and 1 time in the evening.
8. Protect sampling plot from the rain by clear plastic cover

9. Record the result on the 5th, 10th, 15th, 20th, 25th and 30th days
10. Make 3 replicates in each group

3.2.4 Test for heavy metal accumulation in Chinese kale

This study will determine total concentration of heavy metals accumulated in the shoot and root of plants. The shoot and root will be harvested and used for the analysis of total concentration of Pb, Ni, and Cu.

1. The sample was prepared by taking two grams of dry shoot from each group, then digested with acid.
2. This sample was analyzed for lead, nickel, and copper using Atomic Absorption Spectrophotometer (AA)
3. The procedure was repeated using 0.5 grams of root instead.

3.3 Statistical Analysis

ONE-WAY ANOVA and Non-parametric statistics were used to explain differential of concentrations of leaching and heavy metals that accumulated in plant compared between each group.

3.4 Experimental Strategy

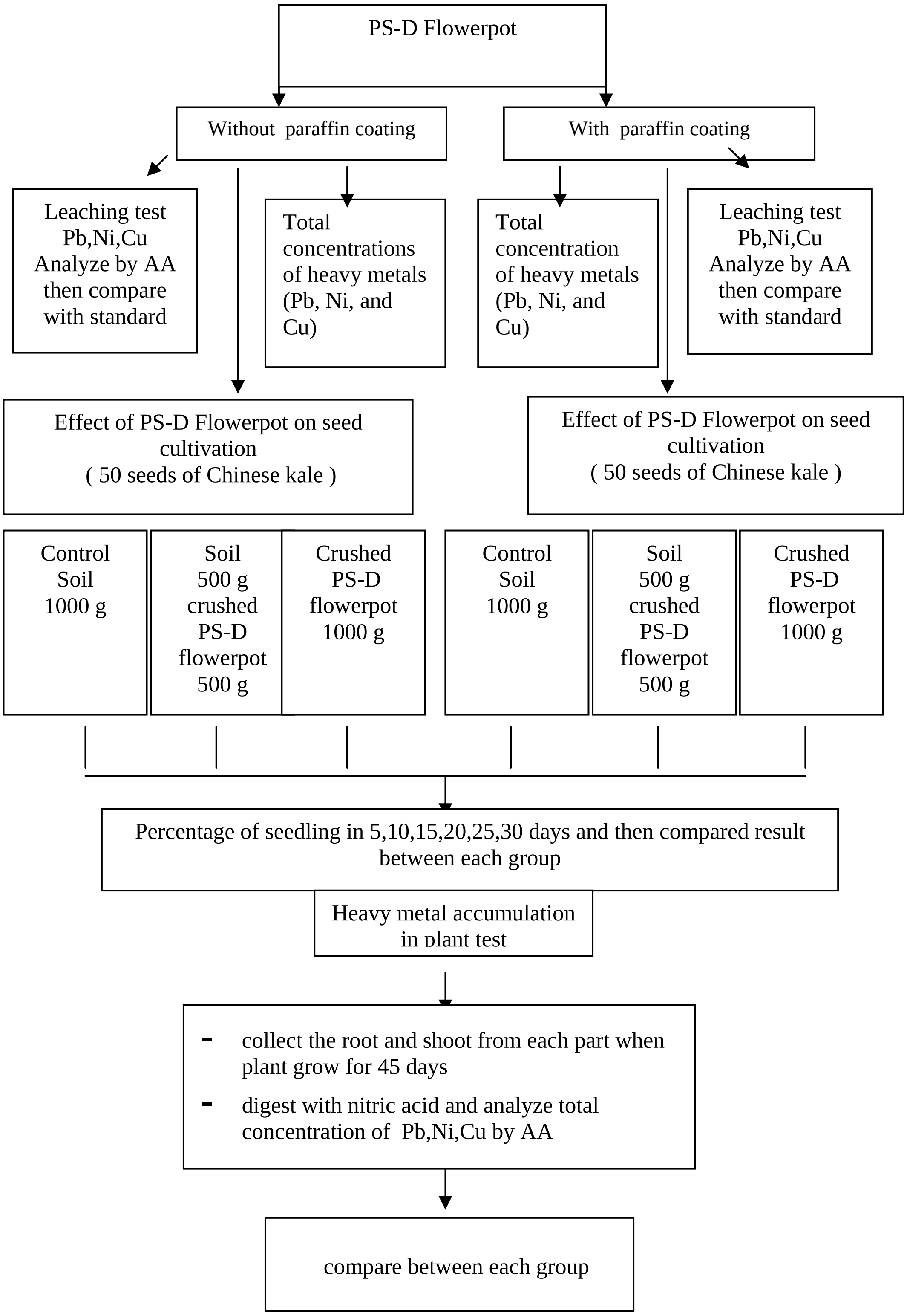


Figure 3.1 Experimental Strategy

CHAPTER IV

RESULTS

4.1 Determination of Total Concentrations of Pb, Ni, and Cu in PS-D flowerpot and Soil

This experiment was conducted for testing the concentrations of heavy metals in 3 groups of sample as follows:

1. **PS-D flowerpot without paraffin coating:** to test background concentrations of heavy metals in PS-D flowerpot without paraffin coating that will be used in the next 2 parts (Test for the effect of PS-D flowerpot on seed cultivation and Test for heavy metal accumulation in plant).
2. **PS-D flowerpot with paraffin coating:** to test background concentrations of heavy metals in PS-D flowerpot with paraffin coating that will be used in the next 2 parts (Test for the effect of PS-D flowerpot on seed cultivation and Test for heavy metal accumulation in plant).
3. **Soil:** to test background concentrations of heavy metals in soil used to grow Chinese kale in the next 2 parts (Test for the effect of PS-D flowerpot on seed cultivation and Test for heavy metal accumulation in plant).

Referring to Table 4.1, the concentrations of Pb, Ni, and Cu in PS-D flowerpot without paraffin coating were 10.64, 1.73, and 10.57 mg/kg, respectively. In another type of PS-D Flowerpot (with paraffin coating), the concentrations of Pb, Ni, and Cu were 9.55, 1.29, and 10.36 mg/kg, respectively. The soil used for this experiment showed low concentrations of heavy metals (Pb, Ni and Cu were 2.85, 2.02 and 7.68 mg/kg, respectively). The concentrations of lead and nickel were lower than the value of soil quality standards for habitat and agriculture as determined by the Notification of National Environmental Board (2004). Copper is not regulated in this Act. The results of this part is used as the background data.

Table 4.1 Results of heavy metals concentrations

Sample	***Concentrations of heavy metals (mg/kg)***		
	Pb	***Ni***	***Cu***
Soil	*2.85*	*2.02*	*7.68*
PS-D Flowerpot without paraffin coating	*10.64*	*1.73*	*10.57*
PS-D Flowerpot with paraffin coating	*9.55*	*1.29*	*10.36*
*Soil Quality Standard**	*400*	*1600*	*-*

* Notification of National Environmental Board No. 25, B.E. 2547 (2004) under the Enhancement and Conservation of National Environmental Quality Act B.E. 2535 (1992)

- not regulated

4.2 Leaching Test for PS-D Flowerpot and Soil

Experiment of leaching test was important to study potential of using PS-D flowerpot in growing plant. Since PS-D flowerpot was contaminated with heavy metals, the measurement of heavy metals concentrations must be necessary. Concentrations of heavy metals (Pb, Ni, and Cu) leached out into the environment were compared with the Standard value for selected heavy metals and other toxic elements based on leaching test to determine the effect of PS-D flowerpot on the environment.

The results in Table 4.5 show amount of Pb that leached out from 3 groups ; (1) soil, (2) PS-D Flowerpot without paraffin coating and (3) PS-D Flowerpot with paraffin coating were 0.35×10^{-3}, 0.75×10^{-3} and 0.55×10^{-3} mg/l , respectively. In terms of Ni, concentrations that leached out from all groups were less than the detection limit of analysis. Concentrations of Cu leached out from soil, PS-D Flowerpot without paraffin coating and PS-D Flowerpot with paraffin coating were 0.45×10^{-3}, 0.63×10^{-3} and 0.37×10^{-3} mg/l, respectively. The comparison of concentrations of Pb, Ni, and Cu between groups are shown in Figure 4.1 and 4.2. The results in Table 4.2 described about statistical analysis of the result of leaching test. Mean of metal concentrations leached out were shown. From non- parametric test: Kruskal Wallis test showed leaching of Pb and Cu in each group were different significantly ($p<0.05$), but leaching of Ni was not different due to the very low concentrations.

Table 4.2 Descriptive Statistical Analysis of Leaching test

Parameter	*group*	*N*	*Mean ($x 10^{-3}$) mg/l*	*Std. Deviation ($x 10^{-5}$)*	*Range (Minimum – Maximum) ($x 10^{-3}$) mg/l*	*P-value.**
leaching of Pb	*soil*	*3*	*0.35*	*0*	*0.35 – 0.35*	*.019*
	PS-D Flowerpot without paraffin coating	*3*	*0.75*	*8.66*	*0.70 – 0.85*	
	PS-D Flowerpot with paraffin coating	*3*	*0.55*	*0*	*0.55 – 0.55*	
leaching of Ni	*soil*	*3*	*0.01*	*0*	*0.01 – 0.01*	*1.000*
	PS-D Flowerpot without paraffin coating	*3*	*0.01*	*0*	*0.01 – 0.01*	
	PS-D Flowerpot with paraffin coating	*3*	*0.01*	*0*	*0.01 – 0.01*	
leaching of Cu	*soil*	*3*	*0.45*	*0*	*0.45 – 0.45*	*.014*
	PS-D Flowerpot without paraffin coating	*3*	*0.63*	*2.89*	*0.60 – 0.65*	
	PS-D Flowerpot with paraffin coating	*3*	*0.37*	*2.89*	*0.35 – 0.40*	

* from non-parametric test ; Kruskal Wallis Test

Table 4.3 shows different means of Pb concentrations in each group. The mean concentrations of Pb leached out from soil was lower than those leached out from PS-D flowerpot with paraffin coating and PS-D flowerpot without paraffin coating. The highest of the mean concentration was the one leached out from PS-D flowerpot without paraffin coating group. In terms of Cu leaching results, the mean concentrations of Cu leached out from soil and PS-D flowerpot with paraffin coating were less than that from PS-D flowerpot without paraffin coating (Table 4.4).

Table 4.3 Homogeneous Subset of leaching of Pb

group for leaching	*N* [a]	*Subset for alpha = .05* *Leching of Pb (x 10^{-3}) mg/l*		
		1	*2*	*3*
soil	*3*	*0.35*		
PS-D Flowerpot with paraffin coating	*3*		*0.55*	
PS-D Flowerpot without paraffin coating	*3*			*0.75*

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table 4.4 Homogeneous Subset of leaching of Cu

group for leaching	*N* [a]	*Subset for alpha = .05* *Leaching of Cu (x 10^{-3}) mg/l*		
		1	*2*	*3*
PS-D Flowerpot with paraffin coating	*3*	*0.37*	*0.37*	
soil	*3*		*0.45*	
PS-D Flowerpot without paraffin coating	*3*			*0.63*

Means for groups in homogeneous subsets are displayed.

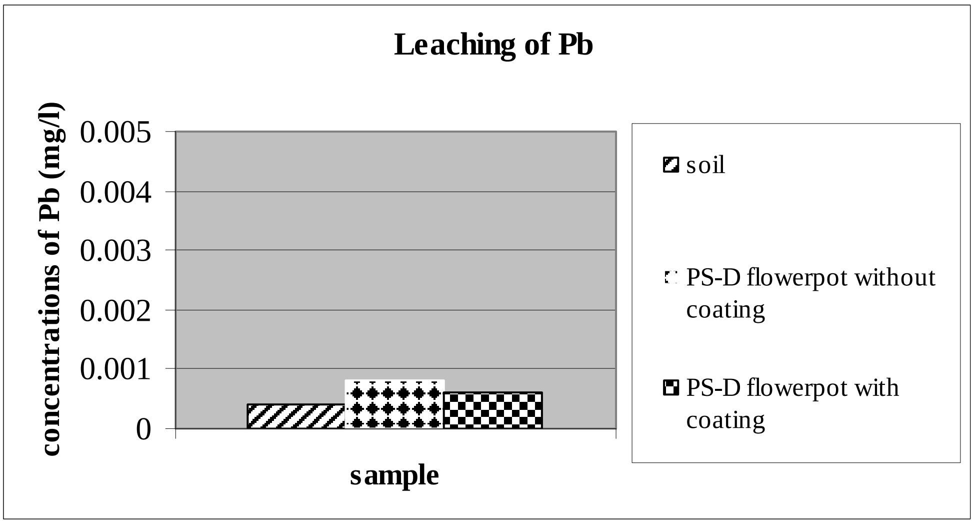
a Uses Harmonic Mean Sample Size = 3.000.

Table 4.5 Results of heavy metals concentrations leaching into the environment compared with standard.

Sample	***Leaching of metals*** *(x 10^{-3}) mg/l*			***Standard value of leaching*** *(x 10^{-3}) mg/l***		
	Pb	***Ni***	***Cu***	***Pb***	***Ni***	***Cu***
soil	*0.35*	*ND**	*0.45*	*5000*	*20000*	*25000*
PS-D Flowerpot without paraffin coating	*0.75*	*ND*.*	*0.63*	*5000*	*20000*	*25000*
PS-D Flowerpot with paraffin coating	*0.55*	*ND*.*	*0.37*	*5000*	*20000*	*25000*

* not detected

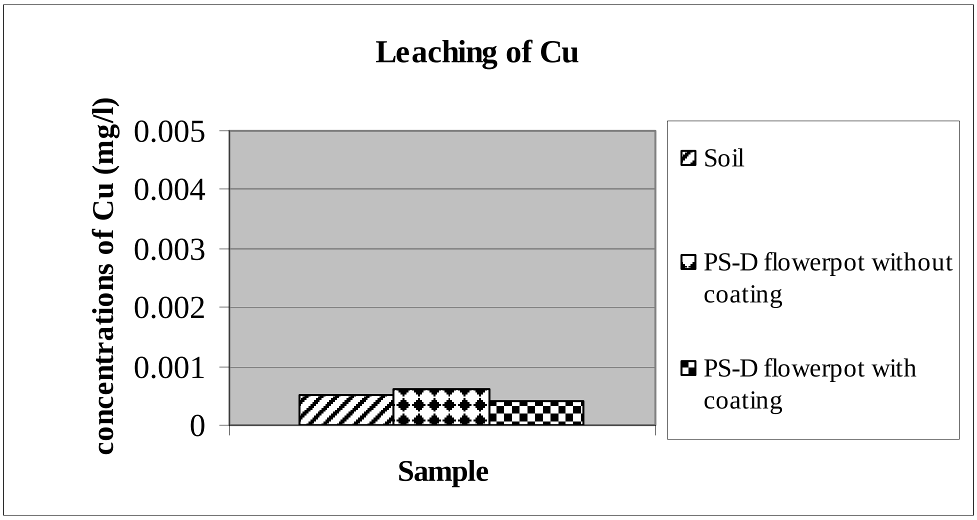
**The Notification of the Ministry of Industry Subject: Disposal of Wastes or Unusable Materials B.E 2548 (2005)



Standard value of leaching : Pb < 5 mg/l*

*The Notification of the Ministry of Industry Subject: Disposal of Wastes or Unusable Materials B.E 2548 (2005)

Figure 4.1 Leaching of Pb



Standard value of leaching : Cu < 25 mg/l*

*The Notification of the Ministry of Industry Subject: Disposal of Wastes or Unusable Materials B.E 2548 (2005)

Figure 4.2 Leaching of Cu

4.3 Effect of Heavy Metals from PS-D Flowerpot on Seed cultivation of Chinese Kale

4.3.1 Results of Seed Cultivation

After cultivating seeds of Chinese kale (*Brassica oleracea var. alboglabra*) in different groups of material to grow, number of growing seeds were observed on days 5, 10, 15, 20, 25 and 30. Seed cultivation results are presented by time as well as by group in Table 4.6. Results indicate that after day 20, number of seedling were stable in all of groups. Thus, percentage of seedling must be calculated from number of seedling on day 20 and were shown in Table 4.6. Comparisons between seedling on day 5, 10, 15, 20, 25 and 30 in different group are presented in Figure 4.18. Results of percentage of seedling (Table 4.7) from group 1 to group 5 were 84, 67.3, 89.3, 61.3 and 66, respectively. Figure 4.19 shows that percentage of seedling from highest to lowest were group 3, group1, group 2, group 5, and group 4, respectively.

Table 4.6 Results of Seed cultivation of Chinese kale

Group	***Number of seed cultivation*(*** From 50 seeds)					
	5 day	***10 day***	***15 day***	***20 day***	***25 day***	***30 day***
1	*37*	*42*	*42*	*42*	*42*	*42*
2	*31*	*34*	*34*	*34*	*34*	*34*
3	*31*	*42*	*43*	*45*	*45*	*45*
4	*17*	*30*	*31*	*31*	*31*	*31*
5	*25*	*32*	*33*	*33*	*33*	*33*

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

* average from 3 replication of results

Figure 4.3 to 4.7 show growing of Chinese kale after cultivated 10 day for group1to group5, respectively. Growths of Chinese kale were similar in each group.

Figure 4.3 Growing of Chinese kale in group 1 on day 10

Figure 4.4 Growing of Chinese kale in group 2 on day 10

Figure 4.5 Growing of Chinese kale in group 3 on day 10

Figure 4.6 Growing of Chinese kale in group 4 on day 10

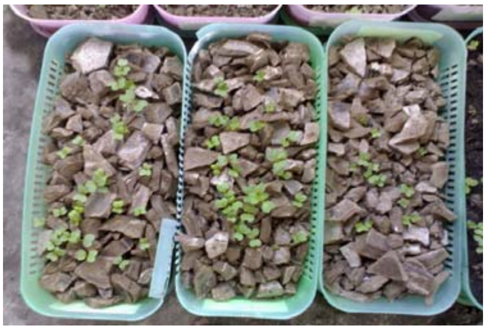


Figure 4.7 Growing of Chinese kale in group 5 on day 10

Growth of Chinese kale on day 20 in group 1, 2 and 3 were better than group 4 and 5 presented in Figure 4.8 to 4.12.

Figure 4.8 Growing of Chinese kale in group 1 on day 20

Figure 4.9 Growing of Chinese kale in group 2 on day 20

Figure 4.10 Growing of Chinese kale in group 3 on day 20

Figure 4.11 Growing of Chinese kale in group 4 on day 20

Figure 4.12 Growing of Chinese kale in group 5 on day 20

Figure 4.13 to 4.17 show growth of Chinese kale after 30 days of cultivation. Result of growth in group 2 and 3 were better than those in group 4 and 5. Group 4 and 5, show undersized of Chinese kale according to Figure 4.16 and 4.17. This section presents the last data before harvest to analyze total concentrations of Pb, Ni, and Cu accumulated in the shoot and root of Chinese kale.

Figure 4.13 Growing of Chinese kale in group 1 on day 30

Figure 4.14 Growing of Chinese kale in group 2 on day 30

Figure 4.15 Growing of Chinese kale in group 3 on day 30

Figure 4.16 Growing of Chinese kale in group 4 on day 30

Figure 4.17 Growing of Chinese kale in group 5 on day 30

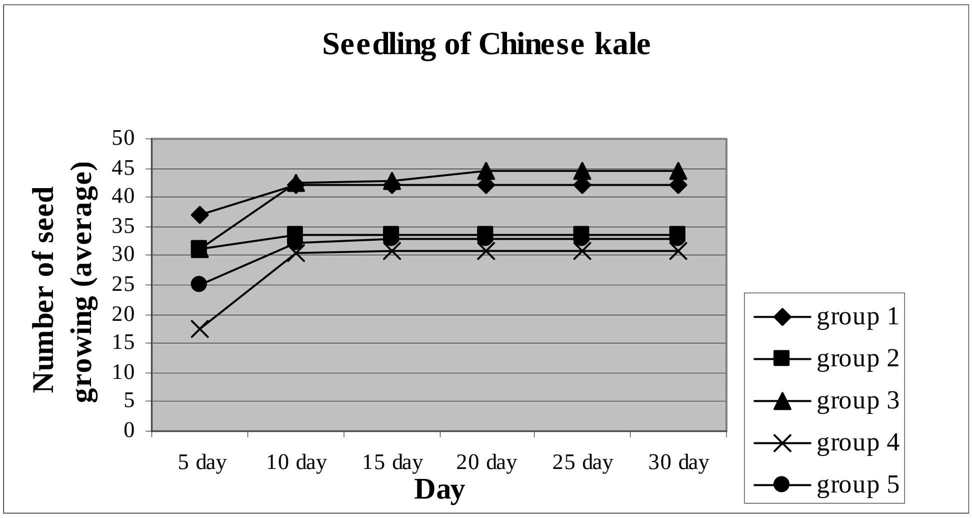


Figure 4.18 Relation between number of Chinese kale seedlings and duration from cultivating day compared in each group

Table 4.7 Percentage of Chinese kale seed cultivation

Group	***Percentage of Chinese kale seed cultivation*** (*Brassica oleracea var. alboglabra*)
1	*84*
2	*67.3*
3	*89.3*
4	*61.3*
5	*66*

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3 Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

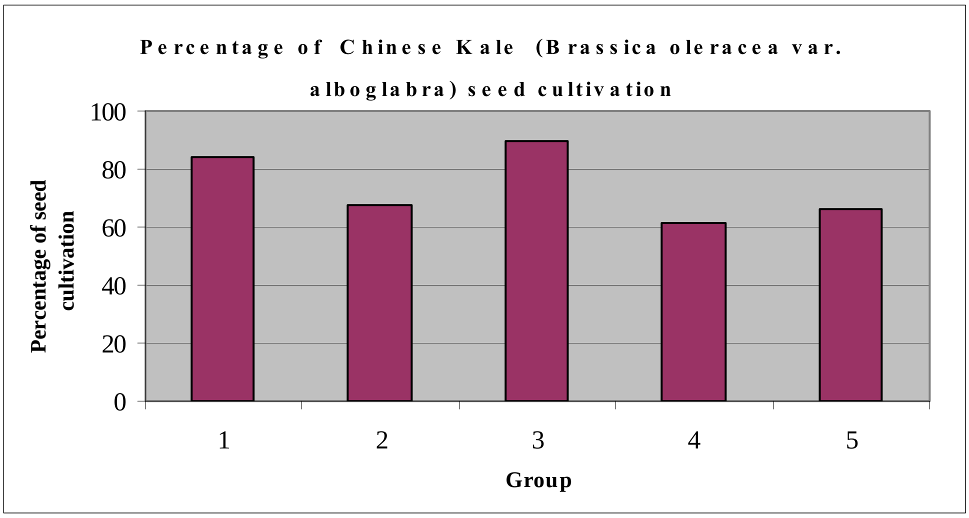


Figure 4.19 Percentage of Chinese kale (*Brassica oleracea var. alboglabra*) seed cultivation

4.4 Heavy metals accumulation in Chinese Kale (*Brassica oleracea var. alboglabra*)

4.4.1 Concentrations of Pb, Ni, and Cu in the shoot of Chinese kale

In this part, the shoot of Chinese kale (*Brassica oleracea var. alboglabra*) were harvested to analyze for the concentrations of Pb, Ni, and Cu. Average concentrations of Pb, Ni, and Cu are presented in Table 4.8. Figure 4.20, 4.21 and 4.22 presented concentrations of heavy metals compared between 5 groups of material that used for growing Chinese kale.

Table 4.8 Results of heavy metals accumulation in the shoot of Chinese kale

Group	*Concentrations of heavy metals in the shoot (mg/kg)*		
	Pb	***Ni***	***Cu***
1	*0.47*	*0.09*	*1.34*
2	*2.44*	*0.14*	*3.16*
3	*1.93*	*0.13*	*3.72*
4	*0.90*	*0.14*	*2.42*
5	*1.03*	*0.25*	*5.00*
standard value*	***0.30***	***67.90***	***73.30***

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

Ranges of Pb mean concentrations in the shoot were 0.47 mg/kg to 2.45 mg/kg. Pb contents in the shoot from all groups were higher than the recommended maximum level of metal concentrations in leafy vegetables. Whereas Ni and Cu contents in the shoot of Chinese kale were lower than the recommended maximum level of metal concentrations in leafy vegetables. Statistical analysis in Table 4.9 showed that Pb, Ni, and Cu in the shoot compared between groups were different significantly ($p<0.05$). Results in Table 4.10 describe the mean concentrations of Pb accumulated in the shoot of Chinese kale. The mean concentration of Pb from the shoot growing in

PS-D flowerpot with paraffin coating mixed with soil and that in PS-D flowerpot with paraffin coating were in the same group which were different from that in PS-D flowerpot without paraffin coating. While the mean concentration of Pb from the shoot growing in PS-D flowerpot without paraffin coating mixed with soil group was the highest.

In terms of Ni from the shoot, the mean concentrations of the one growing in PS-D flowerpot without paraffin coating, PS-D flowerpot without paraffin coating mixed with soil, and PS-D flowerpot with paraffin coating mixed with soil group were in the same group, but different from that in PS-D flowerpot with paraffin coating (Table 4.11). The mean concentrations of Cu accumulated in the shoot of Chinese kale in all groups were different as presented in Table 4.12. The pictures of laboratory analysis are presented in Appendix C.

Table 4.9 Descriptive Statistical Analysis of metals in the shoot of Chinese kale

Parameter	*group*	*N*	*Mean Mg/kg*	*Std. Deviation (x 10^{-3})*	*Range (Minimum – Maximum) mg/kg*	*Asymp Sig.**
Pb in shoot	*control group*	*3*	*0.47*	*3.35*	*0.47 – 0.48*	*.009*
	PS-D Flowerpot without paraffin coating +soil	*3*	*2.44*	*78.46*	*2.39 – 2.54*	
	PS-D Flowerpot coating without paraffin	*3*	*1.93*	*82.83*	*1.84 – 2.00*	
	PS-D Flowerpot with paraffin coating +soil	*3*	*0.90*	*9.80*	*0.89 - 0.90*	
	PS-D Flowerpot with paraffin coating	*3*	*1.03*	*5.10*	*1.03 – 1.04*	
Ni in shoot	*control group*	*3*	*0.09*	*0.70*	*0.09 – 0.10*	*.018*
	PS-D Flowerpot without paraffin coating +soil	*3*	*0.14*	*16.58*	*0.12 - 0.15*	
	PS-D Flowerpot without paraffin coating	*3*	*1.13*	*13.63*	*0.12 – 0.14*	
	PS-D Flowerpot with paraffin coating +soil	*3*	*0.14*	*0.30*	*0.14 – 0.14*	
	PS-D Flowerpot with paraffin coating	*3*	*0.25*	*1.20*	*0.25 – 0.25*	
Cu in shoot	*control group*	*3*	*1.34*	*44.95*	*1.29- 1.38*	*.009*
	PS-D Flowerpot without paraffin coating +soil	*3*	*3.16*	*97.43*	*3.05 – 3.24*	
	PS-D Flowerpot without paraffin coating	*3*	*3.72*	*75.81*	*3.63 – 3.78*	
	PS-D Flowerpot with paraffin coating +soil	*3*	*2.42*	*6.15*	*2.42 – 2.43*	
	PS-D Flowerpot with paraffin coating	*3*	*5.00*	*49.25*	*4.95 – 5.05*	

* from non-parametric test ; Kruskal Wallis Test

Table 4.10 Homogeneous Subset of concentrations of Pb in the shoot

group	*N* ^{a}	*Subset for alpha = .05 Concentrations of Pb in the shoot (mg/l)*			
		1	*2*	*3*	*4*
control group	*3*	*0.47*			
PS-D Flowerpot with paraffin coating +soil	*3*		*0.90*		
PS-D Flowerpot with paraffin coating	*3*		*1.03*		
PS-D Flowerpot without paraffin coating	*3*			*1.93*	
PS-D Flowerpot without paraffin coating +soil	*3*				*2.44*

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table 4.11 Homogeneous Subset of concentrations of Ni in the shoot

group	*N* ^{a}	*Subset for alpha = .05 Concentrations of Ni in the shoot (mg/l)*		
		1	*2*	*3*
control group	*3*	*0.09*		
PS-D Flowerpot without paraffin coating	*3*		*0.13*	
PS-D Flowerpot without paraffin coating +soil	*3*		*0.14*	
PS-D Flowerpot with paraffin coating +soil	*3*		*0.14*	
PS-D Flowerpot with paraffin coating	*3*			*0.25*

Means for groups in homogeneous subsets are displayed.

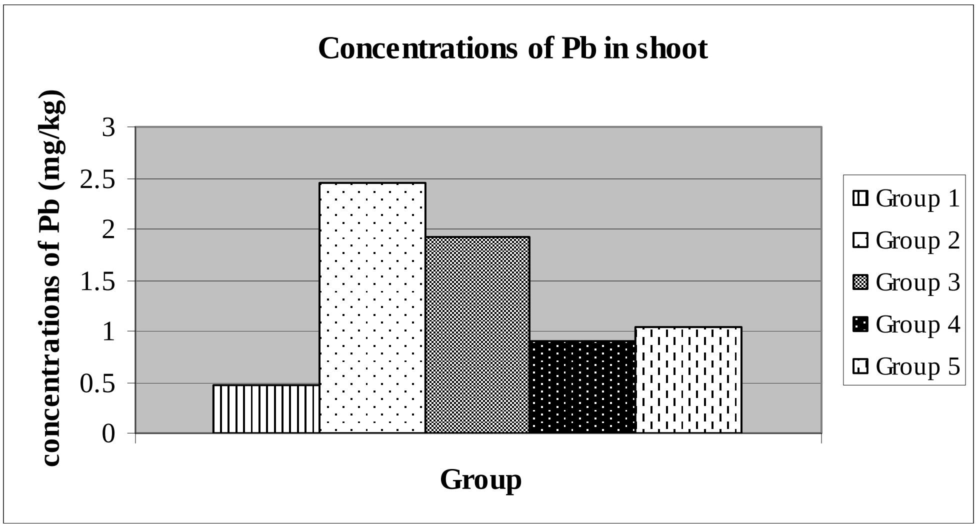
a Uses Harmonic Mean Sample Size = 3.000.

Table 4.12 Homogeneous Subset of concentrations of Cu in the shoot

group	*N* [a]	*Subset for alpha = .05* *Concentrations of Cu in the shoot (mg/l)*				
		1	*2*	*3*	*4*	*5*
control group	*3*	*1.34*				
PS-D Flowerpot with paraffin coating +soil	*3*		*2.42*			
PS-D Flowerpot without paraffin coating +soil	*3*			*3.16*		
PS-D Flowerpot without paraffin coating	*3*				*3.72*	
PS-D Flowerpot with paraffin coating	*3*					*5.00*

Means for groups in homogeneous subsets are displayed.

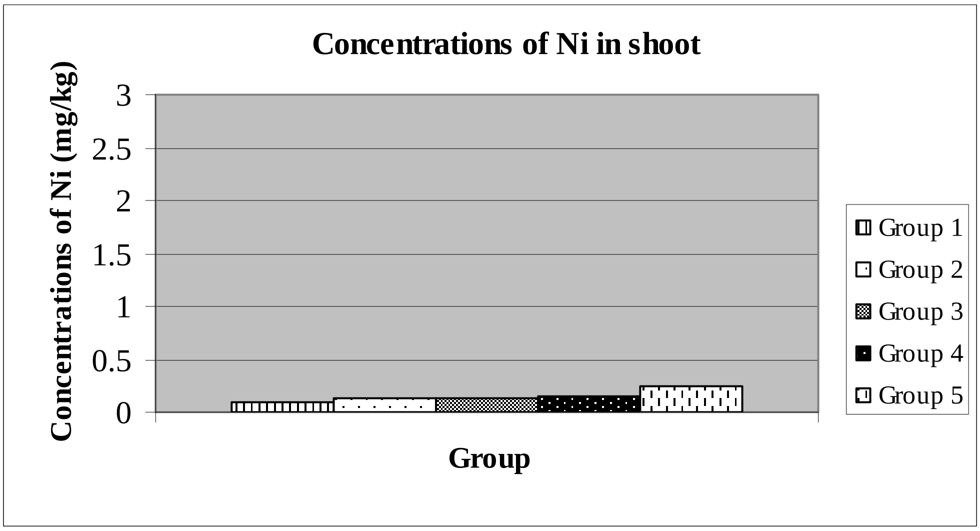
a Uses Harmonic Mean Sample Size = 3.000.



Standard Value : Pb < 0.30*

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

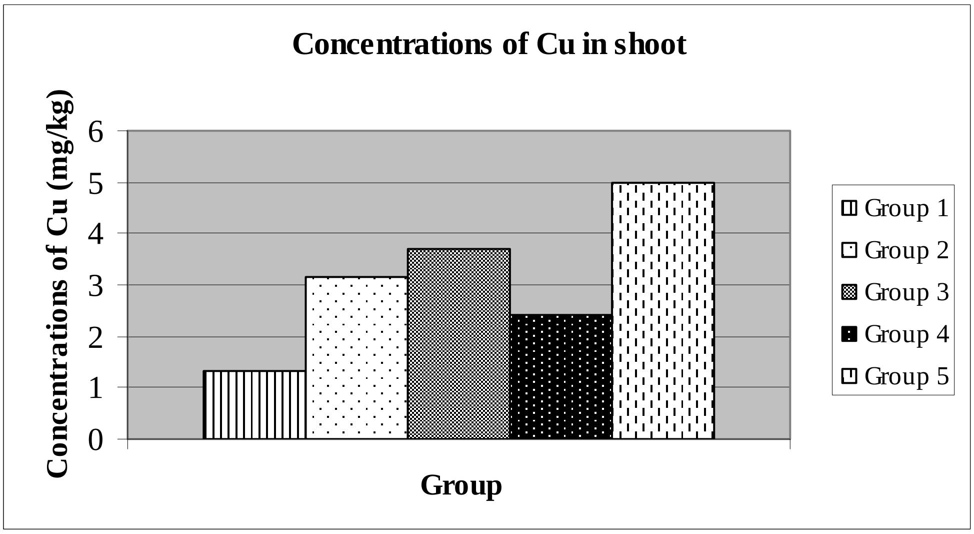
Figure 4.20 Concentrations of Pb in the shoot



Standard Value : Ni < 67.90*

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

Figure 4.21 Concentrations of Ni in the shoot



Standard Value : Cu < 73.30*

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

Figure 4.22 Concentrations of Cu in the shoot

4.4.2 Concentrations of Pb, Ni, and Cu in the root of Chinese kale

Table 4.13 shows Pb, Ni, and Cu accumulated in the root of Chinese kale. Pb obtained in the root to be arranged of group were 1.78, 1.08, 2.70 and 0.40 mg/kg all of which were higher than the recommended maximum level of metal concentrations in leafy vegetables (0.30 mg/kg). Nevertheless, the results of Ni and Cu were within the recommended level. The ranges of Ni were 0.20 mg/kg to 0.51 mg/kg and Cu were 1.41mg/kg to 5.95 mg/kg. Pictures of laboratory analysis are presented in Appendix C.

In terms of statistical analysis, Table 4.14 shown descriptive statistical analysis of metals in the root of Chinese kale.

Table 4.13 Results of Heavy metals accumulation in the root of Chinese kale

Group	***Concentrations of heavy metals in the root (mg/kg)***		
	Pb	***Ni***	***Cu***
1	*ND***	*ND***	*ND***
2	*1.78*	*0.51*	*3.38*
3	*1.08*	*0.20*	*5.95*
4	*2.70*	*0.36*	*2.45*
5	*0.40*	*0.47*	*1.41*
standard value*	***0.30***	***67.90***	***73.30***

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

** not detected

Table 4.14 Descriptive Statistical Analysis of metals in the root of Chinese kale

Parameter	*group*	*N*	*Mean mg/kg*	*Std. Deviation (x 10^{-3})*	*Range (Minimum – Maximum) mgkg*	*Asymp. Sig.**
Pb in root	*control group*	*3*	*0.02*	*0.00*	*0.02 – 0.02*	*.009*
	PS-D Flowerpot without paraffin coating +soil	*3*	*1.78*	*212.35*	*1.54 – 1.92*	
	PS-D Flowerpot without paraffin coating	*3*	*1.08*	*124.15*	*1.00 – 1.22*	
	PS-D Flowerpot with paraffin coating +soil	*3*	*2.70*	*153.05*	*2.55 – 2.86*	
	PS-D Flowerpot with paraffin coating	*3*	*0.40*	*102.5*	*0.29 – 0.50*	
Ni in root	*control group*	*3*	*0.02*	*0.00*	*0.02 – 0.02*	*.011*
	PS-D Flowerpot without paraffin coating +soil	*3*	*0.51*	*58.29*	*0.47 – 0.58*	
	PS-D Flowerpot without paraffin coating	*3*	*0.20*	*2.37*	*0.20 – 0.20*	
	PS-D Flowerpot with paraffin coating +soil	*3*	*0.36*	*51.05*	*0.31 – 0.41*	
	PS-D Flowerpot with paraffin coating	*3*	*0.47*	*29.40*	*0.44 – 0.50*	
Cu in root	*control group*	*3*	*0.20*	*0.00*	*0.20 – 0.20*	*.009*
	PS-D Flowerpot without paraffin coating +soil	*3*	*3.38*	*193.12*	*3.17 – 3.56*	
	PS-D Flowerpot without paraffin coating	*3*	*5.95*	*220.41*	*5.70 – 6.12*	
	PS-D Flowerpot with paraffin coating +soil	*3*	*2.45*	*102.05*	*2.35 – 2.55*	
	PS-D Flowerpot with paraffin coating	*3*	*1.41*	*88.25*	*1.32 – 1.50*	

* From non-parametric test ; Kruskal Wallis Test

The descriptive statistical analysis of metals in the root of Chinese kale (Table 4.14) summarized that concentrations of Pb, Ni, and Cu accumulated in the root were significantly different ($p<0.05$). Although mean concentrations of Pb from the shoot in PS-D flowerpot with paraffin coating group were not different with that in control group, it was different from other groups (Table 4.15). In terms of Ni from the root, the mean concentrations from the one in PS-D flowerpot with paraffin coating group were in same group with that in PS-D flowerpot without paraffin coating mixed with soil group shown in Table 4.16. As for the mean concentrations of Cu in the root, all were different between groups as shown in Table 4.17.

Compared concentrations of Pb, Ni, and Cu in the root of Chinese kale are presented in Figure 4.23, 4.24 and 4.25. Since the concentrations of all heavy metals (Pb, Ni, and Cu) in control group were lower than the detection limit of analysis, therefore, bar of control group (group 1) it cannot be shown by the bar graph.

Table 4.15 Homogeneous Subset of concentrations of Pb in the root

		Subset for alpha = .05 Concentrations of Pb in the root (mg/l)			
group	*N*[a]	*1*	*2*	*3*	*4*
control group	*3*	*0.02*			
PS-D Flowerpot with paraffin coating	*3*	*0.40*			
PS-D Flowerpot without paraffin coating	*3*		*1.08*		
PS-D Flowerpot without paraffin coating +soil	*3*			*1.78*	
PS-D Flowerpot with paraffin coating +soil	*3*				*2.70*

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table 4.16 Homogeneous Subset of concentrations of Ni in the root

group	N [a]	Subset for alpha = .05 Concentrations of Ni in the root (mg/l)			
		1	2	3	4
control group	3	0.02			
PS-D Flowerpot without paraffin coating	3		0.20		
PS-D Flowerpot with paraffin coating +soil	3			0.36	
PS-D Flowerpot with paraffin coating	3				0.47
PS-D Flowerpot without paraffin coating +soil	3				0.51

Means for groups in homogeneous subsets are displayed.

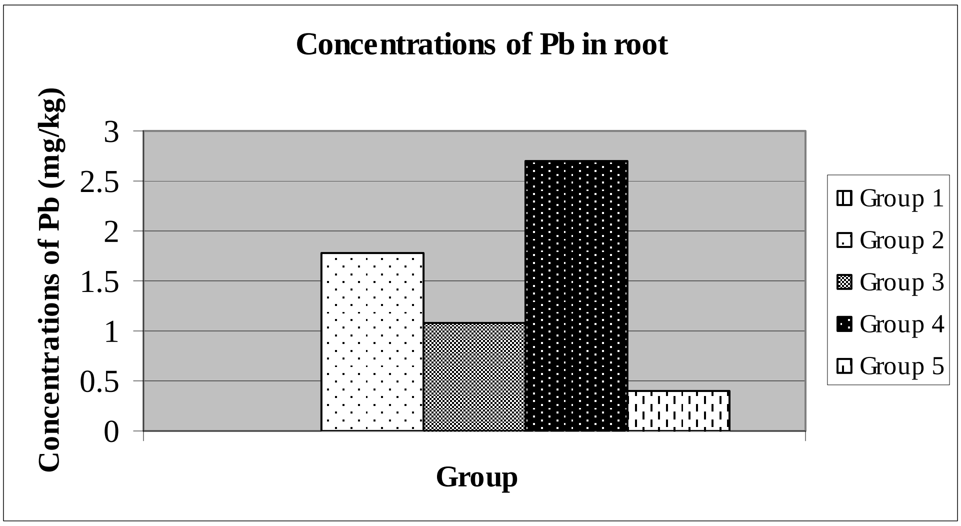
a Uses Harmonic Mean Sample Size = 3.000.

Table 4.17 Homogeneous Subset of concentrations of Cu in the root

group	N [a]	Subset for alpha = .05 Concentrations of Ni in the root (mg/l)				
		1	2	3	4	5
control group	3	0.20				
PS-D Flowerpot with paraffin coating	3		1.41			
PS-D Flowerpot with paraffin coating +soil	3			2.45		
PS-D Flowerpot without paraffin coating +soil	3				3.38	
PS-D Flowerpot without paraffin coating	3					5.95

Means for groups in homogeneous subsets are displayed.

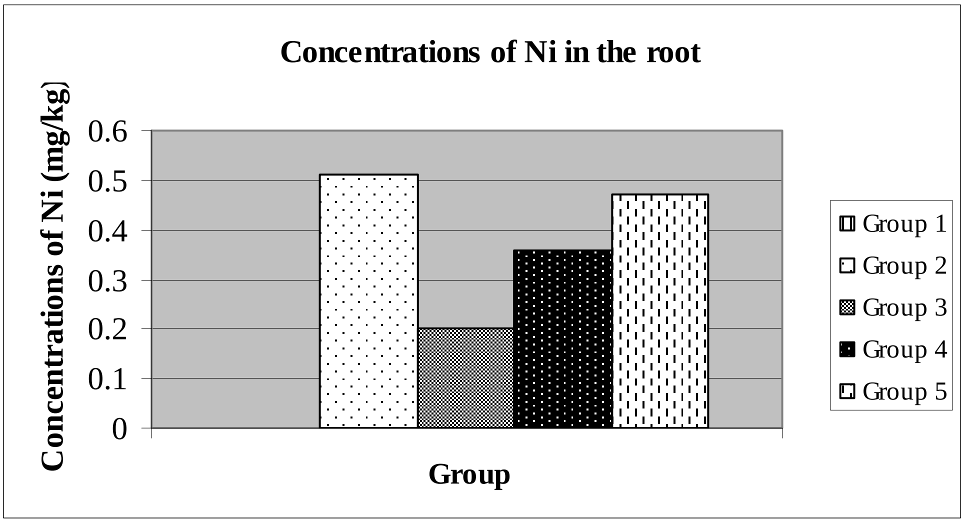
a Uses Harmonic Mean Sample Size = 3.000.



Standard Value: Pb < 0.3*

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

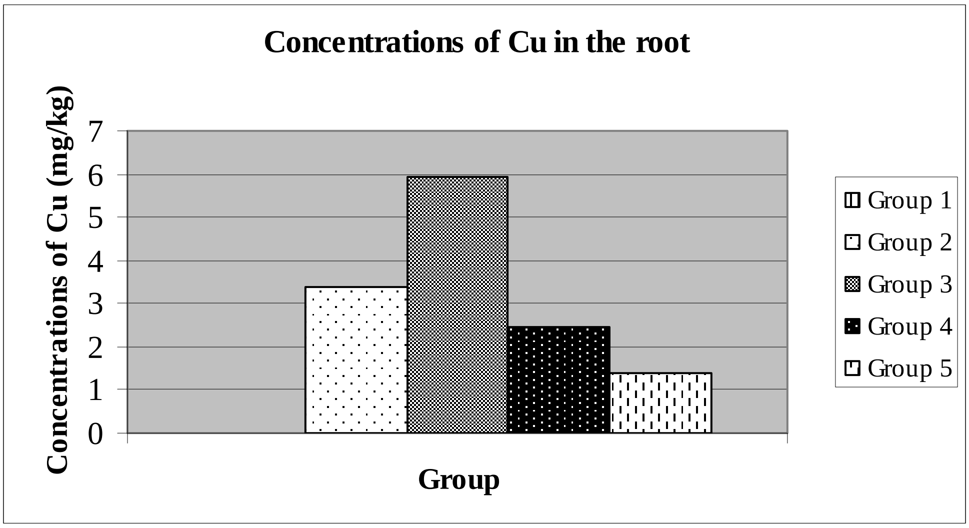
Figure 4.23 Concentrations of Pb in the root



Standard Value: Ni < 67.90*

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

Figure 4.24 Concentrations of Ni in the root



Standard Value: Cu < 73.30*

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

Figure 4.25 Concentrations of Cu in the root

CHAPTER V

DISCUSSION

5.1 Characteristics of PS-D flowerpot

The results from chapter 4 showed total concentrations of Pb, Ni, and Cu in both types of PS-D flowerpot before growing plant. For PS-D flowerpot without paraffin coating, the concentrations of Pb, Ni, and Cu were 10.64, 1.73, and 10.57 mg/kg, respectively. While PS-D flowerpot with coating paraffin, the content of Pb, Ni, and Cu were 9.55, 1.29, and 10.36 mg/kg , respectively. With these considerable amount of heavy metals contained in the PS-D flowerpot, it is interesting to study about leaching concentrations and the effect of heavy metals accumulated in plant.

5.2 Effect of heavy metals leaching from PS-D flowerpot

The appropriateness of using the PS-D flowerpot and its impact on the environment was assessed by the concentrations of heavy metals leaching from the flowerpot. The amount of lead (Pb) leaching from 3 groups ranged from 0.0004 – 0.0008 mg/l (Table 5.1). It can be noticed that higher Pb concentrations were leached from PS-D flowerpot without paraffin coating than from the one with paraffin coating.The amount of Ni leached from both types of PS-D flowerpot was below the detection limit. For Cu, the metal leached from PS-D flowerpot without paraffin coating was higher than the other one. In summary, the result of Pb and Cu leaching from the PS-D flowerpot without paraffin coating was higher than those from the one with paraffin coating. This finding agreed with hypothesis#1 of the study. However, the concentrations of heavy metals leaching were lower than the standard values for selected heavy metal and other toxic elements based on leaching test showed in Table

5.1.Thus, utilized PS-D flowerpot will not seriously affect groundwater, infiltration or receiving water resource located close to where PS-D flowerpot buried underground.

Table 5.1 Concentrations of heavy metals in PS-D flowerpot and leaching results

Sample	***Total concentrations of heavy metals (mg/kg)***			***Leaching concentrations of heavy metals (mg/l)***		
	Pb	***Ni***	***Cu***	***Pb***	***Ni***	***Cu***
soil	*2.85*	*2.02*	*7.68*	*0.0004*	*N.D**	*0.0005*
PS-D Flowerpot without paraffin coating	*10.64*	*1.73*	*10.57*	*0.0008*	*N.D**	*0.0006*
PS-D Flowerpot with paraffin coating	*9.55*	*1.29*	*10.36*	*0.0006*	*N.D**	*0.0004*
Standard Value for leaching (mg/l)**				5	20	25

* not detected

**The Notification of the Ministry of Industry Subject: Disposal of Wastes or Unusable Materials B.E 2548 (2005)

5.3 Effect of using PS-D flowerpot on growing plant

5.3.1 Study of seed cultivation

From the results of seeds cultivation (Table 4.6), the percentage of seedling showed feasibility of using PS-D flowerpot to grow Chinese kale.The impact of heavy metals on seed cultivation was assessed assuming that PS-D flowerpot were blended with soil in the environment. According to the results, at 20 days after cultivating 50 seeds of Chinese kale, number of seedling in group 3 (crushed PS-D flowerpots without paraffin coating) was the highest (89.3 %). The seedling growth percentages in PS-D flowerpot without paraffin coating group (group 2 and group 3) were higher than those in PS-D flowerpot with paraffin coating group(group 4 and group 5) due to insoluble property of paraffin that coated on flowerpot, therefore, percolation of water into soil was protected ; thus, seeds were difficult to grow. Furthermore, growth of

Chinese kale in group 2 and group 3 were higher than group 4 and group 5 due to the same reason with seedling percentage result.

According to seedling result, percentage of seedling was increasing when the ration of crushed PS-D flowerpot was increased. This result is in contrast with the study hypothesis due to organic matter in PS-D flowerpot. According to result of seedling percentage, it was assumed that utilization of PS-D flowerpot without paraffin coating on growing plant had no adverse effect on seed cultivation. However, PS-D flowerpot with paraffin coating (group 4 and group 5) have the tendency to cause an adverse effect to decrease seedling and the growth of Chinese kale.

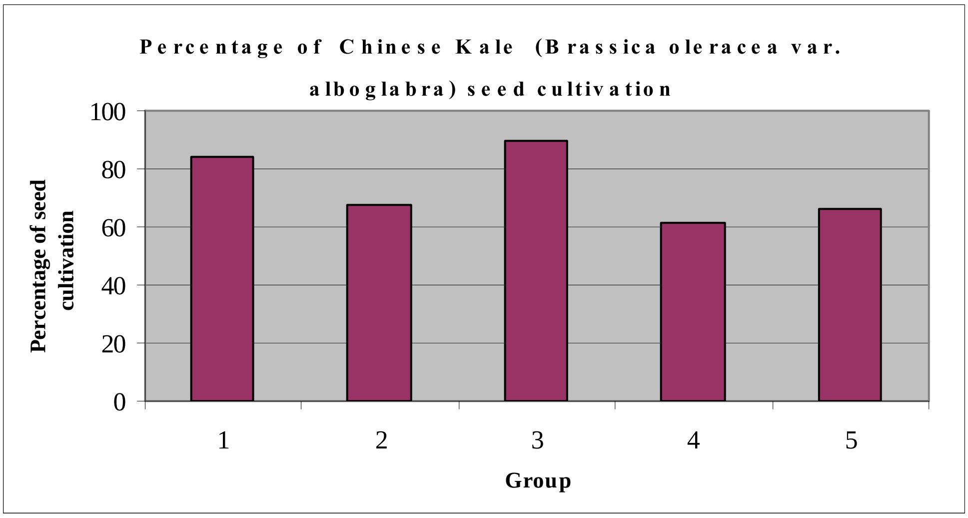


Figure 5.1 Percentage of Chinese kale *(Brassica oleracea var. alboglabra)* seed cultivation

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

5.4 Heavy metal accumulation on plant

In this part, the effect of PS-D Flowerpot application and concentrations of heavy metals (Pb, Ni, and Cu) accumulated in Chinese kale was assessed, including the adverse effect on plant growth and human health.

5.4.1Pb in the shoot and root of Chinese kale

According to Pb level that accumulated in the shoot compared with the recommended maximum level of metal concentrations in leafy vegetables, all groups were higher than maximum value. The highest Pb accumulated in the shoot of Chinese kale that cultivated in crushed PS-D flowerpot without paraffin coating mixed with soil likewise concentrations in the shoot of Chinese kale on PS-D flowerpot with coating paraffin will be lower. The result of high concentrations of Pb accumulated in the shoot of Chinese kale indicated that the plants cultivated in PS-D flowerpot would not be safe for consumption.

In terms of Pb levels in the root of Chinese kale, it followed the same pattern as the one in the shoot. All groups were higher than the recommended maximum level of metal concentrations in leafy vegetables. Thus, Pb levels in the root supported the summary that Pb levels in Chinese kale cultivated in PS-D flowerpot were not safe for human uptake.

Table 5.2 Results of Pb accumulated in the shoot and root of Chinese kale compared with the recommended maximum level of metal concentrations in leafy vegetables

Group	***Concentrations of Pb in the shoot (mg/kg)***	***Concentrations of Pb in the root (mg/kg)***
1	*0.4729*	*ND*
2	*2.4448*	*1.7828*
3	*1.9261*	*1.0816*
4	*0.895*	*2.7041*
5	*1.0345*	*0.3971*
standard value*	***0.3***	***0.3***

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

5.4.2 Ni in the shoot and root of Chinese kale

Ni contents in the shoot and root of Chinese kale were less than the recommended maximum level of metal concentrations in leafy vegetables (67.9 mg/kg). Table 5.3 shows comparison of the result with the standard value of Ni accumulated in the shoot and root. In summary, using PS-D flowerpot to grow Chinese kale would be safe for Ni levels uptake.

Table 5.3 Result of Ni accumulated in the shoot and the root of Chinese kale compared with the recommended maximum level of metal concentrations in leafy vegetables

Group	***Concentrations of Ni in the shoot (mg/kg)***	***Concentrations of Ni in the root (mg/kg)***
1	*0.0945*	*ND*
2	*0.1363*	*0.5098*
3	*0.1258*	*0.2027*
4	*0.1432*	*0.3572*
5	*0.2463*	*0.4706*
standard value*	***67.9***	***67.9***

Group 1 Control group: using 1000 g of soil.
Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.
Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.
Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.
Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

5.4.3 Cu in the shoot and root of Chinese kale

With regard to Cu concentrations in the shoot, all groups in this study were lower than the recommended maximum level of metal concentrations in leafy vegetables so level of Cu were safe for human and plant.

The levels of Cu in the root were lower than the standard as well as Cu in the shoot; thus, it can be assumed that using of PS-D flowerpot was no adverse effect to plant and human. Table 5.4 shows supporting data of the summary.

Table 5.4 Results of Cu accumulated in the shoot and root of Chinese kale compared with the recommended maximum level of metal concentrations in leafy vegetables

Group	***Concentrations of Cu in the shoot (mg/kg)***	***Concentrations of Cu in the root (mg/kg)***
1	*1.3361*	*ND*
2	*3.1605*	*3.3757*
3	*3.7186*	*5.9476*
4	*2.4225*	*2.449*
5	*5.0003*	*1.4118*
standard value*	***73.3***	***73.3***

Group 1 Control group: using 1000 g of soil.

Group 2 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots without paraffin coating.

Group 3Using 1000 g of crushed PS-D flowerpots without paraffin coating.

Group 4 Using 500 g of soil mixed with 500 g of crushed PS-D flowerpots with paraffin coating.

Group 5 Using 1000 g of crushed PS-D flowerpots with paraffin coating.

* Recommended Maximum Level of Metal concentrations in leafy vegetables adapted from Australia New Zealand Food standard 2001-2003 (The Australia and New Zealand Food Regulation Ministerial Council, 2001)

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

6.1.1 Pb and Cu leaching from PS-D flowerpot without paraffin coating were higher than those from PS-D flowerpot with paraffin coating but not higher than the standard value for selected heavy metals and other toxic elements based on leaching test. So it should not have an adverse effect when leaching into groundwater.

6.1.2 Pb accumulated in the shoot and root of Chinese kale were higher than the recommended maximum level of metal concentrations in leafy vegetables; thus, using PS-D flowerpot to grow plant will not be safe for human consumption.

6.1.3 Levels of Ni and Cu in the shoot and root of Chinese kale were lower than the recommended maximum level of metal concentrations in leafy vegetables.

6.1.4 Both PS-D flowerpot without paraffin coating and coating with paraffin were not suitable for growing edible plant, due to the fact that concentrations of Pb in Chinese kale was higher than the recommended maximum level of metal concentrations in leafy vegetables.

6.2 Recommendations

Based on the results of this study, the following recommendations have been offered:

6.2.1 Other heavy metals should be considered in future study.

6.2.2 In future study, a specific type of metal should be emphasized.

6.2.3 Other kinds of plants should be tested in future study such as fruit tree.

6.2.4 In this study, the materials for making PS-D flowerpot came from paper kraft industry. In the future, sludge from other kinds of paper industry should be considered.

6.2.5 Application of PS-D flowerpot which using sludge from paper kraft industry for growing plant in the actual practice would not be safe for growing edible leafy vegetable.

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APPENDIX

APPENDIX A: RESULT

Table A.1 Results of concentrations of heavy metals leaching into environmental compared with standard values.

Sample	Replicate	Concentrations of metals (mg/l)			Standard values (mg/l)		
		Pb	Ni	Cu	Pb	Ni	Cu
soil							
	1	0.0004	ND.	0.0005	0.05	0.05	1
	2	0.0004	ND.	0.0005	0.05	0.05	1
	3	0.0004	ND.	0.0005	0.05	0.05	1
	avg	0.0004		0.0005			
sludge							
	1	0.0007	ND.	0.0002	0.05	0.05	1
	2	0.0007	ND.	0.0003	0.05	0.05	1
	3	0.0008	ND.	0.0003	0.05	0.05	1
	avg	0.0007		0.0003			
PS-D flowerpot without coating paraffin	1	0.0007	ND.	0.0007	0.05	0.05	1
	2	0.0007	ND.	0.0006	0.05	0.05	1
	3	0.0007	ND.	0.0007	0.05	0.05	1
	avg	0.0007		0.0007			
PS-D flowerpot with coating paraffin	1	0.0006	ND.	0.0004	0.05	0.05	1
	2	0.0006	ND.	0.0004	0.05	0.05	1
	3	0.0006	ND.	0.0004	0.05	0.05	1
	avg	0.0006		0.0004			

Table A.2 Results of seed cultivation

Group	Replicate	number of seed cultivation(initial 50 seeds)					
		5 days	10 days	15 days	20 days	25 days	30 days
1							
	1	42	43	43	43	43	43
	2	39	44	44	44	44	44
	3	30	39	39	39	39	39
	avg	37	42	42	42	42	42
2							
	1	33	34	34	34	34	34
	2	34	38	38	38	38	38
	3	27	29	29	29	29	29
	avg	31.33	33.67	33.67	33.67	33.67	33.67
3							
	1	28	45	45	45	45	45
	2	35	42	43	49	49	49
	3	31	40	40	40	40	40
	avg	31.33	42.33	42.67	44.67	44.67	44.67
4							
	1	18	31	32	32	32	32
	2	21	35	35	35	35	35
	2	13	25	25	25	25	25
	avg	17.33	30.33	30.67	30.67	30.67	30.67
5							
	1	25	32	33	33	33	33
	2	30	36	36	36	36	36
	3	20	29	30	30	30	30
	avg	25	32.33	33	33	33	33

Table A.3 Results of Heavy metals accumulation in the shoot of Chinese Kale

Date	Group	Replicate	Concentrations of heavy metals in the shoot (mg/kg)		
24/5/2006			Pb	Ni	Cu
	1				
		1	0.4695	0.0939	1.2911
		2	0.4762	0.0953	1.381
		3	1.3433	0.2985	4.3284
		avg	0.4729	0.0945	1.3361
	2				
		1	2.3944	0.1485	3.2394
		2	2.4048	0.1429	3.1905
		3	2.5352	0.1174	3.0516
		avg	2.4448	0.1363	3.1605
	3				
		1	1.934	0.1415	3.75
		2	1.8396	0.1179	3.7736
		3	2.0047	0.1179	3.6321
		avg	1.9261	0.1258	3.7186
	4				
		1	0.9048	0.1429	2.4286
		2	0.8852	0.1435	2.4163
		3	1.6316	0.3158	5.1053
		avg	0.895	0.1432	2.4225
	5				
		1	1.0396	0.2475	5.0495
		2	1.0294	0.2451	4.951
		3	4.5833	1.0417	20.2083
		avg	1.0345	0.2463	5.0003
	standard values		0.3	67.9	73.3

Table A.4 Results of heavy metals accumulation in the root of Chinese Kale

Date	Group	Replicate	Concentrations of heavy metals in the root (mg/kg)		
24/5/2006			Pb	Ni	Cu
	1				
		1	ND	ND	ND
		2	ND	ND	ND
		3	ND	ND	ND
		avg			
	2				
		1	1.9231	0.5769	3.5577
		2	1.8868	0.4717	3.3962
		3	1.5385	0.4808	3.1731
		avg	1.7828	0.5097	3.3757
	3				
		1	1.2245	0.2041	6.1224
		2	1.0204	0.2041	6.0204
		3	1	0.2	5.7
		avg	1.0816	0.2027	5.9476
	4				
		1	2.8571	0.3061	2.551
		2	2.551	0.4082	2.3469
		3	5.6818	0.9091	4.8455
		avg	2.7041	0.3572	2.449
	5				
		1	0.2941	0.4412	1.3235
		2	0.5	0.5	1.5
		3	1.6667	2.5	6.6667
		avg	0.1478	0.4706	1.4118
	standard values		0.3	67.9	73.3

APPENDIX B: STATISTICAL ANALYSIS

Table B.1 Descriptive statistical analysis of Leaching test

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
leaching of Pb	soil	3	.0003500	.00000000	.00000000	.0003500	.0003500
	sludge	3	.0007333	.00005774	.00003333	.0005899	.0008768
	PS-D flowerpot without paraffin coating	3	.0007500	.00008660	.00005000	.0005349	.0009651
	PS-D flowerpot with paraffin coating	3	.0005500	.00000000	.00000000	.0005500	.0005500
	Total	12	.0005958	.00017511	.00005055	.0004846	.0007071
leaching of Ni	soil	3	.0000100	.00000000	.00000000	.0000100	.0000100
	sludge	3	.0000100	.00000000	.00000000	.0000100	.0000100
	PS-D flowerpot without paraffin coating	3	.0000100	.00000000	.00000000	.0000100	.0000100
	PS-D flowerpot with paraffin coating	3	.0000100	.00000000	.00000000	.0000100	.0000100
	Total	12	.0000100	.00000000	.00000000	.0000100	.0000100

Table B.1 Descriptive statistical analysis of leaching test (continue)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
leaching of Cu	soil	3	.0004500	.00000000	.00000000	.0004500	.0004500
	sludge	3	.0002667	.00005774	.00003333	.0001232	.0004101
	PS-D flowerpot without paraffin coating	3	.0006333	.00002887	.00001667	.0005616	.0007050
	PS-D flowerpot with paraffin coating	3	.0003667	.00002887	.00001667	.0002950	.0004384
	Total	12	.0004292	.00014375	.00004150	.0003378	.0005205

Table B.2 Test of Homogeneity of Variances of leaching test

	Levene Statistic	df1	df2	Sig.
leaching of Pb	11.077	3	8	.003
leaching of Ni	.	3	.	.
leaching of Cu	7.111	3	8	.012

Table B.3 Homogeneous subsets of Leaching of Pb

group for leaching	N$_a$	Subset for alpha = .05		
		1	2	3
soil	3	.0003500		
PS-D flowerpot with paraffin coating	3		.0005500	
sludge	3			.0007333
PS-D flowerpot without paraffin coating	3			.0007500
Sig.		1.000	1.000	.984

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.4 Homogeneous subsets of Leaching of Cu

group for leaching	N$_a$	Subset for alpha = .05		
		1	2	3
sludge	3	.0002667		
PS-D flowerpot with paraffin coating	3	.0003667	.0003667	
soil	3		.0004500	
PS-D flowerpot without paraffin coating	3			.0006333
Sig.		.052	.110	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.5 Descriptive Statistical analysis of leaching test

NPar Tests

	N	Mean	Std. Deviation	Minimum	Maximum
leaching of Pb	12	.0005958	.00017511	.00035	.00085
leaching of Ni	12	.0000100	.00000000	.00001	.00001
leaching of Cu	12	.0004292	.00014375	.00020	.00065
group for leaching	12	2.50	1.168	1	4

Table B.6 Statistical analysis of leaching test by Kruskal-Wallis Test

Kruskal-Wallis Test
Test Statistics(a,b)

	leaching of Pb	leaching of Ni	leaching of Cu
Chi-Square	9.988	.000	10.645
df	3	3	3
Asymp. Sig.	.019	1.000	.014

a Kruskal Wallis Test

b Grouping Variable: group for leaching

Table B.7 Descriptive statistical analysis of Pb, Ni and Cu accumulation in plant test

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Pb in the shoot	control group	3	.472867	.0033501	.0019342	.464544	.481189
	PS-D flowerpot without paraffin +soil coating	3	2.444800	.0784612	.0452996	2.249892	2.639708
	PS-D flowerpot without paraffin coating	3	1.926100	.0828330	.0478237	1.720331	2.131869
	PS-D flowerpot with paraffin coating +soil	3	.895000	.0098000	.0056580	.870655	.919345
	PS-D flowerpot with paraffin coating	3	1.034500	.0051000	.0029445	1.021831	1.047169
	Total	15	1.354653	.7485794	.1932824	.940104	1.769203
Ni in the shoot	control group	3	.094567	.0007024	.0004055	.092822	.096311
	PS-D flowerpot without paraffin +soil coating	3	.136267	.0165772	.0095708	.095087	.177447

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
	PS-D flowerpot without paraffin coating	3	.125767	.0136255	.0078667	.091919	.159614
	PS-D flowerpot with paraffin coating +soil	3	.143200	.0003000	.0001732	.142455	.143945
	PS-D flowerpot with paraffin coating	3	.246300	.0012000	.0006928	.243319	.249281
	Total	15	.149220	.0537333	.0138739	.119463	.178977
Cu in the shoot	control group	3	1.336067	.0449500	.0259519	1.224405	1.447729
	PS-D flowerpot without paraffin +soil coating	3	3.160500	.0974280	.0562501	2.918475	3.402525
	PS-D flowerpot without paraffin coating	3	3.718567	.0758064	.0437668	3.530253	3.906880
	PS-D flowerpot with paraffin coating +soil	3	2.422467	.0061501	.0035507	2.407189	2.437744
	PS-D flowerpot with paraffin coating	3	5.000267	.0492500	.0284345	4.877923	5.122610
	Total	15	3.127573	1.2742357	.3290062	2.421925	3.833222
Pb in the root	control group	3	.020000	.0000000	.0000000	.020000	.020000
	PS-D flowerpot without paraffin +soil coating	3	1.782800	.2123471	.1225987	1.255301	2.310299
	PS-D flowerpot without paraffin coating	3	1.081633	.1241459	.0716757	.773238	1.390029

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
	PS-D flowerpot with paraffin coating+soil	3	2.704067	.1530500	.0883635	2.323869	3.084264
	PS-D flowerpot with paraffin coating	3	.397067	.1029500	.0594382	.141325	.652809
	Total	15	1.197113	1.0052677	.2595590	.640415	1.753812
Ni in the root	control group	3	.020000	.0000000	.0000000	.020000	.020000
	PS-D flowerpot without paraffin coating+soil	3	.509800	.0582882	.0336527	.365004	.654596
	PS-D flowerpot without paraffin coating	3	.202733	.0023671	.0013667	.196853	.208614
	PS-D flowerpot with paraffin coating +soil	3	.357167	.0510500	.0294737	.230351	.483982
	PS-D flowerpot with paraffin coating	3	.470600	.0294000	.0169741	.397566	.543634
	Total	15	.312060	.1897956	.0490050	.206955	.417165
Cu in the root	control group	3	.200000	.0000000	.0000000	.200000	.200000
	PS-D flowerpot without paraffin +soil coating	3	3.375667	.1931204	.1114981	2.895929	3.855404
	PS-D flowerpot without paraffin coating	3	5.947600	.2204094	.1272534	5.400073	6.495127
	PS-D flowerpot with paraffin coating +soil	3	2.448967	.1020500	.0589186	2.195460	2.702473

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
	PS-D flowerpot with paraffin coating	3	1.411767	.0882500	.0509512	1.192542	1.630992
	Total	15	2.676800	2.0200436	.5215730	1.558137	3.795463

Table B.8 Test of Homogeneity of Variances of Pb, Ni and Cu accumulation in plant test

	Levene Statistic	df1	df2	Sig.
Pb in the shoot	5.186	4	10	.016
Ni in the shoot	9.477	4	10	.002
Cu in the shoot	2.746	4	10	.089
Pb in the root	2.836	4	10	.083
Ni in the root	3.719	4	10	.042
Cu in the root	2.706	4	10	.092

Robust tests of equality of means cannot be performed for Pb in the root because at least one group has 0 variance.

Table B.9 Homogeneous subsets of Pb accumulation in the shoot

group		Subset for alpha = .05			
		1	2	3	4
control group	3	.472867			
PS-D flowerpot with paraffin coating +soil	3		.895000		
PS-D flowerpot with paraffin coating	3		1.034500		
PS-D flowerpot without paraffin coating	3			1.926100	
PS-D flowerpot without paraffin coating +soil	3				2.444800
Sig.		1.000	.087	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.10 Homogeneous subsets of Ni accumulation in the shoot

group	N_a	Subset for alpha = .05		
		1	2	3
control group	3	.094567		
PS-D flowerpot without paraffin coating	3		.125767	
PS-D flowerpot without paraffin coating +soil	3		.136267	
PS-D flowerpot with paraffin coating +soil	3		.143200	
PS-D flowerpot with paraffin coating	3			.246300
Sig.		1.000	.357	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.11 Homogeneous subsets of Cu accumulation in the shoot

group	N_a	Subset for alpha = .05				
		1	2	3	4	5
control group	3	1.336067				
PS-D flowerpot with paraffin coating + soil	3		2.422467			
PS-D flowerpot without paraffin coating +soil	3			3.160500		
PS-D flowerpot without paraffin coating	3				3.718567	
PS-D flowerpot with paraffin coating	3					5.000267
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.12 Homogeneous subsets of Pb accumulation in the root

group	N a	Subset for alpha = .05			
		1	2	3	4
control group	3	.020000			
PS-D flowerpot with paraffin coating	3	.397067			
PS-D flowerpot without paraffin coating	3		1.08163 3		
PS-D flowerpot without paraffin coating +soil	3			1.782800	
PS-D flowerpot with paraffin coating +soil	3				2.704067
Sig.		.084	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.13 Homogeneous subsets of Ni accumulation in the root

group	N a	Subset for alpha = .05			
		1	2	3	4
control group	3	.020000			
PS-D flowerpot without paraffin coating	3		.202733		
PS-D flowerpot with paraffin coating +soil	3			.357167	
PS-D flowerpot with paraffin coating	3				.470600
PS-D flowerpot without paraffin coating +soil	3				.509800
Sig.		1.000	1.000	1.000	.791

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.14 Homogeneous subsets of Cu accumulation in the root

group	N$_a$	Subset for alpha = .05				
		1	2	3	4	5
control group	3	.200000				
PS-D flowerpot with paraffin coating	3		1.411767			
PS-D flowerpot with paraffin coating +soil	3			2.448967		
PS-D flowerpot without paraffin coating +soil	3				3.375667	
PS-D flowerpot without paraffin coating	3					5.947600
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Table B.15 Descriptive Statistical of Pb, Ni and Cu accumulation in plant test

NPar Tests

	N	Mean	Std. Deviation	Minimum	Maximum
Pb in the shoot	15	1.354653	.7485794	.4695	2.5352
Ni in the shoot	15	.149220	.0537333	.0939	.2475
Cu in the shoot	15	3.127573	1.2742357	1.2911	5.0495
Pb in the root	15	1.197113	1.0052677	.0200	2.8571
Ni in the root	15	.312060	.1897956	.0200	.5769
Cu in the root	15	2.676800	2.0200436	.2000	6.1224
group	15	3.00	1.464	1	5

Table B.16 Statistical analysis of Pb, Ni and Cu accumulation in plant test by Kruskal-Wallis Test

Test Statistics(a,b)

	Pb in shoot	Ni in shoot	Cu in shoot	Pb in root	Ni in root	Cu in root
Chi-Square	13.500	11.951	13.500	13.597	13.151	13.597
df	4	4	4	4	4	4
Asymp. Sig.	.009	.018	.009	.009	.011	.009

a Kruskal Wallis Test

b Grouping Variable: group

APPENDIX C: DETERMINATION OF HEAVY METALS IN CHINESE KALE

Part 1 Digestion

Figure C.1.1 Sample Weight

Figure C.1.2 Preparation sample to flask

Figure C.1.3 Preparation sample to flask (shoot and root of Chinese kale)

Figure C.1.4 Digestion with nitric acid

Figure C.1.5 Digestion with nitric acid

Figure C.1.6 Filter with filter paper

Part 2 Analysis

Figure C.2.1 Analyzed heavy metal with AA instrument

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