PHYTOEXTRACTION OF ARSENIC FROM CONTAMINATED SOIL BY *Colocasia esculenta* (L.) Schott; TARO AND WILD TARO

Mr. Witchanan Tambamroong

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การดูดดึงสารหนูที่ปนเปื้อนในดินโดยใช้เผือกและบอน

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาและเปรียบเทียบการสะสมของสารหนูในเผือกและบอนที่ความเข้มข้นของสารหนูในดินที่แตกต่างกัน และศึกษาประสิทธิภาพของ ethylenediaminetetraacetic acid (EDTA) ในการเพิ่มประสิทธิภาพของการสะสมสารหนูในพืชทั้งสองชนิด พืชทั้งสองชนิดจะปลูกในกระถางทดลองและผสม $Na_2HAsO_4.7H_2O$ ให้มีความเข้มข้นเป็น 0 100 200 และ 400 มิลลิกรัมของสารหนู/กิโลกรัมของดิน หลังจากนั้นสองอาทิตย์จึงเติม EDTA 5 มิลลิโมล/กิโลกรัมของดิน โดยทำการเก็บตัวอย่างทุก 20 วันจนครบ 100 วัน และวิเคราะห์สารหนูที่สะสมในพืชโดยแบ่งเป็น 4 ส่วน ได้แก่ ราก หัว ก้านใบ และใบ

ผลการศึกษาพบว่าพืชทั้งสองชนิดสามารถเจริญเติบโตได้ดีและอยู่รอดทั้งหมดในความเข้มข้น 100 และ 200 มิลลิกรัม/กิโลกรัม โดยสารหนูสามารถสะสมได้มากที่สุดใน ราก มากที่สุด รองลงมา คือ หัว ก้านใบ และใบ ตามลำดับ ซึ่งจากการศึกษาพบว่าการเติม EDTA สามารถเพิ่มการสะสมของสารหนูในพืชทั้งสองชนิดได้ การสะสมของสารหนูมีค่ามากที่สุดที่ความเข้มข้น 400 มิลลิกรัม/กิโลกรัม พบว่าในเผือกมีค่ามากที่สุด คือ 40.34 มิลลิกรัม ส่วนในบอนพบว่ามีค่า 46.79 มิลลิกรัม

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The purposes of this study were to study and compare the accumulation of arsenic in *Colocasia esculenta* (L.) Schott (taro and wild taro) among different concentrations of arsenic in soil and study the efficiency of ethylenediaminetetraacetic acid (EDTA) solution to enhance the accumulation of arsenic in both plant species. The plants were planted in the experimental pots and amended with disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) solution with 4 different concentrations (control, 100, 200, and 400 mg As/kg soil). Ethylenediaminetetraacetic acid (EDTA) solution 5 mmol/kg was apply to the soil surface 2 weeks after amending with disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) solution. The plants were harvested and cut to sample every 20 days for 100 days and analyzed for total arsenic accumulation in 4 parts that are corm, roots, petioles, and laminas.

The results indicate that both plants could growth well in concentration of 100 and 200 mg As/kg soil. Amount of arsenic accumulated in root was more than in corm, petiole, and lamina, respectively. And the research shows that the accumulation of arsenic in both plants can be enhanced through the application of EDTA to the soil. The maximum arsenic accumulations in plants were 40.34 mg for taro and 46.79 mg for wild taro in concentration of 400 mg As/kg soil.

Department Inter-Department of Environmental Management Student's signaturea……
Field of study Environmental Management..... Advisor's signature……..
Academic year 2002..... Co-advisor's signature…….

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Chapter I

Introduction

1.1 Theoretical background

Industrial and other human activities can cause the releasing of hazardous substances such as heavy metals and other toxic substances to the public and the environment. Soils contaminated with heavy metals such as Cd, As, Zn, Pb, Cr and Se caused many of environmental and health concern because of the potential for contamination of surface or ground water, off-site contamination by wind-transported material, redistribution of contaminated soil or sediment, uptake by plant, and bioaccumulation in food chains (Chaney et al., 1998). Among various metals, arsenic (As) is a toxic metal ubiquitously encountered in the environment and well known for its toxicity to humans and animals when ingested or inhaled, and also raises serious concern in many contaminated sites. The largest sink for man-made arsenic in the environment is the soil (National Research Council, 1977). Mining and smelter activities have traditionally introduced large amounts of arsenic into the environment especially in soil (National Research Council, 1977). Remediation of arsenic that still contaminated in soil is required to reduce or eliminate any risk to humans and the environment.

The problem in Ronphiboon district, Nakhon Si Thammarat province, Thailand, is an example of an area contaminated with arsenic and causes many of health problems was first recognized in 1987. This problem occurred by arsenic spreading from tin ore mining activities over the past 50 years. At present, those mining sites are closed, but still distribute arsenic to the environment. Then it leaches and contaminates shallow wells used by people nearby who become sick and many of them are infected with skin diseases including alternate pigmentation, small corns on palms and soles, purplish-red flush, and even skin cancer. Those infected people were counted upto 1,500 persons in 1987 (Pollution Control Department [PCD], 1998). The concentration of arsenic in soil at this site has been found from 50 – 5,000 mg/kg, so significant level of arsenic is still contaminated in the soil.

The remediation of large volumes of such soil by conventional technologies previously developed for small, heavily contaminated sites would be very expensive (Ebbs et al., 1997). Recently, phytoremediation has emerged as an alternative to the engineering-based methods (Ebbs et al., 1997). Phytoremediation is the technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water (United States Environmental Protection Agency [U.S. EPA], 2000). In this new approach, plants are used to absorb contaminants from the soil and translocate them to the shoots. Pollutants are then removed by harvesting the aboveground tissue for subsequent volume reduction such as ashing or drying. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues. This technology has been receiving attention lately as an innovative, cost effective alternative to the more established treatment methods used at hazardous waste sites (U.S. EPA, 2000).

The ideal plant specie to remediate a toxic metal contaminated soil would be a high biomass crop that can both tolerate and accumulate high concentration of metal in harvestable tissue. Some of the most likely plant species for phytoremediation of arsenic are members of the Araceae family, *Colocasia esculenta* L. Schott (taro and wild taro) because of its high biomass, large corm and tuberous roots. But the low solubility of heavy metals in soil is often a limiting factor in metal extraction by plants (Huang et al., 1998). Increasing metal solubility in soil and the bioavailability of metals to the plants are important to phytoextraction of heavy metals from contaminated soil. Chelating compounds have been used in soils and nutrient solutions to increase the solubility of metals in soil for the plants. But there is little information in the literature concerning the use of these compounds to enhance arsenic accumulation in plants. So in this study, the use of ethylenediaminetetraacetic acid (EDTA) was introduced as chelating agent applied to the arsenic contaminated soil to enhance arsenic hyperaccumulation in plant species.

1.2 Objectives

1.2.1 To study the efficiency of arsenic accumulation in both plants among different concentrations of arsenic in soil.

1.2.2 To study and compare the efficiency of arsenic accumulation in both plants.

1.2.3 To study the efficiency of ethylenediaminetetraacetic acid (EDTA) solution to enhance the accumulation of arsenic in both plant species.

1.3 Hypothesis

1.3.1 The arsenic accumulation in taro would higher than in wild taro.

1.3.2 The arsenic accumulation in plants would be increasing when the concentration of arsenic in soil is increasing.

1.3.3 The addition of ethylenediaminetetraacetic acid (EDTA) solution would increase the arsenic accumulation in both plant species.

1.4 Scope of the study

This study was conducted in the experimental pots containing soil that amended with sodium arsenate ($Na_2HAsO_4.7H_2O$) solution for 4 concentrations (control, 100, 200, and 400 mg As/kg soil) and ethylenediaminetetraacetic acid (EDTA) solution (5 mmol/kg of soil) by using three replications of each treatment. And planted with *Colocasia esculenta* L. Schott (taro and wild taro) at ambient temperature and illuminated with natural light. The plant was harvested to sample every 20 days for 100 days and analyze for total arsenic accumulation in 4 parts that are corm, root, petiole, and lamina.

Chapter II

Literature Review

2.1 Properties of arsenic

Arsenic is classified as a metalloid element, with symbol **As** and atomic number 33. Its properties are similar to phosphorus and antimony, with oxidation state of -3, 0, +3 and +5. Its atomic weight is 74.92158, melting point is 817 ^{o}C, and boiling point is 616 ^{o}C. The chemistry of arsenic is complex and there are many difference compounds of both inorganic and organic arsenic. The most important compounds that have potential environmental importance are presented in Table 2-1.

In the natural environment, arsenic is rarely encountered as the free element. More frequently it is a component of sulfidic ores, in which it occurs as metal arsenides, e.g., nickel diarsenide, cobalt diarsenide sulfide, and iron diarsenide. Arsenates of aluminum, barium, bismuth, calcium, cobalt, copper, iron, lead, magnesium, manganese, uranium, and zinc also occur naturally, along with arsenic trioxide, which is formed as the weathering product of arsenides. Realgar (tetraarsenic tetrasulfide, As_4S_4), orpiment (arsenic trisulfide, As_2S_3) and arsenopyrite (FeAsS) are naturally occurring sulfides of arsenic. In one form or another, arsenic is present in rocks, in soils, in water, and in living organisms in concentrations of parts per billion to part per million. The commercial use and production of inorganic and organic arsenic compounds have raised local concentrations of this element in the environment much above the natural background concentrations.

Arsenic is an extremely toxic metal that poses such a significant environmental health hazard. Its historical and current uses have been primarily as a biocide. It was introduced as Paris green, an insecticide, in 1867 to combat the Colorado potato beetle. Lead arsenate and calcium arsenate were introduced to control the gypsy moth, codling moth, and cotton pests such as the boll weevil. Arsenic was also used in the nineteenth century as a coloring agent for dyes, in fireworks, in tanning, as a depilatory, a preservative for furs, and even in health tonics.

Table 2-1 Some arsenic compounds of environmental importance

Name	Synonyms	Formula
Inorganic arsenic		
- Arsenic	Metallic arsenic	As_4
- Arsenic (III) oxide	Arsenic trioxide Arsenous oxide White arsenic	As_2O_3 (or As_4O_6)
- Arsenous acid	-	H_3AsO_3
- Arsenenous acid,arsenites, salts of arsenous acid	Arsenious acid	$HAsO_2$, $H_2AsO_3^-$, $HAsO_3^{-2}$ or AsO_3^{-3}
- Arsenic (III) chloride	Arsenic trichloride Arsenous trichloride	$AsCl_3$
- Arsenic (III) sulfide	Arsenic trisulfide Orpiment, Auripigment	As_2S_3
- Arsenic (V) oxide	Arsenic pentoxide	As_2O_5
- Arsenic acid	Orthoarsenic acid	H_3AsO_4
- Arsenenic acid arsenates, salts of arsenic acid (ortho)	Metaarsenic acid	$HAsO_3$, $H_2AsO_4^-$, $HAsO_4^{-2}$ or AsO_4^{-3}
Organic arsenic		
- Methylarsonic acid	Methanearsonic	$CH_3AsO(OH)_2$
- Dimethylarsinic acid	Cacodylic acid	$(CH_3)_2AsO(OH)$
- Trimethylarsine oxide	-	$(CH_3)_3AsO$
- Methylarsine	-	CH_3AsH_2
- Dimethylarsine	-	$(CH_3)_2AsH$
- Trimethylarsine		$(CH_3)_3As$

2.1.1 Arsenites and arsenates

Arsenites of the formulas MH_2AsO_3, M_2HasO_3, and M_3AsO_3 are known. In these formulas, M represents a univalent metal cation or one equivalent cation. The alkali-metal arsenites are freely soluble in water, the alkaline-earth arsenites are slightly soluble, and the heavy-metal arsenites are insoluble.

Condensed arsenates or arsenites, which are salts of polyarsenic or polyarsenous acids or a corresponding *meta* acid, are known in the solid state, such as disodium hydrogen arsenate, tetrapotassium diarsenate, and potassium *m*-arsenate. The arsenic-oxygen-arsenic bond in these compounds has extreme hydrolytic instability. It is therefore very unlikely that any species containing an arsenic-oxygen-arsenic group can be present in aqueous media in appreciable concentration. (National Research Council, 1977)

Among the different species of As(V), $H_2AsO_4^-$ is dominant in the pH range 2 to 7, $HAsO_4^-$ is important between pH 7 to 11 as presented in Figure 2-1. (Nriagu, 1994)

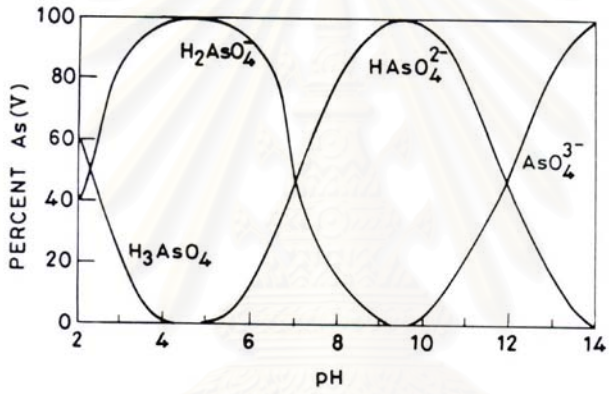


Figure 2-1 Predominance diagram for As(V) as a function of pH

2.1.2 Arsenic in soil environment

Arsenic is present in all soils, and the geologic history of a particular soil determines its arsenic content. The natural arsenic content in virgin soil varies from 0.1 to 40 ppm. The average is about 5 - 6 ppm, but it varies considerably among geographic regions. Soil overlying sulfide ore deposits commonly contain arsenic at several hundred parts per million; the reported maximum is 8,000 ppm (National Research Council, 1977). This arsenic may be present in unweathered sulfide minerals or in an inorganic anion state. The most common sulfide is arsenopyrite, although arsenosulfides of almost any metal cation can be found inorganic arsenate may be bound to iron and aluminum cations or oxides or to any other cation present (such as calcium, magnesium, lead, and zinc).

Soils are usually contaminated with arsenic through the use of pesticides, although some contamination occurs from smelting operations. Arsenic in the environment can undergo oxidation, reduction, methylation, and demethylation in soil. It contained high concentrations of hydrous iron and aluminum oxides or their cation. Arsenic may also be bound to the organic matter in soils, in which case it is released into the soil solution as the organic matter is oxidized and is then available for plant uptake or fixation by soil cations. Some arsenic from other inorganic forms is also available for plant uptake, in as much as the slightly soluble iron and aluminum arsenates and the soil solution are in equilibrium. The amount released for plant uptake is a function of the particular chemical and physical forms of individual arsenic compounds. (National Research Council, 1977; O' Neill, 1993)

Arsenic (As) exists in the soil environment as arsenate, As(V), or as arsenite, As(III), both are toxic; however, arsenite is more toxic form and arsenate is the most common form (Federation Remediation Technologies Roundtable [FRTR], 2001). The behavior of arsenate in soil seems analogous to that of phosphate because of their chemical similarity. Like phosphate, arsenate is fixed to soil, and thus is relatively immobile. Arsenite compounds are 4 to 10 times more soluble than arsenate compounds. Under anaerobic conditions, arsenate may be reduced to arsenite. Arsenite is more subject to leaching because of its higher solubility. (FRTR, 2001; Pickering et al., 2000)

Chemical forms of arsenic and their transformation in soils can be illustrated (Figure 2-2). Oxidation, reduction, adsorption, dissolution, precipitation, and volatilization of arsenic reactions commonly occur. Some soil reactions are associated with bacterial and fungal microorganisms. The volatile organic arsines are extremely toxic. (Nriagu, 1994)

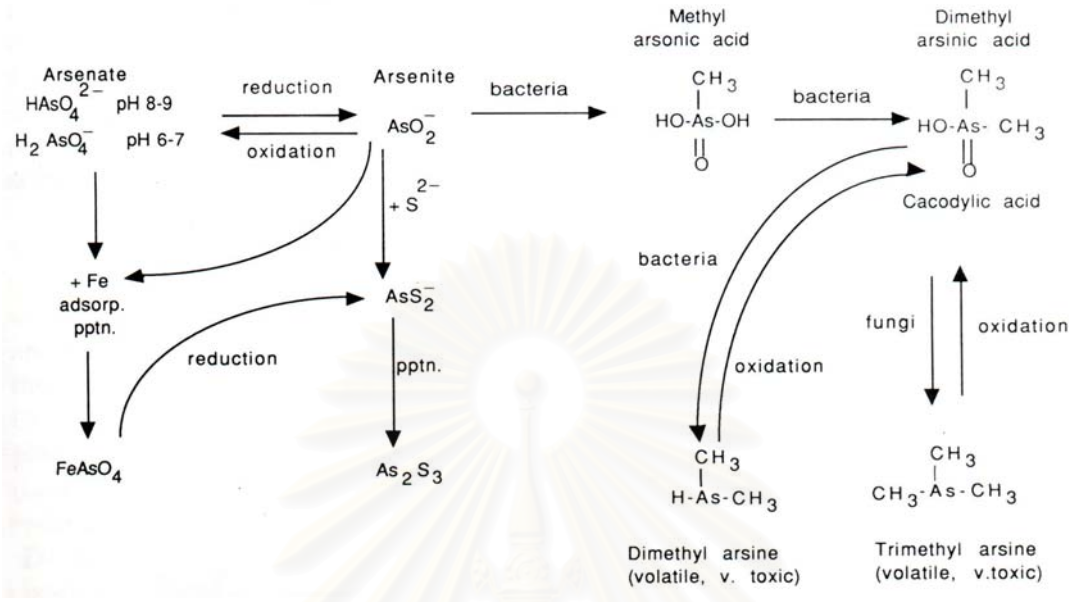


Figure 2-2 **Chemical forms of arsenic and their transformations in soil**

Source: Nriagu, 1994

The range of Eh and pH in soils can lead to either As(V) or As(III) with microbial activity causing methylation, demethylation and/or change in oxidation state and the presence of S species. If the redox potential is low enough, it would favour the formation of arsenic sulfide minerals. A further complicating factor may be the presence of clay minerals, Fe and Al oxides and organic matter that can influence solubility and rate of oxidation. (National Research Council, 1977; O' Neill, 1993; Nriagu, 1994)

A change in ratio of As(V) and As(III) can be brought not only with inorganic mechanism with Eh/pH changes, but the presence of microorganisms can also influence the reaction pathway (O' Neill, 1993). Soluble arsenic concentrations are usually controlled by redox conditions, pH, biological activity, and adsorption reactions, but not by solubility equilibria (Nriagu, 1994). At high Eh values, As(V) exists as H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{-2}$, and AsO_4^{-3}, whereas at low Eh values, the corresponding As(III) species is present along with AsS_2^- (Ferguson and Gavis, 1972)

In guideline of World Health Organization (WHO), standard of arsenic contamination in soil (max. allowable) should not exceed 40 mg As/kg soil (PCD, 1998). And the New Jersey Department of Environmental Protection has established soil cleanup criteria of arsenic for residential and nonresidential uses at 20 mg/kg soil. (New Jersey Department of Environmental Protection, 1996)

2.1.3 Arsenic and plants relationship

Arsenic is chemically similar to phosphorus, an essential plant nutrient; it behaves very much like phosphate in the plant-soil system that it can substitute for phosphorus in plant nutrition, but it is phytotoxic. Arsenate can enter into reactions in place of phosphorus, thereby becoming a toxicant. There is strong evidence that arsenate is normally absorbed in a manner similar to the phosphate uptake mechanism. (National Research Council, 1977; Bieleski and Ferguson, 1983; Nriagu, 1994)

Arsenic is ubiquitous in the plant kingdom. It's concentration varies from less than 0.01 to about 5 ppm (dry-weight basis). Differences in arsenic content probably reflex species differences in plants and, in larger sense and environment in a particular geographic region. Plants growing in arsenic-contaminated soils generally have higher residues than plants grown in normal soils. Arsenic concentrations are less than 5.0 ppm (dry weight) or 0.5 ppm (fresh weight) for untreated vegetation. Whereas treated plants may have much higher concentrations. However, values for some non-treated plants are as high as or higher than those for plants that were treated with arsenic or grown in arsenic-contaminated soil. Natural variations among plants, plant species, available soil arsenic, and growing conditions are all responsible in part for these discrepancies. There appears to be little chance that animals would be poisoned by consuming plants that contain arsenic residues from contaminated soils, because plant injury occurs before toxic concentrations could appear. Arsenic accumulates in plants growth on soils contaminated

by arsenic is not readily translocated to shoots, most is found in the roots. In order for plant levels to reach 1 mg As/kg on a fresh weight basis, soil levels must exceed 200 - 300 mg As/kg (Aten et al., 1980; National Research Council, 1977)

The relationship between the soil arsenic and growth of plants depends on the form and availability of the arsenic. The toxicity of arsenic varies with its form and valence, its toxic order being AsH_3 > As(III) > As(V) > organic As (National Research Council, 1977; Nriagu, 1994).

Arsenite and arsenate are the major forms of As intoxication, and these anions are readily taken up by plants (Schmoger, Oven, and Grill, 2000). The bioaccumulation of arsenic by plants may provide a means of removing this element from contaminated soil and water (Pickering et al., 2000). According to the data from Pickering et al. (2000) which studied the biochemical fate of arsenic taken up by Indian mustard (*Brassica juncea*), suggest that arsenate enters the roots as a phosphate analog possibly via the phosphate transport mechanism and is promptly reduced to arsenite. And little arsenic is transported to the aboveground tissues, but the addition of the arsenic chelator dimercaptosuccinate to the hydroponic growth solution caused significant amounts of arsenic to move into the shoot, perhaps offering a way of removing arsenate from contaminated soil.

When arsenic in solution penetrates the cuticle and enters the apoplast system (the nonliving cell-wall phase), it bathes external surface of the plasmolemma of the symplast. This is the location of at least some of the enzymes of the living plant. One of the first symptoms of injury is wilting, caused by loss of turgor, and this immediately suggests an alteration in membrane integrity. Reaction of trivalent arsenic with sulfhydryl enzymes could well explain the effects of membrane degradation-injury and eventually death. (National Research Council, 1977)

In general, arsenates are less toxic than arsenites. The arsenate symptoms involve chlorosis, but not rapid loss of turgor (at least in the early expression of toxicity), and the

contact action of the arsenates is more subtle. Arsenate is known to uncouple phosphorylation. Thus, the couple phosphorylation of adenosine diphosphate (ADP) is abolished, the energy of adenosine triphosphate (ATP) is not available, and the plant must slowly succumb. (National Research Council, 1977)

2.2 Phytoremediation

Phytoremediation is the direct use of living green plants for *in situ*, or in place, risk reduction for contaminated soil, sludge, sediments, and ground water, through contaminant removal, degradation, or containment. Growing and, in some cases, harvesting plants on a contaminated site as a remediation method is an aesthetically pleasing, solar-energy driven, passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination. This technique can be used along with or, in some cases, in place of mechanical cleanup methods. (U.S. EPA, 1998)

Phytoremediation of metals is a cost-effective "green" technology based on the use of specially selected metal-accumulating plants to remove toxic metals, including radionuclides, from soils and water (as shown in Figure 2-3 and 2-4). Phytoremediation takes advantage of the fact that a living plant can be compared to a solar driven pump, which can extract and concentrate particular elements from the environment. The metals targeted for phytoremediation include lead, cadmium, chromium, arsenic and various radionuclides. The harvested plant tissue, rich in accumulated contaminant, is easily and safely processed by drying, ashing or composting. The volume of toxic waste produced as a result is generally a fraction of that of many current, more invasive remediation technologies, and the associated costs are much less. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues. This new technology can be used to clean up metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, and landfill leachates. Phytoremediation has been studied extensively in research and small scale demonstrations, but full-scale applications are currently limited to a small number of projects. (Raskin, 1997; U.S. EPA, 1998)

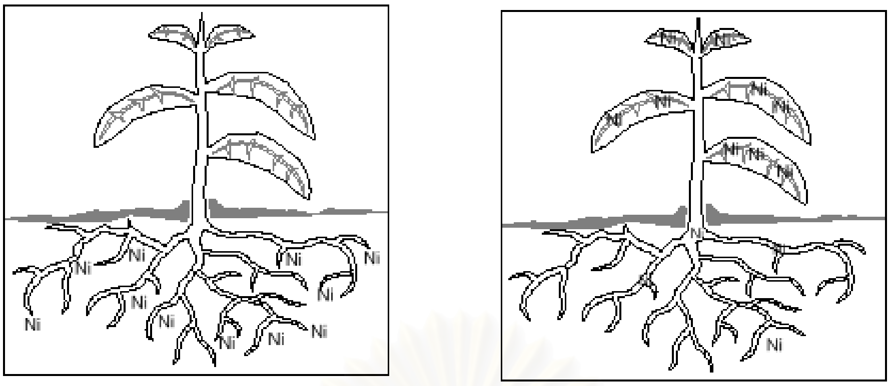


Figure 2-3 Uptake of metals (nickel) by phytoextraction
Source: U.S. EPA, 2000

Nickel is removed from soil by moving up into plant roots, stems, and leaves. The plant is then harvested and disposed of and the site replanted until the nickel in the soil is lowered to acceptable level.

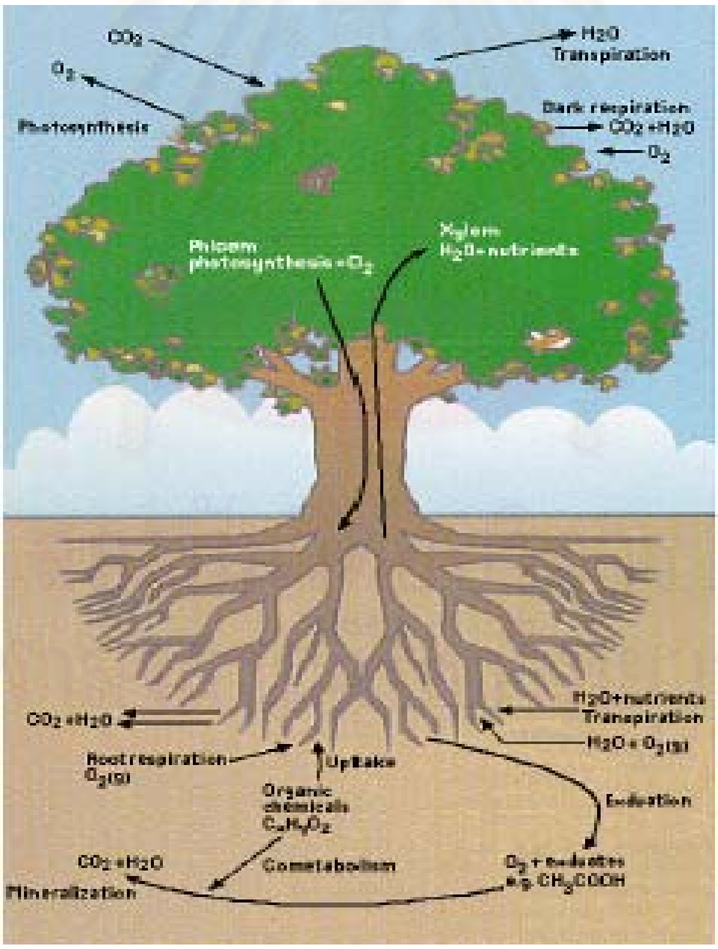


Figure 2-4 The uptaking process of the plant
Source: Schnoor, 1997

2.2.1 Phytoremediation of metals

Soils and waters contaminated with toxic metals pose a major environmental and human health problems which is still in need of an effective and affordable technological solution. Microbial bioremediation has been somewhat successful for the degradation of certain organic contaminants, but ineffective at addressing the challenge of toxic metal contamination, particularly in soils. So phytoremediation can be used for this situation. At sites contaminated with metals, plants are used to either stabilize or remove the metals from the soil and ground water through three mechanisms: phytoextraction, rhizofiltration, and phytostabilization.

2.2.1.1 Phytoextraction

Phytoextraction, also called phytoaccumulation, refers to the uptake and translocation of metal contaminants in the soil by plant roots into the aboveground portions of the plants (Figure 2-3). Certain plants, called hyperaccumulators, absorb unusually large amounts of metals in comparison to other plants. One or a combination of these plants is selected and planted at a particular site based on the type of metals present and other site conditions. After the plants have been allowed to grow for some time, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfill, but the volume of ash will be less than 10% of the volume that would be created if the contaminated soil itself were dug up for treatment. (U.S. EPA, 2000)

2.2.1.2 Rhizofiltration

Rhizofiltration is the adsorption or precipitation onto plant roots or absorption into the roots (*rhizo-* means *root*) of contaminants that are in solution surrounding the root zone. Rhizofiltration is similar to phytoextraction, but the plants are used primarily to address contaminated ground water rather than soil. The plants to be used for cleanup are raised in greenhouses with their roots in water rather than in soil. To acclimate the plants once a large root system has been developed, contaminated water is

collected from a waste site and brought to the plants where it is substituted for their water source. The plants are then planted in the contaminated area where the roots take up the water and the contaminants along with it. As the roots become saturated with contaminants, they are harvested.

2.2.1.3 Phytostabilization

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

2.2.2 Phytoextraction process

Phytoextraction utilizes the roots of plants to absorb, translocate, and concentrate toxic metals from the soil to the aboveground harvestable plant tissues. (Raskin, 1997; U.S. EPA, 1998; Raskin and Ensley, 2000)

In developing phytoextraction, plant species must have such desirable characteristics as high biomass production, handling ease, accumulating high concentration of metals, and easy to harvest. If the plant produces a significant amount of biomass while accumulating high concentrations, an important quantity of metal can be removed from the soil via plant accumulation. The metal-rich plant material can be collected and removed from the site, without the loss of topsoil associated with traditional remediation practices. The metal bioaccumulation and concentration in the plant shoots above that of the soil concentration coupled with subsequent biomass reduction processes

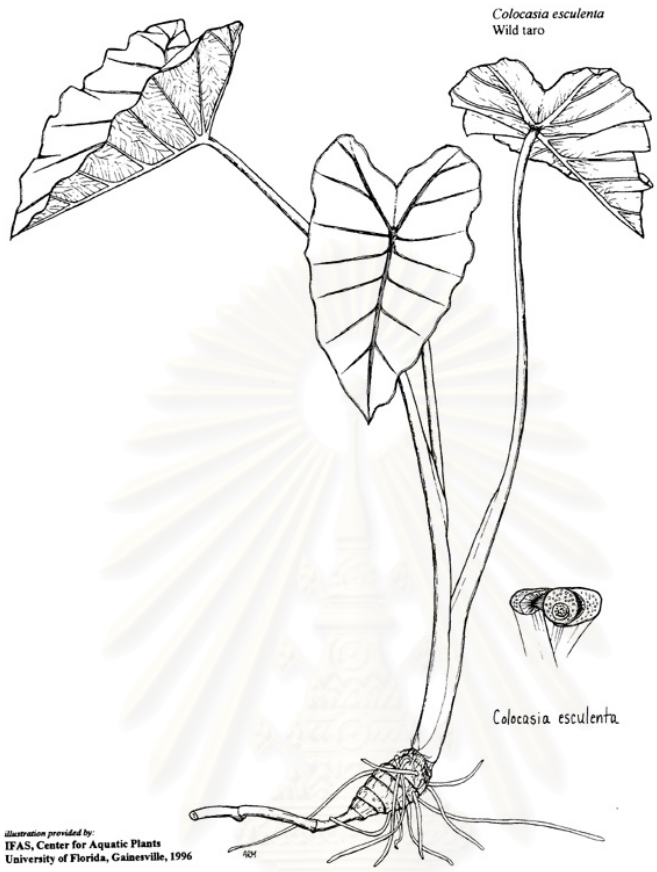
can greatly reduce the amount of contaminated material requiring disposal compared to soil excavation. (Raskin and Ensley, 2000; Huang, 1998)

The main factors influence or determine the ability of phytoextraction are metal solubility, metal uptake by roots, metal translocation from roots to harvestable plant tissues, and plant tolerance to toxic metals. (Raskin and Ensley, 2000)

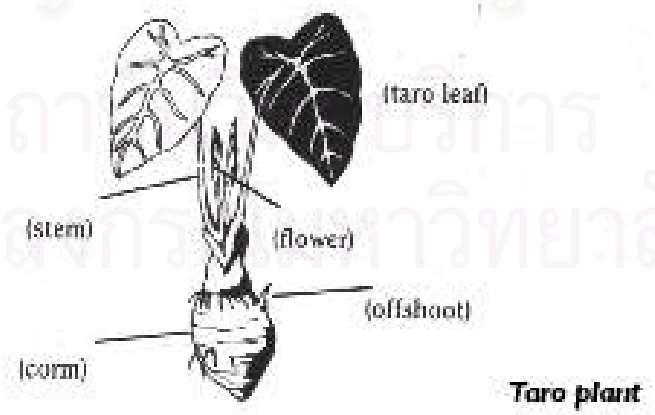
2.3 Taro and wild taro

The selected plants, *Colocasia esculenta* L. Schott (taro and wild taro) have very large corms, tuberous-roots, can tolerate in extreme condition, and can be found widely naturalized in every part of Thailand. They are commonly known as Elephant Ears, mostly found along pond shoreline, marshy shores, and other muddy shallow water areas. They can grow in a wide range of dry to wet sites. They have heart- to arrow-shaped, smooth green leaves. The leaves of these plants are held perpendicular to the stem (called a peltate leaf), so that they face outward and point to the ground (Figure 2-5). Wild taro mostly has larger lamina with more arrow-shaped than in taro (Figure 2-6). But taro has larger corm and mostly has rounded lamina than in wild taro. And there was no other difference characteristics between two types.

These plants are perennial herbs which 1.5 m. (4 ft.) tall, with thick shoots from a large corm; slender stolons also often produced, along with offshoot corms. Seed production considered uncommon, with low viability and difficulty in germination. The tuberous roots can be divided at potting time and small plant that grow on the terminal ends of the stolons may be detached and planted. They are suitable for growing in containers, borders, and also bog gardens because they tolerate wet soil, even standing water. They can grow in all kinds of soil types but prefers deep soil, well drained, friable loams, especially alluvial loams with high water table. They primarily adapted to moist environment but can be grown in wide range of dry to wet sites. Planting time is not a major factor, but they need adequate moisture and sunlight. They also like little acidic soil (pH 5.5-6.5), so the addition of weak acid chelator would not cause an adverse effect to the plants.



a)



b)

Figure 2-5 *Colocasia esculenta* (L.) Schott: a) wild taro b) taro

Source: University of Florida, 1996

a)

b)

Figure 2-6 Lamina of taro (a) and wild taro (b)

2.4 Role of soil amendments in metal phytoextraction

Plants grown on heavy-metal-contaminated soils generally do not accumulate high levels of the targeted metals in the plant tissue, the major limitations to the phytoextraction of heavy metals are the low metal bioavailability in the soil and the poor metal translocation from roots to shoots. The application of soil amendments (such as synthetic chelates and organic acids) could increases metal desorption from soil to soil solution and metal translocation from roots to shoots. (Raskin, 1997; U.S. EPA, 1998; Raskin and Ensley, 2000)

The availability of metal in the soil for plant uptake is another limitation for successful phytoremediation. However, since metals may be bound too tightly to soil components, genetic potential to accumulate metals does not always translate into effective phytoextraction (Raskin, 1997). The low solubility of heavy metals in the soil is often a limiting factor in phytoextraction by plants. A key to the success of metal phytoextraction is to increase and maintain metal concentration in the soil solution (Raskin and Ensley, 2000). Increasing metal solubility in soil and bioavailabity of metals to the plants by chelating agent are important to phytoextraction of heavy metals from contaminated soils (Blaylock et al., 1997). Chelates and other chemical compounds have been used in soils and nutrient solutions to increase the solubility of metals in plant growth media, and could significantly increase metal accumulation in plants (Blaylock et al., 1997; Huang et al., 1998). Metal chelators and other soil amendments, which release metals to plant roots and facilitate metal uptake and translocation are extremely effective in improving phytoextraction in the field and make this process cost effective (Raskin, 1997). The diagram of metal phytoextraction by adding chelating agent is shown on Figure 2-7.

2.4.1 Chelating agent

A chelating agent is a substance whose molecules can form several bonds to a single metal ion. A chelate is a chemical compound composed of a metal ion and a chelating agent. Many essential biological chemicals are chelates. Chelates play important roles in oxygen transport and in photosynthesis. Furthermore, many biological

catalysts (enzymes) are chelates. In addition to their significance in living organisms, chelates are also economically important, both as products in themselves and as agents in the production of other chemicals.

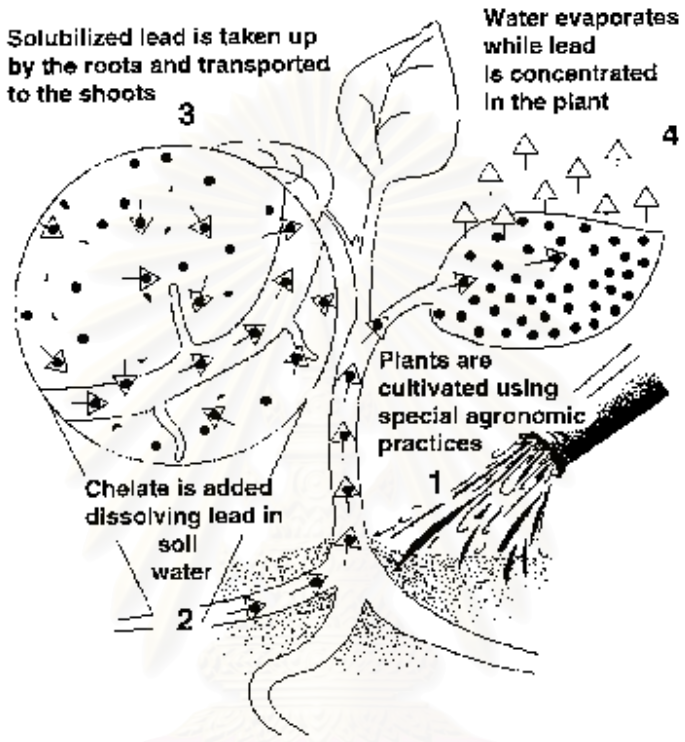


Figure 2-7 Phytoextraction of lead from soils by adding chelating agent
Source: Raskin, 1997

2.4.2 Ethylenediaminetetraacetic acid (EDTA)

Ethylenediaminetetraacetic acid (EDTA) is a versatile chelating agent. It can form four or six bonds with a metal ion, and it forms chelates with both transition-metal ions and main-group ions. EDTA is frequently used in soaps and detergents, because it forms a complexes with calcium and magnesium ions. These ions are in hard water and interfere with the cleaning action of soaps and detergents. The EDTA binds to them, sequestering them and preventing their interference. In the calcium complex, $[Ca(EDTA)]^{2-}$, EDTA is a tetradentate ligand, and chelation involves the two nitrogen atoms and two oxygen atoms in separate carboxyl ($-COO^-$) groups. EDTA is also used

extensively as a stabilizing agent in the food industry. Food spoilage is often promoted by naturally-occurring enzymes that contain transition-metal ions. These enzymes catalyze the chemical reactions that occur during spoilage.

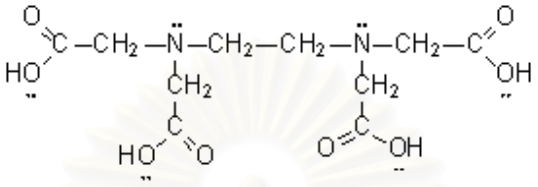


Figure 2-8 Chemical structure of ethylenediaminetetraacetic acid (EDTA)

2.4.3 Mechanisms of soil-amendment

Major limiting factors for phytoextraction of metals from contaminated soils are the lower metal bioavailability in the soil and poor metal translocation from roots to shoots. Application of soil amendments could partially eliminate these limiting steps in the metal phytoextraction. There are several mechanisms involved in the soil amendment triggered metal hyperaccumulation in plants.

First, the increase in metal level in soil solution is required, soil-amendment-induced surge of metal concentration in soil solution could be the chelation between metals and the chelating compounds.

Second, chelating compounds could buffer metal activity near the root surface and thus maintain a constant supplying of free metal to the uptake sites of roots.

Third, the complex of metal chelate could be directly absorbed by roots and translocate to shoots. Finally, it may also be possible that chelating compounds at higher levels alter plant ion transport system and/or root-cell membrane structure such that metal uptake and translocation are facilitated.

2.5 Review of the studies

The study concerning the accumulation of arsenic contaminated soil by plants was conducted by Jirawan (2000) studying the level of arsenic in parts of *Colocasia esculenta* L. Schott (dark violet and green) in soil contaminated with $Na_2HAsO_4.7H_2O$ at 6 different concentration (control, 50, 75, 100, 125, and 150 mg As/kg soil). Then she found that both species can grow well in all concentration of arsenic and amount of arsenic accumulated in root is more than that in corm, lamina, and petiole. The maximum arsenic removal rate was 0.07% from *Colocasia esculenta* L. Schott (dark violet) in 100 mg As/kg soil and 0.06% from *Colocasia esculenta* L. Schott (green) in 125 mg As/kg soil. The results showed that the efficiency of both plants was not different and all plants in this experiment can survive in arsenic contaminated soil up to 150 mg As/kg soil.

There is little information in the literature concerning the use of chelating agent to enhance arsenic accumulation in plants, but there is some information about the chelating agents applied for the phytoremediation of the other contaminants.

Blaylock et al. (1997) conducted the pot experiment and studied the accumulation of lead (Pb) in Indian mustard (*Brassica juncea*), and also enhanced the accumulation of Pb by using synthetic chelating agents such as EDTA (ethylene dinitrilo tetra acetic acid) and citric acid applied to the soil surface. And found that concentrations of 1.5% Pb in the shoots of *B. juncea* were obtained from soils containing 600 mg of Pb/kg amended with synthetic chelates such as EDTA. The research indicated that the accumulation of metal in the shoots of *B. juncea* can be enhanced through the application of synthetic chelates to the soil, facilitating high biomass accumulation as well as metal uptake.

Huang et al. (1998) have found that some organic acids can be added to soils to increase Uranium (U) desorption from soil to soil solution and to trigger a rapid U accumulation in plants. Of the organic acids (acetic acid, citric acid, and malic acid) tested, citric acid was the most effective in enhancing U accumulation in plants. Shoot U concentrations of *Brassica juncea* and *Brassica chinensis* grown in a U-contaminated soil (total soil U, 750 mg/kg) increased from less than 5 mg/kg to more than 5,000 mg/kg in citric acid-treated soils.

Vassil et al. (1998), studied the role of EDTA in lead (Pb) transport and accumulation by Indian mustard (*Brassica juncea*) plant by exposing to Pb and EDTA in hydroponic solution. They found that it was able to accumulate up to 55 mmol/kg Pb in dry shoot tissue. The accumulation of EDTA in shoot tissue was also observed to be directly correlated with the accumulation of Pb. These studies clearly demonstrate that coordination of Pb transport by EDTA enhances the mobility within the plants of this otherwise insoluble metal ion, allowing plants to accumulate high concentrations of Pb in shoots.

Chapter III

Methodology

3.1 Experimental design

3.1.1 Soil preparation

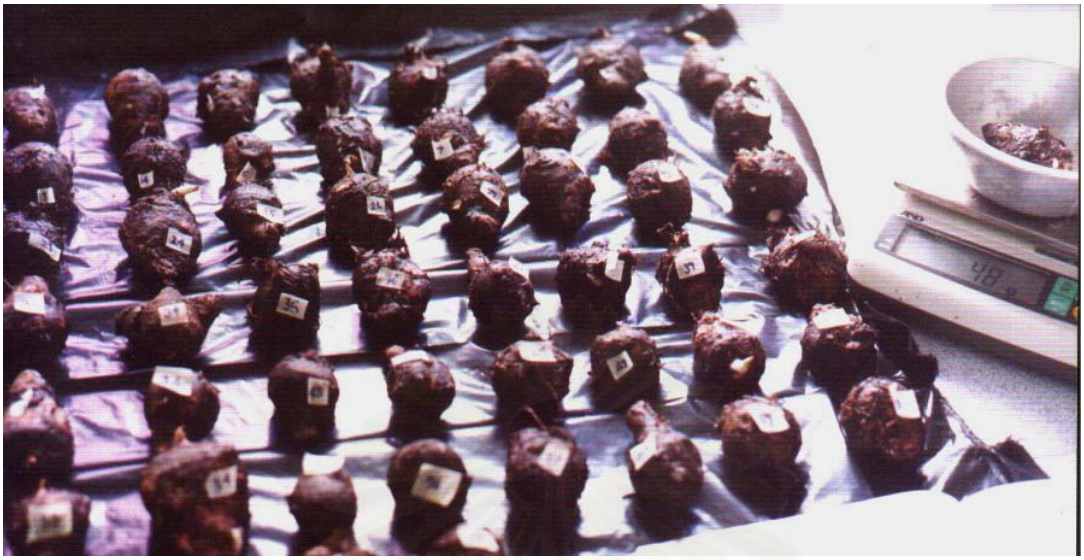
The soil used in this study is silt loam, which is excavated from upper layer (15 cm) of the soil, and thoroughly mixed to homogeneous, and then analyzed for particle size, pH, cation exchange capacity (CEC) and total arsenic (as presented in Table 3-1).

Table 3-1 Physical and chemical characteristics of the experimental soil.

Soil properties		***Methods***
soil texture	silt loam	*Hydrometer method*
- sand (%)	26	
- silt (%)	51	
- clay (%)	23	
soil pH	6.15	*1:1 soil/water ratio*
cation exchange capacity (CEC, me/100 g)	16.88	*Ammonium acetate*
total soil arsenic (mg As/kg soil)	0.03	*EPA-3052*

3.1.2 Plant preparation

Colocasia esculenta (L.) Schott (wild taro) were collected from natural area of Bangkok, while corms of taro were collected from the market. After analyzed for arsenic in plant, have found that arsenic is non-detectable. Both corms of plants were cleaned and weighted. The weight of both corms was determined in the range of 35 - 50 g., Figure 3-1 illustrated the corms of both plants.



a)

b)

Figure 3-1 The corms of *C. esculenta* (L.) Schott; a) taro b) wild taro

3.2 Plant culture and experimental set up

Both of the plants were grown from corms. After the emergence of the corms, then they was planted in the experimental pots and amended with disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) solution with 4 different concentrations (control, 100, 200, and 400 mg As/kg soil) by using 3 replications for each concentration (as shown in Table 3-2). Then the soil was placed in 30 cm diameter round pots with no holes under the pots to prevent loss of amendments from leaching. Ethylenediamine tetraacetic acid (EDTA) solution 5 mmol/kg will apply to the soil surface 2 weeks after amending with disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$).

Table 3-2 Experimental plan for each species and harvested time

Days **As concentration (mg As/kg soil)**	***20***	***40***	***60***	***80***	***100***
Control	20 Feb. 2002	12 Mar. 2002	1 Apr. 2002	21 Apr. 2002	11 May 2002
100	21 Apr.2002	11 May 2002	31 May2002	20 Jun. 2002	10 Jul. 2002
200	20 Feb. 2002	12 Mar. 2002	1 Apr. 2002	21 Apr. 2002	11 May 2002
400	20 Feb. 2002	12 Mar. 2002	1 Apr. 2002	21 Apr. 2002	11 May 2002

3.3 Samplings

The plants were harvested and cut to sample every 20 days for 100 days and analyzed for total arsenic accumulation in 4 parts that are corm, root, petiole, and lamina.

Figure 3-2 Both plants were ready to be contaminated with arsenic

3.4 Sample preparation and analysis

Samples were taken out of the pots and cleaned. Then separated into four parts and dried at 70 ^{o}C for 48 hours in the oven. The dry weight was analyzed and extracted dry samples with acid digestion technique by the EPA-3052 method (microwave assisted acid digestion of siliceous and organically based matrices). Then the extracted solution was analyzed for total arsenic with inductively couple plasma atomic emission spectroscopy (ICP-AES).

3.5 Data analysis

Arsenic accumulations in plants were calculated from amount of arsenic in each part of the plants (mg) per dry weight (kg) as followed equation.

$$\text{As accumulation in each part of the plants (mg/kg)} = \frac{\text{Amount of arsenic in each part of the plants (mg)}}{\text{Dry weight (kg)}}$$

The amount of arsenic in plants were calculated from total arsenic accumulated in plant (mg) per amount of arsenic concentration in pot (mg) as followed equation.

$$\text{Arsenic in plant (\%)} = \frac{\text{Amount of arsenic in each part of the plants (mg) x 100}}{\text{Amount of arsenic concentration in pot (mg)}}$$

3.6 Statistical analysis

The data from the pot experiment with 3 replications were subjected to statistical test. The effect of various arsenic concentrations in soil on arsenic accumulation in each part and the effect of EDTA on total arsenic accumulation in plants were compared by analysis of variance (One-way ANOVA). Total arsenic accumulation in plants was compared by analysis of variance (two-way ANOVA), using two factors that are harvest time and concentration of arsenic in soil. Differences among treatments were compared with Duncan multiple range test (DMRT). All of the statistical analysis was calculated by using the 95% confidential level ($P < 0.05$).

Chapter IV

Results and Discussion

4.1 General observation

During the experimental period, *Colocasia esculenta* (L.) Schott (wild taro) could survive under all concentrations of arsenic in soil and growth as well as control, except the concentration of 400 mg As/kg soil with EDTA which all died in 13 days after the second harvest. While *C. esculenta* (taro) could survive all in the concentrations of 100 and 200 mg As/kg soil and growth normally. But in the concentration of 400 mg As/kg soil they all died in 7 days after the third harvest and in the concentration of 400 mg As/kg soil with EDTA they died all in 10 days after the second harvest.

4.2 Arsenic accumulation in various parts of *C. esculenta* (L.) Schott

Arsenic accumulations in *C. esculenta* (taro and wild taro) were analyzed in 4 parts that are lamina, petiole, corm, and root. The results were expressed in milligram of arsenic per kilogram of plant dry weight (mg As/kg). The data indicated that the accumulation of arsenic in parts of plant was in root > corm > petiole > lamina, respectively. The accumulation of arsenic in all control pots was not detected. And from statistical analysis, it was shown that there was no significant difference between harvest time in all experimental tests ($P < 0.05$).

4.2.1 Arsenic accumulation in lamina

The accumulations of arsenic in lamina of taro were in the range of 14.58 - 425.84 mg As/kg. Table 4-1 and Figure 4-1 illustrated the accumulation of arsenic depending on the concentration of arsenic in soil and on the periods of harvest time, it can be seen that the amounts of arsenic accumulated in lamina was higher by time during the growth period and corresponding with level of arsenic in soil. The highest accumulation was in day 60 from treatment of 400 mg As/kg. From statistical

analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-1.

While arsenic accumulations in lamina of wild taro was little higher than in taro, and were in the range of 22.24 - 186.67 mg As/kg. Table 4-2 and Figure 4-2 illustrated that arsenic in lamina tended to increase by level of arsenic concentration in soil. The highest accumulation was in day 40 from treatment of 400 mg As/kg with EDTA. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-5.

Table 4-1 Arsenic accumulation in lamina of *C. esculenta* (L.) Schott (Taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	52.56[a]	56.51[a]	68.33[a]	103.20[a]	80.44[a]
100 with EDTA	72.21[a]	75.28[a]	81.21[a]	223.94[a]	95.67[a]
200	67.50[a]	55.50[a]	14.58[a]	64.28[a]	20.50[a]
200 with EDTA	68.87[a]	67.56[a]	383.50[a]	94.64[a]	36.25[a]
400	53.45[b]	98.24[b]	425.84[b]	-	-
400 with EDTA	80.50[a]	125.76[a]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

Table 4-2 Arsenic accumulation in lamina of *C. esculenta* (L.) Schott (Wild taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	61.15[a]	41.50[a]	22.24[a]	85.20[a]	94.56[a]
100 with EDTA	74.85[a]	43.65[a]	35.81[a]	94.56[a]	156.44[a]
200	95.62[a]	120.21[a]	126.50[a]	139.59[a]	84.85[a]
200 with EDTA	128.55[a]	171.32[a]	131.58[a]	148.33[a]	66.13[a]
400	62.83[a]	95.50[a]	166.60[a]	121.15[a]	86.28[a]
400 with EDTA	85.50[b]	186.67[b]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

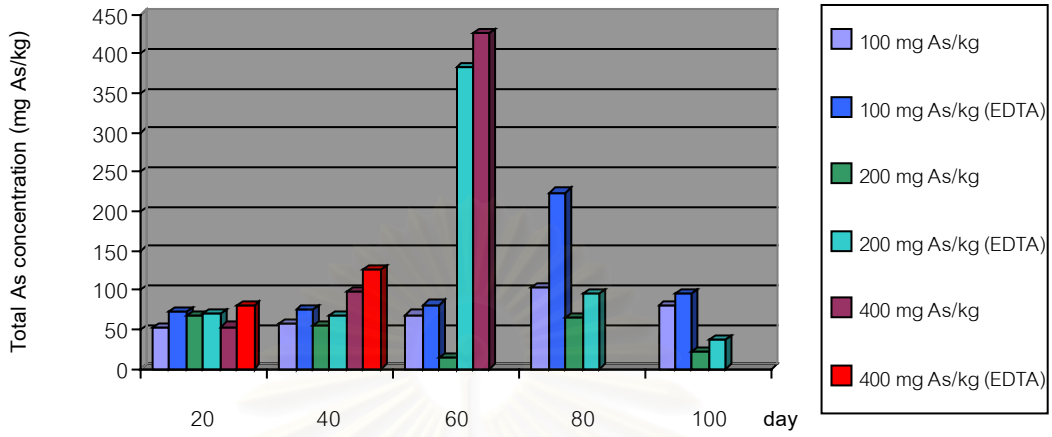


Figure 4-1 Arsenic accumulation in lamina of taro

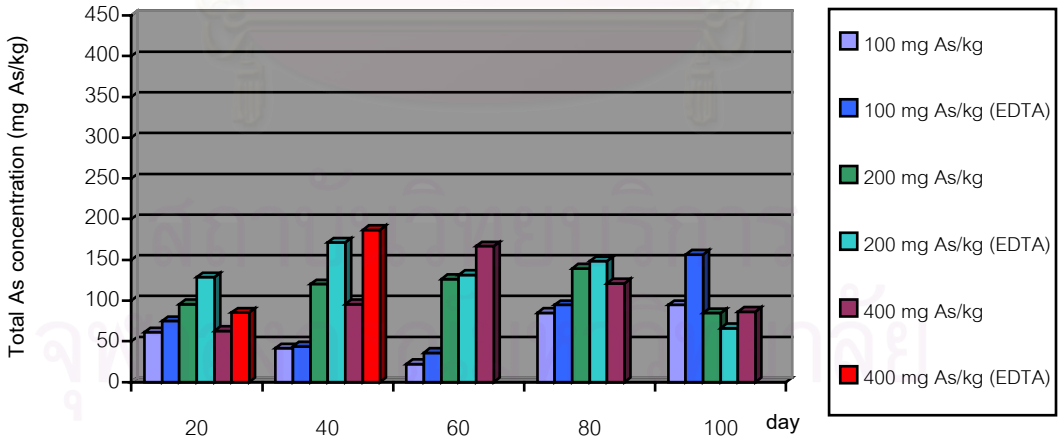


Figure 4-2 Arsenic accumulation in lamina of wild taro

4.2.2 Arsenic accumulation in petiole

The accumulations of arsenic in petiole of taro were in the range of 15.12 - 498.21 mg As/kg. Table 4-3 and Figure 4-3 illustrated the accumulation of arsenic depending on the concentration of arsenic in soil, but not on the periods of harvest time. It can be seen that the amounts of arsenic accumulated in petiole was higher in the middle period time during the growth period and corresponding with level of arsenic in soil. The highest accumulation was in day 40 from treatment of 400 mg As/kg with EDTA. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-2.

Consider the arsenic accumulations in petiole of wild taro were little higher than in taro, and were in the range of 13.56 - 624.43 mg As/kg. Table 4-4 and Figure 4-4 illustrated that arsenic in petiole tended to increase by level of arsenic concentration in soil. The highest accumulation was in day 20 from treatment of 400 mg As/kg with EDTA. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-6.

Table 4-3 Arsenic accumulation in petiole of *C. esculenta* (L.) Schott (Taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in petiole at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	49.25[a]	35.66[a]	15.12[a]	52.25[a]	68.48[a]
100 with EDTA	81.88[abc]	103.84[abc]	92.85[abc]	63.95[abc]	132.50[abc]
200	45.50[ab]	41.83[ab]	63.84[ab]	86.62[ab]	85.26[ab]
200 with EDTA	87.04[c]	304.56[c]	173.24[c]	121.48[c]	95.78[c]
400	200.56[bc]	96.68[bc]	111.75[bc]	-	-
400 with EDTA	463.50[d]	498.21[d]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

Table 4-4 Arsenic accumulation in petiole of *C. esculenta* (L.) Schott (Wild taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in petiole at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	13.56[a]	27.50[a]	51.23[a]	22.56[a]	31.87[a]
100 with EDTA	78.14[ab]	57.89[ab]	62.51[ab]	50.73[ab]	42.84[ab]
200	126.50[ab]	297.94[ab]	62.84[ab]	75.46[ab]	91.52[ab]
200 with EDTA	174.86[bc]	378.72[bc]	71.51[bc]	81.75[bc]	77.08[bc]
400	211.57[c]	252.13[c]	356.50[c]	264.31[c]	126.64[c]
400 with EDTA	624.43[d]	584.87[d]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

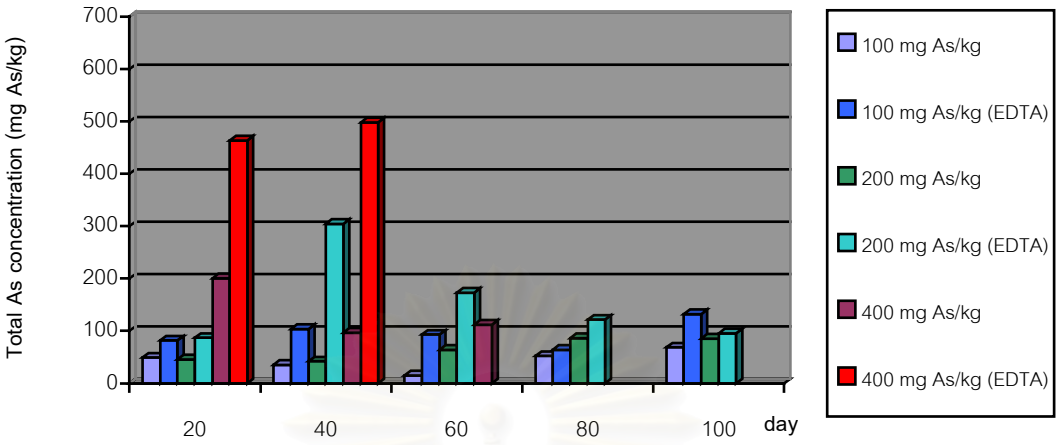


Figure 4-3 Arsenic accumulation in petiole of taro

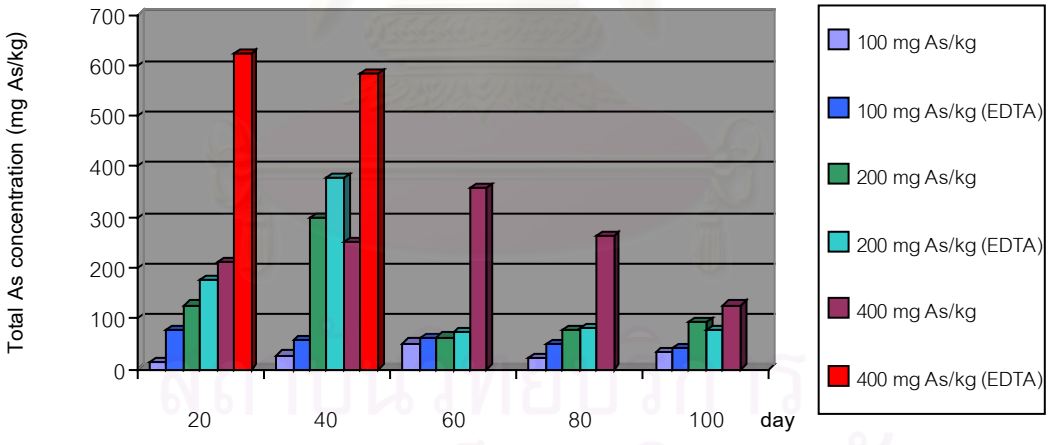


Figure 4-4 Arsenic accumulation in petiole of wild taro

4.2.3 Arsenic accumulation in corm

The accumulations of arsenic in corm of taro were in the range of 24.56 - 404.33 mg As/kg. Table 4-5 and Figure 4-5 illustrated the accumulation of arsenic depending on the concentration of arsenic in soil, it can be seen that the amounts of arsenic accumulated in corm were higher in day 20 and 40 and corresponding with level of arsenic in soil. The highest accumulation was in day 60 from treatment of 400 mg As/kg. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-3.

Consider the arsenic accumulations in petiole of wild taro were little higher than in taro, and were in the range of 13.56 - 624.43 mg As/kg. Table 4-6 and Figure 4-6 illustrated that arsenic in corm tended to increase by level of arsenic concentration in soil. It can be seen that the amounts of arsenic accumulated in corm were higher in day 20 and 40 and corresponding with level of arsenic in soil. The highest accumulation was in day 20 from treatment of 400 mg As/kg with EDTA. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-7.

Table 4-5 Arsenic accumulation in corm of *C. esculenta* (L.) Schott (Taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in corm at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	30.58[a]	24.56[a]	29.59[a]	36.45[a]	25.57[a]
100 with EDTA	65.24[a]	111.16[a]	64.30[a]	35.50[a]	39.61[a]
200	131.68[a]	321.43[a]	59.81[a]	39.30[a]	26.58[a]
200 with EDTA	275.57[a]	98.59[a]	83.52[a]	50.41[a]	69.15[a]
400	264.22[b]	197.24[b]	404.33[b]	-	-
400 with EDTA	315.50[b]	254.02[b]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

Table 4-6 Arsenic accumulation in corm of *C. esculenta* (L.) Schott (Wild taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in corm at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	111.25[a]	150.58[a]	70.08[a]	31.50[a]	41.39[a]
100 with EDTA	235.57[abc]	384.46[abc]	133.92[abc]	56.59[abc]	105.33[abc]
200	226.51[ab]	201.54[ab]	64.48[ab]	94.54[ab]	118.57[ab]
200 with EDTA	317.12[bc]	318.79[bc]	216.25[bc]	204.72[bc]	201.48[bc]
400	401.08[c]	211.34[c]	441.50[c]	160.03[c]	165.64[c]
400 with EDTA	561.20[d]	512.05[d]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

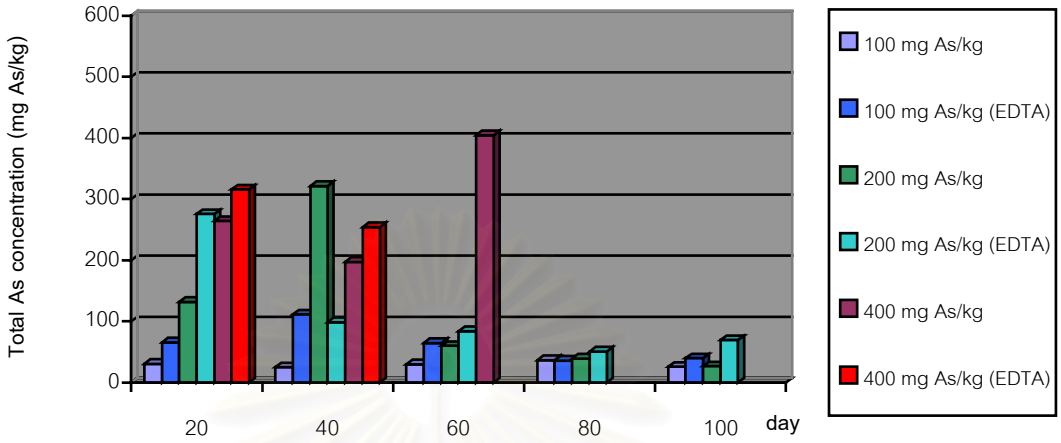


Figure 4-5 Arsenic accumulation in corm of taro

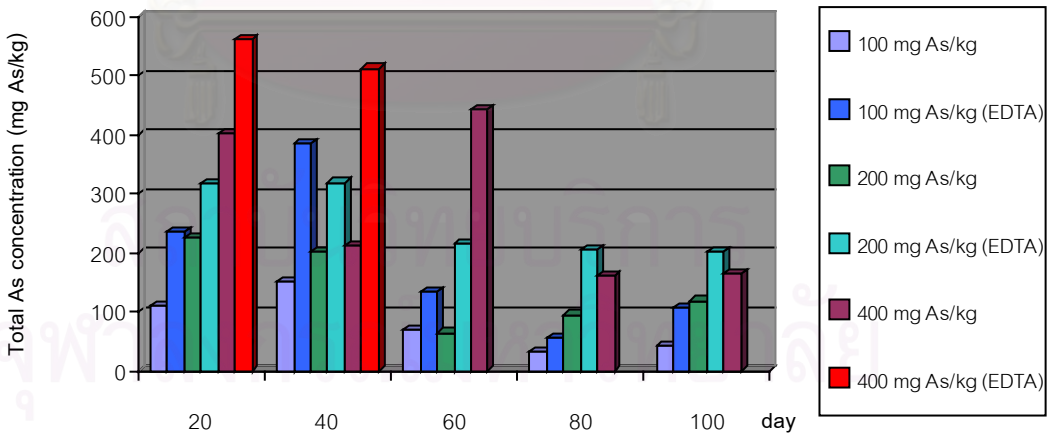


Figure 4-6 Arsenic accumulation in corm of wild taro

4.2.4 Arsenic accumulation in root

The accumulations of arsenic in root of taro were in the range of 232.58 - 2005.56 mg As/kg. Table 4-7 and Figure 4-7 illustrated the accumulation of arsenic depending on the concentration of arsenic in soil, it can be seen that the amounts of arsenic accumulated in root were increased from day 20 to day 60, and corresponding with level of arsenic in soil. The highest accumulation was in day 60 from treatment of 400 mg As/kg. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-4.

Consider the arsenic accumulations in root of wild taro were little higher than in taro, and were in the range of 175.40 - 2089.29 mg As/kg. Table 4-8 and Figure 4-8 illustrated that arsenic in corm tended to increase by level of arsenic concentration in soil. It can be seen that the amounts of arsenic accumulated in root were higher from day 20 to day 80 and corresponding with level of arsenic in soil. However, there were fluctuations in some harvest time. The highest accumulation was in day 80 from treatment of 400 mg As/kg. From statistical analysis, it was shown that there was significant difference between treatments ($P < 0.05$) as shown in APPENDIX A-8.

Table 4-7 Arsenic accumulation in root of *C. esculenta* (L.) Schott (Taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in root at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	232.58[a]	495.83[a]	668.33[a]	950.96[a]	448.33[a]
100 with EDTA	592.56[ab]	646.88[ab]	1038.04[ab]	894.83[ab]	1252.20[ab]
200	562.50[a]	597.57[a]	340.21[a]	462.56[a]	797.92[a]
200 with EDTA	868.41[abc]	1256.92[abc]	1245.08[abc]	917.35[abc]	821.43[abc]
400	500.04[bc]	585.64[bc]	2005.56[bc]	-	-
400 with EDTA	939.52[c]	1585.87[c]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

Table 4-8 Arsenic accumulation in root of *C. esculenta* (L.) Schott (Wild taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in root at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	184.38[a]	787.70[a]	251.45[a]	466.51[a]	175.40[a]
100 with EDTA	301.24[a]	942.59[a]	281.25[a]	483.65[a]	548.15[a]
200	550.21[a]	950.27[a]	820.76[a]	827.45[a]	733.58[a]
200 with EDTA	664.56[b]	1017.56[b]	1327.57[b]	1511.67[b]	1856.82[b]
400	961.08[b]	1070.02[b]	1885.25[b]	2089.29[b]	1106.76[b]
400 with EDTA	1265.94[b]	1254.94[b]	-	-	-

Note:The same alphabet on the right corner means there is no significant difference ($P < 0.05$)

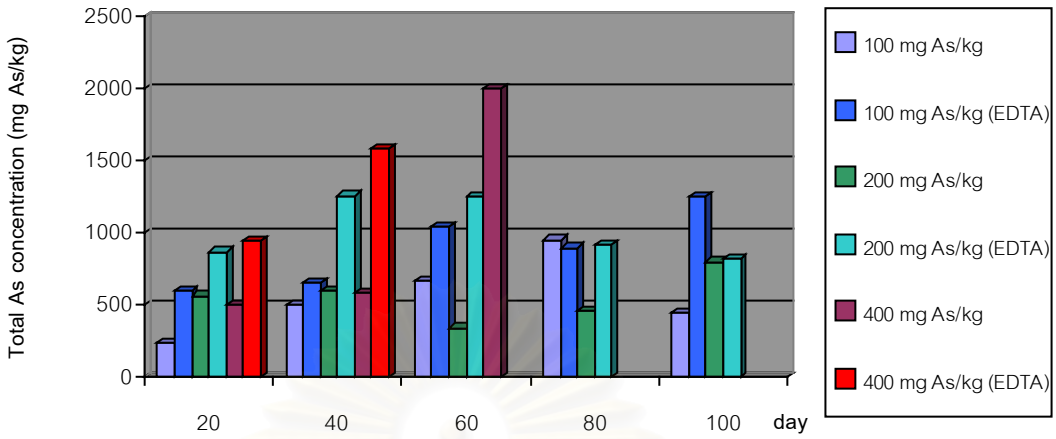


Figure 4-7 Arsenic accumulation in root of taro

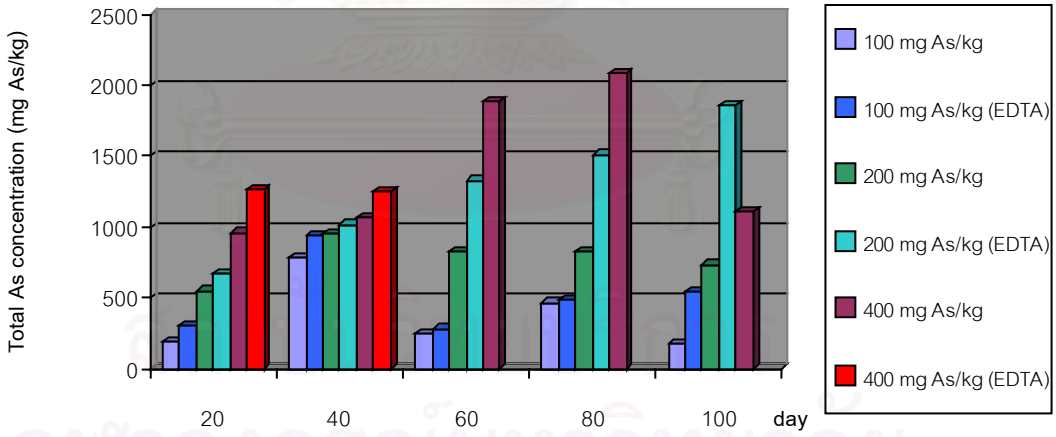


Figure 4-8 Arsenic accumulation in root of wild taro

4.3 Arsenic accumulation in all parts of *C. esculenta* (L.) Schott

The amount of arsenic accumulation in all parts of both plants was expressed as milligram of arsenic that accumulated in total of plant dry weight.

The accumulations of arsenic in all part of taro were in the range of 4.99 - 40.34 mg. Table 4-9 and Figure 4-9 illustrated the accumulation of arsenic depending on the concentration of arsenic in soil and on harvest time, it can be seen that the amounts of arsenic were increased from day 20 to day 100, and corresponding with level of arsenic in soil. The highest accumulation was in day 60 from treatment of 400 mg As/kg with EDTA. From statistical analysis, it was shown that there was significant difference between concentration of arsenic in soil and harvest time ($P < 0.05$) as shown in APPENDIX A-10.

The accumulations of arsenic in all part of wild taro were in the range of 6.43 - 46.79 mg. Table 4-10 and Figure 4-10 illustrated the accumulation of arsenic depending on the concentration of arsenic in soil and on harvest time, it can be seen that the amounts of arsenic were increased from day 20 to day 100, and corresponding with level of arsenic in soil. The highest accumulation was in day 100 from treatment of 400 mg As/kg with EDTA. From statistical analysis, it was shown that there was significant difference between concentration of arsenic in soil and harvest time ($P < 0.05$) as shown in APPENDIX A-10.

In comparison of the accumulations of arsenic in all part of *C. esculenta*, it can be seen that wild taro can accumulated arsenic slightly higher than taro. And it was found that there was significant difference between both type of *C. esculenta* as shown in APPENDIX A-11.

From the observation it was found that wild taro has much more root and has weight of root higher than root of taro, so it could suggest that wild taro has root surfaces for uptaking arsenic in soil more than taro. And this reason could explain the difference arsenic accumulation between both plants.

Table 4-9 Arsenic accumulation in all parts of *C. esculenta* (L.) Schott (Taro)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in all parts at harvested time (mg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	4.99	8.38	10.69	15.64	8.52
100 with EDTA	11.11	12.83	16.67	17.47	20.80
200	11.26	13.91	17.55	18.93	22.73
200 with EDTA	17.79	23.63	25.80	26.20	24.99
400	13.38	12.94	40.34	-	-
400 **with EDTA**	24.62	33.72	-	-	-

Table 4-10 **Arsenic accumulation in all parts of *C. esculenta*** (**L.**) **Schott** (**Wild taro**)

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in all parts at harvested time (mg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	6.43	7.49	6.86	10.52	9.96
100 with EDTA	8.98	14.81	8.92	11.90	14.84
200	17.34	18.26	18.66	19.74	17.86
200 with EDTA	22.31	30.76	32.33	33.80	38.23
400	28.42	28.29	39.48	45.75	46.79
400 with EDTA	44.05	44.08	-	-	-

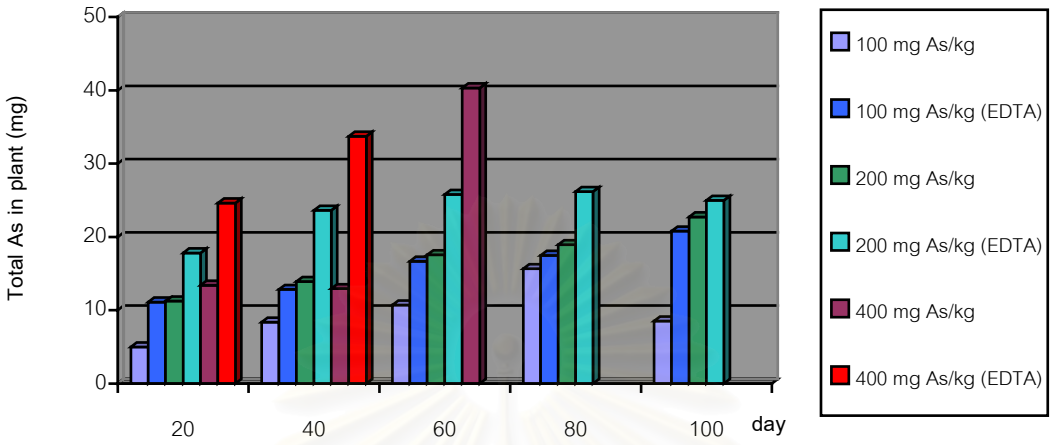


Figure 4-9 Arsenic accumulation in all parts of *C. esculenta* (L.) Schott (Taro)

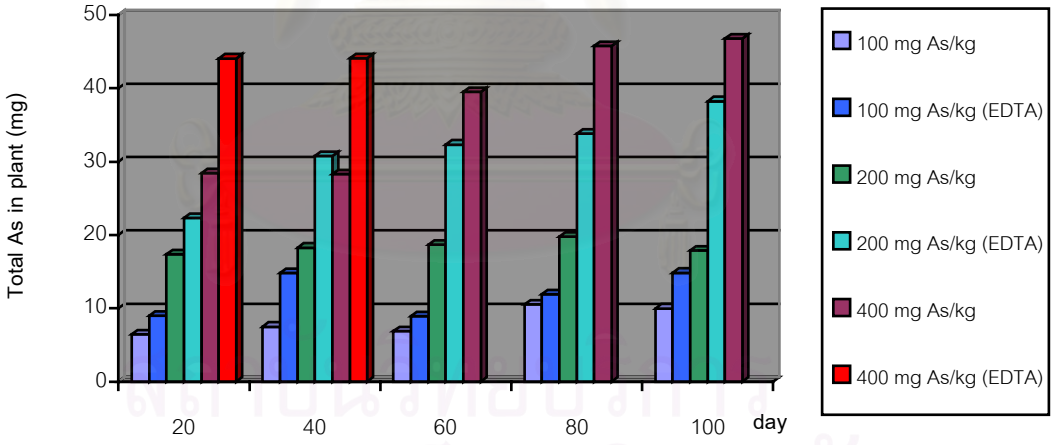


Figure 4-10 Arsenic accumulation in all parts of *C. esculenta* (L.) Schott (Wild taro)

4.4 Amount of arsenic in *C. esculenta* (L.) Schott

Amount of arsenic in plants was calculated from total arsenic accumulated in plant (mg) per amount of arsenic concentration in pot (mg) and presented as percentage of arsenic plants.

The amount of arsenic in taro was in the range of 0.67 % - 4.16 %. Table 4-11 and Figure 4-11 illustrated the amount of arsenic in taro which can be seen that the amounts of arsenic were increased from day 20 to day 100, but not corresponding with level of arsenic in soil. The highest percentage was 4.16 % in day 100 from treatment of 100 mg As/kg with EDTA.

While in wild taro the amount of arsenic was in the range of 1.29 % - 3.82 %. Table 4-12 and Figure 4-12 illustrated the amount of arsenic in wild taro which can be seen that the amounts of arsenic were increased from day 20 to day 100, but not corresponding with level of arsenic in soil. The highest percentage was 3.82 % in day 100 from treatment of 200 mg As/kg with EDTA.

From the results, the maximum amount of arsenic in taro was occurred in the treatment of 100 mg As/kg with EDTA, while in wild taro was in the treatment of 200 mg As/kg with EDTA instead of 400 mg As/kg with EDTA. It occurred because the proportion of total arsenic in plant per amount of arsenic in pot was so different at high level of arsenic in soil. For this reason, the percentage of high level of arsenic concentration was less than the lower level of arsenic concentration. And it may be suggested that *C. esculenta* (wild taro) have higher limit of arsenic accumulation than *C. esculenta* (taro).

Table 4-11 Amount of arsenic in *C. esculenta* (L.) Schott (Taro)

Arsenic concentration (mg As/kg soil)	% As in plant				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	1.00	1.68	2.14	3.13	1.70
100 with EDTA	2.22	2.57	3.33	3.49	4.16
200	1.13	1.39	1.76	1.89	2.27
200 with EDTA	1.78	2.36	2.58	2.62	2.50
400	0.67	0.65	2.02	-	-
400 **with EDTA**	1.23	1.69	-	-	-

Table 4-12 **Amount of arsenic in *C. esculenta*** (**L.**) **Schott** (**Wild taro**)

Arsenic concentration (mg As/kg soil)	% As in plant				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	1.29	1.50	1.37	2.10	1.99
100 **with EDTA**	1.80	2.96	1.78	2.38	2.97
200	1.73	1.83	1.87	1.97	1.79
200 **with EDTA**	2.23	3.08	3.23	3.38	3.82
400	1.42	1.41	1.97	2.29	2.34
400 **with EDTA**	2.20	2.20	-	-	-

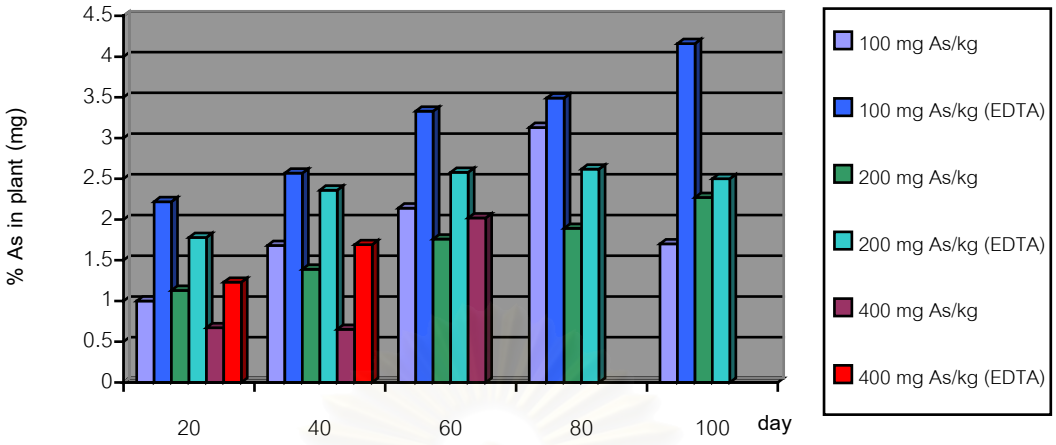


Figure 4-11 **Amount of arsenic in *C. esculenta* (L.) Schott (Taro)**

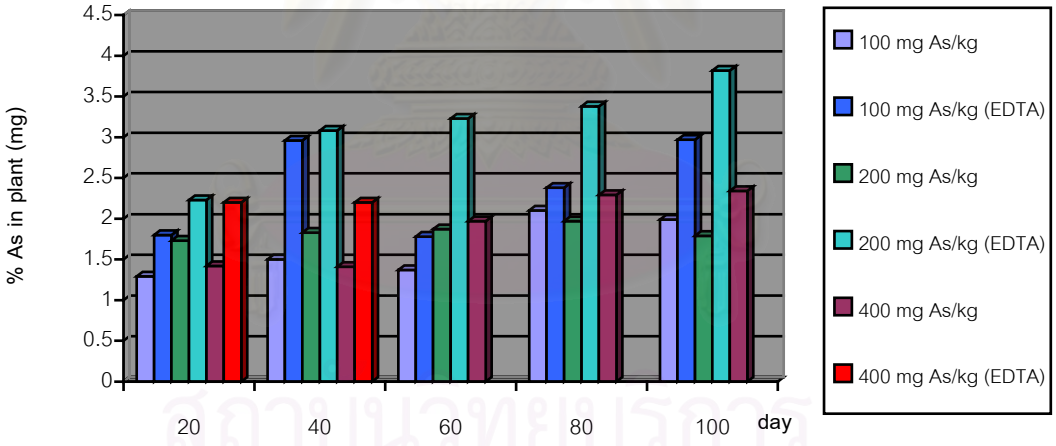


Figure 4-12 Amount of arsenic in *C. esculenta* (L.) Schott (Wild taro)

4.5 Effect of soil-applied chelating agents on arsenic accumulation in plants

Through the addition of EDTA chelator to arsenic contaminated soil, accumulation of arsenic in *C. esculenta* was enhanced. The application of EDTA to the soil may solubilized arsenic in the soil and also increased arsenic uptake and translocation to the above ground tissues. Table 4-13 illustrated the difference of arsenic accumulation between with and without amended with EDTA chelator. It can be seen that the addition of EDTA increased arsenic uptake in all of experimental tests.

In soil, the applied chelate acts first to complex the soluble metals in the soil solution. As the free metal activity decreases, dissolution of bound metal ions begins to compensate for the shift in equilibrium (Blaylock et al., 1997). But for this research EDTA may competed with others ion in soil such as Fe or Al, which can complex very well with arsenic, and form complex with that ion. Then these ions would form complex with EDTA instead of arsenic, so it could have more arsenic in the form that available for plants. Or it dropped pH in soil and made little acidic soil, and have some effects on the uptaking process of the plants. But it could not conclude that, what is the main mechanism of EDTA that effected the accumulation of arsenic in plants.

Table 4-13 Difference of arsenic accumulation between with and without EDTA

Arsenic concentration	Difference between with and without EDTA (%)				
(mg As/kg soil)	Day 20	Day 40	Day 60	Day 80	Day 100
Taro					
100	55.09	34.68	35.87	10.48	59.04
200	36.71	41.13	31.98	27.75	9.04
400	45.65	61.63			
Wild taro					
100	28.40	49.43	23.09	11.60	32.88
200	22.28	40.64	42.28	41.60	53.28
400	35.48	35.82			

Chapter V

Conclusions and Recommendations

5.1 Conclusions

From the experimental results, the following conclusions can be presented.

5.1.1 Arsenic accumulation in parts of plants

Arsenic accumulations were measured into concentration, amount of arsenic per dry weight of plants. The results indicated that the level of arsenic accumulation was in root > corm > petiole > lamina, respectively. And from statistical test shown that arsenic accumulations had significant different among concentration of arsenic in soil. Moreover the accumulation was dependent on the arsenic concentration in soil and increased with the higher of arsenic in soil concentration.

5.1.2 Arsenic accumulation in lamina

The accumulation of arsenic in both *C. esculenta* tended to increase at high concentration of arsenic in soil. The maximum arsenic accumulated in lamina of taro was 383.50 mg As/kg at day 60 in the concentration of 400 mg As/kg soil, while in lamina of wild taro was 186.67 mg As/kg at day 40 in the concentration of 400 mg As/kg soil with EDTA. But from all results indicated that arsenic was accumulated in lamina of wild taro more than in lamina of taro.

5.1.3 Arsenic accumulation in petiole

Arsenic accumulation in petiole of both of *C. esculenta* tended to increase at high concentration of arsenic in soil. The maximum arsenic accumulated in petiole of taro was 498.21 mg As/kg at day 40 in the concentration of 400 mg As/kg soil with EDTA, while in petiole of wild taro was 624.43 mg As/kg at day 20 in the

concentration of 400 mg As/kg soil with EDTA. And from all results indicated that arsenic was accumulated in petiole of wild taro more than in petiole of taro.

5.1.4 Arsenic accumulation in corm

Arsenic accumulation in corm of both of *C. esculenta* tended to increase at high concentration of arsenic in soil. The maximum arsenic accumulated in corm of taro was 404.33 mg As/kg at day 60 in the concentration of 400 mg As/kg soil, while in corm of wild taro was 561.20 mg As/kg at day 20 in the concentration of 400 mg As/kg soil with EDTA. And from all results indicated that arsenic was accumulated in corm of wild taro more than in corm of taro.

5.1.5 Arsenic accumulation in root

Significantly, the root of both plants was the best part for arsenic accumulation. Arsenic accumulation in root of both of *C. esculenta* tended to increase at high concentration of arsenic in soil. The maximum arsenic accumulated in root of taro was 2005.56 mg As/kg at day 60 in the concentration of 400 mg As/kg soil, while in root of wild taro was 2089.29 mg As/kg at day 20 in the concentration of 400 mg As/kg. And from all results indicated that arsenic was accumulated in root of wild taro more than in root of taro.

5.1.6 Arsenic accumulation and the application of EDTA

The research indicates that the accumulation of arsenic in both plants can be enhanced through the application of EDTA to the soil. The results can be seen that the amendment of EDTA could enhanced the accumulation of arsenic in both plants in all most concentration. With the maximum of 61.63% enhanced the accumulation for taro in the concentration of 400 mg As/kg soil at day 40 and 53.28% for wild taro in the concentration of 200 mg As/kg soil at day 100.

5.2 Recommendations

From the results, several recommendations for further study are given as follow.

5.2.1 From the arsenic accumulation and the survival of the plants, it may suggest that the total harvest time only for 100 days does not effect the analysis of arsenic accumulation. So it should plant more than 100 days to determine overall.

5.2.2 The concentration of EDTA is fixed in this studied, so it should be culture with different concentrations of EDTA in further study.

5.2.3 Arsenic species that accumulated in plant should be also study.

5.2.4 The effect of soil pH on the efficiency of EDTA is interesting for further research.

References

Aten, C.F., Bourke, J.B., Martini, J.H., and Walton, J.C. Arsenic and lead in an orchard environment. **Environ. Toxicol.** (1980): 108-115.

Bieleski, R. L., and Ferguson, I. B. Physiology and metabolism of phosphate and its compounds. **Encyclopaedia of Plant Physiology.** (1983): 422-449.

Blaylock, M. J., et al. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. **Environ. Sci. Technol.** 31,3 (1997): 860-865.

Chaney, R. L.; Brown, S. L.; and Angle, J. S. Soil root interface: Food chain contamination and ecosystem health. Madison, WI, 1998.

Ebbs, S. D., et al. Phytoextraction of cadmium and zinc from a contaminated soil. **J. Environ. Qual.** 26 (1997): 1424-1430.

Federation Remediation Technologies Roundtable. **Remediation technologies screening matrix and reference guide.** [electronic reference guide], 2001 [cited 27 April 2001]Available from http://www.frtr.gov/matrix2/top_page.html [internet]

Furguson, J. F., and Gavis, J. A review of the arsenic cycle in natural waters. **Water Res.** 6 (1972): 1259-1274.

Jirawan Jampanil. **Efficiency of arsenic removal from soil by *Colocasia esculenta* (L.) Schott (dark violet and green).** Master's Thesis, Inter-Department of Environmental science, Graduate School, Chulalongkorn University, 2000.

Huang, J. W. et al. Phytoremediation of lead from contaminated soils: Role of synthetic chelates in lead phytoextraction. **Environ. Sci. Technol.** 31 (1997): 800-805.

Huang, J. W.; Blaylock, M. J.; Kapulnik, Y.; and Ensley, B. D. Phytoremediation of uranium-contaminated soils: Role of organic acids in triggering uranium hyperaccumulation in plants. **Environ. Sci. Technol.** 32,13 (1998): 2004-2008.

Mattusch, J.; Wennrich, R.; Schmidt, A. C.; and Reisser, W. Determination of arsenic species in water, soils and plants. **Fresenius. J. Anal. Chem.** 366 (2000): 200-203.

National Research Council. **Arsenic.** Washington, DC: National Academy Press, 1977.

New Jersey Department of Environmental Protection. **Soil cleanup criteria.** New Jersey: 1996.

Nriagu, J. O. **Arsenic in the environment.** NY: A Wiley-interscience publication, 1994.

O' Neill, P. Arsenic. In B.J. Alloway (ed.). **Heavy metal in soil.** UK: Blackie Academy & Professional, 1993.

Pickering, I. J., et al. Reduction and coordination of arsenic in Indian mustard. **Plant Physiol.** 122 (2000): 1171-1177.

Raskin, I. **Phytoremediation: Using plants to remove pollutants from the environment.** NJ: American Society of Plant Physiologists, 1997.

Raskin, I.; and Ensley, B. D. **Phytoremediation of toxic metals: Using plants to clean up the environment.** NY: A Wiley-interscience publication, 2000.

Schmoger, M. E. V.; Oven, M.; and Grill, E. Detoxification of arsenic by phytochelatins in plants. **Plant Physiol.** 122 (2000): 793-801.

Schnoor, L. J. **Phytoremediation.** Iowa: Ground-water remediation technologies analysis center, 1997.

Thailand. Pollution Control Department. **Full report of project for investigate and analysis of the remediation plan for arsenic contamination in Ronphiboon district, Nakhon Si Thammarat province.** Bangkok, 1998.

University of Florida, **Aquatic and wetland plants of Florida.** USA, 1996

U.S. Environmental Protection Agency. **Introduction to phytoremediation.** Cincinnati, OH, 2000.

U.S. Environmental Protection Agency. **A citizen's guide to phytoremediation.** Cincinnati, OH, 1998.

Vassil, A. D.; Kapulnik, Y.; Raskin, I.; and Salt, D. E. The role of EDTA in lead transport and accumulation by Indian mustard. **Plant Physiol.** 117 (1998), 447-453.

APPENDICES

APPENDIX A

STATISTICAL ANALYSIS

A-1 Test of arsenic concentration in lamina of taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	177294.6	5	35458.913	3.372	.019
Within Groups	252412.5	24	10517.189		
Total	429707.1	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05	
CONC	N	1	2
3	5	44.4720	
1	5	72.2080	
2	5	109.6620	
6	5	116.7080	
4	5	130.1640	
5	5		285.8420
Sig.		.249	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-2 Test of arsenic concentration in petiole of taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	871690.0	5	174337.994	71.416	.000
Within Groups	58587.99	24	2441.166		
Total	930278.0	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05			
CONC	N	1	2	3	4
1	5	44.1520			
3	5	64.6100	64.6100		
2	5	95.0040	95.0040	95.0040	
5	5		129.5900	129.5900	
4	5			156.4200	
6	5				544.1440
Sig.		.136	.059	.074	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-3 Test of arsenic concentration in corm of taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	414486.6	5	82897.327	12.710	.000
Within Groups	156536.0	24	6522.331		
Total	571022.6	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05	
CONC	N	1	2
1	5	29.3500	
2	5	63.1620	
4	5	115.4480	
3	5	115.7600	
6	5		280.9120
5	5		354.6500
Sig.		.134	.162

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-4 Test of arsenic concentration in root of taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4219907	5	843981.423	5.352	.002
Within Groups	3784729	24	157697.029		
Total	8004636	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05		
CONC	N	1	2	3
3	5	552.1520		
1	5	559.2060		
2	5	884.9020	884.9020	
4	5	1021.654	1021.654	1021.654
5	5		1373.884	1373.884
6	5			1544.324
Sig.		.099	.077	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-5 Test of arsenic concentration in lamina of wild taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	521783.2	5	104356.646	5.995	.001
Within Groups	417758.5	24	17406.603		
Total	939541.7	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05	
CONC	N	1	2
1	5	60.9300	
2	5	81.0620	
5	5	106.4720	
3	5	113.3540	
4	5	129.1820	
6	5		447.0540
Sig.		.472	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-6 Test of arsenic concentration in petiole of wild taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1105774	5	221154.887	38.903	.000
Within Groups	136434.0	24	5684.751		
Total	1242208	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05			
CONC	N	1	2	3	4
1	5	29.3440			
2	5	58.4220	58.4220		
3	5	130.8520	130.8520		
4	5		156.7840	156.7840	
5	5			242.2300	
6	5				604.5200
Sig.		.054	.061	.086	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-7 Test of arsenic concentration in corm of wild taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	652235.3	5	130447.064	16.731	.000
Within Groups	187119.1	24	7796.629		
Total	839354.4	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05			
CONC	N	1	2	3	4
1	5	80.9600			
3	5	141.1280	141.1280		
2	5	183.1740	183.1740	183.1740	
4	5		251.6720	251.6720	
5	5			275.9180	
6	5				541.5280
Sig.		.095	.072	.129	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-9 Test of arsenic concentration in root of wild taro

Oneway

ANOVA

ACCUMULA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6149656	5	1229931.103	9.999	.000
Within Groups	2952137	24	123005.718		
Total	9101793	29			

Post Hoc Tests

Homogeneous Subsets

ACCUMULA

Duncan[a]

		Subset for alpha = .05	
CONC	N	1	2
1	5	373.0880	
2	5	511.3760	
3	5	776.4540	
4	5		1275.636
5	5		1422.480
6	5		1543.422
Sig.		.097	.265

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

A-10 Test of arsenic accumulation in all part by comparing between concentrations and time

Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: ACCUMULA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5832.474[a]	26	224.326	7.205	.000
Intercept	25280.189	1	25280.189	811.945	.000
CONC	5255.363	5	1051.073	33.758	.000
TIME	859.446	4	214.861	6.901	.001
CONC * TIME	655.396	17	38.553	1.238	.306
Error	778.383	25	31.135		
Total	29114.378	52			
Corrected Total	6610.857	51			

a. R Squared = .882 (Adjusted R Squared = .760)

Post Hoc Tests

CONC

Homogeneous Subsets

ACCUMULA

Duncan[a,b,c]

CONC	N	Subset 1	Subset 2	Subset 3	Subset 4
1	10	8.9480			
2	10	13.8330	13.8330		
3	10		17.6240		
4	10			27.5840	
5	8			31.9238	31.9238
6	4				36.6175
Sig.		.097	.193	.139	.110

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 31.135.

a. Uses Harmonic Mean Sample Size = 7.742.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

A-11 Test of arsenic accumulation in all part by comparing between type and concentrations

Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: ACCUMULA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5316.152[a]	11	483.287	14.931	.000
Intercept	23124.681	1	23124.681	714.438	.000
CONC	4076.674	5	815.335	25.190	.000
TYPE	378.613	1	378.613	11.697	.001
CONC * TYPE	647.291	5	129.458	4.000	.005
Error	1294.706	40	32.368		
Total	29114.378	52			
Corrected Total	6610.857	51			

a. R Squared = .804 (Adjusted R Squared = .750)

Post Hoc Tests

CONC

Homogeneous Subsets

ACCUMULA

Duncan[a,b,c]

CONC	N	Subset 1	2	3	4
1	10	8.9480			
2	10	13.8330	13.8330		
3	10		17.6240		
4	10			27.5840	
5	8			31.9238	31.9238
6	4				36.6175
Sig.		.099	.197	.141	.112

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 32.368.

a. Uses Harmonic Mean Sample Size = 7.742.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

APPENDIX B

ARSENIC ACCUMULATION IN *C. esculenta* (L.) Schott.

Table B-1 Arsenic accumulation in lamina of taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	64.23	47.98	49.16	98.21	78.85
	53.84	59.34	72.31	105.66	75.90
	39.61	62.21	83.52	105.73	86.57
Average	52.56	56.51	68.33	103.20	80.44
100 with EDTA	56.58	94.35	105.15	256.34	94.18
	85.57	52.38	43.56	198.20	89.24
	74.48	79.11	94.92	217.28	103.59
Average	72.21	75.28	81.21	223.94	95.67
200	76.70	11.50	8.08	68.34	9.46
	56.55	65.65	15.36	59.34	26.35
	69.25	69.25	20.30	65.16	25.69
Average	67.50	55.50	14.58	64.28	20.50
200 with EDTA	64.65	41.61	433.33	102.06	43.34
	72.61	90.25	295.61	96.41	40.35
	69.35	70.82	421.56	85.45	25.06
Average	68.87	67.56	383.50	94.64	36.25
400	51.74	102.68	425.84	-	-
	49.28	95.66	-	-	-
	59.33	96.38	-	-	-
Average	53.45	98.24	425.84	-	-
400 with EDTA	96.54	135.65	-	-	-
	76.95	115.87	-	-	-
	68.01	-	-	-	-
Average	80.50	125.76	-	-	-

Table B-2 Arsenic accumulation in lamina of wild taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	65.24	68.08	36.94	35.95	86.59
	55.66	36.21	23.59	103.26	76.24
	62.55	20.21	6.19	116.39	120.85
Average	61.15	41.50	22.24	85.20	94.56
100 with EDTA	87.60	56.32	65.94	126.35	154.21
	85.57	32.65	26.35	86.32	98.65
	51.38	41.98	15.14	71.01	216.46
Average	74.85	43.65	35.81	94.56	156.44
200	69.25	97.26	165.33	145.90	96.54
	95.34	135.34	118.08	124.00	79.05
	122.27	128.03	96.09	148.87	78.96
Average	95.62	120.21	126.50	139.59	84.85
200 with EDTA	129.65	184.76	138.65	198.36	45.59
	136.97	171.11	159.48	145.66	68.94
	119.03	158.09	96.61	100.97	83.86
Average	128.55	171.32	131.58	148.33	66.13
400	59.33	106.54	126.54	165.24	84.69
	64.57	135.66	195.48	122.01	95.32
	64.59	44.30	177.78	76.20	78.83
Average	62.83	95.50	166.60	121.15	86.28
400 with EDTA	75.54	198.37	-	-	-
	85.62	65.84	-	-	-
	95.34	295.80	-	-	-
Average	85.50	186.67	-	-	-

Table B-3 Arsenic accumulation in petiole of taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	52.98	35.84	20.64	65.49	86.44
	46.42	51.2	8.56	49.14	72.36
	48.35	19.94	16.16	42.12	46.64
Average	49.25	35.66	15.12	52.25	68.48
100 with EDTA	76.14	99.08	89.62	69.22	152.24
	95.02	106.47	96.57	76.11	96.87
	74.48	105.97	92.36	46.52	148.39
Average	81.88	103.84	92.85	63.95	132.50
200	49.10	53.95	49.24	86.60	94.15
	57.20	35.64	76.85	95.64	86.31
	30.20	35.90	65.43	77.62	75.32
Average	45.50	41.83	63.84	86.62	85.26
200 with EDTA	96.32	326.11	138.65	116.65	96.24
	76.24	296.54	195.49	102.37	86.34
	88.56	291.03	185.58	145.42	104.76
Average	87.04	304.56	173.24	121.48	95.78
400	196.52	115.22	124.87	-	-
	231.87	95.34	-	-	-
	173.29	79.48	-	-	-
Average	200.56	96.68	124.87	-	-
400 with EDTA	85.62	538.21	-	-	-
	95.34	259.84	-	-	-
	1029.54	-	-	-	-
Average	463.50	498.21	-	-	-

Table B-4 Arsenic accumulation in petiole of wild taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	25.94	27.70	54.95	16.47	36.54
	10.35	22.56	62.15	24.86	10.25
	4.39	32.24	36.59	26.35	48.82
Average	13.56	27.50	51.23	22.56	31.87
100 with EDTA	95.62	67.73	54.18	50.63	65.12
	65.44	42.09	95.47	76.11	35.46
	73.36	63.85	37.88	25.45	27.94
Average	78.14	57.89	62.51	50.73	42.84
200	153.43	300.05	66.21	86.95	86.71
	96.42	299.10	76.85	96.54	89.64
	129.65	294.67	45.46	42.89	98.21
Average	126.50	297.94	62.84	75.46	91.52
200 with EDTA	96.32	366.74	76.35	46.31	98.20
	176.45	368.41	69.58	102.37	85.24
	251.81	401.01	68.60	96.57	47.80
Average	174.86	378.72	71.51	81.75	77.08
400	198.75	326.10	324.96	271.21	95.54
	231.88	264.31	322.02	264.78	165.24
	204.08	165.98	422.52	256.94	119.14
Average	211.57	252.13	356.50	264.31	126.64
400 with EDTA	725.35	588.82	-	-	-
	629.08	652.34	-	-	-
	518.86	513.45			-
Average	624.43	584.87	-	-	-

Table B-5 Arsenic accumulation in corm of taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	35.64	24.14	35.24	33.65	28.64
	32.15	23.68	25.87	36.66	34.56
	23.95	25.86	27.66	39.04	13.51
Average	30.58	24.56	29.59	36.45	25.57
100 with EDTA	69.84	123.54	62.60	36.54	24.52
	65.82	109.66	75.62	53.21	32.00
	60.06	100.28	54.68	16.75	62.31
Average	65.24	111.16	64.30	35.50	39.61
200	136.85	360.01	53.34	26.50	24.41
	132.55	298.63	79.34	45.28	21.05
	125.64	305.65	46.75	46.12	34.28
Average	131.68	321.43	59.81	39.30	26.58
200 with EDTA	282.27	99.02	88.71	65.84	79.84
	278.95	102.35	96.48	42.72	51.46
	265.49	94.40	65.37	42.67	76.15
Average	275.57	98.59	83.52	50.41	69.15
400	268.88	190.28	404.33	-	-
	286.73	201.54	-	-	-
	237.05	199.90	-	-	-
Average	264.22	197.24	404.33	-	-
400 with EDTA	365.24	268.72	-	-	-
	298.54	239.32	-	-	-
	282.72	-	-	-	-
Average	315.50	254.02	-	-	-

Table B-6 Arsenic accumulation in corm of wild taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	125.65	167.15	75.23	27.56	40.59
	96.35	138.96	69.36	40.80	45.96
	111.75	145.63	65.65	26.14	37.62
Average	111.25	150.58	70.08	31.50	41.39
100 with EDTA	233.12	393.43	132.46	48.75	103.25
	248.77	389.54	135.24	60.01	108.23
	224.82	370.41	134.06	61.01	104.51
Average	235.57	384.46	133.92	59.59	105.33
200	220.13	203.66	61.89	87.93	125.03
	236.41	202.31	63.12	95.63	119.45
	222.99	198.65	68.43	100.06	111.23
Average	226.51	201.54	64.48	94.54	118.57
200 with EDTA	318.90	312.34	216.87	215.22	203.64
	320.12	320.12	234.12	198.76	197.17
	312.34	310.45	197.76	200.18	203.64
Average	317.12	318.79	216.25	204.72	201.48
400	397.21	211.56	439.12	149.27	144.32
	410.25	214.41	452.87	172.35	185.02
	395.78	208.05	432.51	158.47	167.58
Average	401.08	211.34	441.50	160.03	165.64
400 with EDTA	514.10	510.99	-	-	-
	554.81	524.84	-	-	-
	614.69	500.32	-	-	-
Average	561.20	512.05	-	-	-

Table B-7 Arsenic accumulation in root of taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	220.13	496.94	670.35	932.50	436.27
	254.89	500.24	664.05	967.14	458.20
	222.72	490.31	670.59	953.24	450.52
Average	232.58	495.83	668.33	950.96	448.33
100 with EDTA	601.33	643.30	1027.22	904.40	1269.70
	587.45	640.11	1014.08	895.48	1248.21
	588.90	657.23	1072.82	884.61	1238.69
Average	592.56	646.88	1038.04	894.83	1252.20
200	537.41	632.25	356.21	619.63	803.45
	570.38	600.33	345.71	237.18	793.21
	579.71	560.13	318.71	422.87	797.10
Average	562.50	597.57	340.21	426.56	797.92
200 with EDTA	853.70	1273.80	1268.34	908.63	810.30
	879.23	1239.41	1284.56	924.77	817.74
	872.30	1257.55	1182.34	918.65	836.25
Average	868.41	1256.92	1245.08	917.35	821.43
400	512.50	576.22	2005.56	-	-
	498.98	591.05	-	-	-
	488.64	589.65	-	-	-
Average	500.04	585.64	2005.56	-	-
400 with EDTA	957.25	1596.54	-	-	-
	914.56	1575.20	-	-	-
	946.75	-	-	-	-
Average	939.52	1585.87	-	-	-

Table B-8 Arsenic accumulation in root of wild taro

Arsenic concentration (mg As/kg soil)	Arsenic accumulation in lamina at harvest time (mg/kg)				
	Day 20	Day 40	Day 60	Day 80	Day 100
100	175.63	780.77	235.29	456.28	174.84
	190.45	798.21	255.98	470.96	187.08
	187.06	784.12	263.08	472.29	164.28
Average	184.38	787.70	251.45	466.51	175.40
100 with EDTA	311.64	936.90	272.32	478.08	581.23
	298.78	954.12	293.31	477.32	895.74
	293.30	936.75	278.12	495.55	275.48
Average	301.24	942.59	281.25	483.65	584.15
200	562.37	896.47	817.26	843.02	724.19
	548.99	967.20	834.84	805.64	734.08
	539.27	987.14	810.18	833.69	742.47
Average	550.21	950.27	820.76	827.45	733.58
200 with EDTA	678.25	1019.49	1337.88	1409.06	1842.58
	645.32	1024.84	1342.50	1502.32	1847.36
	670.11	1008.35	1302.33	1542.63	1880.52
Average	664.56	1017.56	1327.57	1511.67	1856.82
400	958.31	1074.75	1847.23	2127.38	1112.96
	975.52	1045.78	1896.35	2069.24	1103.57
	949.41	1089.53	1912.17	2071.25	1103.75
Average	961.08	1070.02	1885.25	2089.29	1106.76
400 with EDTA	1252.79	1148.84	-	-	-
	1274.20	1352.41	-	-	-
	1270.83	1263.57	-	-	-
Average	1265.94	1254.94	-	-	-

BIOGRAPHY

Witchanan Tambamroong was born on the 27th of March 1979 in Bangkok. He started to study at Mahidol University in 1996 and graduated the Bachelor Degree of Environmental Science and Technology in 1999 from Faculty of Environmental and Resource Studied. Then, he continued his further education for Master degree at International Post-graduated Program in Environmental Management, a joint program of National Research Center for Environmental and Hazardous Waste Management in 2000.