THE EFFECT OF EDTA-APPLICATION TO HEAVY METALS PHYTOREMEDIATION BY VETIVER

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ผลกระทบของ EDTA ต่อการดูดซับโลหะหนักในดินโดยหญ้าแฝก

นางสาวรมิดา วะสิโน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ภาวะดินปนเปื้อนโลหะหนักที่ประกอบด้วยตะกั่ว สังกะสี และแคดเมียม เป็นปัญหาหนึ่งที่เกิดขึ้นในประเทศไทย ซึ่งในงานวิจัยนี้ปัญหาดังกล่าวถูกแก้ไขโดยการบำบัดด้วยหญ้าแฝก เพื่อเปรียบเทียบความสามารถในการดูดซับโลหะหนักจากดินปนเปื้อนของหญ้าแฝกสองชนิด ได้แก่ หญ้าแฝกลุ่ม สายพันธุ์สงขลา3 และหญ้าแฝกดอน สายพันธุ์ประจวบคีรีขันธ์ นอกจากนี้ยังมีการศึกษาผลจากใช้ EDTA ในการช่วยชะละลายโลหะหนักจากดิน เพื่อเพิ่มประสิทธิภาพในการดูดซับโลหะหนักในหญ้าแฝก ดินที่บำบัดแล้วจะถูกนำมาทำการชะละลายด้วยวิธี TCLP พร้อมทั้งประเมินความเป็นพิษของดินด้วยวิธี Allium test และการงอกรากของเมล็ดผักกาด

ในการวิเคราะห์การย่อยด้วยกรดโดยใช้เครื่องไมโครเวฟ พบว่าความเข้มข้นของโลหะหนักในดินลดลง และมีการเพิ่มสูงขึ้นในบริเวณรากและลำต้นของหญ้าแฝกทั้งสองสายพันธุ์ พบว่าการใช้ EDTA ทำให้มีการเพิ่มขึ้นอย่างมีนัยสำคัญของการสะสมสังกะสีในรากและลำต้นของหญ้าแฝกสายพันธุ์สงขลา3 ส่วนสายพันธุ์ประจวบคีรีขันธ์ไม่พบว่ามีการเพิ่มขึ้นอย่างมีนัยสำคัญของการสะสมสังกะสีในส่วนราก การทดลองการชะละลายด้วยวิธี TCLP พบว่าการชะละลายโลหะหนักมีค่าต่ำกว่าเกณฑ์ของเสียอันตรายหลังจากบำบัดด้วยหญ้าแฝกในเวลาสามเดือน และภายหลังจากการใช้ EDTA ในเดือนที่สี่ ปริมาณแคดเมียมเพิ่มขึ้นสูงกว่าเกณฑ์มาตรฐาน ผลจากการทดสอบความเป็นพิษของดินหลังจากการบำบัดด้วยหญ้าแฝกด้วยวิธี Allium test ไม่พบความผิดปกติในระดับโครโมโซม และพบว่าเปอร์เซ็นต์การงอกของเมล็ดผักกาดในน้ำที่สกัดจากดินที่บำบัดด้วยหญ้าแฝกพันธุ์สงขลา3มีค่าสูงเมื่อเปรียบเทียบกับชุดควบคุม ผลการศึกษาในครั้งนี้สามารถยืนยันได้ว่าหญ้าแฝกทั้งสองสายพันธุ์มีความสามารถในการลดความเข้มข้นของโลหะหนักในดินปนเปื้อนและสามารถสะสมโลหะหนักในส่วนรากและลำต้นได้

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Contaminated soil with heavy metals includes Pb, Zn, and Cd were remediated by phytoremediation with vetiver to solved the problem in Thailand. This study compared the ability of two species of vetiver (i.e., Songkhla 3 and Prachuap Khiri Khan) in absorbing Pb, Zn, and Cd in contaminated soils and study the effect of EDTA application. After the experiment, Toxicity Characteristic Leaching Procedure (TCLP) test, Allium test, and seed germination were used to determined the toxicity of remediated soil. In this study, 5 mmol of ethylenediaminetetraacetic acid (EDTA) was used to improve the solubility of heavy metals in soil which leads to an increase in translocation factor and bioaccumulation factor.

According to microwave digestion method, the concentration of heavy metals includes Pb, Zn, and Cd was lower in soil and higher in both shoot and root parts of two ecotypes of vetiver in period of studied. While the EDTA application significantly increased the bioaccumulation of Zn in shoot and root part of Songkhla3, it did not significantly increase the total uptake of Zn in root parts of Prachuap Khiri Khan. TCLP results showed that the concentration of heavy metals was lower than regulatory level after 3 months of remediation,but concentration increased after EDTA application in fourth month. To confirm the ability of vetiver in phytoremediation, Allium test showed no effect of heavy metals in chromosomal aberration and high percentage of seed germination was observed in aliquot extracted from remediated soil by Songkhla3. The results from experiment confirmed that two ecotypes of vetiver grass have ability to reduce heavy metal concentration in contaminated soil and accumulated the in their shoot and root.

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CHAPTER 1
INTRODUCTION

1.1 State of problem

Nowadays, the rapid growth of industries, the global increase in urbanization, the use of fertilizers and pesticide in agricultural activities (Wuana & Okieimen, 2011), and mining activities are causing serious effects to humans and the environment. Many industries mismanage their waste handling and dispose of their solid and liquid wastes inappropriately (Lestan, Luo, & Li, 2008). Oftentimes, good technologies for waste handling are quite expensive and economically infeasible in developing countries. As a result, heavy metal contaminants end up in soil and water in many countries. The most common heavy metals found in contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) (Wuana & Okieimen, 2011). In soil, these heavy metals have various forms with different physical and chemical properties including mobility, biological availability, and toxicity. Toxic heavy metals in soil are not biodegradable and will remain in soils for a long time (Lestan et al., 2008). For these reasons, contaminated soil is a global environmental concern, and a local concern in Thailand, because of its effects on human health and ecosystem (Chen, Shen, & Li, 2004).

Heavy metal contaminated soil is encountered in Thailand in both urban and rural areas. Some examples include cadmium (Cd) contamination in rice at Mae Sot (Chantana, Wittaya, Muneko, Werawan, & Thawangon), and lead (Pb) contamination in Kanchanaburi province. An example of contamination from mining activities is lead (Pb) contamination found in Klity Village, Kanchanaburi province (Human Rights Watch, 2014). Leaking of wastewater from tailing ponds and mineral processing activities are other major anthropogenic sources of heavy metals contamination (2009). All these types of contamination can seriously affect living organisms in the areas they are present. Lead (Pb) in the environment can threaten human, plants, animals, and microorganisms. Pb^{2+} is a non-biodegradable form of lead which can remain in the environment for a long time and acts as a long-term source of lead exposure. High amounts of lead can cause acute toxic effects to human health (Pehlivan, Ozkan, Dinc, & Parlayici, 2009).

In the United States, the toxicity characteristic leaching procedure (TCLP) (EPA, 1992) is commonly used to assess the toxicity of pollutant in the environment (Kiran D Ladwani, Krishna D Ladwani, Vivek S Manik, & Dilip S Ramteke, 2012). This method was developed by the US Environmental Protection Agency (USEPA1992) to determine the mobility of organic and inorganic compounds. Moreover, TCLP can imitate landfill conditions when the water or other liquids leach through landfills and causes a higher risk to environment due to the contaminants in it.

Therefore, TCLP analysis can determine concentration of heavy metal leachate and help to predict toxic components of waste while they mobilized with the leachate (Todd A. Kimmell, Llewellyn R. Williams, & Susan S. Sorini, 2011). In conjunction to assessment of pollutant toxicity, there are many other methods that have been listed as best demonstrated available technologies (BDATs) for remediating soil contaminated with heavy metals. Some of these methods include immobilization, soil washing, and phytoremediation (Wuana & Okieimen, 2011). Specifically, in the countryside where contamination often covers large areas, phytoremediation has a potential to be an effective remediation method.

Recently, many researchers have claimed that phytoremediation as an alternative way to solve the problem of soil contamination (Ali, Khan, & Sajad, 2013; Chen et al., 2004; Ghosh, Paul, Jana, De, & Mukherjee, 2015; I. Raskin, 1999; Kumsorn & Chairak, 2013; Tangahu et al., 2011). Specifically, this method can be used as a practical technique to remove heavy metals from soil and water because this technique is both environmentally friendly and cost effective. Previous studies on using phytoremediation to treat heavy metal contaminated soil have used several plants (Bhattacharya T, Banerjee DK, & B., 2006 ; I. Raskin, 1999; Kumsorn & Chairak, 2013; Rotkittikhun, Chaiyarat, Kruatrachue, Pokethitiyook, & Baker, 2007).Vetiver grass is one of these plants which has been studied. Vetiver grass is very useful and environmental friendly because it can prevent soil erosion and tolerate up to a high concentration of heavy metals with no observable effects on its growth and development (Chou, Shih, & Lin, 2016). Vetiver grass is fast growing and requires low maintenance. Its root system can grow up to one to three meters into the ground and forms tight weaves as it penetrates the ground. As a result, its root system can efficiently absorb water and act as a habitat for soil microbes (Land Development Department). Many researchers have studied the plant's ability to remediate contaminated soil. In 2004, Chen Y. et al. found that using vetiver grass in soil doused with EDTA could increase the translocation of Pb from roots to shoots. Moreover, soils planted with vetiver grass could re-adsorb heavy metals which might reduce the penetration of heavy metals downward in the soil layer and reduced leaching of heavy metals through groundwater (Chen et al., 2004). In 2006, Rotkittikhun et al. studied heavy metals uptake and compared the effects between pig manure and inorganic fertilizer on four ecotypes of vetiver. The results showed that the application of pig manure reduced Pb uptake. (Rotkittikhun et al., 2007). In 2015, Ghosh et al. used vetiver grass to phytoremediate soil contaminated with fly ash sourced from a thermal power plant (Ghosh et al., 2015). The results from both studies showed that vetiver grass has ability to decrease the concentration of heavy metals in soil and that the contaminants accumulated in the vetiver root more than in its shoot. While the studies listed above investigated the ability of vetiver to remediate soil contaminated with heavy metals, our current study concentrated on comparing the ability of two species of *Vetiveria zizanioides* (vetiver grass) - which are Songkhla 3 (*Chrysopogon zizanioides*) and

Prachuap Khiri Khan (*Chrysopogon nemoralis*) - to reduce the concentration of Pb, Zn, and Cd in contaminated soil and water, and examine the toxicity in soil after remediation with vetiver by the Allium test and seed germination.

1.2 Hypothesis

1) Vetiver grass that is planted in soil with EDTA application can accumulate a higher Pb, Zn, and Cd concentration in its plant structure than vetiver planted in soil without EDTA application.
2) Two species of vetiver grass which are Songkhla 3 (*Chrysopogon zizanioides*) and Prachuap Khiri Khan (*Chrysopogon nemoralis*) can reduce concentration of Pb, Zn, and Cd in contaminated soil.
3) Soil toxicity can be reduced by phytoremediation.

1.3 Objectives

1) Study the effect of EDTA on reducing Pb, Zn, and Cd in soil by phytoremediation with vetiver.
2) Compare the ability of Songkhla 3 (*Chrysopogon zizanioides*) and Prachuap Khiri Khan (*Chrysopogon nemoralis*) vetiver in absorbing Pb, Zn, and Cd in contaminated soils.
3) Use the Allium test and Green oak seed germination to study the toxicity of soils after they have been phytoremediated with vetiver grass.

1.4 Scope of Study

1) This study considers 3 heavy metals include Pb ($PbCO_3$), Zn ($ZnCO_3$), and Cd ($CdCl_2$)
2) Two species of vetiver were studied which are Songkhla3 (*Chrysopogon zizanioides*) and Prachuap Khiri Khan (*Chrysopogon nemoralis*) to compare the ability in absorbing Pb, Zn, and Cd by determining the concentration of heavy metals that vetiver can accumulate in their shoots and roots.
3) 5 mmol of EDTA were added into 1 kg soil, and EDTA effect was considered on the increasing in translocation of heavy metals from roots to shoots of vetiver.

CHAPTER 2
LITERATURE REVIEW

During the 1960s to 1980s, the Bo Ngam lead mine located in Klity Village, Cha Lea sub-district, Thong Pha Phum district, Kanchanaburi province, was an important lead producer in Thailand. Lead in this area is in the form of cerussite ($PbCO_3$). Mining activities at the mine ended in 1999 and the ore dressing plant was closed by the Thailand Department of Mineral Resources and Pollution Control Department due to the tailing leaked from the mine operation's sediment pond into the Klity canal (Poopa, Pavasant, Kanokkantapong, & Panyapinyopol, 2015). The tailing leak caused heavy metal (lead, zinc, cadmium, and chromium) contamination in the Klity canal both in stream and sediment. The contamination became a concern because these heavy metals can accumulate in environment by vegetation and animals through their mobility and bioavailability (Nannoni, Protano, & Riccobono, 2011), causing long-term adverse health effects for people who live in the agricultural and residential zones neighboring the mining area.

2.1 Toxicity

Lead is classified as a probable human carcinogen. Predominant insoluble compounds of Pb are lead phosphates, lead carbonates, and lead (hydr)oxides which can form when pH is higher than 6 (I. Raskin, 1999). There are two routes of lead exposure: inhalation and ingestion. Pb can accumulate in human organs and has effects on the gastrointestinal tract, kidneys, and central nervous system (NSC, 2009).

Zinc usually occurs naturally in soil and is a trace element that is essential for human health. A lack of zinc can cause birth defects. However, Zn can also disturb the activities of microorganism and earthworms which live in soil and cause a delay in the breakdown of organic matter in soil (Greany, 2005).

Cadmium does not have much toxicological properties but it is very biopersistent. When organisms absorbed cadmium, the metal can remain for many years (Wuana & Okieimen, 2011). This will eventually lead to human exposure to the metal as it is bioaccumulated in the food chain before human ingestion (Campbell, 2006). Chronic cadmium exposure is a major threat to human health as the metal accumulates in the kidneys and causes kidney dysfunction (Manahan, 2003).

Table 2-1 Standard limits of heavy metals Agricultural soil (μg/g)

Heavy metals	**Pb**	**Zn**	**Cd**
PCD standard	400		37
Indian standard	250-500	300-600	3-6
European union Standard	3	300	3

Table 2-2 Toxic effects of heavy metals and its limitation in ppm (mg/kg)

Heavy Metals	EPA	WHO	Toxic Effects
Lead (Pb)	15	0.01	- In children, the most sensitive organ system for excess lead exposure is the nervous system. It also causes developmental problems, delayed learning, short-term memory loss, and lower IQ - Lead exposure can lead to renal effects and increased risk for hypertension and its consequences, disruption of the heme synthesis process results in lower blood hemoglobin, intervene the vitamin D formation. For pregnant women, it reduces fetal growth and lower birth weight.
Zinc (Zn)	0.5		Exposed to zinc impair of growth and reproduction
Cadmium (Cd)	5.0	0.05	Classified as probable human carcinogen and effected to cardiovascular, developmental, gastrointestinal, neurological, renal, reproductive, and respiratory systems.

Source: ("Agency for Toxic Substances and Disease Registry," 2017; Edao, 2017)

Due to these heavy metals are determined in form of available to plants uptake (Ettler, Vaněk, Mihaljevič, & Bezdička, 2005). Then, one alternative method that can restore the health of contaminated soils is phytoremediation by revegetation of plant species that are tolerant to high concentrations of heavy metals. The availability of heavy metals for plant uptake is affected by the solubility of the heavy metals in the affected soil. heavy metals in soil cannot be extracted if they are not at least tinily soluble. Heavy metals have to be in form of the soluble fraction which can be taken up by plants. The availability can be divided into three categories: available, unavailable, and exchangeable fraction (Sheoran, Sheoran, & Poonia, 2016). Plants can absorb heavy metals that are available and exist in soil solution as free metal ions and soluble metal complexes. Moreover, they can extract some part of heavy metals which are in exchangeable fractions. However, plants difficultly absorb unavailable heavy metals. Moreover, in polluted area heavy metals which can be transferred from soil to plant should be concern. Only total concentration of heavy metals in soil is not enough to assess the adverse effects which actually occur in soil ecosystem(R. Y. Kim et al., 2015) and does not provide predictive comprehension on their bioavailability, mobility and fate(Chengo Katana, Murungi Jane, & Harun*, 2013). A bioavailable form of heavy metals in soil can enter the environment and capable of causing bioaccumulation in other species increasing their toxicity. Moreover, it can accumulate in human body through food chain; therefore, the chemical forms of heavy metals are important factor in assessing their impact on environment. Due to various forms of heavy metals and different behaviors, the mechanisms of heavy metals in soil can be studied by monitoring speciation/ bioavailability concentration of heavy metals in soil. Normally, heavy metals in soil are present in precipitate forms from bounding to organic and inorganic soil components (Ilya Raskin, PBA Nanda Kumar, Slavik Dushenkov, & Salt, 1994). To enhance the solubility of heavy metals in soil, chelating agents such as EDTA (ethylenediaminetetraacetic acid), CDTA (trans-1,2-dia-minocyclohexane-*N*,*N*,*N*',*N*'-tetraacetic acid), and EGTA [ethyleneglyclo-bis(β-aminoethyl ether), *N*,*N*,*N*',*N*'-tetraacetic acid] can be used to desorb metals from the soil matrix and dissolve them into the soil solution and also increase the translocation of heavy metals from the roots to the shoots of plants (Evangelou, Ebel, & Schaeffer, 2007; Luo, Shen, & Li, 2005).

Various species of plants can remove and accumulate metal in their roots and shoots, so there are selective considerations of the proper plant to use (Wong, 2003). There are three key factors in determining the success of phytoremediation of contaminated soils: the amount of biomass, heavy metals concentration in plant tissues, and the bioavailable fraction of heavy metals in roots (Saifullah et al., 2009; Shahid et al., 2013).

Previous studies studied a various species of plant that can remove or remediate heavy metals in contaminated soil. A study from Cho-Ruk *et al*. in 2006, revealed the ability of *Brassica juncea*, *Brassica rapa*, and *Brassica napus* in accumulation Cd, Cr, Cu, Ni, Pb, and Zn in soil contaminated. The results from their study showed that *Brassica rapa* has the highest affinity to accumulated Cd ang Pb. Moreover, the results also suggested the accumulation in leaves > stems > root > fruit shell > seed (K. Cho-Ruk, J. Kurukote, P. Supprung, & S. Vetayasuporn, 2006). In contrast, Chandra *et al*. (2009) evaluated the potential of wheat (*Triticum aestivum* L. and *Brassica campestris* L.) in soil remediation in agricultural area. They studied soil contaminated with Cu, Cd, Cr, Zn, Fe, Ni, Mn, and Pb. The results showed that leachant and soil sample had higher metals concentration than the standard limit, except for Pb. Plant sample analysis showed that metal uptake and accumulation in root is more than shoot, leaves, and seeds of Fe followed by Mn and Zn (Chandra, Bharagava, Yadav, & Mohan, 2009).

In addition, vetiver is the one of those plant species which has been studied. Many researchers have studied the ability of vetiver grass and have claimed that this plant is not only has unique characteristics but it also can reduce the concentration of heavy metals in contaminated soils by accumulating heavy metals in their root and shoot (Ghosh et al., 2015; Ondo Zue Abaga, Dousset, Mbengue, & Munier-Lamy, 2014).

2.2 Vetiver

In Thailand, there are two species of vetiver: *Chrysopogon nemoralis* (Balansa) Holttum, and *Chrysopogon zizaniodes* (L.) Roberty. These species have different characteristics that help them to survive in different habitations. They also have many ecotypes. Vetiver ecotypes found in Thailand are named based on the first province where they were found. Thailand's Department of Land Development has studied 28 ecotypes of vetiver, 11 of which are *Chrysopogon nemoralis* and 17 are *Chrysopogon zizaniodes*. 10 of these ecotypes were found to be suitable for growth in different soil types and regions (ORDPB, 2000) as shown in Table 2-3 and Table 2-4. The ecotypes Prachuap Khiri Khan *(Chrysopogon nemoralis)* and Songkhla 3 *(Chrysopogon zizaniodes)* were selected for further investigation in this current study. For their suitability to grow in loamy clay and lateritic soil. In addition, Songkhla 3 has suitability to grow in sandy soil as well (Land Development Department). Therefore, these two species represent suitable candidates to remediate contaminated soils like the types found in Klity Village in Thailand.

Table 2-3 Suitability of vetiver ecotypes in various soil types

Soil type	**Chrysopogon nemoralis**	**Chrysopogon zizaniodes**
Sandy soil	Nakhon Sawan, Kamphaeng Phet 1, Roi Et, Ratchaburi	Kamphaeng Phet 2, Songkhla 3
Clay loam soil	Loei, Nakhon Sawan, Ratchaburi, Kamphaeng Phet 1, Prachuap Khiri Khan	Surat Thani, Songkhla 3
Laterite soil	Prachuap Khiri Khan, Loei	Kamphaeng Phet 2, Songkhla 3, Surat Thani, Sri Lanka

Table 2-4 Suitability of vetiver ecotypes in various regions

Region	**Chrysopogon nemoralis**	**Chrysopogon zizaniodes**
North	Nakhon Sawan, Kamphaeng Phet 1	Sri Lanka
Northeast	Roi Et	Songkhla 3
Central and East Region	Ratchaburi, Kamphaeng Phat 1, Prachuap Khiri Khan	Kamphaeng Phet 2, Songkhla 3, Surat Thani
South		Songkhla 3, Surat Thani

Complicating the application of phytoremediation to treat heavy metal soil contamination, heavy metals in soil are generally bound to organic and inorganic soil components, creating insoluble precipitates which make the heavy metals unavailable for plant uptake (Ilya Raskin et al., 1994). Even if the concentrations of heavy metals are high, the metals' low solubility result in low availability for phytoremediation (Shahid et al., 2013). There are both soil-associated and plant-associated factors that influence the availability of heavy metals in soil. Soil-associated factors include soil type, soil moisture, soil pH, cation exchange capacity (CEC), and biochemical processes. Plant-associated factors include plant root depth, root absorption factor, acidification of rhizosphere, and growth and biomass of plant species (Sheoran et al., 2016). Moreover, acid rain can also affect phytoavailability by increasing the release of soil heavy metals into their surrounding area (A.-Y. Kim, Kim, Ko, & Kim, 2010). In order to increase the mobility and availability of heavy metals in soils and to enhance the translocation of heavy metals from root to shoot of the vetiver, the application of synthetic chelating agents such as EDTA has been suggested (Saifullah et al., 2009).

2.3 Factors controlling solubility of heavy metals in soil

Availability of heavy metal in soil can indicated by the free ions of heavy metals which was available for plant uptake rather than the total heavy metals concentration (Gray & McLaren, 2006). Although the total concentration of heavy metals cannot show the exact value of metals due to unextractable lattice-bound metal. It can refer to the fraction of metal in soil that is removed by strong extractants such as hydrochloric and nitric acids (Rieuwerts, Thornton, Farago, & Ashmore, 2015). Several researchers (Gray & McLaren, 2006; M. McBride, S. Sauve, & Hendershot, 1997; Xingfuxian & Gholamhoss In Shokohifard, 1989) reported that the heavy metal forms in soils are affected by pH, cation exchange capacity (CEC), electrode potential (Eh), and other factors. Soil not only has higher adsorption capacity for heavy metals such as Cd and Zn when pH values increase, but plants can also uptake lower metals such as Cd, Zn, and Pb (Xingfuxian & Gholamhoss In Shokohifard, 1989). Moreover, it has been informed that the soluble fraction of heavy metals in soil and rate of metals uptake by plants are intimately related.

Release of heavy metal in soil could be affected by the solubility of heavy metal in soil and the immobilization of heavy metals through adsorption process which makes them harmless and inert.

2.3.1 Solubility process

The solubility of each metal varies significantly. Cd and Zn are rather mobile as compared to Pb (Iain Thornton, 1972). Therefore, the aqueous phase of heavy metals is mobile and toxic. Free metal ions in soil solution complex with organic and inorganic (CO_3^{2-}, Cl^-) ligands (Ogundiran & Osibanjo, 2015). If the metal is in soluble form, they can leach into soil profile and contaminate groundwater or be accumulated by plant, animals or human. Usually, the concentration of heavy metals in soil solution can be affected by soil pH. A decrease in soil pH can increase the concentration of heavy metals in soil solution due to an increase in adsorption by exchangeable capacity between metal cations and H^+ on soil surface.

2.3.2 Sorption process

Sorption process is an important process which can indicate the transport of heavy metals in soil. pH or Eh alteration can affect metal mobility in this mechanism which has an impact sorption and desorption equilibrium (The Global CCS Institute).

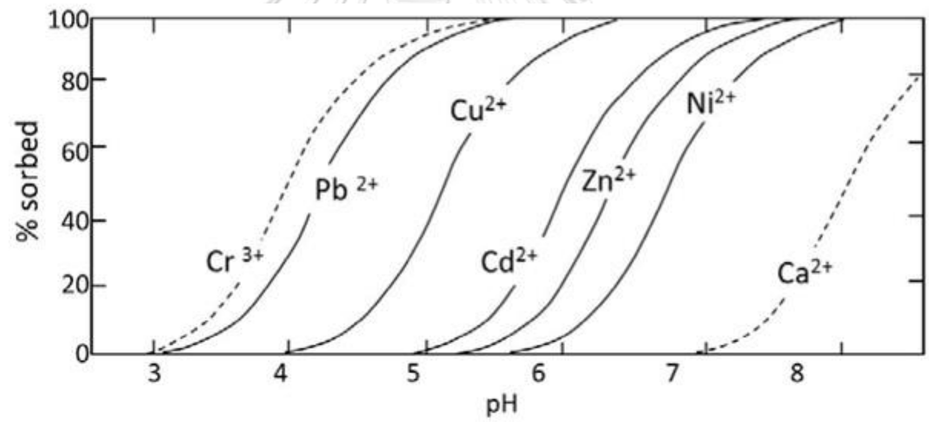


Figure 2-1 Adsorption of heavy metals on ferrihydrite's surface as a function of pH

Normally, six different forms of geochemical including soluble, exchangeable, iron-manganese oxide bound, carbonate bound, organic matter bound and residual were present in soil (Ogundiran & Osibanjo, 2015). Therefore, if metals are in available form for plant uptake, it can lead to the change in translocation and bioaccumulation of heavy metals in the vetiver.

2.4 Bioavailability and Bioaccumulation of Heavy metals in Plants

To investigating the phytoremediation ability of vetiver, bioaccumulation factor (BF) and translocation factor (TF) were used to assess and can define as equations below.

$$\mathrm{BF}_{root} = \frac{[Metal]_{root}}{[metal]_{soil}}$$

$$\mathrm{BF}_{shoot} = \frac{[Metal]_{shoot}}{[metal]_{soil}}$$

$$TF = \frac{[Metal]_{shoot}}{[metal]_{root}}$$

2.5 EDTA-application

Generally, metal in the form of metal oxides, hydroxides and others are either poorly applied or not available. Therefore, the application of organic chelating agents is use to compensate low heavy metal availability in soil (BERND NOWACK & ROBINSON, 2006) because these agents can increase heavy metals uptake and translocation of heavy metal from root to shoot of the plants (Evangelou et al., 2007). Several synthetic and natural organic chelating agents have been used in the last decade in studies for heavy metal remediation; these agents include EDTA, ethylene diamine dissuccinate (EDDS), low molecular weight organic acids (LMWOA), humic substances (HSs), and nitrilo triacetic acid (NTA) (BERND NOWACK & ROBINSON, 2006). Among these chelating agents, several studies have suggested that EDTA is the most effective and efficient organic chelate in solubilizing heavy metals in soil (Evangelou et al., 2007; Leleyter, Rousseau, Biree, & Baraud, 2012) due to the formation of soluble metal-EDTA complexes which are most advantageous for plant species to take up, especially by hyper-accumulator plants (Shahid et al., 2013). EDTA is known as a strong chelator and has an influence on chemical speciation. It has an effect on the mobility, solubility, and bioavailability of heavy metals in soil, which can change the uptake of the metals by plant roots and the accumulation of metals in each part of plants (Evangelou et al., 2007; Saifullah et al., 2009).

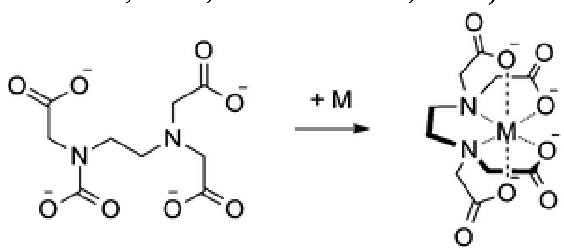


Figure 2-2 Chemical structure of deprotonated EDTA and its complexation with a metal ion M in 1:1 stoichiometry.

EDTA increases the mobility of heavy metals in soils through two mechanisms: thermodynamically, and EDTA-promoted dissolution (Shahid et al., 2013). Heavy metals can adsorb on free EDTA and as a consequence, the EDTA helps destabilize the weak bond between heavy metals and oxygen in mineral structures. Furthermore, EDTA deteriorates some parts of soil minerals and structures, promoting heavy metal mobilization in the soil. In agriculture, EDTA is used to increase bioavailability and plant uptake of nutrients from the soil (Abbas & Abdelhafez, 2013).

There are several existing studies about EDTA-application with several plants. In a 2013 study, the uptake of heavy metals (Zn, Pb, and Cu) in sunflowers was increased when EDTA was applied to a contaminated soil (Kumsorn & Chairak, 2013). A 2004 study showed that the application of EDTA with vetiver caused an increase in the translocation ratio of Pb from the root to the shoot of the vetiver (Chen et al., 2004). Applying high levels of EDTA to soil can dissolve mineral components; unfortunately, the drawback from using EDTA is that it can interrupt the physical structure and chemical properties of soil (Shahid et al., 2013). EDTA and metal-EDTA complexes are non-biodegradable and have a high toxicity towards soil bacteria, fungi, and plants (Shahid et al., 2013). Moreover, the leaching of heavy metals to groundwater is increased after EDTA application to soil (BERND NOWACK & ROBINSON, 2006). Therefore, precaution must be taken in the use of EDTA and it must be applied in proper ways.

2.5.1 Relationship between speciation and bioavailability of heavy metals with metal-EDTA complexes

Generally, heavy metals in soil are present in various forms, and their forms can be influenced by the appearance of EDTA. Rate of complex formation significantly relies on the competition between cations and soil types (Udovic & Lestan, 2009). EDTA can form soluble complexes with heavy metals which leads to an increase in exchangeable fraction. EDTA can also enhance the mobility of heavy metals by increasing the concentration of heavy metal in soil solution (Voglar & Lestan, 2012). Certainly, a high binding constant of metal-EDTA complexes are 18.00, 18.10, 18.16, 19.71, 20.11, 20.49 for Zn, Cd, Co, Pb, Ni, and Cu, respectively (Meers et al., 2008). Previous study indicated that the extraction of Cd and Pb is more favorable to EDTA in soil contaminated with several metals (Gheju, Pode, & Manea, 2011).

Soil solution pH is also known as an important factor which can control the amount of heavy metals between soil solution and soil solid phase (Bennedsen, Krischker, Jorgensen, & Sogaard, 2012; Peng, Song, Yuan, Cui, & Qiu, 2009). For example, alkaline pH leads to a decrease in efficiency of metal removal in calcareous soils while mobilization and uptake can be increased by soil acidification.

CHAPTER 3
METHODOLOGY

3.1 Material

1) Vetiver grass (Songkhla 3 and Prachuap Khiri Khan)
2) Nitric acid 65% QREC (CAS: 7697-37-2)
3) Hydrochloric acid 37% QREC (CAS: 7647-01-0)
4) Hydrogen peroxide
5) Acetic acid
6) Ethanol
7) Petri dish
8) DI water
9) Glass fiber filter paper 4.7 cm GF/F WHATMAN

3.2 Instruments

1) Inductive Couple Plasma- Optical Emission Spectrometer (ICP-OES), ULTIMA2, HORIBA used to analyze the concentrations of chromium, lead, and zinc in leachate from leaching methods
2) Microwave
3) Analytical Balances
4) Oven used to dry soil and plant samples at 60°C
5) pH meter

3.3 Method

3.3.1 Preparation of soil sample

Soil samples were sieved through a 2-mm mesh sieve (sieve number 10) and sent for characterization according to cation exchange capacity (CEC), total N, P, and K, pH, organic matter in soil, and metal elements (Pb, Zn, and Cd) at the Department of Soil Science, Faculty of Agriculture, Kasetsart University. Afterwards, soil sample particle size distribution was determined via sieve analysis by the Geotechnical Research Unit, Chulalongkorn University.

3.3.2 Preparation of vetiver root

Two species of vetiver (Songkhla 3 and Prachuap Khiri Khan) were obtained from the Land Development Department, Thailand. Their shoots were soaked in buckets for one week for roots to sprout before the plants were transplanted into soils to ensure that the plants can survive.

3.3.3 Study of the accumulation of heavy metals in vetiver

Soils were air-dried and sieved through a 2-mm mesh sieve (sieve number 10). Afterwards, soils were characterized for total N, P, and K, organic matter, CEC, and pH. In order to simulate heavy metal contamination, 200 ppm $PbCO_3$, $ZnCO_3$, and $CdCl_2$ were directly added to soil samples. pH was measured. Soil samples without heavy metals, vetiver planted in soil without heavy metals, soil samples with heavy metals, and soil samples with heavy metals composed of EDTA-application were kept as control. Soils were placed in separate plastic pots (25 cm diameter, 22.5 cm height). Plants were selected and their roots and shoots were pruned to 5 cm and 20 cm respectively before the plants were transplanted into the pots (2 plants per pot with 10 cm of space between the plants). Triplicate samples were created for replicability. The pots were placed in plant nurseries and watered with deionized water every two days. Vetiver plants were planted in the pots for four months. Every month, soil and plant samples were collected to analyze for concentrations of Pb, Zn, and Cd that still existed in the soil and that has accumulated in the plant shoots and roots.

3.3.4 Digestion of soil and plant samples

Five spots of soil samples were collected per pot: 3 spots collected at soil surface and other 2 spots collected at 10 cm and 20 cm depth, respectively to find out the concentration of heavy metals in different soil layers. Soil samples were digested with conc. HNO_3 and conc. HCl by microwave digestion (3 replicates). After the digestion, the concentration of Pb, Zn, and Cd were determined by ICP-OES.

Plant samples were washed with water and rinsed with deionized water then divided into shoot and root. These were oven-dried at 60°C for 48 hrs., and their dry weights were recorded. Plant samples were digested in HNO_3 and H_2O_2 in microwave digestion and analyzed for Pb, Zn, and Cd by using ICP-OES (3 replicates).

3.3.5 Toxicity Characteristic Leaching Proceduce (TCLP)

Hazardous wastes were determined for leaching of heavy metals in high acidic condition (pH<3) by using TCLP test. This test used 1:20 (solid: liquid) ratio. In this research, 1 g of soil samples added with 20 mL of acetic acid at pH~2.88 in 50 mL centrifuge tube and rotated by rotary agitation device at 30±2 rpm for 18±2 hours and DI water was used as a leachant for control test. After rotated, soil samples were filtered and supernatant was collected and stored at 4ºC for further chemical analysis. (EPA, 1992)

3.3.6 Study of soil toxicity after remediation with vetiver by Allium test

The genotoxicity of soil samples after remediation by vetiver was estimated by growing allium in pots that contained treated soil samples.

3.3.7 Study the effect of remediated soil on seed germination

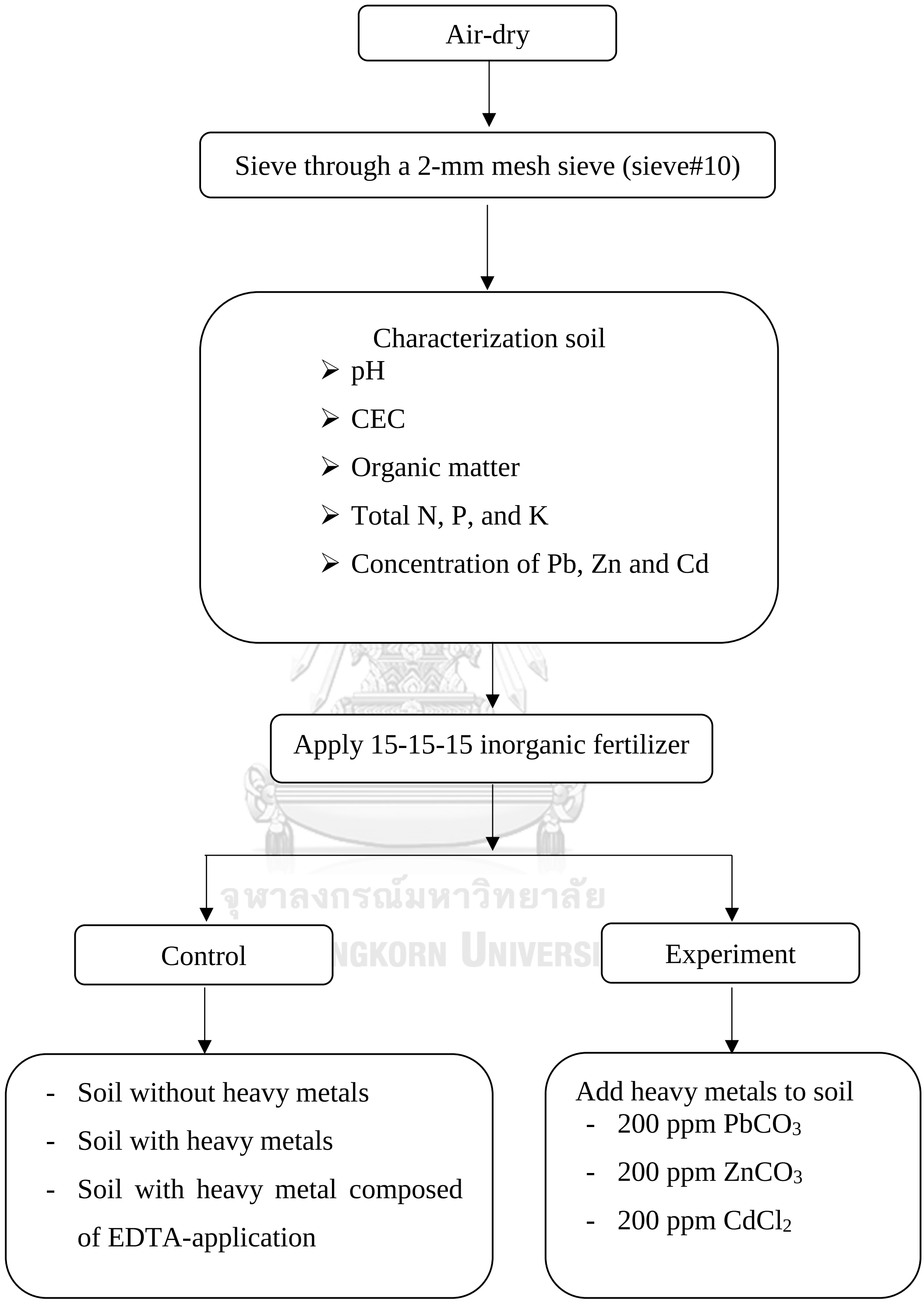
2,800 seeds of green oak were used to study the effect of remediated soil by two species of vetiver. Seeds of these green oak were purchased from Or Tor Kor Market, Bangkok, Thailand which is a local market contains food and product from Royal project.

Seeds of green oak were germinated on filter paper (110mm Whatman No.1 filter) in Petri dishes and covered with another filter paper. Each dish contained distilled water (control) and soil solution after fourth month of phytoremediation. Solutions were subsequently added to Petri dishes which are containing selected seeds. Seeds which used to test for germination using 5 replicates of 80 seeds. Seeds were obtained to geminate for 4 days in the dark. Percent of seed germination was calculated.

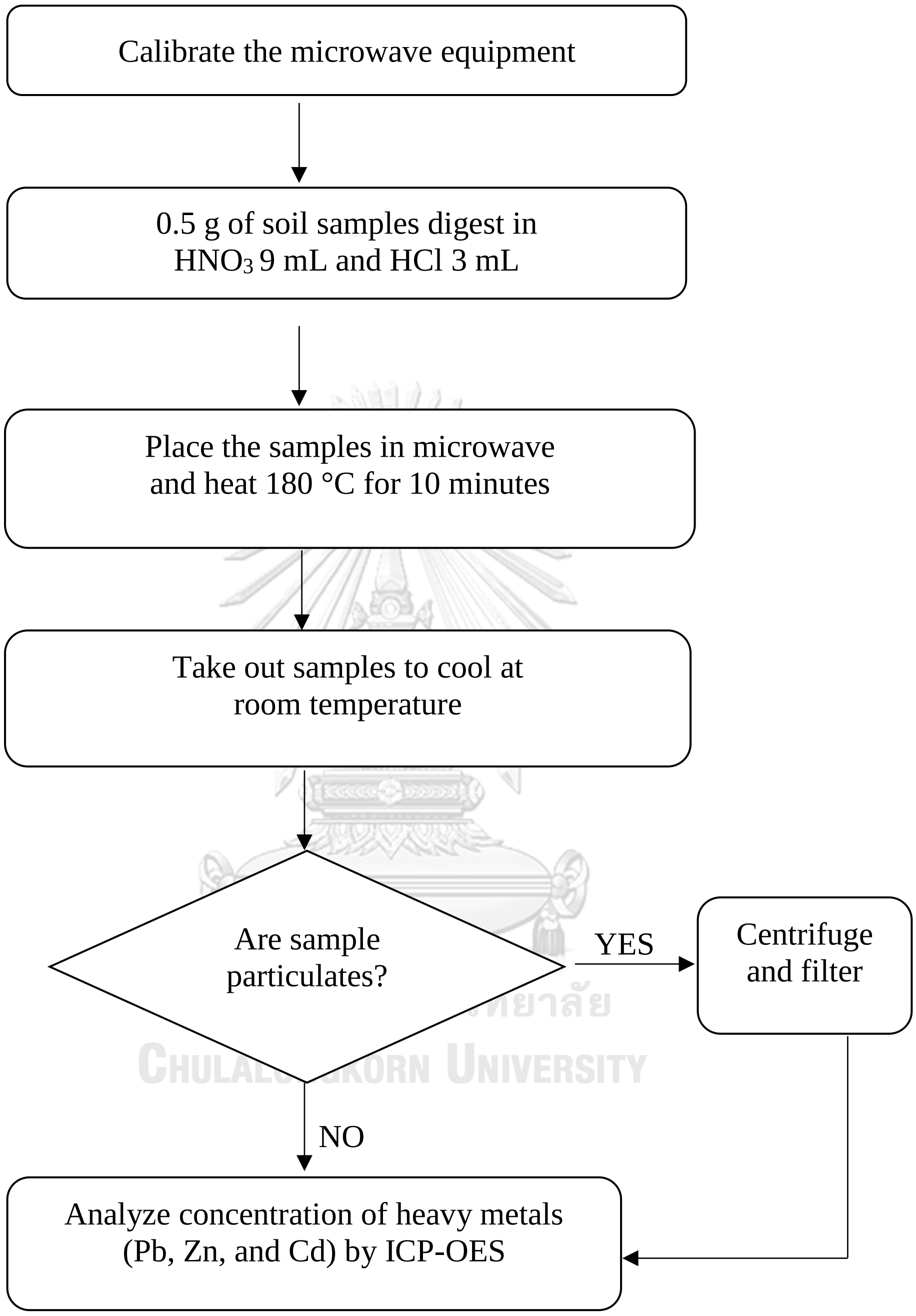
3.3.8 Data analyses

The statistical analysis was used to approximate the results from experiments in research methodology. In this research, the experiments used t-test analysis to indicate the difference between one result’s values from other experiments at the different statistical significant level of 0.05 with other values.

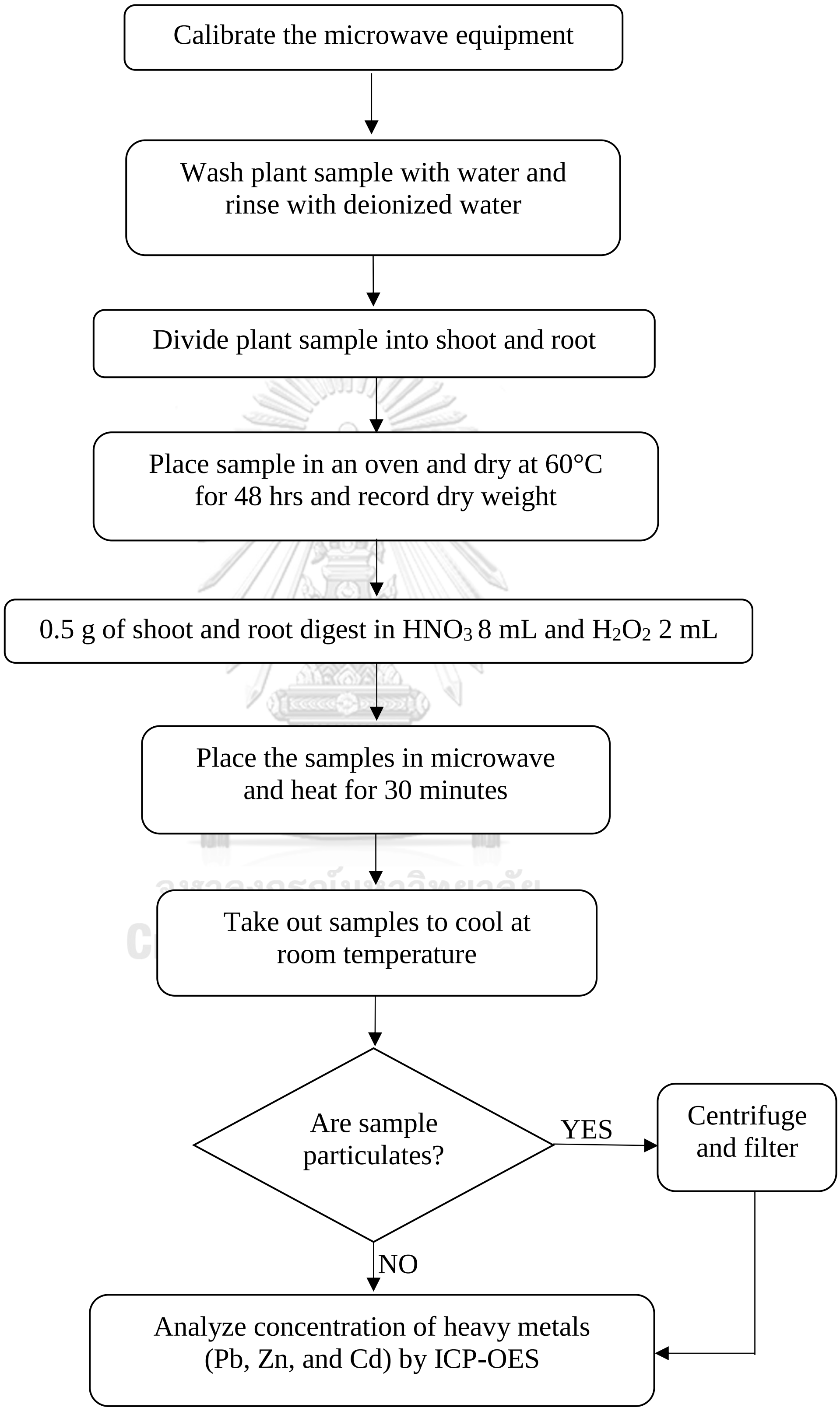
Soil preparation



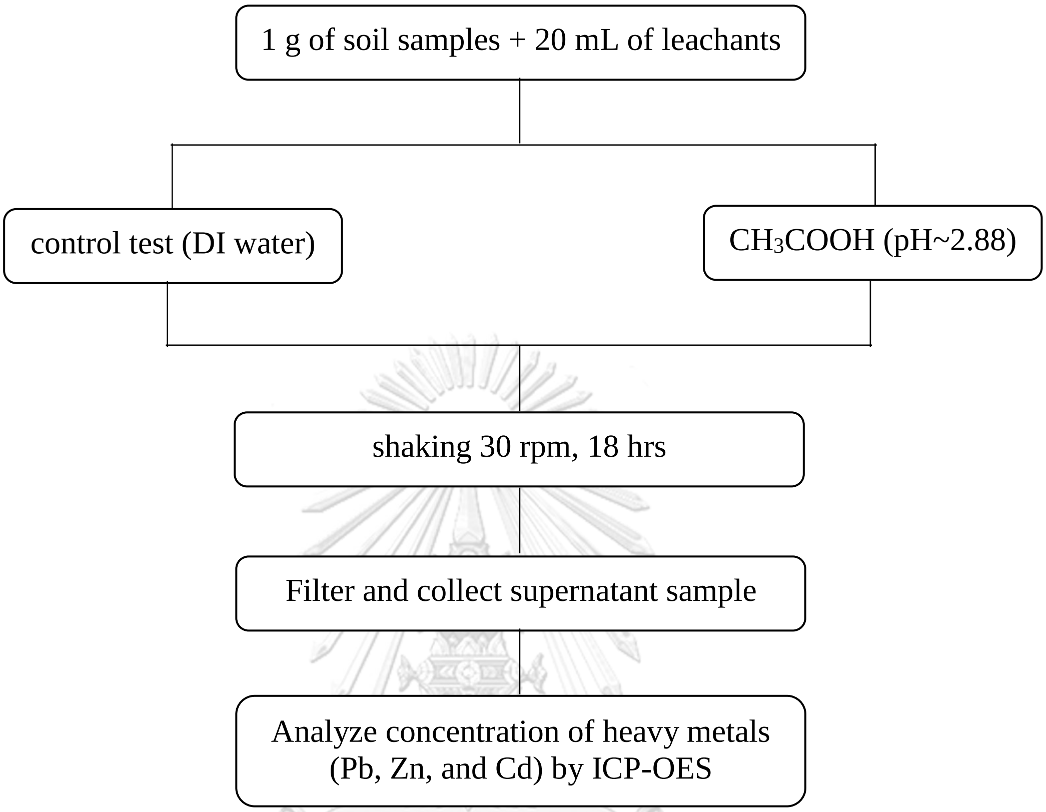
Microwave digestion for analyses of heavy metals in soil samples (EPA, 2007)



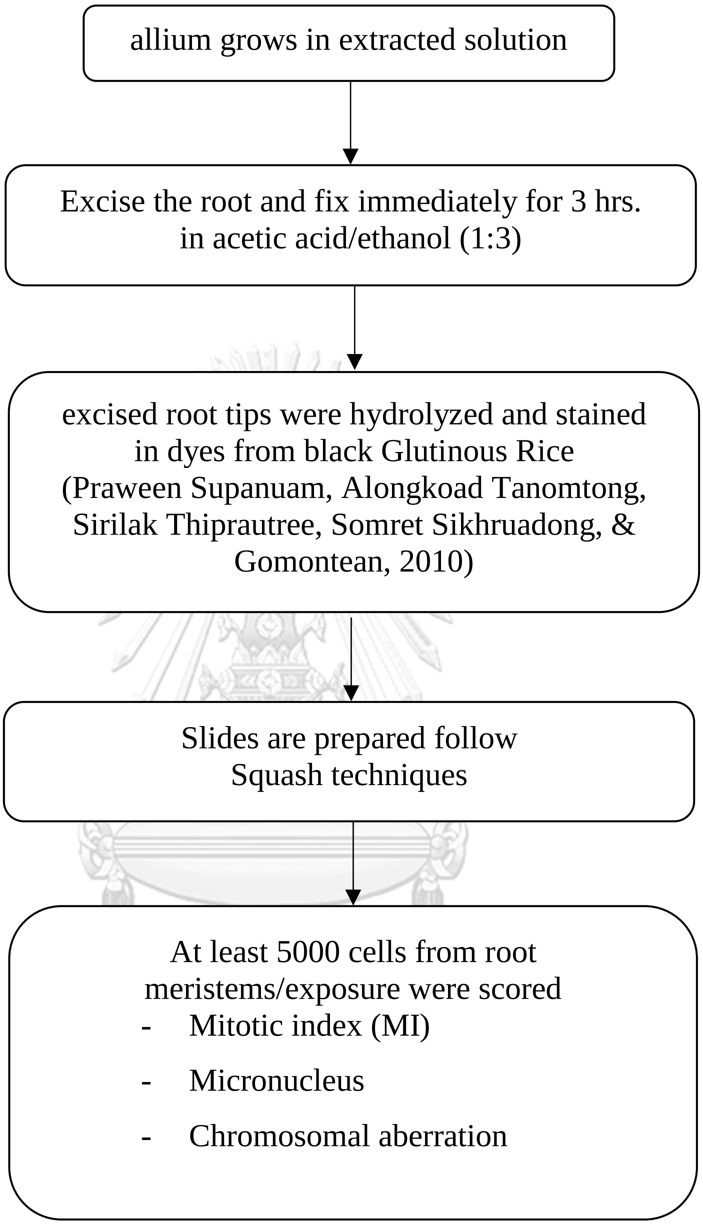
Microwave digestion for analyze heavy metal in plant samples (EPA, 1996)



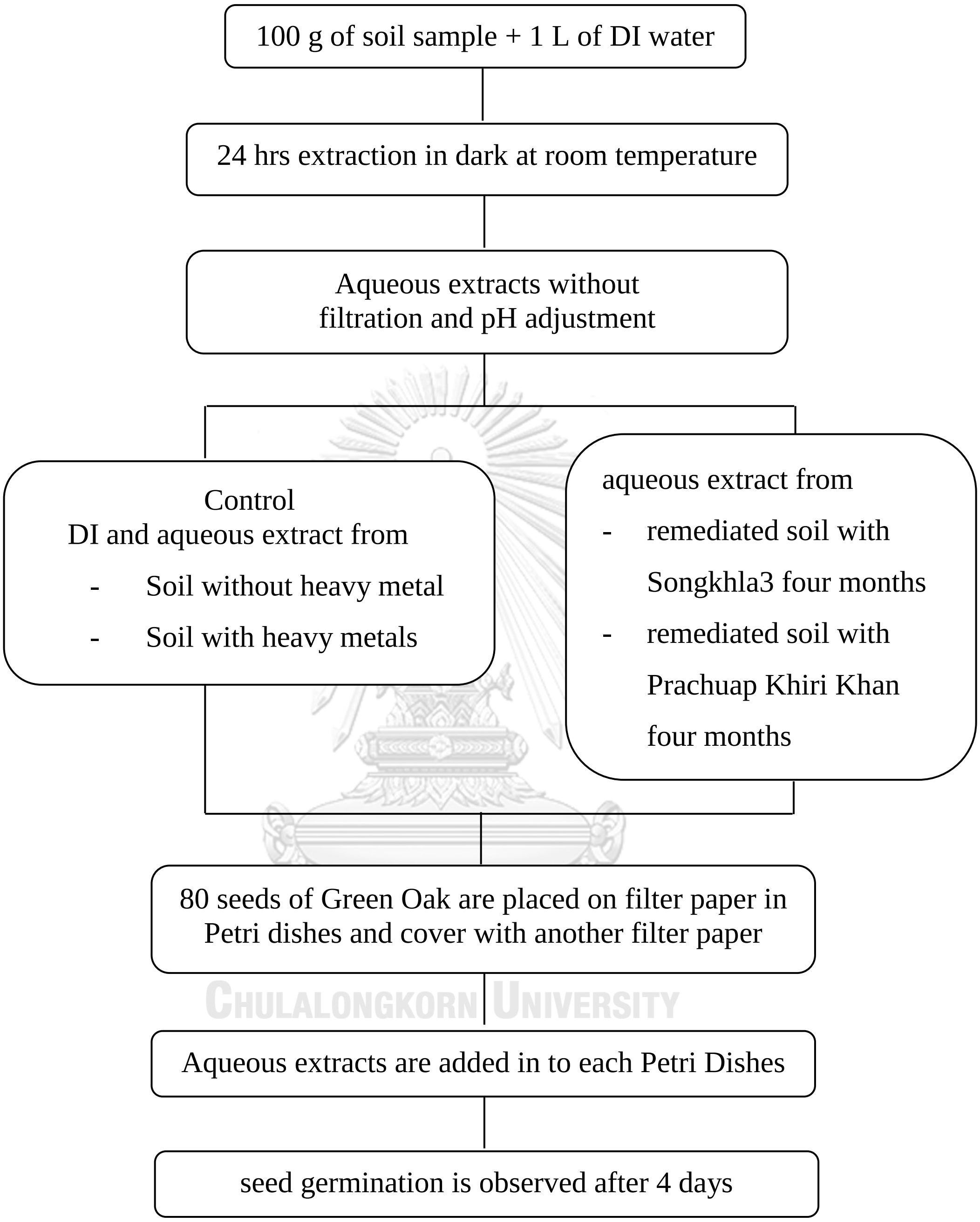
Toxicity Characteristic Leaching Procedure (TCLP) test (EPA, 1992)



Allium test (Ghosh et al., 2015)



Study the effect of remediated soil on seed germination



CHAPTER 4
RESULTS AND DISCUSSION

4.1 Soil sample characterization

Soils were sent for characterization according to cation exchange capacity (CEC), total N, P, K, pH of soil, organic matter in soil and metal elements (Pb, Zn, and Cd) at Department of Soil Science, Faculty of Agriculture, Kasetsart University.

The results are shown in Table 4-1. Analytical result suggested that soil samples are silty clay which is acid (pH~5) and have 2.42% organic matter, cation exchange capacity 11.13 $cmol_e/kg$. The concentration of heavy metals includes Pb, Zn, Cd in soil samples are 8.87, 1.75, 0.07 mg/kg, respectively.

Table 4-1 The physical properties of soil sample

pH	organic matter (%)	Texture	CEC ($cmol_e/kg$)	Metal concentration (mg/kg)		
				Pb	Zn	Cd
5.03	2.42	Silty clay[a]	11.13	8.87	1.75	0.07

[a] sand 47%, silt 14%, and clay 39%.

The final concentration of Pb, Zn, and Cd in soil after spiked heavy metals into soil studied were 180.42, 153.43, 103.57 mg/kg soil, respectively.

4.2 Phytoremediation potential of vetiver grass

4.2.1 Effect on root quality of vetiver after remediated with vetiver

To observe the effect of heavy metals (Pb, Zn, and Cd) on root of vetiver, vetiver grass was moved out of the pot after the end of the experiment period (1, 2, 3, and 4 month) and the root quality were observed. The root quality indicated the effect of heavy metals to root of vetiver compared to the vetiver which was grown in normal soil. The results showed that roots which is grown in contaminated soil were thicker and shorter than roots which were grown in normal soil. It might because of toxicity of heavy metals in soil which can obstruct the root's growth and it also has an effect on the length of lateral root zone. (V. B. Ivanov, E. I. Bystrova, & Seregin, 2003)

Table 4-2 Root quality of two species of vetiver

	Songkhla 3 (*Chrysopogon zizaniodes*)	Prachuap Khiri Khan (*Chrysopogon nemoralis*)
Normal soil		
initial month		
1st month		
2nd month		

Table 4-2 Root quality of two species of vetiver

	Songkhla 3 (*Chrysopogon zizaniodes*)	Prachuap Khiri Khan (*Chrysopogon nemoralis*)
3rd month		
4th month		
Soil contaminated with heavy metals (Pb, Zn, and Cd)		
1st month		

Table 4-2 Root quality of two species of vetiver

	Songkhla 3 (*Chrysopogon zizaniodes*)	Prachuap Khiri Khan (*Chrysopogon nemoralis*)
2nd month		
3rd month		
4th month		
4th month +EDTA		

4.2.2 Reduction of heavy metals (Pb, Zn, and Cd) in contaminated soil

To investigate the heavy metals uptake by each part of vetiver. The concentration of heavy metals in soil can reveal efficient uptake of heavy metals by vetiver. The results showed a significant decrease (p<0.05) in concentration of Pb, Zn, and Cd in soils over the period of 4 months as shown in the Figure 4-1 and Figure 4-2. Specifically, Pb concentration in soil was 180.42 mg/kg at the beginning after remediation with Songkhla3 and Prachuap Khiri Khan the concentration decreased to 57.49 mg/kg and 53.13 mg/kg, respectively. Likewise, Zn concentration is continued decreased from 153.43 mg/kg to 92.68 mg/kg in Songkhla3 and 94.19 mg/kg in Prachaup Khiri Khan. It showed the same trend with Cd concentration that reduced from 103.57 mg/kg to 57.75 mg/kg and 63.37 mg/kg in Songkhla3 and Prachuap Khiri Khan, respectively. Similarly, the results of this research are consistent with other authors such as in 2004, Chen Y. *et al.* and in 2006, Rotkittikhun *et al.* studied heavy metals uptake by using vetiver grass, the results from both studies showed that vetiver grass has ability to decrease the concentration of heavy metals in soil contaminated with heavy metals.

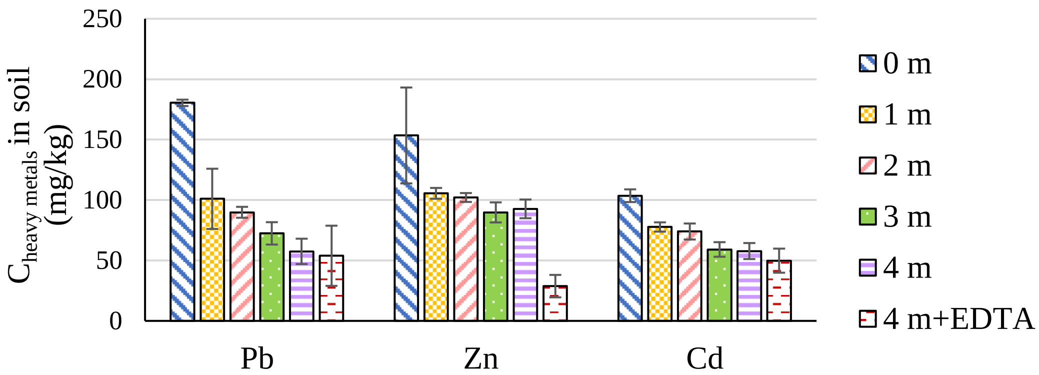


Figure 4-1 Concentration of heavy metals in soil after phytoremediation with Songkhla3

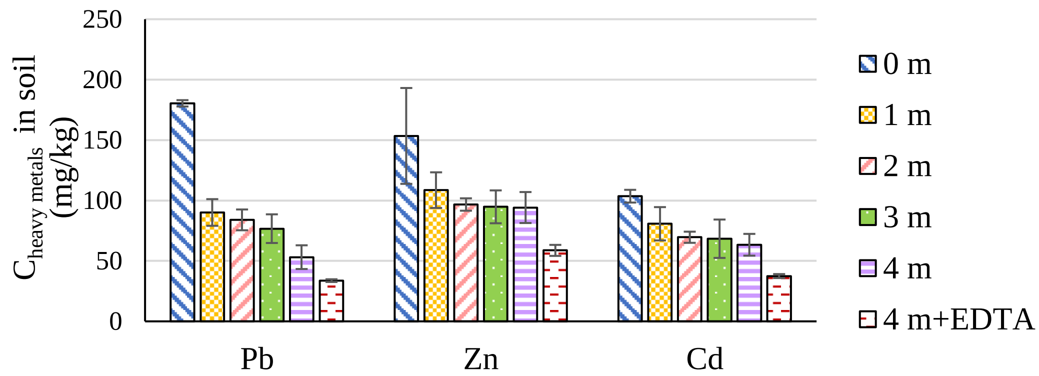


Figure 4-2 Concentration of heavy metals in soil after phytoremediation with Prachuap Khiri Khan

Moreover, the concentration of soil in vertical line was measured to confirm the effect from blended soil samples with heavy metals and watered them during period experiment. The results from Table 4-3 showed that all heavy metals are dispersed throughout in pots except Pb concentration in first month the concentration in the middle of pots is higher than concentration in soil surface and soil at the bottom of pots experiment. Therefore, it can confirm that soils and heavy metals are blended homogeneously and watered vetiver didn't effect on heavy metals concentration in different layer.

Table 4-3 Concentration of heavy metals in each layer of pot

species of vetiver	month	depth	heavy metal concentration (mg/kg)		
			Pb	Zn	Cd
	Initial month	surface soil	190.19	110.88	102.57
		10cm	173.58	117.76	103.87
		20 cm	175.26	117.55	100.22
Songkhla3	1 m	surface soil	100.98	105.52	72.41
		10 cm	173.44	4.72	8.41
		20 cm	86.16	14.29	8.74
	2 m	surface soil	89.84	102.11	89.76
		10 cm	81.71	14.36	8.97
		20 cm	102.86	12.57	9.88
	3 m	surface soil	72.41	89.76	59.10
		10 cm	91.12	13.39	8.54
		20 cm	90.59	12.83	8.72
Prachuap Khiri Khan	1 m	surface soil	90.09	83.98	76.70
		10 cm	95.14	13.16	7.44
		20 cm	57.55	16.08	7.69
	2 m	surface soil	108.58	96.68	94.77
		10 cm	68.04	14.10	8.43
		20 cm	69.63	15.88	7.15
	3 m	surface soil	80.74	69.59	68.37
		10 cm	89.02	9.77	8.36
		20 cm	84.79	9.98	8.65

4.2.3 Effect of EDTA on phytoremediation by vetiver

Figure 4-1 and Figure 4-2 shows the concentration of heavy metals (mg/kg) in pots which is applied EDTA into soils compared to the control group without EDTA application. The application of EDTA results in the higher decrease in concentration of Pb, Zn, and Cd in both species of vetiver. Due to EDTA application, it competently changed form of heavy metals in soil to be soluble or exchangeable fraction which was available to plants (Lai & Chen, 2004). Therefore, the concentration of heavy metals except Pb in Songkhla3 vetiver decrease significantly with EDTA application while it showed significantly decrease (p<0.05) in Prachuap Khiri Khan.

Moreover, the data shown in Table 4-4 and Table 4-5 indicate that EDTA application was related with the increase in heavy metals concentration in shoot and the decrease in root of vetiver. Moreover, translocation from root to shoot of vetiver increased with EDTA-application. For Pb, the translocation factor (TF) is more than 1 indicated that species of Songkhla3 specially accumulated Pb in shoots more than in root. Because after application of EDTA to soil, Pb tends to form Pb-EDTA complex which is more available for plant's uptake (Andrew D. Vassil, Yoram Kapulnik, Ilya Raskin, & Salt*, 1998).

Table 4-4 Effect of EDTA on bioaccumulation factors and translocation factor of Songkhla3

EDTA treatment (mmol/kg)	HM	concentration (mg/kg)		BF (roots)	BF (shoots)	TF
		roots	shoots			
0.00	Pb	54.27 ± 24.14	23.21 ± 16.06	0.94	0.40	0.58
5.00		37.62 ± 8.36	51.75 ± 36.04	0.70	0.96	1.58
0.00	Zn	368.15 ± 71.54	113.51 ± 32.69	3.97	1.22	0.32
5.00		346.93 ± 80.84	259.60 ± 111.26	13.10*	10.50*	0.74
0.00	Cd	530.69 ± 76.56	140.28 ± 37.34	9.19	2.43	0.27
5.00		472.67 ± 108.97	315.69 ± 149.84	9.49	6.34	0.70

*indicates a significant increase at $p<0.05$

Table 4-5 Effect of EDTA on bioaccumulation factors and translocation factor of Prachuap Khiri Khan

EDTA treatment (mmol/kg)	HM	concentration (mg/kg)		BF (roots)	BF (shoots)	TF
		roots	shoots			
0.00	Pb	24.22 ± 12.35	13.35 ± 7.37	0.46	0.25	0.76
5.00		28.32 ± 11.37	16.46 ± 11.58	0.84	0.49	0.58
0.00	Zn	309.14 ± 54.81	98.51 ± 36.68	3.28	1.05	0.33
5.00		277.23 ± 70.66	136.65 ± 39.59	4.72	2.33*	0.51
0.00	Cd	412.27 ± 103.10	132.39 ± 85.93	6.51	2.09	0.32
5.00		430.32 ± 182.47	136.59 ± 64.81	11.48	3.58	0.35

*indicates a significant increased at $p<0.05$

4.2.4 pH of soil in the period of studied

pH of soil studied after phytoremediation by two species of vetiver was shown in Table 4-6.

Table 4-6 pH of soil studied

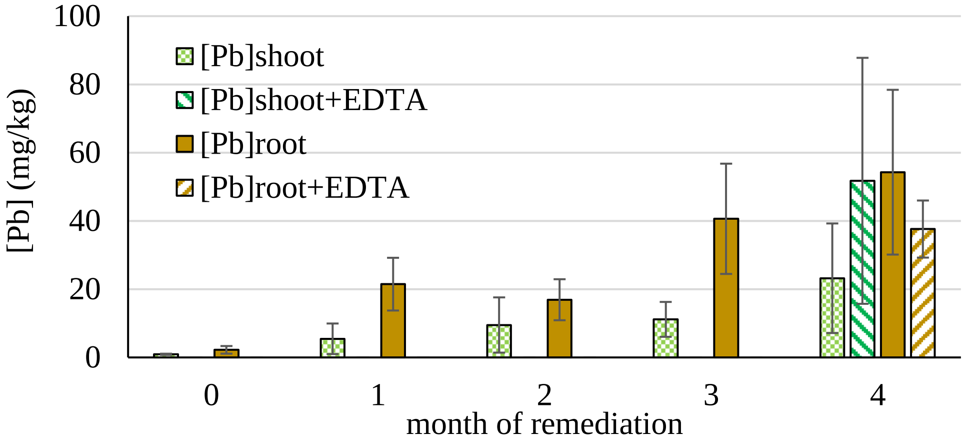
month	pH of soil remediated by Songkhla3 vetiver	pH of soil remediated by Prachuap Khiri Khan vetiver
0	5.03	5.03
1	4.83	4.65
2	4.99	4.82
3	4.77	4.65
4	4.73	4.63
4 with EDTA-application	3.23	3.76

The result showed a higher pH of soil after heavy metals were added into soil. Because the major retention mechanism of heavy metals in soil related to a change in pH of soil solution. At high pH values, dominatingly mechanisms of heavy metals in soil is precipitation. During the time that pH decreases, dominant mechanism becomes cation exchanges (Raymond N. Yong & Yuwaree Phadungchewit, 1993). The changed in pH of soil can lead to the change in metals uptake by vetiver. Due to the increase in large amount of heavy metals ions which desorbed from soil particles and tends to enter in solution which was available for plant uptake.

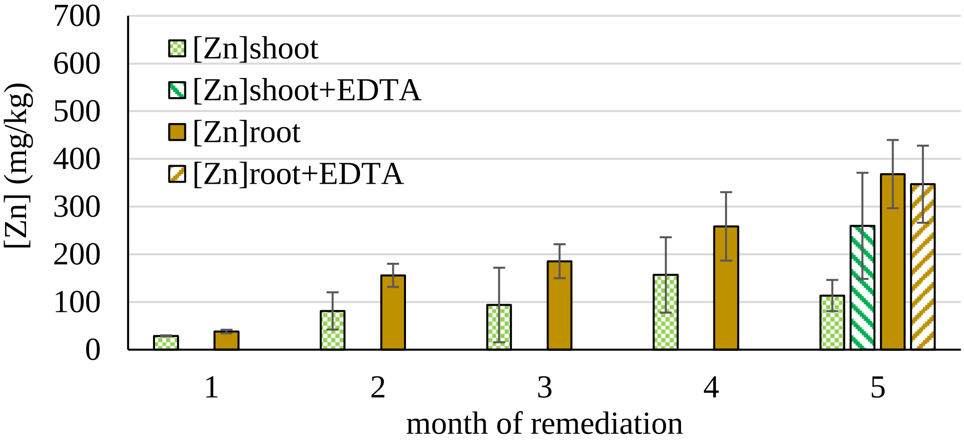
4.2.5 Heavy metals (Pb, Zn, and Cd) uptake by roots and shoots of vetiver

The role of vetiver in phytoremediation can be confirmed by higher heavy metals concentration in each part of plants such as the 24-fold increasing of Pb concentration in root of Songkhla3 during four months of remediation. The heavy metals estimation revealed higher heavy metals including lead, zinc, and cadmium accumulation in the root of the vetiver as compared to shoot. Comparative concentration of lead, zinc, and cadmium in contaminated soil which is accumulated in root and shoot of Songkhla3 and Prachuap Khiri Khan were shown in Figure 4-3 and Figure 4-5.

(a)



(b)



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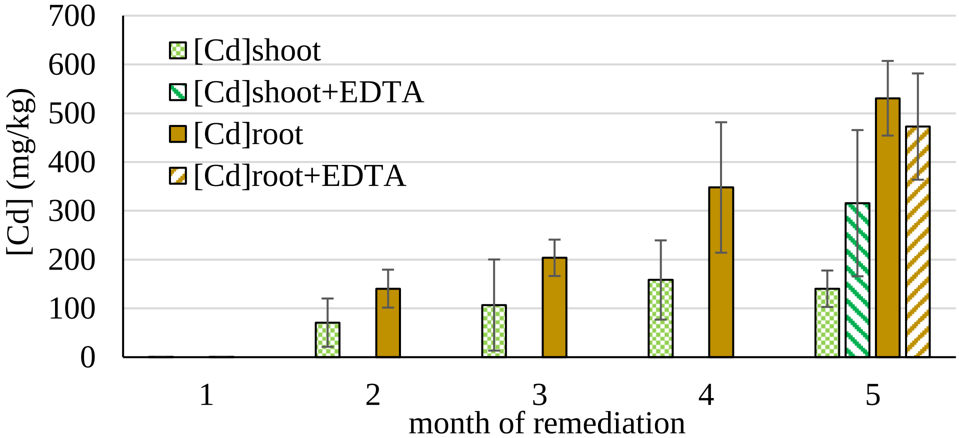


Figure 4-3 Concentration of heavy metals in shoot and root of Songkhla3 vetiver; (a) concentration of lead (Pb); (b) concentration of zinc (Zn); (c) concentration of cadmium (Cd)

From Figure 4-3, the graph shows an increase in concentration of heavy metals includes lead, zinc, and cadmium in both shoot and root of Songkhla3 vetiver. For Pb concentration in root part is increased from 2.22 mg/kg to 54.27 mg/kg and it showed the higher accumulation as compared to the accumulation in shoot part of vetiver which also continued to increase from 0.91 mg/kg to 23.21 mg/kg. For Zn and Cd, they showed similar trend with Pb.

Moreover, the application of EDTA reduced the concentration of lead, zinc, and cadmium in root lower than those in pots which were not applied EDTA. The concentration of Zn that didn't apply EDTA in fourth month was 368.15 mg/kg, while the concentration of Zn in root part was 346.93 mg/kg with EDTA application. On the other hand, concentration of heavy metals in shoot increased after EDTA application.

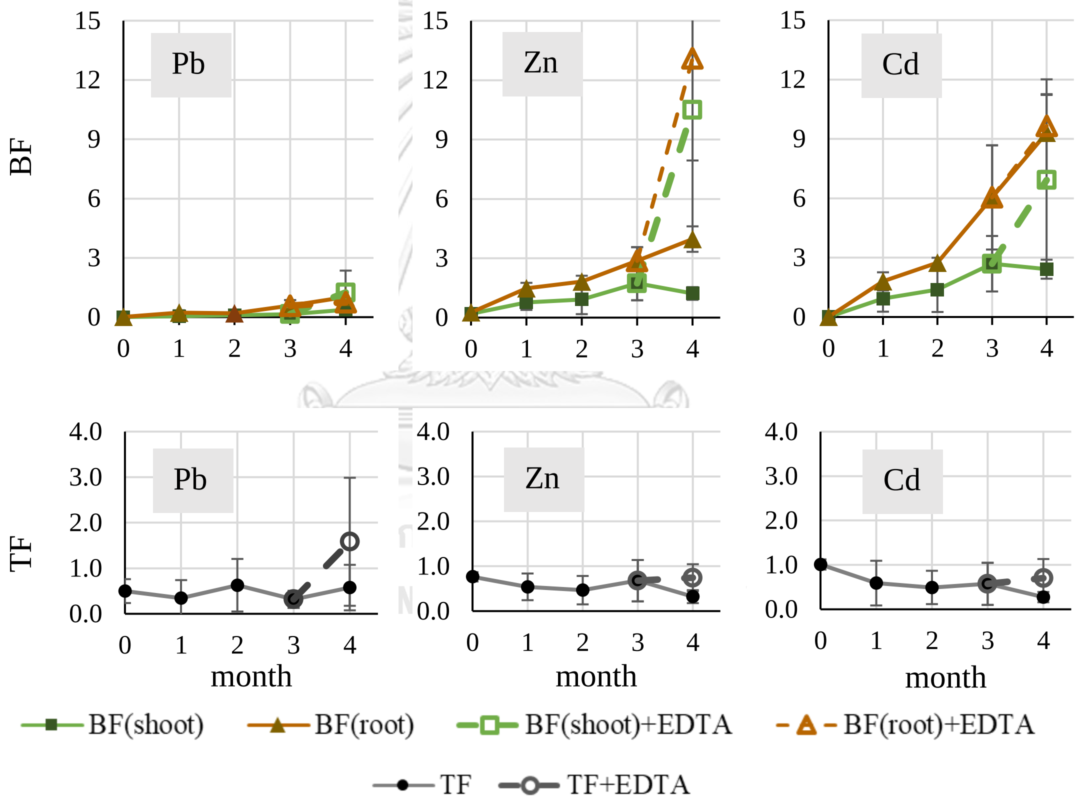
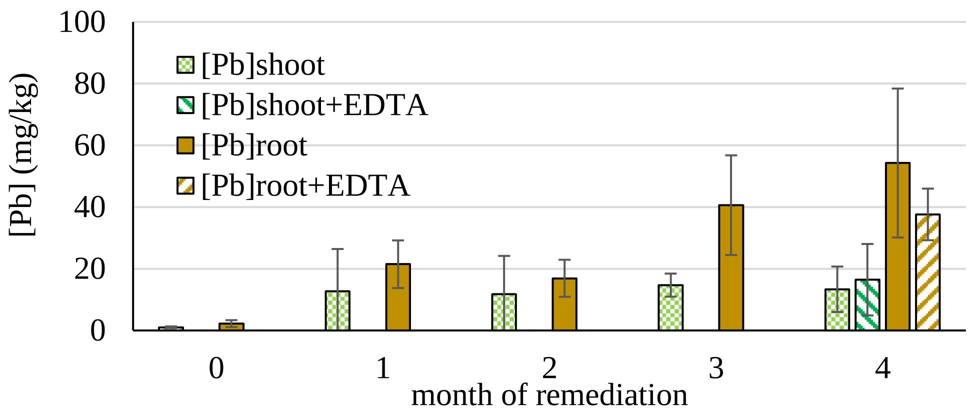


Figure 4-4 Bioaccumulation factor in both shoot and root and translocation factor of Songkhla3

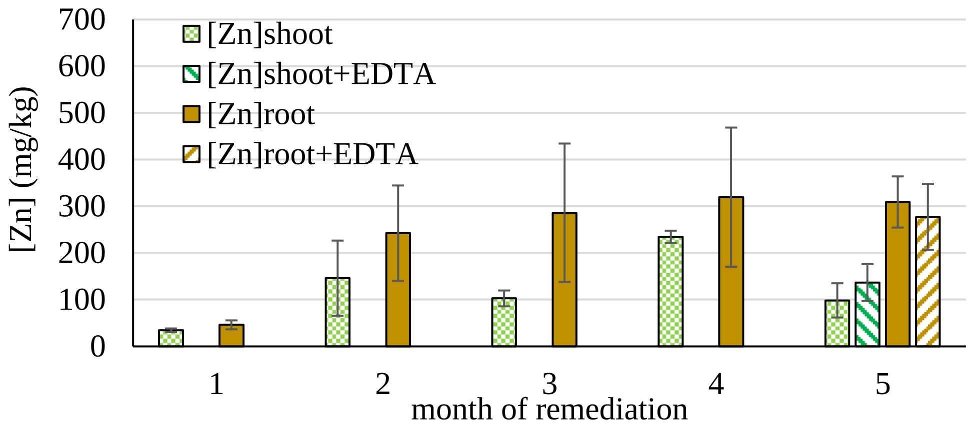
EDTA application also affected the translocation factor and bioaccumulation factor which indicated potential uptake of heavy metals by Songkhla3 as shown in Figure 4-4. Translocation factor increased with application of EDTA to contaminated soil, and it led to an increase of heavy metal concentrations in shoot of Songkhla3.

Likewise, an increase in concentration of heavy metals accumulated in both shoot and root of Prachuap Khiri Khan vetiver, bioaccumulation factor and translocation factor were shown in Figure 4-5 and Figure 4-6,respectively.

(a)



(b)



(c)

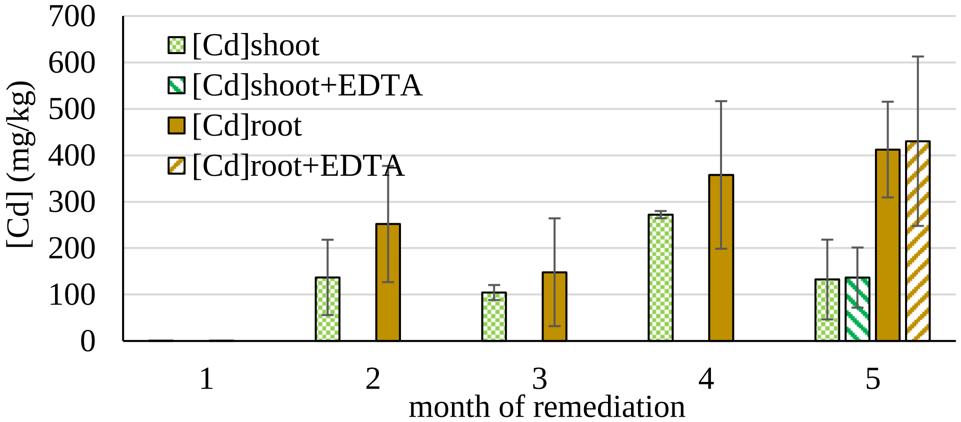


Figure 4-5 Concentration of heavy metals in shoot and root of Prachuap Khiri Khan vetiver; (a) concentration of lead (Pb); (b) concentration of zinc (Zn); (c) concentration of cadmium (Cd)

From Figure 4-5, the graph shows a higher accumulation of heavy metals includes lead, zinc, and cadmium in both shoot and root of Prachuap Khiri Khan vetiver. For Pb, Zn, and Cd concentration in root part are continually increased from 1.62 mg/kg to 24.22 mg/kg, 46.17 mg/kg to 277.23 mg/kg, and 0.20 mg/kg to 430.32 mg/kg, respectively. Moreover, it showed the lower accumulation in shoot part which also continued to increase. Except Cd concentration that continually increased from 0.19 mg/kg in initial month to 272.15 mg/kg and dropped to 132.39 mg/kg in fourth month of remediation.

Moreover, the application of EDTA slightly increased the concentration of lead, zinc, and cadmium in both part of vetiver, except the concentration of Zn in root part which was 309.14 mg/kg without EDTA application and 277.23 mg/kg with EDTA application.

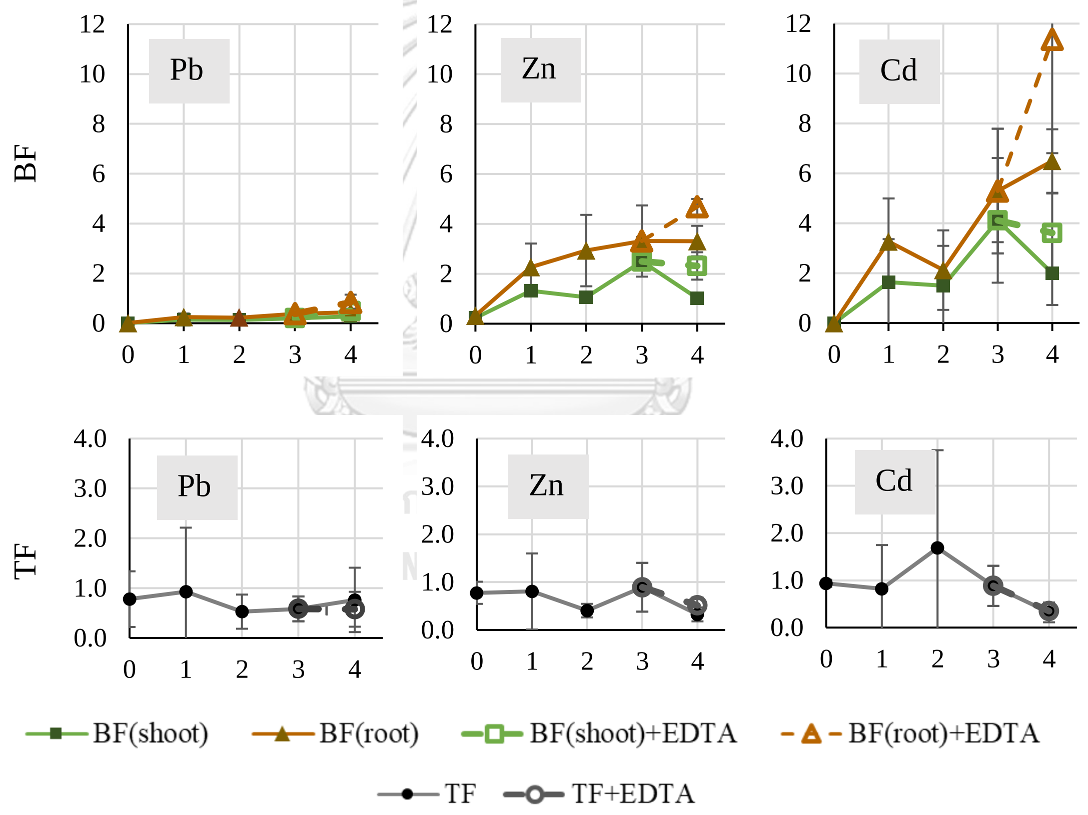


Figure 4-6 Bioaccumulation factor in both shoot and root and translocation factor of Prachuap Khiri Khan

From the results of phytoremediated soil contaminated with Pb, Zn, and Cd, lead (Pb) showed a poor uptake by two species of vetiver as compared to other heavy metals because Pb^{2+} has higher ionic potential to soil which causes stronger adsorb than Cd^{2+} and Zn^{2+} (M. B. McBride, 1989). Moreover, a decrease in pH leads to higher transportation of Cd in soil (pH 4.5-5.5) while the transportation of Zn decreases when pH is less than 7. Therefore, vetiver can uptake and accumulate Cd > Zn > Pb in their shoot and root. A study by X. Xian (1988) showed the similar results on uptake in cabbage plant. Application of EDTA could increase translocation of heavy metals from root to shoot of vetiver, especially for Pb. Resulted in the translocation factor of Pb increased from 0.58 to 1.58 in Songkhla3 which means Pb was largely stored in shoot than in the root (TF>1). The application of EDTA did not have an effect on translocation factor by Prachuap Khiri Khan. Although, it showed no significant increase in both translocation factor and bioaccumulation factor in Pb and Cd uptake, bioaccumulation of Zn significantly increased ($p<0.05$) from 1.22 to 10.50 and 3.97 to 13.10 in shoot and root of Songkhla3 and 1.05 to 2.33 in shoot of Prachaup Khiri Khan.

Furthermore, the results indicated that both species of vetiver have potential to reduce the concentration of heavy metals in contaminated soil and accumulated them in their root more than their shoot.

4.3 Comparison the ability between Songkhla3 and Prachuap Khiri Khan vetiver in absorbing Pb, Zn, and Cd

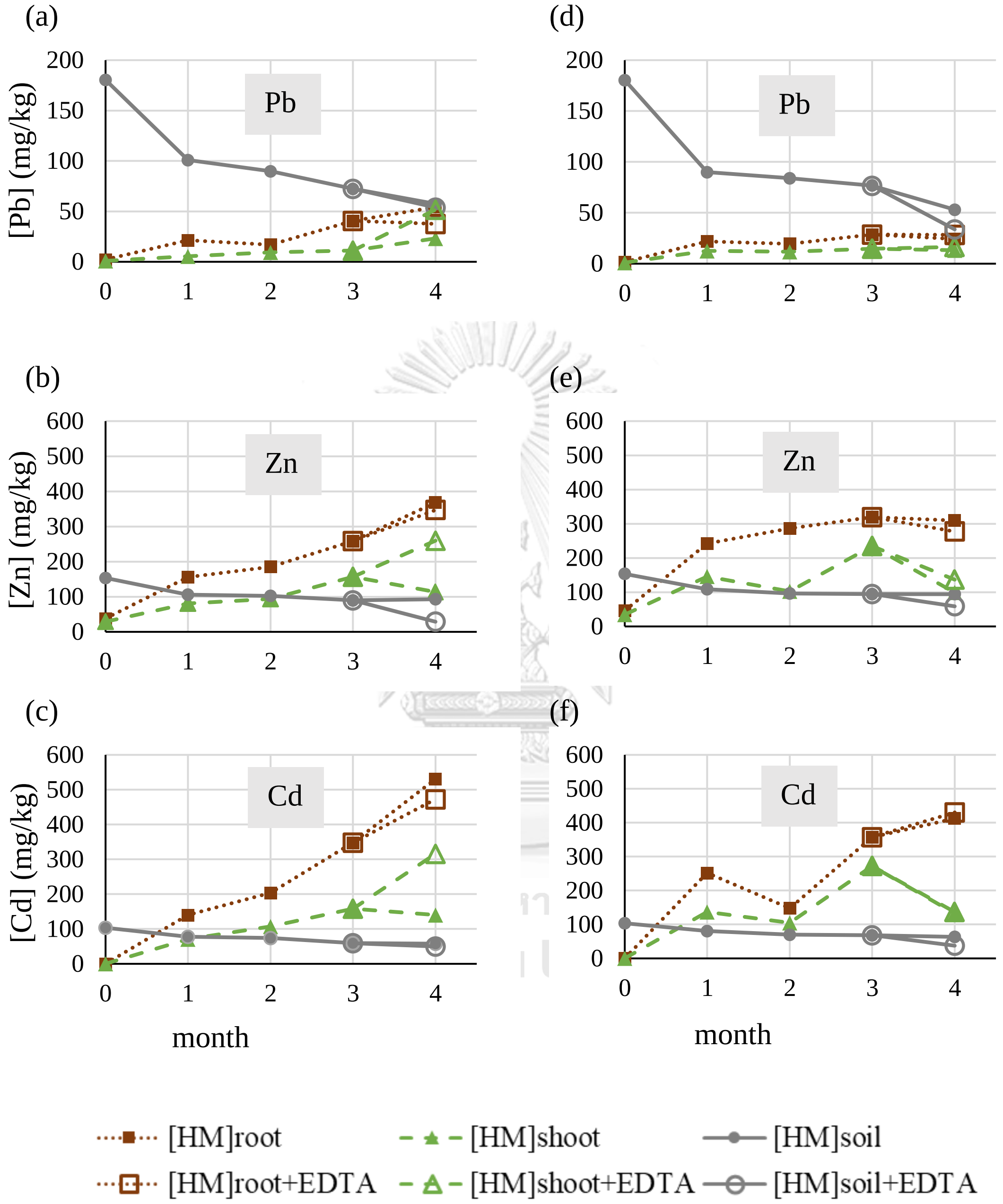


Figure 4-7 Concentration of heavy metals and time in root, shoot of vetiver, and in soil; (a,b,c) concentration of Pb, Zn, and Cd from phytoremediation with Songkhla3; (d,e,f) concentration of Pb, Zn, and Cd from phytoremediation with Prachuap Khiri Khan

Results show a significant decrease in concentration of heavy metals in soil ($p<0.01$) includes Pb, Zn, and Cd in both species of vetiver.

Moreover,

Figure 4-7 showed a significant increase in heavy metals uptake in root and shoot of Songkhla3. The highest efficiency is Cd uptake up to 315.69 mg/kg in shoot and 530.69 mg/kg in root followed by Zn and Pb. Similarly, Prachuap Khiri Khan also has a significant increase in concentration of Cd in shoot and root which is accumulated up to 136.59 mg/kg in shoot and 430.32 mg/kg in root. For Pb and Zn, concentration in root has a significantly increase at $p<0.05$ while concentration in shoot showed no significant. From Pearson correlation, the correlation between bioaccumulation of heavy metals in shoot and root can be observed. Species of vetiver also have an effect on translocation factor of Pb. Moreover, period of the study shows the relationship not only with bioaccumulate of Pb, Zn, and Cd in both shoot and root of vetiver, but also with the translocation of Zn and Cd.

4.4 Toxicity Characteristic Leaching Procedure (TCLP)

The leachate concentration of heavy metals includes Pb, Zn, and Cd by using DI water as a leachant are shown in Figure 4-8, and Figure 4-9 which are reported in mg/L. All of heavy metals concentration were below the regulatory level for TCLP as shown in the Table 4-7, except Cd in fourth month with and without EDTA application.

Table 4-7 Toxicity Characteristic Leaching Procedure (TCLP) Regulatory level

Heavy metals	TCLP Regulatory level (mg/L)
Arsenic (As)	5.0
Barium (Ba)	100.0
Cadmium (Cd)	1.0
Chromium (Cr)	5.0
Lead (Pb)	5.0
Mercury (Hg)	0.2
Selenium (Se)	1.0
Silver (Ag)	5.0

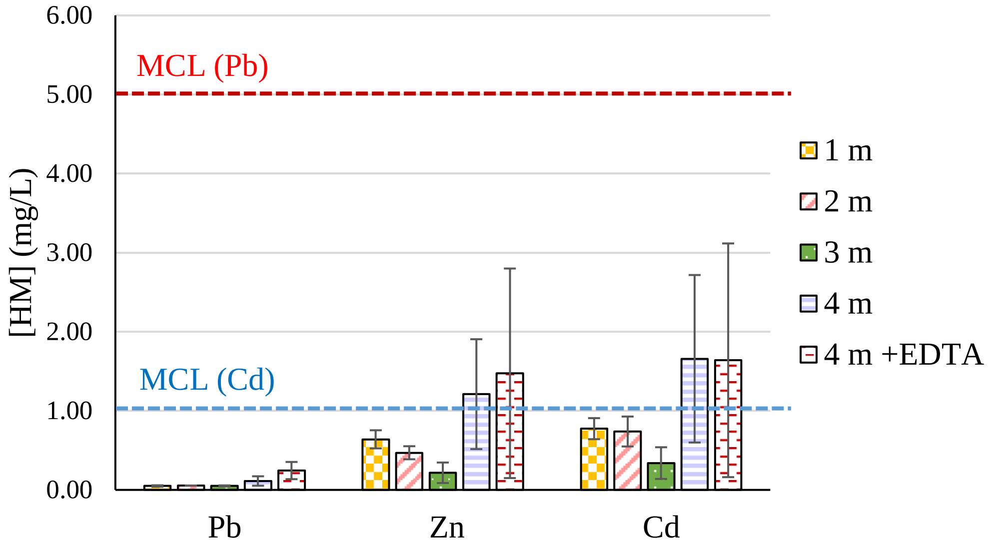


Figure 4-8 Total concentration of heavy metals in soil after phytoremediation with Songkhla3 vetiver according to TCLP method (using DI water as a leachant)

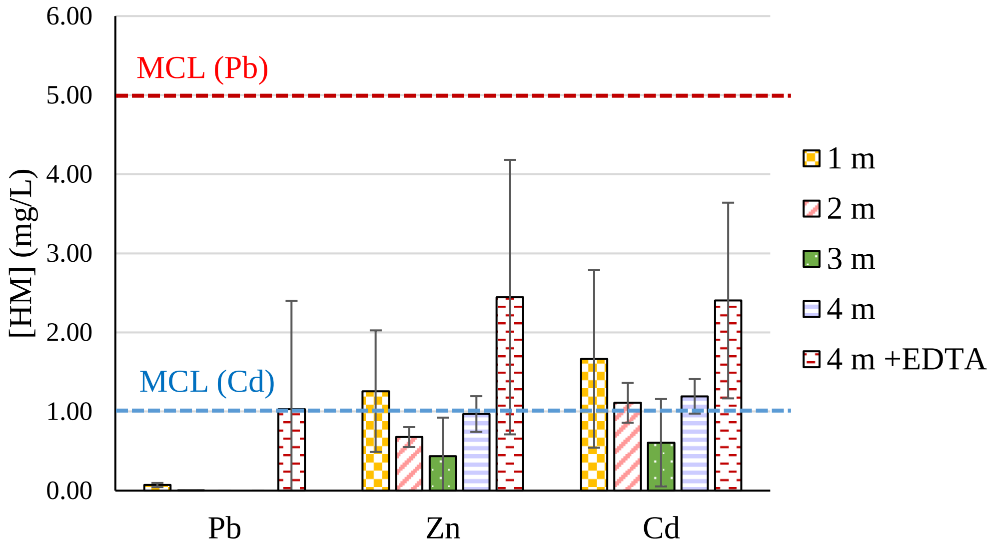


Figure 4-9 Total concentration of heavy metals in soil after phytoremediation with Prachuap Khiri Khan vetiver according to TCLP method (using DI water as a leachant)

Moreover, the results indicated that after applied 5 mmol of EDTA, the leachate concentration of heavy metals in soils was higher. This is because EDTA has ability to increase metal concentration in soil solution, however; Pb concentration was still below the TCLP regulatory level. In the other hand, Cd concentration is higher than the standard limit.

4.5 Reduction of genotoxic of contaminated soil by vetiver

Genotoxic study was used to confirm the potential of vetiver system to phytoremediate heavy metal-contaminated soil (Pb, Zn, and Cd) and monitored a change in toxicity of contaminated soil over period of study. Allium test was used for this study.

4.5.1 Allium test

The genotoxic effects of phytoremediated soil were evaluated by Allium test. Results showed no difference in chromosome aberration between allium which were grown in normal soil solution, and contaminated soil solution as shown in Figure 4-10.

(a) (b) (c) (d)

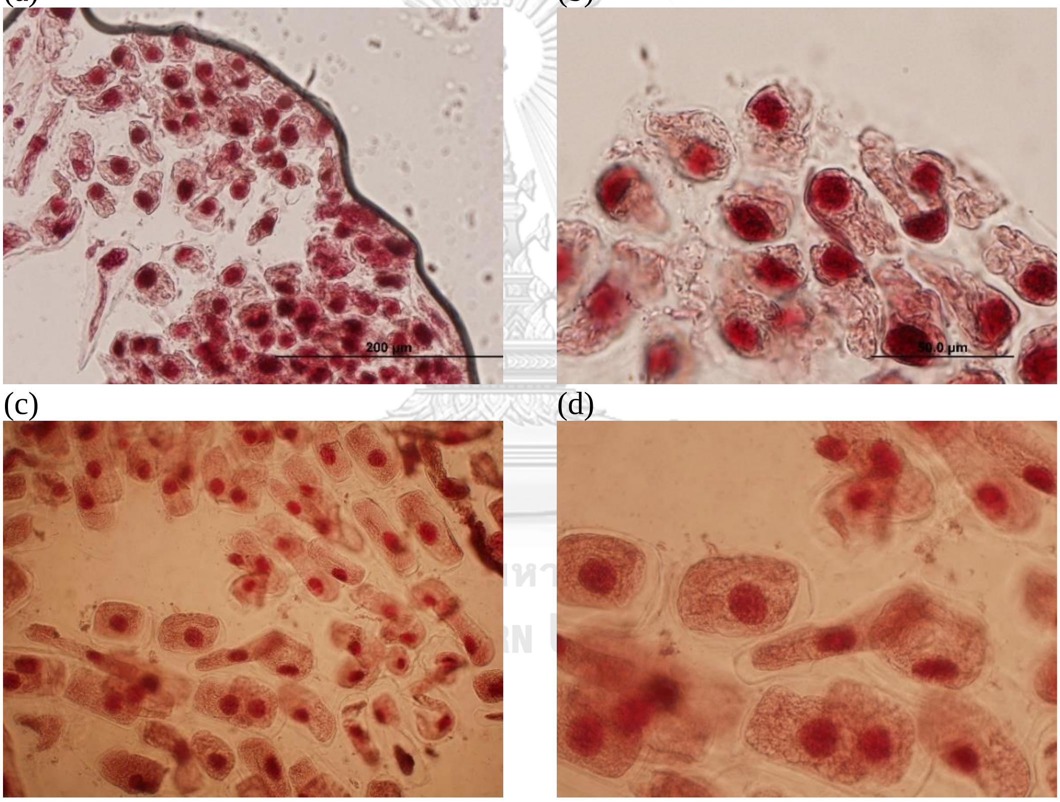


Figure 4-10 Microphotograph showed nuclease in allium test; (a,b) control group without heavy metals; (c,d) control group with heavy metals, microphotographs at 40x and 100x, respectively

Though results showed no different in chromosomal observation, the different external characteristic in allium’s root was shown between each group.

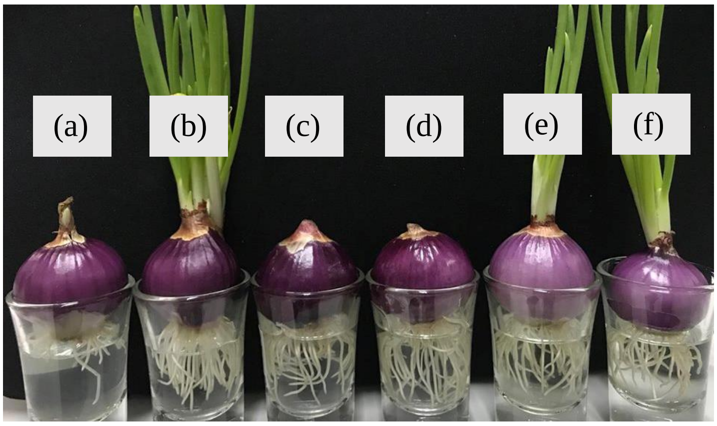


Figure 4-11 External characteristic of allium’s root; extracted solutions from (a,b) soil with heavy metals and without heavy metals; (c,d) soil after four months remediation with Songkhla3 and Prachuap Khiri Khan; (e,f) soil after four months remediation with Songkhla3 and Prachuap Khiri Khan with EDTA application, respectively

From Figure 4-11, roots of allium grown well in control without heavy metals and solutions which are extracted from contaminated soil after 4 months of phytoremediation by two species of vetiver. On the other hand, some external characteristics such as short, small, distorted, and a few number of roots represented as root aberration in control solutions with heavy metals. Moreover, the color of allium root which were grown in remediated soil with EDTA- application became darker yellow as compared to others groups, obviously because of toxic effect that causes cell death. (Sheila O'Hare & Atterwill, 1995; V. B. Ivanov et al., 2003)

4.6 Effect of remediated soil solution on Green Oak seed germination

From the experiments, its showed the different results in the seed germination percentage of the six groups tested between 7.25%-60.87% compared to the seed germination in DI water as shown in Figure 4-12.

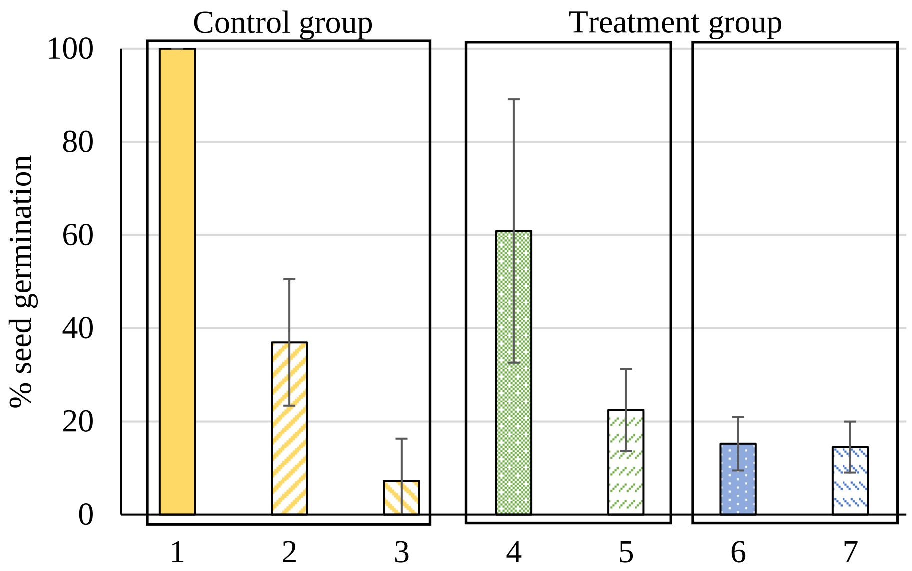
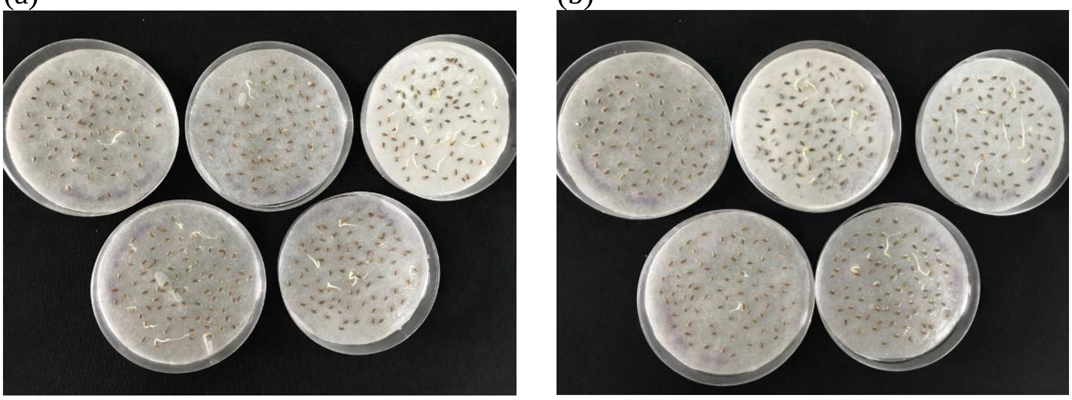


Figure 4-12 Effect of heavy metal on seed germination; (1) DI water; aqueous extracted from; (2) soil without heavy metals; (3) soil with heavy metals; (4) soil after four months remediation by Songkhla3; (5) soil after four months remediation by Songkhla3 with EDTA application; (6) soil after four months remediation by Prachuap Khiri Khan; (7) soil after four months remediation by Prachuap Khiri Khan with EDTA application

Effect on seed germination revealed the potential of phytoremediation by two species of vetiver grass. The highest germination percentage is 60.87% in remediated soil solution from Songkhla3, while the lowest percentage of seed germination was shown in solution extracted from soil control with heavy metals. Although, the percentage of seed germination in soil after phytoremediation with Prachuap Khiri Khan is not much higher than soil control with heavy metals, no seed germination was shown in one Petri dish. However, it showed no significance between each treatment (p<0.05).

(a) (b)



(c) (d)

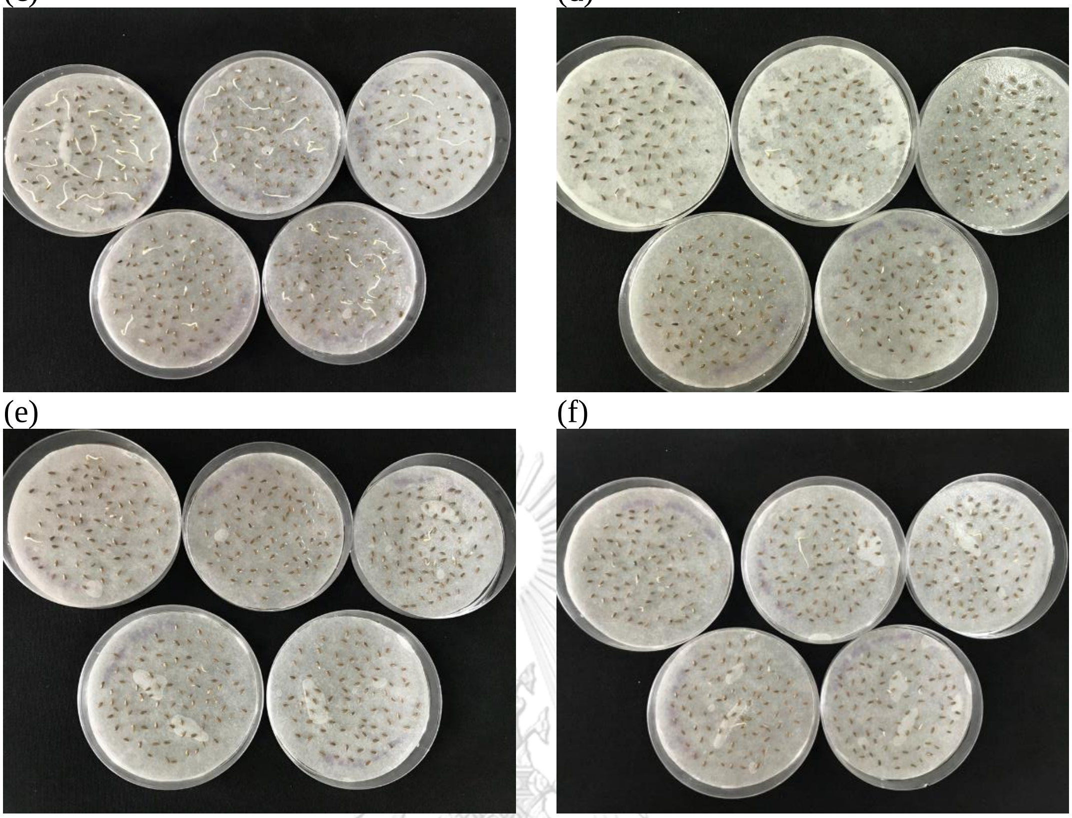


Figure 4-13 Seed germination on different solutions after 4 days in dark at room temperature (5 replicates with 80 seeds); solutions extracted from (a) control soil without heavy metals; (b) control soil with heavy metals; (c,d) soil after four months remediation by Songkhla3 without and with EDTA application, respectively; (e,f) soil after four months remediation by Prachuap Khiri Khan without and with EDTA application, respectively

Percentage of seed germinated in each treatment was compared to the seed germinated in DI water (control). The results showed that seedling in solution extracted from soil after phytoremediation with Songkhla3 vetiver is highest as compared to seedling in other solutions. It can reflect that soil remediated by Songkhla3 species has more suitable properties to use in other agricultural activities.

Study by Ivanov *et al.* showed the similar results of no observe on root hair and growing of root were stopped when seeding was put on the solution which contains heavy metals salts (V. B. Ivanov et al., 2003). Therefore, the low percentage of root germination in aqueous extracted from control group with heavy metal, soil after phytoremediation with Prachuap Khiri Khan, and remediated soil with EDTA application might resulted from the effect of heavy metals which inhibit seed germination.

CHAPTER 5
CONCLUSIONS

Pots experiment in this study showed that two species of vetiver grass which are Songkhla3 and Prachuap Khiri Khan have ability to reduce the concentration of Pb, Zn, and Cd in soil and accumulates them in their roots more than shoots over the period of study. Thus, this study indicated that vetiver grass can be used to phytoremediate contaminated soil. To compare the ability between two species of vetiver, the results showed that Songkhla3 has a higher bioaccumulation factor and translocation than Prachuap Khiri Khan vetiver, especially for Pb.

Consequently, bioaccumulation and translocation factors which indicate the efficiency in transfer of heavy metals from root to shoot of vetiver revealed the ability of two species of vetiver to phytoremediate soil contaminated with heavy metals. The results showed an increase in bioaccumulation factor in shoot, root and translocation factor for all heavy metals. On the other hand, the bioaccumulation of Pb in root is lower after application of EDTA in Songkhla3, which leads to the considerably increase in translocation factor. Pearson correlation also suggested a relation between the bioaccumulation of heavy metal in shoot and root of vetiver. Species of vetiver also have an effect on the transportation of Pb from root to shoot part of vetiver. Moreover, period of study has a relationship not only with the bioaccumulation in both shoot and root part but also with translocation factor of Zn and Cd.

Moreover, the results from experiment confirm the probability to increase the solubility of heavy metals in soil that leads to an increase in translocation factor and bioaccumulation of heavy metals in shoot and root of vetiver when EDTA was applied to soils. Although, the application of EDTA revealed many drawbacks including heavy metal leaching through groundwater, EDTA application could be an alternative method to enhance metal uptake in phytoremediation.

The results from Toxicity Characteristic Leaching Procedure (TCLP) showed that the leachability of heavy metals from remediated soil was lower after first three months of phytoremediation and the results showed that concentration of metal leachate was below the regulatory level except Cd in fourth month after EDTA application.

For Allium test, the chromosomal aberration cannot be observed but the aberration in external characteristics of allium's root can be noticed via the presence in shape and color of their roots. In addition, soil after four months of remediation with Songkhla3 had the highest of percentage of seed germination at 60.87%. It can imply that soil remediated by Songkhla3 is more suitable to utilize than other types of soil.

Suggestion for further research

The results from this research suggested that Songkhla3 vetiver may have a higher efficiency to remediate soil contamination with heavy metal in practice because it significantly decreases the heavy metals in soil and increases the accumulation of metal uptake. EDTA-application also has a higher effect to increase the bioaccumulation and translocation in Songkhla 3. Although, EDTA had a potential to increase bioaccumulation and translocation of heavy metals from root to shoot, it also increases bioavailability of heavy metals in soil which leads to a higher risk of heavy metals leaching to groundwater. Consequently, heavy metals that leach into groundwater can accumulate in consumable plants and animals which will further cause an adverse health effect on human.

In the future, bioavailability of heavy metals in soil should be analyzed to give more comprehensive understanding of phytoremediation.

APPENDIX A
Soil digestion results

Table A-1 Concentration of Pb, Zn and Cd in soil after phytoremediation with Songkhla3 measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
0	1	182.73	127.47	97.60
	2	180.97	199.09	107.53
	3	177.56	133.74	105.58
	Average	180.42	153.43	103.57
	SD	2.63	39.66	5.26
1	1	80.14	108.37	82.14
	2	128.59	107.85	75.59
	3	94.22	100.35	75.36
	Average	100.98	105.52	77.70
	SD	24.92	4.49	3.85
2	1	93.68	105.34	81.58
	2	84.84	102.87	69.41
	3	90.99	98.11	70.89
	Average	89.84	102.11	73.96
	SD	4.53	3.67	6.64
3	1	72.55	99.29	53.10
	2	81.59	84.10	65.17
	3	63.08	85.89	59.03
	Average	72.41	89.76	59.10
	SD	9.26	8.30	6.04
4	1	45.44	84.58	50.10
	2	64.58	93.46	61.40
	3	62.44	100.00	61.76

Table A-1 Concentration of Pb, Zn and Cd in soil after phytoremediation with Songkhla3 measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
	Average	57.49	92.68	57.75
	SD	10.49	7.74	6.63
4 with EDTA application	1	82.50	38.72	60.05
	2	37.33	20.36	40.13
	3	41.75	27.21	49.25
	Average	53.86	28.76	49.81
	SD	24.90	9.28	9.97

Table A-2 Concentration of Pb, Zn and Cd in soil after phytoremediation with Prachuap Khiri Khan measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Prachuap Khiri Khan**		
		Pb	**Zn**	**Cd**
0	1	182.73	127.47	97.60
	2	180.97	199.09	107.53
	3	177.56	133.74	105.58
	Average	180.42	153.43	103.57
	SD	2.63	39.66	5.26
1	1	79.76	91.55	66.58
	2	101.73	116.87	81.54
	3	88.79	117.33	94.10
	Average	90.09	108.58	80.74
	SD	11.04	14.76	13.78
2	1	83.56	99.84	73.17

Table A-2 Concentration of Pb, Zn and Cd in soil after phytoremediation with Prachuap Khiri Khan measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Prachuap Khiri Khan**		
		Pb	**Zn**	**Cd**
	2	75.60	90.76	64.40
	3	92.80	99.45	71.20
	Average	83.98	96.68	69.59
	SD	8.61	5.13	4.60
3	1	86.95	109.60	86.23
	2	63.71	91.82	63.05
	3	79.45	82.89	55.84
	Average	76.70	94.77	68.37
	SD	11.86	13.60	15.88
4	1	51.05	103.90	71.22
	2	63.83	79.66	53.55
	3	44.50	99.01	65.35
	Average	53.13	94.19	63.37
	SD	9.83	12.82	9.00
4 with EDTA application	1	34.81	59.18	37.87
	2	32.56	53.96	35.69
	3	33.74	63.04	38.89
	Average	33.71	58.73	37.48
	SD	1.12	4.56	1.63

APPENDIX B
Plants digestion results

Table B-1 Concentration of Pb, Zn and Cd in root of Songkhla3 measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	Zn	Cd
0	1	3.50	41.64	0.19
	2	1.41	34.25	0.18
	3	1.76	38.34	0.24
	Average	2.22	38.07	0.20
	SD	1.12	3.70	0.03
1	1	25.25	165.09	169.03
	2	12.59	128.29	96.17
	3	26.59	174.12	156.19
	Average	21.47	155.83	140.46
	SD	7.73	24.28	38.90
2	1	22.65	226.12	246.60
	2	17.41	159.89	184.79
	3	10.66	170.35	179.64
	Average	16.91	185.45	203.68
	SD	6.01	35.60	37.26
3	1	48.94	304.74	401.43
	2	22.00	175.82	195.68
	3	50.92	294.97	446.55
	Average	40.62	258.51	347.88
	SD	16.15	71.78	133.73
4	1	79.23	357.50	553.65
	2	52.54	302.54	445.28
	3	31.05	444.42	593.14
	Average	54.27	368.15	530.69

Table B-1 Concentration of Pb, Zn and Cd in root of Songkhla3 measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	Zn	Cd
	SD	24.14	71.54	76.56
4 with EDTA application	1	45.48	276.53	440.99
	2	28.83	329.04	383.05
	3	38.54	435.22	593.97
	Average	37.62	346.93	472.67
	SD	8.36	80.84	108.97

Table B-2 Concentration of Pb, Zn and Cd in shoot of Songkhla3 measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
0	1	0.71	27.55	0.19
	2	0.99	29.75	0.20
	3	1.05	29.22	0.22
	Average	0.91	28.84	0.20
	SD	0.18	1.15	0.02
1	1	0.99	36.09	13.93
	2	9.95	103.31	104.64
	3	5.45	104.15	93.64
	Average	5.46	81.18	70.74
	SD	4.48	39.05	49.50
2	1	15.70	179.00	201.93
	2	0.35	25.52	15.16
	3	12.46	76.60	103.47

Table B-2 Concentration of Pb, Zn and Cd in shoot of Songkhla3 measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
	Average	9.50	93.70	106.85
	SD	8.09	78.16	93.43
3	1	16.42	203.64	198.03
	2	10.84	201.06	211.86
	3	6.20	65.50	64.73
	Average	11.15	156.73	158.21
	SD	5.12	79.02	81.25
4	1	4.67	83.17	110.19
	2	32.18	148.13	182.07
	3	32.77	109.24	128.59
	Average	23.21	113.51	140.28
	SD	16.06	32.69	37.34
4 with EDTA application	1	19.68	137.35	155.86
	2	90.75	354.91	452.99
	3	44.82	286.56	338.21
	Average	51.75	259.60	315.69
	SD	36.04	111.26	149.84

Table B-3 Concentration of Pb, Zn and Cd in root of Prachuap Khiri Khan measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
0	1	2.30	56.53	0.22
	2	0.96	37.75	0.19
	3	1.59	44.22	0.20
	Average	1.62	46.17	0.20
	SD	0.67	9.54	0.01
1	1	32.65	245.33	261.75
	2	21.19	343.07	371.90
	3	11.83	138.85	122.14
	Average	21.89	242.42	251.93
	SD	10.43	102.14	125.17
2	1	16.83	231.25	262.35
	2	11.71	173.18	151.66
	3	30.25	453.95	30.25
	Average	19.60	286.13	148.09
	SD	9.58	148.21	116.09
3	1	41.08	383.68	363.81
	2	29.77	425.68	513.45
	3	15.23	149.23	195.75
	Average	28.69	319.53	357.67
	SD	12.96	148.97	158.94
4	1	21.44	271.87	402.26
	2	37.72	283.48	314.54
	3	13.50	372.08	520.02
	Average	24.22	309.14	412.27

Table B-3 Concentration of Pb, Zn and Cd in root of Prachuap Khiri Khan measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
	SD	12.35	54.81	103.10
4 with EDTA application	1	38.91	326.95	573.75
	2	16.31	196.34	224.95
	3	29.75	308.41	492.26
	Average	28.32	277.23	430.32
	SD	11.37	70.66	182.47

Table B-4 Concentration of Pb, Zn and Cd in shoot of Prachuap Khiri Khan measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
0	1	0.92	32.88	0.19
	2	1.36	39.00	0.20
	3	0.83	31.62	0.18
	Average	1.04	34.50	0.19
	SD	0.92	32.88	0.19
1	1	4.92	96.62	86.08
	2	4.79	102.41	94.32
	3	28.53	238.96	230.56
	Average	12.75	146.00	136.99
	SD	13.67	80.56	81.14
2	1	2.93	90.56	95.38
	2	6.51	95.51	94.74

Table B-4 Concentration of Pb, Zn and Cd in shoot of Prachuap Khiri Khan measured by ICP-OES (3 replicates)

Month of remediation	**No.**	**Concentration of heavy metals(mg/kg) in soil after phytoremediation with Songkhla3**		
		Pb	**Zn**	**Cd**
	3	25.93	122.12	123.09
	Average	11.79	102.73	104.40
	SD	12.38	16.97	16.19
3	1	12.86	246.77	269.33
	2	19.01	236.27	280.94
	3	12.22	220.61	266.18
	Average	14.70	234.55	272.15
	SD	3.75	13.16	7.77
4	1	15.74	133.17	226.44
	2	5.09	60.10	57.99
	3	19.23	102.25	112.73
	Average	13.35	98.51	132.39
	SD	7.37	36.68	85.93
4 with EDTA application	1	13.74	105.11	98.72
	2	6.48	123.76	99.63
	3	29.16	181.08	211.42
	Average	16.46	136.65	136.59
	SD	11.58	39.59	64.81

Table B-5 Translocation and bioaccumulation of heavy metals by Songkhla3

Heavy metals	Month of remediation	$BF_{(shoot)}$	$BF_{(root)}$	TF
Pb	0	0.01	0.01	0.41
	1	0.05	0.21	0.25
	2	0.11	0.19	0.56
	3	0.15	0.56	0.27
	4	0.40	0.94	0.58
	4+EDTA	1.25	0.70	1.58
Zn	0	0.19	0.25	0.76
	1	0.77	1.48	0.52
	2	0.92	1.82	0.51
	3	1.75	2.88	0.61
	4	1.22	3.97	0.32
	4+EDTA	10.50	13.10	0.74
Cd	0	0.00	0.00	1.00
	1	0.91	1.81	0.50
	2	1.44	2.75	0.52
	3	2.68	5.89	0.57
	4	2.43	9.19	0.27
	4+EDTA	6.34	9.49	0.70

Table B-6 Translocation and bioaccumulation of heavy metals by Prachuap Khiri Khan

Heavy metals	Month of remediation	$BF_{(shoot)}$	$BF_{(root)}$	TF
Pb	0	0.01	0.01	0.64
	1	0.14	0.24	0.93
	2	0.14	0.23	0.53
	3	0.19	0.37	0.58
	4	0.25	0.46	0.76
	4+EDTA	0.49	0.84	0.58
Zn	0	0.22	0.30	0.75
	1	1.34	2.23	0.80
	2	1.06	2.96	0.36
	3	2.47	3.37	0.89
	4	1.05	3.28	0.33
	4+EDTA	2.33	4.72	0.51
Cd	0	0.00	0.00	0.93
	1	1.70	3.12	0.82
	2	1.50	2.13	1.69
	3	4.12	5.23	0.88
	4	2.09	6.51	0.32
	4+EDTA	3.58	11.48	0.35

APPENDIX C
TCLP results

Table C-1 Heavy metals concentration from TCLP method by phytoremediation with Songkhla3 (using DI water as a leachant)

Month of remediation	**No.**	**Weight(g)**	**Concentration of heavy metals (mg/L)**		
			Pb	**Zn**	**Cd**
0	1	1.0556	0.00	1.50	2.16
	2	1.0117	0.00	1.03	1.48
	3	1.0037	0.00	0.16	0.29
	Average		0.00	0.90	1.31
	SD		0.00	0.68	0.95
1	1	1.0586	0.06	0.51	0.62
	2	1.073	0.05	0.73	0.88
	3	1.0503	0.04	0.67	0.82
	Average		0.05	0.64	0.77
	SD		0.01	0.11	0.13
2	1	1.0327	0.06	0.42	0.57
	2	1.0187	0.05	0.57	0.95
	3	1.0312	0.05	0.43	0.70
	Average		0.05	0.47	0.74
	SD		0.00	0.08	0.19
3	1	1.0278	0.05	0.14	0.20
	2	1.0199	0.06	0.14	0.25
	3	1.0034	0.05	0.37	0.57
	Average		0.05	0.22	0.34
	SD		0.01	0.13	0.20
4	1	1.0175	0.18	0.51	0.62
	2	1.0404	0.09	1.22	1.61
	3	1.048	0.06	1.90	2.74

Table C-1 Heavy metals concentration from TCLP method by phytoremediation with Songkhla3 (using DI water as a leachant)

Month of remediation	No.	Weight(g)	Concentration of heavy metals (mg/L)		
			Pb	**Zn**	**Cd**
	Average		0.11	1.21	1.66
	SD		0.06	0.69	1.06
4 with EDTA application	1	1.0303	0.21	0.81	0.90
	2	1.0396	0.16	0.62	0.68
	3	1.0543	0.37	3.00	3.34
	Average		0.24	1.47	1.64
	SD		0.11	1.32	1.48

Table C-2 Heavy metals concentration from TCLP method by phytoremediation with Prachuap Khiri Khan (using DI water as a leachant)

Month of remediation	No.	Weight(g)	Concentration of heavy metals (mg/L)		
			Pb	**Zn**	**Cd**
0	1	1.0556	0.00	1.50	2.16
	2	1.0117	0.00	1.03	1.48
	3	1.0037	0.00	0.16	0.29
	Average		0.00	0.90	1.31
	SD		0.00	0.68	0.95
1	1	1.0041	0.05	1.16	1.32
	2	1.0299	0.07	0.54	0.76
	3	1.0648	0.10	2.07	2.92
	Average		0.07	1.26	1.67
	SD		0.03	0.77	1.12

Table C-2 Heavy metals concentration from TCLP method by phytoremediation with Prachuap Khiri Khan (using DI water as a leachant)

Month of remediation	**No.**	**Weight(g)**	**Concentration of heavy metals (mg/L)**		
			Pb	**Zn**	**Cd**
2	1	1.1559	0.00	0.60	0.96
	2	1.0932	0.00	0.61	0.97
	3	1.068	0.00	0.82	1.40
	Average		0.00	0.68	1.11
	SD		0.00	0.13	0.25
3	1	1.0207	0.00	1.00	1.24
	2	1.0585	0.00	0.12	0.24
	3	1.0304	0.00	0.19	0.34
	Average		0.00	0.43	0.61
	SD		0.00	0.49	0.55
4	1	1.0095	0.00	0.74	0.99
	2	1.0769	0.00	0.98	1.17
	3	1.0655	0.00	1.19	1.42
	Average		0.00	0.97	1.19
	SD		0.00	0.23	0.22
4 with EDTA application	1	1.0092	2.61	4.44	3.77
	2	1.0082	0.16	1.63	2.08
	3	1.0314	0.32	1.27	1.36
	Average		1.03	2.45	2.40
	SD		1.37	1.74	1.24

Table C-3 Heavy metals concentration from TCLP method by phytoremediation with Songkhla3 (using acetic acid as a leachant)

Month of remediation	**No.**	**Weight(g)**	**Concentration of heavy metals (mg/L)**		
			Pb	**Zn**	**Cd**
0	1	1.0022	1.52	8.85	11.65
	2	1.0406	1.43	8.40	10.02
	3	1.1113	0.90	8.61	9.34
	Average		1.28	8.62	10.34
	SD		0.33	0.23	1.19
1	1	1.0700	2.73	9.27	8.93
	2	1.0619	2.43	7.47	7.81
	3	1.0852	2.50	8.54	8.30
	Average		2.55	8.43	8.35
	SD		0.16	0.91	0.56
2	1	1.0266	2.54	7.27	6.82
	2	1.0186	2.07	9.09	10.04
	3	1.0179	3.00	7.76	7.64
	Average		2.54	8.04	8.17
	SD		0.47	0.94	1.67
3	1	1.0283	2.66	7.49	8.38
	2	1.0457	3.59	7.70	8.40
	3	1.0188	2.81	7.23	5.73
	Average		3.02	7.47	7.50
	SD		0.50	0.24	1.54
4	1	1.0426	2.26	5.81	5.35
	2	1.0038	2.35	6.96	6.08
	3	1.0776	1.46	9.86	11.22

Table C-3 Heavy metals concentration from TCLP method by phytoremediation with Songkhla3 (using acetic acid as a leachant)

Month of remediation	No.	Weight(g)	Concentration of heavy metals (mg/L)		
			Pb	Zn	Cd
	Average		2.02	7.54	7.55
	SD		0.49	2.09	3.20
4 with EDTA application	1	1.0472	1.35	6.50	6.24
	2	1.0556	1.90	7.79	7.17
	3	1.0735	0.95	8.73	8.88
	Average		1.40	7.67	7.43
	SD		0.48	1.12	1.34

Table C-4 Heavy metals concentration from TCLP method by phytoremediation with Prachaup Khiri Khan (using acetic acid as a leachant)

Month of remediation	No.	Weight(g)	Concentration of heavy metals (mg/L)		
			Pb	Zn	Cd
0	1	1.0022	1.52	8.85	11.65
	2	1.0406	1.43	8.40	10.02
	3	1.1113	0.90	8.61	9.34
	Average		1.28	8.62	10.34
	SD		0.33	0.23	1.19
1	1	1.0026	5.64	8.94	9.22
	2	1.0174	2.01	8.94	8.87
	3	1.0122	3.16	8.53	8.79
	Average		3.60	8.80	8.96
	SD		1.86	0.24	0.23

Table C-4 Heavy metals concentration from TCLP method by phytoremediation with Prachaup Khiri Khan (using acetic acid as a leachant)

Month of remediation	**No.**	**Weight(g)**	**Concentration of heavy metals (mg/L)**		
			Pb	**Zn**	**Cd**
2	1	1.0268	1.92	6.90	6.99
	2	1.0895	7.85	8.73	7.89
	3	1.092	4.39	9.20	8.28
	Average		4.72	8.28	7.72
	SD		2.98	1.22	0.66
3	1	1.0309	4.12	8.76	8.21
	2	1.031	1.50	5.88	5.78
	3	1.0309	4.64	8.41	7.25
	Average		3.42	7.68	7.08
	SD		1.68	1.57	1.22
4	1	1.039	1.11	5.58	6.25
	2	1.0097	1.00	6.45	6.21
	3	1.0512	0.84	5.99	6.68
	Average		0.98	6.01	6.38
	SD		0.14	0.44	0.26
4 with EDTA application	1	1.0093	4.53	6.45	5.92
	2	1.0391	1.62	6.19	6.74
	3	1.0323	3.58	6.95	5.66
	Average		3.24	6.53	6.11
	SD		1.48	0.39	0.56

APPENDIX D
Statistical analysis

This research use SPSS program (Statistical Package for Social Science for Windows version 22) to analyze the concentration of heavy metals from phytoremediation by two species of vetiver by T-test and Pearson correlation.

Define H0: mean concentration of heavy metals equals in variance groups
H1: mean concentration of heavy metals at least one group is not equals to other groups

If the results from Sig 2-tailed ≥ 0.05 showed that month of remediation, species of vetiver, or EDTA application is not significance to concentration of heavy metals which is increase or decrease.

If the results from Sig 2-tailed ≤ 0.05 showed that month of remediation, species of vetiver, or EDTA application are significance to concentration of heavy metals which is increase or decrease.

Table D-1 concentration of heavy metal from phytoremediation with Songkhla3

		Age when test (m)
Age when test (m)	Pearson Correlation	1
	Sig. (2-tailed)	
	N	15
[Pb]soil	Pearson Correlation	-.881**
	Sig. (2-tailed)	.000
	N	15
[Pb]shoot	Pearson Correlation	.694**
	Sig. (2-tailed)	.004
	N	15
[Pb]root	Pearson Correlation	.812**
	Sig. (2-tailed)	.000
	N	15
[Zn]soil	Pearson Correlation	-.824**
	Sig. (2-tailed)	.000
	N	15
[Zn]shoot	Pearson Correlation	.566*
	Sig. (2-tailed)	.028
	N	15
[Zn]root	Pearson Correlation	.924**
	Sig. (2-tailed)	.000
	N	15
[Cd]soil	Pearson Correlation	-.910**
	Sig. (2-tailed)	.000
	N	15
[Cd]shoot	Pearson Correlation	.688**
	Sig. (2-tailed)	.005
	N	15
[Cd]root	Pearson Correlation	.939**
	Sig. (2-tailed)	.000
	N	15

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table D-2 concentration of heavy metal from phytoremediation with Prachuap Khiri Khan

		Age when test (m)
Age when test (m)	Pearson Correlation	1
	Sig. (2-tailed)	
	N	15
[Pb]soil	Pearson Correlation	-.856**
	Sig. (2-tailed)	.000
	N	15
[Pb]shoot	Pearson Correlation	.424
	Sig. (2-tailed)	.116
	N	15
[Pb]root	Pearson Correlation	.589*
	Sig. (2-tailed)	.021
	N	15
[Zn]soil	Pearson Correlation	-.771**
	Sig. (2-tailed)	.001
	N	15
[Zn]shoot	Pearson Correlation	.414
	Sig. (2-tailed)	.125
	N	15
[Zn]root	Pearson Correlation	.638*
	Sig. (2-tailed)	.010
	N	15
[Cd]soil	Pearson Correlation	-.852**
	Sig. (2-tailed)	.000
	N	15
[Cd]shoot	Pearson Correlation	.581*
	Sig. (2-tailed)	.023
	N	15
[Cd]root	Pearson Correlation	.752**
	Sig. (2-tailed)	.001
	N	15

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table D-3 concentration of heavy metals from phytoremediation with Songkhla3 with EDTA application

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
[Pb]soil	Equal variances assumed	4.243	.108	.232	4	.828
	Equal variances not assumed			.232	2.688	.833
[Pb]shoot	Equal variances assumed	1.782	.253	-1.253	4	.278
	Equal variances not assumed			-1.253	2.764	.306
[Pb]root	Equal variances assumed	1.876	.243	1.129	4	.322
	Equal variances not assumed			1.129	2.473	.356
[Zn]soil	Equal variances assumed	.127	.740	9.164	4	.001
	Equal variances not assumed			9.164	3.875	.001
[Zn]shoot	Equal variances assumed	3.817	.122	-2.182	4	.095
	Equal variances not assumed			-2.182	2.343	.142
[Zn]root	Equal variances assumed	.075	.798	.341	4	.751
	Equal variances not assumed			.341	3.942	.751
[Cd]soil	Equal variances assumed	.259	.638	1.149	4	.315

Table D-3 concentration of heavy metals from phytoremediation with Songkhla3 with EDTA application

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
	Equal variances not assumed			1.149	3.480	.323
[Cd]shoot	Equal variances assumed	3.287	.144	-1.967	4	.121
	Equal variances not assumed			-1.967	2.247	.174
[Cd]root	Equal variances assumed	.561	.496	.755	4	.492
	Equal variances not assumed			.755	3.588	.497

Table D-4 concentration of heavy metals from phytoremediation with Prachaup Khiri Khan with EDTA application

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
[Pb]soil	Equal variances assumed	5.903	.072	3.400	4	.027
	Equal variances not assumed			3.400	2.052	.074
[Pb]shoot	Equal variances assumed	.740	.438	-.392	4	.715
	Equal variances not assumed			-.392	3.391	.718

Table D-4 concentration of heavy metals from phytoremediation with Prachaup Khiri Khan with EDTA application

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
[Pb]root	Equal variances assumed	.046	.841	-.423	4	.694
	Equal variances not assumed			-.423	3.973	.694
[Zn]soil	Equal variances assumed	4.354	.105	4.514	4	.011
	Equal variances not assumed			4.514	2.497	.030
[Zn]shoot	Equal variances assumed	.079	.793	-1.224	4	.288
	Equal variances not assumed			-1.224	3.977	.288
[Zn]root	Equal variances assumed	.432	.547	.618	4	.570
	Equal variances not assumed			.618	3.767	.572
[Cd]soil	Equal variances assumed	11.193	.029	1.020	4	.366
	Equal variances not assumed			1.020	2.043	.413
[Cd]shoot	Equal variances assumed	.252	.642	-.068	4	.949
	Equal variances not assumed			-.068	3.719	.950

Table D-4 concentration of heavy metals from phytoremediation with Prachaup Khiri Khan with EDTA application

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
[Cd]root	Equal variances assumed	1.576	.278	-.149	4	.889
	Equal variances not assumed			-.149	3.159	.890

Table D-5 BF(shoot and root) factor and TF (translocation factor)

		type of plant
type of plant	Pearson Correlation	1
	Sig. (2-tailed)	
	N	10
age when test	Pearson Correlation	0.000
	Sig. (2-tailed)	1.000
	N	10
Pb BF(shoot)	Pearson Correlation	-.007
	Sig. (2-tailed)	.985
	N	10
Pb BF(root)	Pearson Correlation	.228
	Sig. (2-tailed)	.527
	N	10
Pb TF	Pearson Correlation	-.748*
	Sig. (2-tailed)	.013
	N	10
Zn BF(shoot)	Pearson Correlation	-.203
	Sig. (2-tailed)	.573
	N	10
Zn BF(root)	Pearson Correlation	-.144
	Sig. (2-tailed)	.691
	N	10
Zn TF	Pearson Correlation	-.038

Table D-5 BF(shoot and root) factor and TF (translocation factor)

		type of plant
	Sig. (2-tailed)	.917
	N	10
Cd BF(shoot)	Pearson Correlation	-.156
	Sig. (2-tailed)	.666
	N	10
Cd BF(root)	Pearson Correlation	.094
	Sig. (2-tailed)	.796
	N	10
Cd TF	Pearson Correlation	-.223
	Sig. (2-tailed)	.536
	N	10

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table D-6 BF$_{shoot,root}$ (Bioaccumulation factor) and TF (translocation factor) from Songkhla3 with EDTA application

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
Pb BF(shoot)	Equal variances assumed	2.014	.229	-1.331	4	.254
	Equal variances not assumed			-1.331	2.676	.285
Pb BF(root)	Equal variances assumed	1.717	.260	.950	4	.396
	Equal variances not assumed			.950	2.537	.424
Pb TF	Equal variances assumed	3.593	.131	-1.168	4	.308
	Equal variances not assumed			-1.168	2.497	.342

Table D-6 ***$BF_{shoot,root}$ (Bioaccumulation factor) and TF (translocation factor) from Songkhla3 with EDTA application***

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
Zn BF(shoot)	Equal variances assumed	6.796	.060	-3.479	4	.025
	Equal variances not assumed			-3.479	2.033	.072
Zn BF(root)	Equal variances assumed	3.818	.122	-4.807	4	.009
	Equal variances not assumed			-4.807	2.300	.031
Zn TF	Equal variances assumed	2.046	.226	-2.192	4	.094
	Equal variances not assumed			-2.192	2.881	.120
Cd BF(shoot)	Equal variances assumed	3.652	.129	-2.200	4	.093
	Equal variances not assumed			-2.200	2.184	.148
Cd BF(root)	Equal variances assumed	1.078	.358	-.203	4	.849
	Equal variances not assumed			-.203	3.294	.851
Cd TF	Equal variances assumed	4.935	.090	-1.659	4	.172
	Equal variances not assumed			-1.659	2.291	.223

***Table D-7** $BF_{shoot,root}$ (Bioaccumulation factor) and TF (translocation factor) from Prachuap Khiri Khan with EDTA application*

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
Pb BF(shoot)	Equal variances assumed	2.457	.192	-1.108	4	.330
	Equal variances not assumed			-1.108	2.635	.359
Pb BF(root)	Equal variances assumed	.349	.586	-1.625	4	.179
	Equal variances not assumed			-1.625	3.550	.188
Pb TF	Equal variances assumed	.627	.473	.443	4	.681
	Equal variances not assumed			.443	3.082	.687
Zn BF(shoot)	Equal variances assumed	1.426	.298	-2.850	4	.046
	Equal variances not assumed			-2.850	3.201	.060
Zn BF(root)	Equal variances assumed	2.994	.159	-1.864	4	.136
	Equal variances not assumed			-1.864	2.887	.163
Zn TF	Equal variances assumed	.146	.722	-1.464	4	.217
	Equal variances not assumed			-1.464	3.926	.218
Cd BF(shoot)	Equal variances assumed	.498	.519	-1.226	4	.287

***Table D-7** BF$_{shoot,root}$ (Bioaccumulation factor) and TF (translocation factor) from Prachuap Khiri Khan with EDTA application*

		Equality of Variances		t-test for Equality for Means		
		F	Sig.	t	df	Sig. (2-tailed)
	Equal variances not assumed			-1.226	3.785	.291
Cd BF(root)	Equal variances assumed	4.324	.106	-1.679	4	.168
	Equal variances not assumed			-1.679	2.441	.212
Cd TF	Equal variances assumed	.737	.439	-.179	4	.867
	Equal variances not assumed			-.179	3.654	.867

REFERENCES

Abbas, M. H. H., & Abdelhafez, A. A. (2013). Role of EDTA in arsenic mobilization and its uptake by maize grown on an As-polluted soil. *Chemosphere, 90*(2), 588-594. doi:10.1016/j.chemosphere.2012.08.042

Agency for Toxic Substances and Disease Registry. (2017). Retrieved from https://www.atsdr.cdc.gov/

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals--concepts and applications. *Chemosphere, 91*(7), 869-881. doi:10.1016/j.chemosphere.2013.01.075

Andrew D. Vassil, Yoram Kapulnik, Ilya Raskin, & Salt*, a. D. E. (1998). The Role of EDTA in Lead Transport and Accumulation by Indian Mustard. *Plant Physiol, 117*, 447-453.

Bennedsen, L. R., Krischker, A., Jorgensen, T. H., & Sogaard, E. G. (2012). Mobilization of metals during treatment of contaminated soils by modified Fenton's reagent using different chelating agents. *J Hazard Mater, 199-200*, 128-134. doi:10.1016/j.jhazmat.2011.10.068

BERND NOWACK, R. S., & ROBINSON, B. H. (2006). Critical assessment of chelant-enhanced metal phytoextraction. *ENVIRONMENTAL SCIENCE & TECHNOLOGY, 40*, 5225-5232.

Bhattacharya T, Banerjee DK, & B., G. (2006). Heavy metal uptake by Scirpus Littoralis Schrad. from fly ash dosed and metal spiked soils. *Environ Monit Assess, 121(1-3)*, 363-380.

Campbell, P. G. C. (2006). Cadmium—A Priority Pollutant. *Environmental Chemistry, 3*, 387-388. doi:10.1071/EN06075

Chandra, R., Bharagava, R. N., Yadav, S., & Mohan, D. (2009). Accumulation and distribution of toxic metals in wheat (Triticum aestivum L.) and Indian mustard (Brassica campestris L.) irrigated with distillery and tannery effluents. *J Hazard Mater, 162*(2-3), 1514-1521. doi:10.1016/j.jhazmat.2008.06.040

Chantana, P., Wittaya, S., Muneko, N., Werawan, R., & Thawangon, I. Health Risk Management for Cadmium Contamination in Thailand: Are Challenges Overcome ?

Chen, Y., Shen, Z., & Li, X. (2004). The use of vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. *Applied Geochemistry, 19*(10), 1553-1565. doi:10.1016/j.apgeochem.2004.02.003

Chengo Katana, Murungi Jane, & Harun*, M. (2013). Speciation of Zinc and Copper in Open-Air Automobile Mechanic Workshop Soils in Ngara Area-Nairobi Kenya. *Resources and Environment, 3(5)*, 145-154. doi:10.5923/j.re.20130305.06

Chou, S.-T., Shih, Y., & Lin, C.-C. (2016). Vetiver Grass (Vetiveria zizanioides) Oils. 843-848. doi:10.1016/b978-0-12-416641-7.00096-1

Department, P. C. (2009). Publication of Lead Contamination in Klitty Creek to people. Retrieved from http://infofile.pcd.go.th/mgt/situation_kity52.pdf?CFID=1207809&CFTOKEN=74486625

Edao, H. G. (2017). Heavy Metals Pollution Of Soil; Toxicity And Phytoremediation Techniques. *International Journal of Advanced Research and Publications (IJARP), 1*(1), 13.

EPA. (1992). Method 1311 Toxicity Characteristic Leaching Procedure. In: EPA.

EPA. (1996). Method 3052 Microwave assisted acid digetion of siliceous and organically matrices. In: EPA.

EPA. (2007). Method 3051A Microwave assisted acid digestion of sediments sludges, soils and oils. In: EPA.

Ettler, V., Vaněk, A., Mihaljevič, M., & Bezdička, P. (2005). Contrasting lead speciation in forest and tilled soils heavily polluted by lead metallurgy. *Chemosphere, 58*(10), 1449-1459. doi:10.1016/j.chemosphere.2004.09.084

Evangelou, M. W. H., Ebel, M., & Schaeffer, A. (2007). Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere, 68*(6), 989-1003. doi:10.1016/j.chemosphere.2007.01.062

Gheju, M., Pode, R., & Manea, F. (2011). Comparative heavy metal chemical extraction from anaerobically digested biosolids. *Hydrometallurgy, 108*(1-2), 115-121. doi:10.1016/j.hydromet.2011.03.006

Ghosh, M., Paul, J., Jana, A., De, A., & Mukherjee, A. (2015). Use of the grass, Vetiveria zizanioides (L.) Nash for detoxification and phytoremediation of soils contaminated with fly ash from thermal power plants. *Ecological Engineering, 74*, 258-265. doi:10.1016/j.ecoleng.2014.10.011

Gray, C. W., & McLaren, R. G. (2006). Soil Factors Affecting Heavy Metal Solubility in Some New Zealand Soils. *Water, Air, and Soil Pollution, 175*(1-4), 3-14. doi:10.1007/s11270-005-9045-2

Greany, K. M. (2005). *An assessment of heavy metal contamination in the marine sediments of Las Perlas Archipelago, Gulf of Panama.* (M.S.), School of Life Sciences Heriot-Watt University, Edinburgh, Scotland,

Human Rights Watch. (2014). Thailand: Clean Up Klity Creek: Confront Toxic Legacy Before Reopening Lead Mines. Retrieved from https://www.hrw.org/news/2014/12/15/thailand-clean-klity-creek

I. Raskin, B. D. E. (1999). *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment* New York, NY, USA.

Iain Thornton. (1972). Geochemical aspects of the distribution and forms of heavy metals in soil In N. W. LEPP (Ed.), *EFFECT OF HEAVY METAL POLLUTION ON PLANTS* (Vol. 2, pp. 1-34).

Ilya Raskin, PBA Nanda Kumar, Slavik Dushenkov, & Salt, D. E. (1994). Bioconcentration of heavy metals by plants. *Current Opinion in 8iotechnology, 5*, 285-290.

K. Cho-Ruk, J. Kurukote, P. Supprung, & S. Vetayasuporn. (2006). Perennial Plants in the Phytoremediation of Lead-contaminated Soils. *Biotechnology, 5*(1), 1-4.

Kim, A.-Y., Kim, J.-Y., Ko, M.-S., & Kim, K.-W. (2010). Acid Rain Impact on Phytoavailability of Heavy Metals in Soils. *Geosystem Engineering, 13*(4), 133-138. doi:10.1080/12269328.2010.10541320

Kim, R. Y., Yoon, J. K., Kim, T. S., Yang, J. E., Owens, G., & Kim, K. R. (2015). Bioavailability of heavy metals in soils: definitions and practical implementation--a critical review. *Environ Geochem Health, 37*(6), 1041-1061. doi:10.1007/s10653-015-9695-y

Kiran D Ladwani, Krishna D Ladwani, Vivek S Manik, & Dilip S Ramteke. (2012). Assessment of heavy metal contaminated soil near coal minning area in Gujaray by toxicity characteristic leaching procedure. *International Journal of Life Sciences Biotechnology and Pharma Research, 1*, 73-80.

Kumsorn, D., & Chairak, C. (2013). Uptake of Zinc, Lead, and Copper by Sunflower grown in contaminated soil. *KKU Science, 41*.

Lai, H. Y., & Chen, Z. S. (2004). Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass. *Chemosphere, 55*(3), 421-430. doi:10.1016/j.chemosphere.2003.11.009

Land Development Department. Vetiver for King. Retrieved from https://www.ldd.go.th/link_vetiver/index.htm

Leleyter, L., Rousseau, C., Biree, L., & Baraud, F. (2012). Comparison of EDTA, HCl and sequential extraction procedures, for selected metals (Cu, Mn, Pb, Zn), in soils, riverine and marine sediments. *Journal of Geochemical Exploration, 116-117*, 51-59. doi:10.1016/j.gexplo.2012.03.006

Lestan, D., Luo, C. L., & Li, X. D. (2008). The use of chelating agents in the remediation of metal-contaminated soils: a review. *Environ Pollut, 153*(1), 3-13. doi:10.1016/j.envpol.2007.11.015

Luo, C., Shen, Z., & Li, X. (2005). Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere, 59*(1), 1-11. doi:10.1016/j.chemosphere.2004.09.100

M. B. McBride. (1989). Reactions Controlling Heavy Metal Solubility in Soils. In B. A. Stewart (Ed.), *Advances in Soil Science* (Vol. 10).

M. McBride, S. Sauve, & Hendershot, W. (1997). Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *European Journal of Soil Science, 48*, 337-346.

Manahan, S. E. (2003). *Toxicological Chemistry and Biochemistry* (3 ed.): CRC Press, Limited Liability Company (LLC).

Meers, E., Tack, F. M., Van Slycken, S., Ruttens, A., Du Laing, G., Vangronsveld, J., & Verloo, M. G. (2008). Chemically assisted phytoextraction: a review of potential soil amendments for increasing plant uptake of heavy metals. *Int J Phytoremediation, 10*(5), 390-414. doi:10.1080/15226510802100515

Nannoni, F., Protano, G., & Riccobono, F. (2011). Fractionation and geochemical mobility of heavy elements in soils of a mining area in northern Kosovo. *Geoderma, 161*(1-2), 63-73. doi:10.1016/j.geoderma.2010.12.008

NSC. (2009). Lead Poisoning. http://www.nsc.org/NSCDocuments_Advocacy/Fact%20Sheets/Lead-Poisoning-Fact-Sheet.pdf

Ogundiran, M. B., & Osibanjo, O. (2015). Mobility and speciation of heavy metals in soils impacted by hazardous waste. *Chemical Speciation & Bioavailability, 21*(2), 59-69. doi:10.3184/095422909x449481

Ondo Zue Abaga, N., Dousset, S., Mbengue, S., & Munier-Lamy, C. (2014). Is vetiver grass of interest for the remediation of Cu and Cd to protect marketing gardens in Burkina Faso? *Chemosphere, 113*, 42-47. doi:10.1016/j.chemosphere.2014.04.010

ORDPB. (2000). Factual tips about vetiver grass

Pehlivan, E., Ozkan, A. M., Dinc, S., & Parlayici, S. (2009). Adsorption of Cu2+ and Pb2+ ion on dolomite powder. *J Hazard Mater, 167*(1-3), 1044-1049. doi:10.1016/j.jhazmat.2009.01.096

Peng, J. F., Song, Y. H., Yuan, P., Cui, X. Y., & Qiu, G. L. (2009). The remediation of heavy metals contaminated sediment. *J Hazard Mater, 161*(2-3), 633-640. doi:10.1016/j.jhazmat.2008.04.061

Poopa, T., Pavasant, P., Kanokkantapong, V., & Panyapinyopol, B. (2015). Spatial dstribution and mobility factor of heavy metals in agricultural soil in the Vicinity of abandoned lead ore dressing plant, Klity Village, Thailand. *EnvironmentAsia, 8*, 94-108.

Praween Supanuam, Alongkoad Tanomtong, Sirilak Thiprautree, Somret Sikhruadong, & Gomontean, B. (2010). Chromosomal Staining Comparison of Plant Cells with Black Glutinous Rice (*Oryza sativa* L.) and Lac (*Laccifer lacca* Kerr). *Cytologia, 75*(1), 89-97.

Raymond N. Yong, & Yuwaree Phadungchewit. (1993). pH influence on selectivity and retention of heavy metals in some clay soils. *Canadian Geotechnical Journal, 30*(5), 821-833.

Rieuwerts, J. S., Thornton, I., Farago, M. E., & Ashmore, M. R. (2015). Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chemical Speciation & Bioavailability, 10*(2), 61-75. doi:10.3184/095422998782775835

Rotkittikhun, P., Chaiyarat, R., Kruatrachue, M., Pokethitiyook, P., & Baker, A. J. (2007). Growth and lead accumulation by the grasses Vetiveria zizanioides and Thysanolaena maxima in lead-contaminated soil amended with pig manure and fertilizer: a glasshouse study. *Chemosphere, 66*(1), 45-53. doi:10.1016/j.chemosphere.2006.05.038

Saifullah, Meers, E., Qadir, M., de Caritat, P., Tack, F. M. G., Du Laing, G., & Zia, M. H. (2009). EDTA-assisted Pb phytoextraction. *Chemosphere, 74*(10), 1279-1291. doi:10.1016/j.chemosphere.2008.11.007

Shahid, M., Austruy, A., Echevarria, G., Arshad, M., Sanaullah, M., Aslam, M., . . . Dumat, C. (2013). EDTA-Enhanced Phytoremediation of Heavy Metals: A Review. *Soil and Sediment Contamination: An International Journal, 23*(4), 389-416. doi:10.1080/15320383.2014.831029

Sheila O'Hare, & Atterwill, a. C. K. (1995). *Methods in Molecular Biology: in vitro toxicity testing protocols* (Vol. 43).

Sheoran, V., Sheoran, A. S., & Poonia, P. (2016). Factors Affecting Phytoextraction: A Review. *Pedosphere, 26*(2), 148-166. doi:10.1016/s1002-0160(15)60032-7

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. *International Journal of Chemical Engineering, 2011*, 1-31. doi:10.1155/2011/939161

The Global CCS Institute. Sorption processes. from The Global CCS Institute https://vpn.chula.ac.th/+CSCO+0075676763663A2F2F75686F2E7479626F6E79707066766166776676867722E70627A++/publications/potential-impacts-groundwater-resources-co2-geological-storage/33-potential-chemical

Todd A. Kimmell, Llewellyn R. Williams, & Susan S. Sorini. (2011). The RCRA Toxicity Characteristic Leaching Procedure (TCLP): A Concept for a New Method. *Federal Facilities Environmental Journal, 12*(3), 7-24.

Udovic, M., & Lestan, D. (2009). Pb, Zn and Cd mobility, availability and fractionation in aged soil remediated by EDTA leaching. *Chemosphere, 74*(10), 1367-1373. doi:10.1016/j.chemosphere.2008.11.013

V. B. Ivanov, E. I. Bystrova, & Seregin, a. I. V. (2003). Comparative Impacts of Heavy Metals on Root Growth as Related to Their Specificity and Selectivity. *Russian Journal of Plant Physiology, 50*, 398-406.

Voglar, D., & Lestan, D. (2012). Electrochemical treatment of spent solution after EDTA-based soil washing. *Water Res, 46*(6), 1999-2008. doi:10.1016/j.watres.2012.01.018

Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere, 50*, 775-780.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology, 2011*, 1-20. doi:10.5402/2011/402647

Xingfuxian, & Gholamhoss In Shokohifard. (1989). Effect of pH on chemical forms and plant availability of cadmium, zinc, and lead in polluted soils. *Water, Air, and Soil Pollution*, *45*, 265-273.

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