EFFECT OF NITRILOTRIACETIC ACID (NTA) AND ETHYLENEDIAMINETETRAACETIC ACID (EDTA) ON ARSENIC UPTAKE FROM CONTAMINATED SOIL BY *MIMOSA PUDICA* L.

Mr. Khamla Nanthavong

A Thesis Submitted in Partial Fulfillment of the Requirements
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ผลของกรดไนตริโลไทรอะซีติก (เอนทีเอ) และกรดเอทิลีนไดเอมินเททระอะซีติก (อีดีทีเอ) ต่อการดูดดึงสารหนูที่ปนเปื้อนในดินด้วยไมยราบ

นายคำหล้า นันทวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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Thesis Title	EFFECT OF NITRILOTRIACETIC ACID (NTA) AND ETHYLENEDIAMINETETRAACETIC ACID (EDTA) ON ARSENIC UPTAKE FROM CONTAMINATED SOIL BY *MIMOSA PUDICA* L.
By	Mr. Khamla Nanthavong
Field of study	Environmental Management
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คำหล้า นันทวงศ์: ผลของกรดไนตริโลไทรอะซีติก (เอนทีเอ) และกรดเอทิลีนไดเอมินเททระอะซีติก (อีดีทีเอ) ต่อการดูดดึงสารหนูที่ปนเปื้อนในดินด้วยไมยราบ. (EFFECT OF NITRILOTRIACETIC ACID (NTA) AND ETHYLENEDIAMINETETRAACETIC ACID (EDTA) ON ARSENIC UPTAKE FROM CONTAMINATED SOIL BY *MIMOSA PUDICA* L.) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. พันธวัศ สัมพันธ์พานิช, 105 หน้า.

การศึกษาผลของสารเร่งเอนทีเอ และอีดีทีเอ ต่อการดูดดึงและสะสมของสารหนูในราก ลำต้น และใบของต้นไมยราบ การศึกษาเบื้องต้นได้ทำการทดสอบความเป็นพิษของสารหนูที่มีต่อต้นไมยราบ โดยการเติมสารไดโซเดียมไฮโดรเจนอาซิเนท ($Na_2HAsO_4.7H_2O$) ที่มีระดับความเข้มข้นของสารหนูแตกต่างกันคือ 5, 10, 20, 40, 80, 120, 160, 200, 300 และ 400 มิลลิกรัมต่อกิโลกรัมดิน และทดสอบเอนทีเอและอีดีทีเอที่ความเข้มข้น 50, 100 และ 200 มิลลิกรัมต่อกิโลกรัมดิน จากนั้นจึงปลูกต้นไมยราบเป็นเวลา 1 เดือน ซึ่งผลการศึกษาเบื้องต้นแสดงให้เห็นว่าต้นไมยราบสามารถเจริญเติบโตได้ดีในดินที่มีความเข้มข้นของสารหนูน้อยกว่าหรือเท่ากับ 10 มิลลิกรัมต่อกิโลกรัมดิน ในขณะที่ความเข้มข้นอื่นๆ แสดงความเป็นพิษต่อพืช เช่น ใบและลำต้นแห้ง หรือหงิกงอ ส่วนเอนทีเอและอีดีทีเอไม่ส่งผลกระทบต่อการเจริญเติบโตของพืช สำหรับการทดลองหลักได้เติมสารหนูที่ความเข้มข้น 5 มิลลิกรัมต่อกิโลกรัมดิน ทิ้งไว้เป็นเวลา 3 เดือน หลังจากนั้นจึงปลูกต้นไมยราบ 1 ต้นต่อกระถาง และเติมสารเร่งเอนทีเอและอีดีทีเอแยกกันที่ความเข้มข้น 3 ระดับดังกล่าว จากนั้นเก็บตัวอย่างดินและพืชทุกๆ 30 วัน เป็นระยะเวลา 120 วัน เพื่อนำไปวิเคราะห์หาความเข้มข้นของสารหนู ผลการศึกษาแสดงให้เห็นว่าสารหนูที่สะสมอยู่ในรากมีความเข้มข้นสูงกว่าในลำต้นและใบอแตกต่างกันอย่างมีนัยสำคัญ ($p \leq 0.05$) ซึ่งความเข้มข้นสูงสุดอยู่ที่ระยะเวลา 120 วัน พบว่าในรากมีค่าเท่ากับ 29.71 มิลลิกรัมของสารหนูต่อกิโลกรัมพืช ส่วนในลำต้นและใบมีค่าเท่ากับ 6.32 มีลลิกรัมของสารหนูต่อกิโลกรัมพืช ทั้งนี้ค่าเฉลี่ยของสารหนูที่สะสมในทุกส่วนของต้นไมยราบตลอดระยะการศึกษามีค่าอยู่ในช่วง 2.71 - 36.03 มิลลิกรัมของสารหนูต่อกิโลกรัมพืช และพบว่ามีการสะสมมากที่สุดในชุดการทดลองที่มีการเติมสารเร่งอีดีทีเอ 100 มิลลิกรัมต่อกิโลกรัมดิน ซึ่งในการเก็บเกี่ยวที่ระยะเวลาและความเข้มข้นเดียวกันของสารเร่ง พบว่า อีดีทีเอ มีความสามารถในการเร่งการดูดดึงสารหนูเข้าสู่ต้นไมยราบได้ดีกว่าเอนทีเอ ทั้งนี้ผลจากการวิเคราะห์ด้วยเครื่อง Synchrotron μ-X-ray fluorescence spectroscopy (Beamline 6b) ไม่สามารถตรวจพบรูปแบบการแพร่กระจายของสารหนูในส่วนต่างๆของต้นไมยราบที่ระดับความเข้มข้นดังกล่าวได้ เนื่องจากข้อจำกัดของเครื่องมือและความเข้มข้นของสารหนูในตัวอย่างที่น้อยเกินไป อย่างไรก็ตาม การศึกษาในครั้งนี้ไม่ได้ศึกษาถึงรายละเอียดที่เกี่ยวกับปฏิกิริยาระหว่างสารหนูกับสารเร่ง ทั้งนี้หากมีการศึกษาเพิ่มเติม ควรมีการศึกษารายละเอียดในระดับโมเลกุล รวมทั้งปัจจัยจากการใส่ปุ๋ย ซึ่งอาจมีผลต่อการดูดดึงสารหนูของต้นไมยราบ

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต----------

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก----------

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KHAMLA NANTHAVONG: EFFECT OF NITRILOTRIACETIC ACID (NTA) AND ETHYLENEDIAMINETETRAACETIC ACID (EDTA) ON ARSENIC UPTAKE FROM CONTAMINATED SOIL BY *MIMOSA PUDICA* L.
ADVISOR: ASST. PROF. PANTAWAT SAMPANPANISH, Ph.D., 105 pp.

This study investigated the arsenic (As) accumulation in the underground part (root) and aboveground parts (stem and leaves) of *Mimosa pudica* L. and the effects of chelating agents, NTA and EDTA for enhancing As uptake by the plant. Uncontaminated soil and plants were used in this experiment. A preliminary study was also conducted to determine the phytotoxicity using added disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) at different concentrations of As (5, 10, 20, 40, 80, 120, 160, 200, 300 and 400 mg/kg soil) and three doses of NTA and EDTA (50, 100 and 200 mg/kg soil). Then plants were grown on prepared soil and monitored for a month. The preliminary results showed that plants could grow healthy in As contaminated soil up to 10 mg/kg soil while higher concentrations showed phytotoxicity in the plant. The symptoms varied including dry and curly in plant leaves and stems. NTA and EDTA did not produce phytotoxicity during plant growth. For the main experimental procedure, 5 mg As/kg soil was added into uncontaminated soil pots and left for three months. Then one seedling of *Mimosa pudica* L. per pot was grown in As contaminated soil and followed by adding three doses of NTA or EDTA (50, 100 and 200 mg/kg soil) separately. Soil and plant samples were collected every 30 days for a 120 day period and analyzed for As concentration. The results showed that As accumulation in the underground part of the plant (root) was significantly higher than in the aboveground parts (stem and leaves) ($P \leq 0.05$). The maximum As accumulation in the root after 120 days was at 29.71 mg As/kg plant while the combined concentration in the stem and leaves was only 6.32 mg As/kg plant. The average As accumulations in all parts of the plant during four months were in the range of 2.71 - 36.03 mg As/kg plant and set EDTA 100 mg/kg soil showed the highest As accumulation in *Mimosa pudica* L. Overall, at the same harvesting times and application doses of chelating agents, EDTA has more efficiency for enhancing As uptake by this plant than NTA. Moreover, the synchrotron μ-X-ray fluorescence spectroscopy (Beamline 6b) analysis provided an unexpected result on the distribution of As in the plant caused by the limitation of radiation beam line. However, this research did not study the chemical reactions between As and chelating agents. Therefore, for future studies it is recommended to investigate the detail in molecular level and study more on the difference between the application of fertilizers and without fertilizers.

Field of Study: Environmental Management Student's Signature _____

Academic Year: 2012 Advisor's Signature _____

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LIST OF ABBREVATIONS

AAS	Atomic Absorption Spectrometry
As	Arsenic
Atm	Atmospheric Pressure
CRD	Completely Randomized Design
CDTA	Cyclohexyleneditrilotetraacetic acid
cm	Centimeter
°C	Degree Celsius
DMR	Department of Mineral Resources
DMRT	Duncan's new Multiple Range Test
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetriacetic acid
EC	Electrical Conductivity
EGTA	Ethylenebis (Oxyethylenetrinitrilo) tetraacetic acid
GeV	Gigaelectron volt
G	Gram
HCl	Hydrochloric acid
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
HEDTA	N-hydroxyethylenediaminetriacetic acid
HEIDA	N-(2-hydroxyethyl) iminodiacetic acid
Kg	Kilogram
keV	Kilo electron volt
kJ	Kilojoule
L	Liter
ml	Milliliter
mg	Milligram
mm	Millimeter
nm	Nanometer
mol	Mole

mmol	Millimole
MeV	Mega electron volt
mV	Millivolt
meq	Milliequivalents
µS	Micro Siemens
NTA	Nitrilotriacetic acid
ORP	Oxidation reduction potential
PCD	Pollution Control Department
RGR	Relative growth rate
SPS	Siam Photon Source
SCTEE	Scientific Committee on Toxicity, Eco-toxicity and the Environment
SPSS	Statistical Package for the Social Sciences
TCE	Trichloroethylene
USEPA	United States Environmental Protection Agency
V	Volt

CHAPTER I

INTRODUCTION

1.1 Statement of Problem

Arsenic (As) contamination of soil is a widespread problem due to human activities such as mining, past use of arsenical agrochemicals or pesticides and smelting activities. These activities have caused many negative effects to the environment and ultimately on human health. The hazardous substances which have been released into soil, water and ground water are numerous; arsenic is one of the most common. Plants absorb arsenic easily so high concentrations may be present in food.

The concentration of dangerous inorganic arsenic is currently present in surface water and can enhance the change of fish genetics. This is mainly caused by accumulation of arsenic in the bodies of plant-eating freshwater organisms. This poison can move to humans and other animals through the food chain. In addition, arsenic is well known to be toxic when it is encountered in the environment and can cause multiple problems in humans such as cancers and skin diseases through ingestion or inhalation.

Ronphibun District, Nakhon SiThammarat Province, South Thailand, is an example of an arsenic contaminated area producing many health problems to humans. This situation was first recognized in 1987 and occurred through arsenic spreading from tin ore mining activities over the prior 50 years. In 1992 the department of mineral resources investigated and measured the arsenic concentration at this site and they found that the arsenic concentration in soil ranges from 0-1,770 mg As/kg soil. An analysis for species of arsenic showed that As (V) was found to be more than 90% of all arsenic in this site (Department of Mineral Resources [DMR], 1992). Moreover, DMR also reported that the arsenic contamination in soil and sediment is higher than the background concentration 50 mg As/kg soil. This arsenic poison comes from Arsenopyrite mineral (FeAsS) which is dissolved by reacting with air and water and transformed to another form as in the following equation:

$$4FeAsS + 13O_2 + 6H_2O \rightarrow 4FeSO_4 + 4H_3AsO_4 \quad (1.1)$$

At present, those mining sites are closed, but the arsenic contamination still spreads into the environment, especially in agricultural surface soil and water as well as shallow wells which have been used for a long time by the local population nearby. The people have become sick and many of them are infected with skin diseases including alternate pigmentation, small corns on palms and soles, purplish-red flush and skin cancer (Pollution Control Department [PCD], 1998). The concentration of arsenic in surface soil (0-25 cm depth) at Ronna temple which represents soil in the village area in this site has been found from 20 – 62 mg arsenic/kg soil (Visoottisethet et al., 2002), while Jankong (2007) has found 136 – 269 mg arsenic/kg in the soil samples (0-15 cm).

The remediation of large volumes of such soil by conventional technologies previously developed for small, heavily contaminated sites would be expensive (Ebbs et al., 1997). Phytoremediation has been suggested as an effective and low-cost method to clean up contaminated soil (Pilon-Smits, 2005; Salt et al., 1998). This is a technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water (United States Environmental Protection Agency [USEPA], 2000).

Recently, this method has been studied as an inexpensive and appropriate method to apply in developing countries like Thailand. In addition, phytoremediation is an environmental friendly technology that aims to reduce heavy metal contamination in soil. The heavy metal contaminant in soil can be taken up by plants and accumulated in their stems and leaves. After that, contaminated plants can be harvested and transferred for secure landfill treatment, combustion or stabilization which uses the ash from combustion for being a component of cement.

The plant species used to remediate toxic metal contaminated soil would be based on several criteria including: wide distribution, high above ground biomass, high bioaccumulation factors, short life cycles and high propagation rates. *Mimosa pudica* L. (Bashful mimosa) is a plant species that tolerates high arsenic contamination and has a short life cycle. This plant is also found commonly in the arsenic toxic site and it is

ranked as the fourth most suitable plant species for phytoremediation found at arsenic contaminated areas in Ronphibun District, Nakon Si Thammarat Province.

Visoottiviseth et al. (2002) reported that other plants such as *Pityrogrammacalomelanos* (Silver fern), *Pterisvittata* (Chinese brake fern), and *Melastomamalaba-thricum* (Blackmouth plant) can be used in phytoremediation as well. However, the required time to remediate the toxic site is quite long. Thus this research studied the use of chelating agents (NTA and EDTA) to enhance the heavy metal uptake by *Mimosa pudica* L. This would help to clean up the toxic areas faster.

NTA and EDTA have been previously studied to improve phytoremediation efficiency of plants on other heavy metal contaminants and results showed that NTA and EDTA significantly enhanced the heavy metal uptake by various other plants (Chiu et al., 2005). Therefore, the aims of this research are to compare the efficiency of different doses of NTA and EDTA for enhancing arsenic uptake by *Mimosa pudica* L., and determine the arsenic accumulation in underground part (root) and aboveground parts (stem and leaves) of this target plant. The experiment was conducted of 3 main study groups: 1) with arsenic but without chelates (control); 2) with arsenic and NTA; 3) with arsenic and EDTA.

1.2 Objectives

1.2.1 To determine arsenic accumulation in underground parts (root) and aboveground parts (stem and leaves) of *Mimosa pudica* L.

1.2.2 To compare the effect of NTA and EDTA for enhancing arsenic uptake by the plants.

1.2.3 To investigate the distribution of arsenic and other elements inside the plants over different periods of time.

1.3 Hypotheses

1.3.1 The arsenic in soil would be taken up and stored in each part of the plant with different concentrations.

1.3.2 The arsenic uptake by *Mimosa pudica* L. would be higher when EDTA is added compared with adding NTA.

1.3.3 The arsenic would be taken up by plants and slowly move from the root up to the leaves over a period of time.

1.4 Scope of the Study

This study investigated the possibility of increasing arsenic uptake by *Mimosa pudica* L. by adding chelating agents to arsenic contaminated soil during a four month period. Different doses of chelating agents: NTA ($C_6H_9NO_6$) and EDTA ($C_{10}H_{16}N_2O_8$) were tested. This research was divided into two stages as follows:

1.4.1 Preliminary study

This study aims to investigate the tolerance of *Mimosa pudica* L. on different concentrations of arsenic and studies the phytotoxicity from an addition of the two chelating agents (NTA and EDTA). The first, *Mimosa pudica* L. was grown in ten different concentrations of arsenic contaminated soil which was amended by disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) at 5, 10, 20, 40, 80, 120, 160, 200, 300 and 400 mg of arsenic/kg soil. Then a dose of arsenic contaminated soil in which the plant can grow well would be selected for further studies. The second, *Mimosa pudica* L. was grown in the arsenic contaminated soil with three different doses (50, 100 and 200 mg of NTA and EDTA per kg soil) added separately and also grown in uncontaminated soil with the same concentrations added as above for NTA and EDTA.

All the plants were watered by tap water daily and the growth properties and phytotoxicities of plants were recorded during a one month period.

1.4.2 Experimental procedure

This research studied the abilities of NTA and EDTA for enhancing arsenic up take by *Mimosa pudica* L. A concentration of arsenic at 5 mg As/kg soil was selected from the preliminary study and it was mixed with the uncontaminated soil and left for three months in order to allow the arsenic and soil to mix together. Then the *Mimosa pudica* L. was transplanted to the prepared soil, and the chelating agents (NTA and EDTA) at the rates of 50, 100 and 200 mg/kg soil were also applied separately. The plants were grown for four months.

Soil and plant samples were collected at 0, 30, 60, 90 and 120 days after transplanting to analyze the arsenic concentrations. The arsenic accumulation in plants was determined in two parts of the plant: (1) underground parts (root) and (2) aboveground parts (stem and leaves). Soil samples were also analyzed for arsenic concentration, pH level, conductivity and ORP (Oxidation reduction potential). In addition, one sample from each set was used for determining the distribution of arsenic and other elements in the plants at 30 and 120 days. The scope of this research is shown in Figure 1 below:

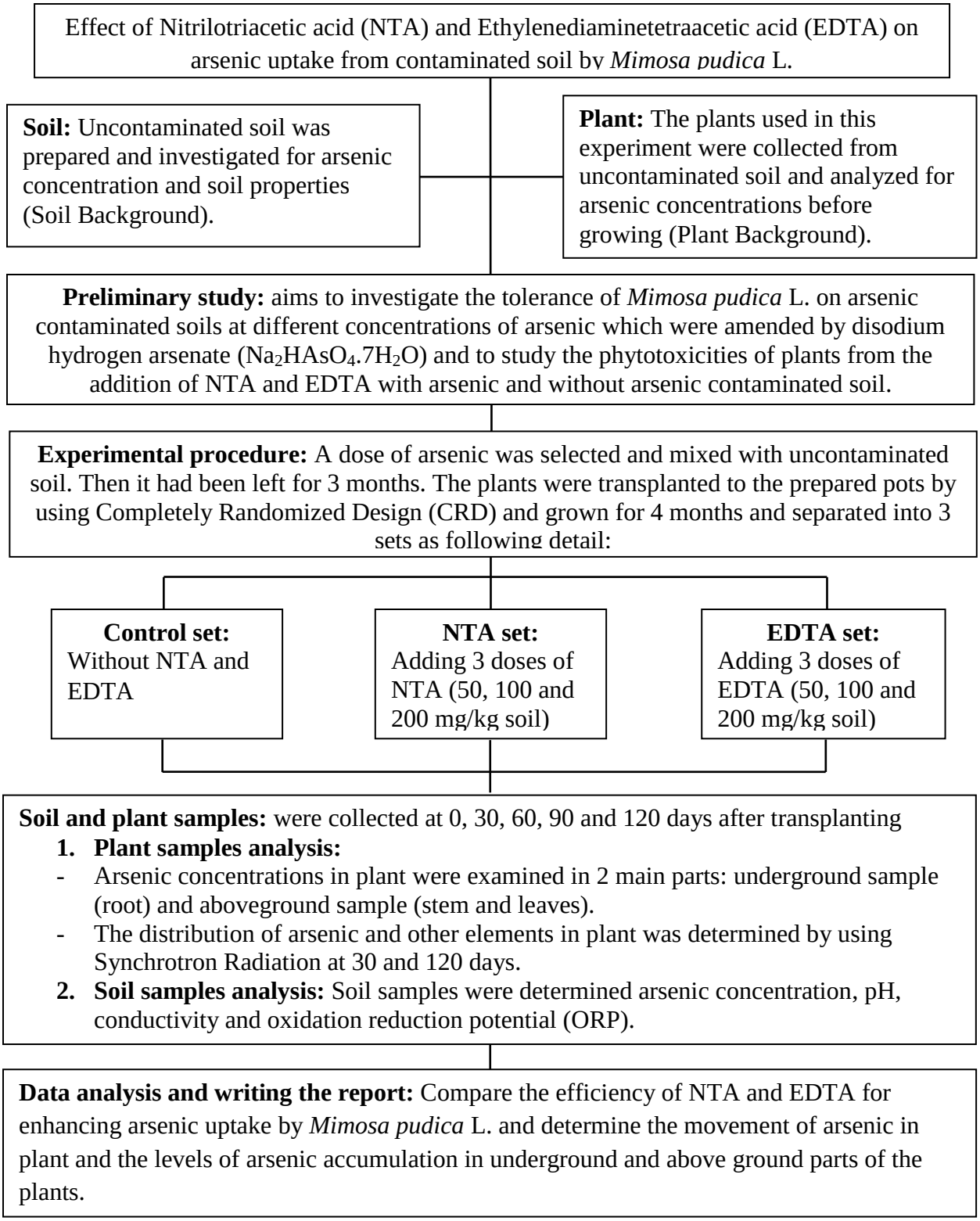


Figure 1.1 Diagram of the study scope

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Properties of arsenic

Arsenic is symbolized as **As**; and it is classified as a metalloid element in the periodic table. Arsenic is in the same group as nitrogen, phosphorus and bismuth, it has four oxidation states (-3, 0, +3 and +5). Its atomic number is 33, and other basic properties of arsenic are shown in (Table 1). Arsenic is the most toxic, followed in order of generally decreasing toxicity by trivalent compound (+3), pentavalent compound (+5), and elemental arsenic (0) (Sridokchan, 2000). Arsenic appears in three colored forms: yellow, black and grey; the stable form is a silver-gray, brittle crystalline solid. Arsenic tarnishes very fast in air and under high temperatures.

There are many forms of arsenic in the environment; the metallic form is brittle, and when heated it rapidly oxidizes to arsenic trioxide which has a garlic-like odor. The non-metallic form is less reactive but dissolves when heated with strong oxidizing acids and alkalis (Technical University of Delft, Netherland. 2011).

Table 2.1 Chemical properties of arsenic

Property	Information
Atomic number	33
Atomic mass	2
Electronegativity according to Pauling	74.9216 $g.mol^{-1}$
Density	5.7 $g.cm^{-3}$ at 14°C
Melting point	814 °C (36 atm)
Boiling point	615 °C (sublimation)
Vanderwaals radius	0.139 nm
Ionic radius	0.222 nm (-2) 0,047 nm (+5) 0,058 (+3)
Isotopes	8
Electronic shell	[Ar] $3d^{10}$ $4s^2$ $4p^3$
Energy of first ionization	947 $kJ.mol^{-1}$
Energy of second ionization	1798 $kJ.mol^{-1}$
Energy of third ionization	2736 $kJ.mol^{-1}$
Standard potential	- 0.3 V (As^{3+}/As)

Source: Technical University of Delft, Netherland, 2011.

In nature arsenic is found in combination with other compounds such as oxygen, chorine, and sulfur; when it is combined with these elements it is known as inorganic arsenic. Whereas it is combined with carbon and hydrogen it is represented as organic arsenic. These two forms of arsenic (organic and inorganic arsenics) are very important to identify because inorganic arsenic is more toxic and moves far easily than organic arsenic.

Arsenic is an extremely toxic metal that poses a significant environmental health hazard. It has been used for many years for making special types of glass, as a wood preservative and, lately, in semiconductors as gallium arsenate, which has the ability to convert electric current to laser light.

Arsine gas (AsH_3) has become an important dopant gas in the microchip industry. Arsenic was used in the nineteenth century as a coloring agent for dyes in fireworks, as a depilatory, a preservative for furs and in health tonics. Arsenic pollution becomes a wider issue because it easily spreads.

Arsenic cannot be mobilized easily when it occurs in an immobile state. When arsenic is released by human activities such as mining, metal melting and use as arsenical agrochemicals or pesticides, it settles in the environment especially in surface water, ground water and soil. Then these compounds of arsenic are spread easily and can be found throughout the environment where they did not naturally exist. Most arsenic is found in conjunction with sulfur in minerals such as arsenopyrite (AsFeS) and other forms (Tambamroong, 2002).

2.1.1 Arsenic in the environment

Arsenic can be found naturally on earth in small concentrations. It appears in soil, minerals, surface water and ground water, and arsenic may occur in the air as well. In nature arsenic can transfer from one place to another through wind, water run-off and volatility. Likewise, arsenic is also released in to the environment by human activities such as mining, burning of fossil fuels, the use of arsenical agrochemicals and industries. Large amounts of arsenic are very hard to change into the soluble form and volatile products. It is true that arsenic is naturally mobile in the environment and can move around freely. Therefore, arsenic pollution can be harmful to humans because it spreads very easily. This problem becomes the main issue; human activities can change some immobile arsenic into the mobile form mainly through mining and smelting. Pure arsenic is rarely found in nature, most arsenic is found in combined elements with sulfur in minerals such as arsenopyrite (AsFeS), realgar, orpiment and enargite. (Technical University of Delft, 2011)

Arsenic occurs everywhere in the environment; especially inorganic arsenic, a major component of natural rock and soil. The concentration of arsenic in general surface soil ranges from 1 to 40 milligrams per kilogram soil (mg/kg), and the average

concentration of natural soil is 5 mg/kg (Argonne National Laboratory, EVS. 2005). Arsenites (As III) and arsenates (As V) are the two most common forms that can dissolve in water more easily than other forms. Moreover, the arsenites can move soil particles 10 to 20 times faster than water in the pore spaces between the soil particles. The standard of arsenic in residential and agricultural soil of Thailand is not greater than 3.9 mg As/kg soil; arsenic is not permitted in quantaties greater than 27 for general land (PCD of Thailand, 2004).

Vaughan (1993) reported that arsenic usually occurs in forms of inorganic and organic compounds in soil. It mainly occurs as inorganic varieties such As V and arsenite As III. Arsenic is commonly found in the +5 oxidation state and As III is also the predominant form which is reduced from As V (Masscheleyn et al., 1991). Both As V and As III can be formed in many compounds through chemical and biological processes especially oxidation, reduction and methylation in soils (Brannon and Patrick, 1987). Therefore, there are many forms of arsenic compounds in the environment and these arsenic compounds are classified into 2 main groups: inorganic arsenic and organic arsenic as show in the following table.

Table 2.2 Some important compounds of arsenic in the environment

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	$HAsO_2$, $H_2AsO_3^-$, HAs_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, Salt of arsenic acid (ortho)	Metaarsenic acid	$HAsO_3$, $H_2AsO_4^-$ $HAsO_4^{-2}$ or AsO_4^{-3}
Organic arsenic:		
- Methylarsonic acid	Methanearrsenic	$CH_3AsO(OH)_2$
- Dimethylarsine acid	Cacodylic acid	$(CH_3)_2AsO(OH)$
- Trimethylarsine oxide	-	$(CH_3)_3AsO$
- Methylarsine	-	CH_3AsH_2
- Dimethylarsine	-	$(CH_3)_2AsH$
- Trimethylarsine	-	$(CH_3)_3As$

Source: Tambamroong, 2002.

2.1.2 Interaction of arsenic in soil

Soil is usually contaminated with arsenic through two main ways, natural processes and human activities. Human activities that release arsenic into the environment include mining, the use of pesticides, insecticides, burning of fossil fuels and smelting. When arsenic is present in soil, many reactions can occur, especially oxidation, reduction, methylation and demethylation.

Smith et al. (2002) reported that two of these microbial processes, oxidation and reduction are the most important because they are possible applications for bioremediation of contaminated soil. Moreover, some organic matters in soil may be bound to arsenic too, and this compound might be up taken by surrounding plants in that particular site. The amount of arsenic that is available for plant uptake is a function of the chemical and physical forms of individual arsenic compounds (O'Neill, 1993). Arsenate (As V) and arsenite (As III) are two toxic forms of arsenic that are commonly found in the soil but arsenite is more toxic than arsenate. However, arsenate is the most common form which exists in the soil environment. Arsenite compounds can dissolve in water 4 to 10 times more than arsenate compounds. Moreover, arsenate can be reduced to arsenite under anaerobic condition (Pickering et al., 2001).

The spreading of arsenic in soil depends on the soil type and the surrounding environment. Biological transformation is also important for arsenic distribution in soil. Arsenic may affect the microbial population in soil and the soil biological population will probably decline due to the heavy metals present. However, arsenic in soil can be accumulated and depleted through plant uptake, leaching, methylation or erosion. The transformation of arsenic in soil from one oxidation phase to another is influenced by many parameters, including pH and microbial activity (Smith et al., 2002; Bisessar, 1982).

2.1.3 Soil arsenic and plant uptake

In general, the concentration of arsenic accumulation in eatable plants to hazardous levels rarely occur due to contaminated plants might be affected and died from phytotoxicity before the concentration in contaminated plants reach to hazardous levels. Plants normally accumulate the arsenic in plant roots more than other parts (Smith et al., 2002). Arsenic in contaminated soil is up taken through the root system. The highest arsenic accumulation in plants is in plant roots and tubers. Therefore, the tuber crops (e.g., carrots, potatoes, taros) could have higher arsenic concentrations than other crops when grow in the same arsenic contaminated soil (Marin et al., 1993).

Arsenic has been used a lot in herbicides and insecticides for agriculture. Therefore, arsenic poison could be found to contaminate soil and crop productions. Arsenic present in soil acts similar to phosphorus, it also interacts with plants like in the process where plants take phosphorus as a nutrient for growing. In these ways, arsenic enters into plants and affects plant growth (Bieleski and Ferguson, 1983; Nriagu, 1994).

Plants that grow in arsenic contaminated soil may have higher arsenic accumulation than the plants that grow in uncontaminated soil. The differences of arsenic uptake by plants also depend on plant varieties, available soil arsenic, characteristics of soil (physical and chemical) and growing conditions.

National Research Council (1977) reported that if 1 mg As/kg is taken up by plants, the level of arsenic in soil in that particular area would be greater than 200 - 300 mg As/kg soil.

Plants normally take arsenic in the forms of arsenate and arsenite. The reduction of arsenic in soil is also influenced by the plants intake. Arsenic which is absobed by plants is rarely transported to the upper parts of plants (stems and leaves). It enters into plant bodies through the absorption of plant roots (Schmoger et al., 2000; Pickering et al., 2000). Arsenate compounds are less toxic than arsenite compounds.

If the plants are affected by arsenate poison, the plants lose their green coloring or the symptoms of chlorosis appear. The swelling of plants does not occur in the early

period of meeting this toxin. Plants would slowly dry and die, these also depend on other factors as well like the concentration of arsenates in soil, pH and the tolerance of each plant variety (National Research Council, 1977). The different species of As (V) depend on compound elements as well such as $H_2AsO^-_4$ which is dominant in the pH 2 to 7 value while $HAsO^-_4$ is dominant pH 7 to 11 as shown in the Figure 2.1 below (Nriagu, 1994)

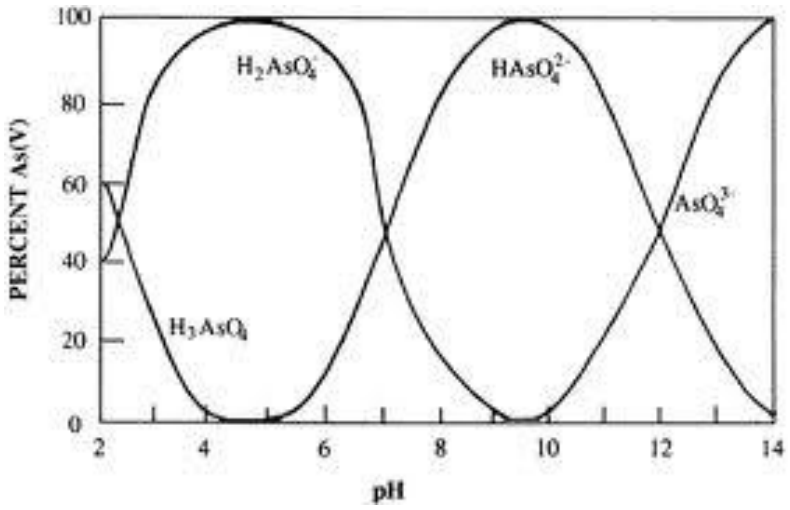


Figure 2.1 Predominance diagram for As (V) as a function of pH

Source: Nriagu, 1994.

2.2 Phytoremediation

Phytoremediation is a technology that uses various plants to accumulate, extract, degrade, or immobilize contaminants from soil and water. Phytoremediation can be also a combination of these techniques. "Phyto" means plants and "Remediation" means removing pollution. This technology has been promoted as an effective and low-cost technology.

Phytoremediation is the direct use of living green plants for in situ removal of toxins. The contaminants in soil, sediments, sludge and water can be reduced by using this method. The idea is that poisons are harvested together with the plants and transferred to other treatment technologies such as secure landfill, combustion and/or stabilization. The targeted

metals for phytoremediation include lead, cadmium, chromium, arsenic and various radionuclides. The contaminants which are up taken by plants, would be accumulated in plant tissue thus these toxins could be easily controlled and be made safe by drying, ashing or combusting. 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; USEPA, 1998).

There are two methods of phytoremediation for removing heavy metal contamination in soil, sediment and water: continuous or natural phytoremediation and chemically enhanced phytoremediation by using chelating agents (Salt et al., 1998). The volume of toxic waste produced as a result is generally a fraction 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 (Raskin, 1997). There are many advantages of phytoremediation for removing arsenic contaminated soil; plants can transform toxic arsenic forms to less or non-toxic forms. The implementation and maintenance costs are low.

Phytoremediation is able to treat the contaminated areas as an *in situ* technology and can also be applied with others treatment technologies. McGrath (1998) reported that the appropriate plants for phytoremediation of heavy metals should have high metal accumulation in their shoots, high shoot biomass and wide distribution. Plants should be able to grow in high metal contaminated soil. Furthermore, plants will have a short life cycle and high propagation.

Cincinnati (2000) has described 6 processes of phytoremediation technologies that have formulated the definitions, availabilities and categorized the required technology. Those sub-technologies of phytoremediation are (1) Phytoextraction, (2) Rhizofiltration, (3) Phytostabilization, (4) Rhizodegradation (Phytostimulation), (5) Phytodegradation, and (6) Phytovolatilization. Each sub-technology might be different in application depending on the prospective remediation.

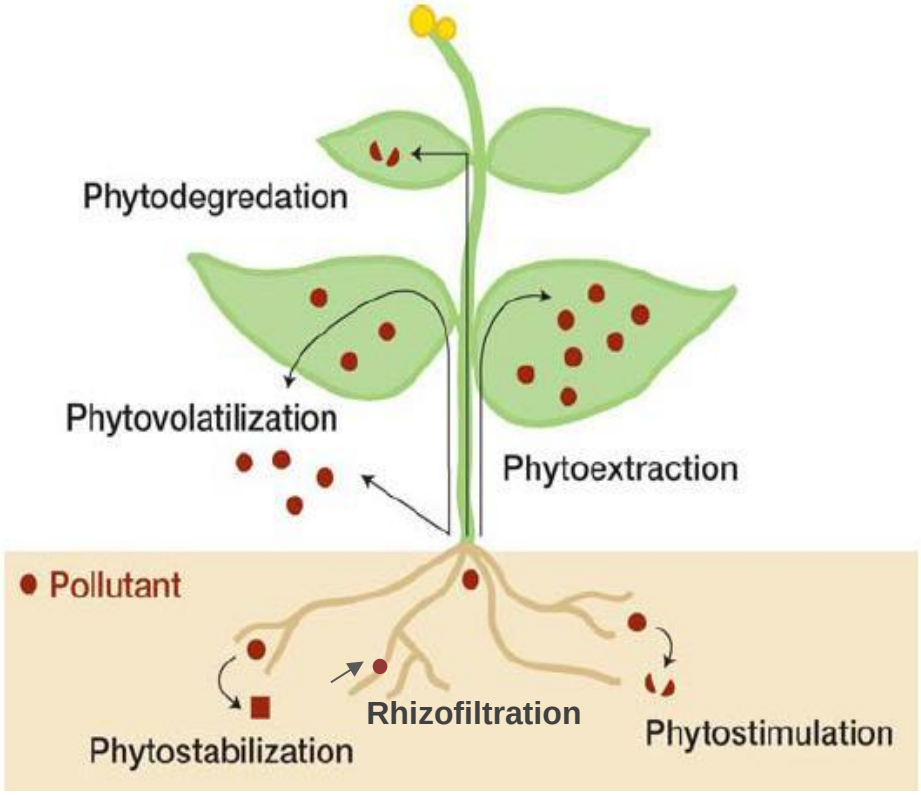


Figure 2.2 Possible fates of pollutants during phytoremediation

Source: Chen, 2010.

2.2.1 Phytoextraction

Contaminants in soil, sediment and sludge are up taken by plant roots and translocate the contaminants in the plant root, stem or leaves. Contaminants are generally removed by harvesting the plants. This technology is safe and rarely does the pollutants that might be exposed to the surrounding environment leak out. This technology is often used to remediate the metal-contaminated soil. Other than the remediation of soil, sediment and sludge; the phytoextraction is also used to treat contaminated water too. The advantage of this technology is that the extracted contaminants from this treatment can be used or recycled for other manufactures. However, each plant variety cannot soak

up all heavy metals. There are only some plant varieties that can up take some specific metals (Cincinnati, 2000).

2.2.2 Rhizofiltration

Rhizofiltration is the absorption into plant roots especially the absorbtion of the contaminants that are in the solution surrounding the root zone. This solution of contaminants might be formed by biotic or abiotic processes in water. Cincinnati (2000) reported that the concentration of metals and their translocation into plant roots occur depending on the contaminant characteristics. Rhizofiltration affects in contaminant containment which the contaminants are immobilized within the plant(??). Then contaminants are removed by physically removing the plant or excavating the contaminated plant for further remediation technologies. Extracted ground water, surface water and waste water can be treated using this technology. Rhizofiltration is generally applicable to low-concentration. This technology does not work well with soil, sediments or sludge because the contaminant needs to be in solution in order to be absorbed by the plant system.

2.2.3 Phytostabilization

Phytostabilization is the process of remediation technology that aims to (i) immobilize the contaminants in soil through absorption and accumulation by roots, absorption on the roots, or precipitation within the root zone of plants, and (ii) to use the plants and plant roots to prevent contaminants migration via wide water erosion, leaching and soil dispersion (Cincinnati, 2000). Phytostabilization occurrs where there are activities of microbiology and chemistry in a soil surround root-zone. The use of CO_2 by bacteria may change the soil pH, metal solubility and the mobility compounds in soil. The plant-affected soil environment convert metals from a soluble to an insoluble oxidation state (Salt et al., 1995). Plants can be used to reduce the erosion of metal contaminated soil. When organic compounds are incorporated into plant lignin; a form of

this process refers to the term phytoligninfication (Cunning et al., 1995b). A similar process, when compounds are incorporated into humic material in soil; this would relate to phytostabilization. Phytostabilization is used in the treatment of soil, sediments and sludge.

2.2.4 Rhizodegradation

Rhizodegradation helps plants to degrade an organic contaminant in the surrounding root zone. The contaminants are degraded by microbial activity that is present in the root zone. Rhizodegradation is also known as plant-assisted degradation, plant-assisted bioremediation, and plant-aided in situ biodegradation and enhanced rhizosphere biodegradation. Root-zone biodegradation is the mechanism for implementing rhizodegradation. The plant root releases many products as the root exudates sugar, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes and other compounds (Shimp et al. 1993; Schnoor et al. 1995a).

These products can increase the microbial populations and organic contaminant biodegradation in soil. Additionally, the rhizosphere substantially increases the surface area where active microbial degradation can be stimulated. Degradation of the exudates can lead to co-metabolism of contaminants in the rhizosphere.

Plant roots can affect soil conditions by increasing soil aeration and moderating moisture content. Therefore, increased microorganism could occur even in the absence of root exudates. The soil pH and contaminants might be also changed through the chemical and physical effects of the exudates (Cincinnati, 2000).

2.2.5 Phytodegradation

Cincinnati (2000) stated that phytodegradation or phytotransfromation is the breakdown of contaminants where contaminant is taken in by plants through metabolic processes within the plant. The contaminant is taken in and metabolized through the

mechanism of the plant. Moreover, degradation may occur outside the plant, due to the release of compounds that cause transformation. If there is any degradation caused by microorganisms associated with or affected by plant roots, this is considered rhizodegradation. Phytodegradation is used in the treatment of soil, sediments, sludge and groundwater. Surface water can also be remediated by using this technology.

2.2.6 Phytovolatilization

Phytovolatilzation is the uptake and transpiration of a contaminant by a plant, with the release of contaminant or a modified form of the contaminant to the atmosphere from the plant through contaminant uptake, plant metabolism and plant transpiration. Phytodegradation is a related phytoremediation process that can occur along with phytovolatilization.

Phytovolatilization has mainly been applied to groundwater, but it can be applied to soil, sediments and sludge. After the contaminant is transformed; the toxicity could be reduced, especially elemental mercury and dimethyl selenite gas. The volatilized contaminant which is released to the atmosphere might be rapidly degraded by photodegradation or the natural degradation processes. However, some unwanted hazardous compounds like trichloroethylene (TCE) might be released in to the atmosphere.

2.3 *Mimosa pudica* L. (Bashful mimosa)

2.3.1 Background of *Mimosa pudica* L.

Mimosa pudica L. is a major weed in 22 crops in 38 countries (Holm et al. 1977). It is a widespread plant in tropical and subtropical areas and also known as an annual precipitation plant which origins from Tropical America. In some Asian countries, *Mimosa pudica* L. is ranked in terms of large distribution to less as Myanmar > Singapore > Indonesia > Malaysia > Philippine > Thailand > Vietnam > Laos >

Cambodia, respectively (Waterhouse, 1994). The scientific classification of *Mimosa pudica* L. is shown below:

Kingdom	***Plantae***
Subkingdom	***Tracheobionta***
Superdivision	***Spermatophyta***
Division	***Magnoliophyta***
Class	***Magnoliopsida***
Subclass	***Rosidae***
Order	***Fabales***
Family	***Fabaceae* or *Mimosaceae***
Genus	***Mimosa***
Species	***Pudica***

Mimosa pudica L. is also known as the "sensitive plant" due to its retraction response when touched or brushed. It has many common names such as bashful mimosa, nimble plant, shame bush, small sensitive plant, touch-me-not, dormidera, humble plant and sleeping grass. *Mimosa pudica* L. is commonly found in waste land and lawns, crops, pastures and roadside. It is a serious weed in Southeast Asia and the Pacific because it appears in areas alongside crops of corn, sorghum, sugarcane, tea, soybean, upland rice, pineapples and cotton. *Mimosa pudica* L. is a plant that can survive with little sunlight; therefore it can also be found in plantation crops, such as rubber, coconuts, bananas, papaya, coffee, oil palm and citrus (Holm et al. 1977).

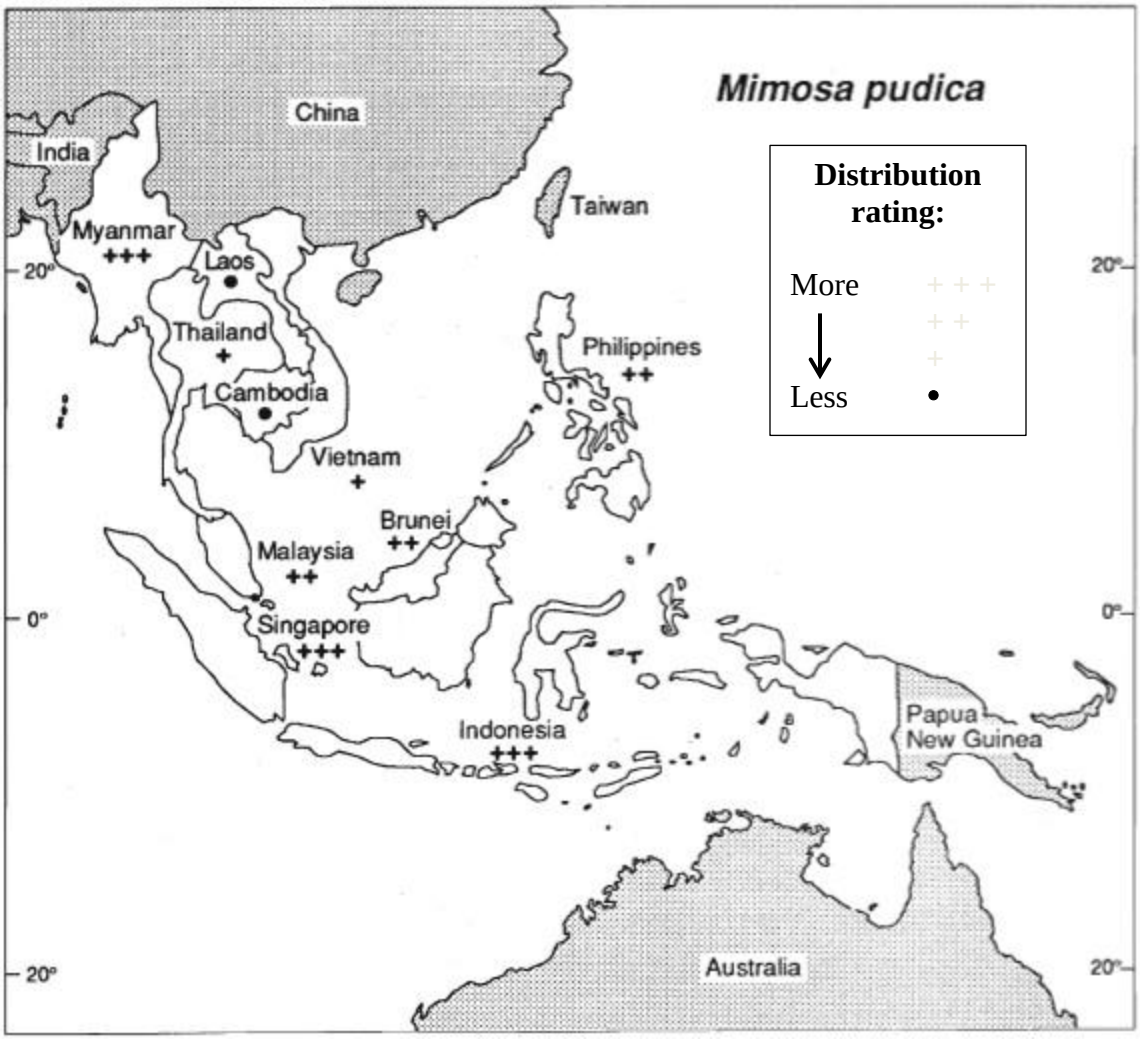


Figure 2.3 *Mimosa pudica* L. in Southeast Asia

Source: Waterhouse, 1994.

2.3.2 Botanical characteristics and chemical constituents of *Mimosa pudica* L.

Mimosa pudica L. is generally a perennial plant that is 15 to 100 cm high and has a six month life cycle. This plant is easily found in all parts of Thailand (Suvannakhun, 2001). It usually appears in open fields, livestock grazing areas, fruit plantation areas, and beside roads. It has brown or purple stems, light green leaves and pink flowers which grow up to form small globular heads. There are dark-red thorns around its stem, and it

has combined leaves which look like fingerprints. The first sub-layer leaf has 2 – 4 pairs (2.5 - 6 cm long), and the secondary-layer leaf has 12 – 25 pairs. The pink-purple colored flower is about 1 cm long and grows up in spherical clusters from the stem.

The peduncle is 2.5 – 3.8 cm long, the secondary flower petals are very small and separated into 4 petals 1.9 – 2.3 mm long (Figures 2.3, 2.4, 2.5). *Mimosa pudica* L. has 4 stamens, the ovary is smooth. The pods are flat, and grow parallel to the edge as globular clusters (Oudhia, 2004). *Mimosa himalayana* and *Mimosa hamata* are two species in the same Genus and look similar to *Mimosa pudica* L. But there are specific parts that can indicate the difference among these species (Table 2.3).

Table 2.3 Botanical differences among the major species of *Mimosa*.

Characters	***M. pudica***	***M. himalayana*** **syn. *M. rubicaulis***	***M. hamata***
Plant	Small woody herbs or low-spreading undershrub with hairy and prickly branches, hairs glandular	A large straggling shrub, studded with straw-coloured, hooked prickels	A much branched, armed shrub, branches downy, with numerous straw-coloured, curced or straight prickles
Leaves	Bipinnate, sensitive to touch, pinnae 1-2 pairs, leaflets 10-20 pairs, linear, glabrous	Bipinnate, main rachis with hooked prickles, pinnae 5-11 pairs, linear-oblong	2-pinante, main rachis pubescent, some timely prickly, leaflets 6-10 pairs
Flowers	Heads small, peduncled, globose, axilalry, pink-purple, Calyx campanulate, Petals crenate towards base	Numerous, in globose heads, peduncles crowded at the ends of branchlets	4-merous in globose heads, peduncles axillary, crowded at the end of branches

Characters	***M. pudica***	***M. himalayana*** **syn. *M. rubicaulis***	***M. hamata***
Pods	1.5-2.5 cm long, closely prickly on the sutures	7-10 cm long, falcate, glabrous, one seeded joints, persistant but not prickly	5-7 cm long, falcate, consisting 4-8 one seeded joints, pubescent
Flowering and Fruiting time	Sept.-March in Indian conditions	August-Sept. and October in Indian conditions	Aug.-Nov. and Dec.-Feb. in Indian conditions

Source: Oudhia, 2004.

This sensitive plant species originated in the tropics and can also be found in all regions of Thailand. It is a weed which can survive in both wet soil and in the open-air. In tropical countries the weed flowers all year and each plant may produce up to 700 seeds.

Mimosa pudica L. is a weed that tolerates toxic elements and commonly found on toxic sites. There are several chemicals contained in *Mimosa pudica* L. such as Ascorbic-acid, crocetin, crocetin-dimethyl-ether, D-glucuronic-acid, D-xylose, linoleic-acid, linolenic-acid, mimosine, mucilage, norepinephrine, oleic-acid, palmitic-acid, sitosterol and stearic-acid.

Figure 2.4 Botanical characteristic of *Mimosa pudica L.*

Source: www.jardins-interieurs.com and www.fotocommunity.de

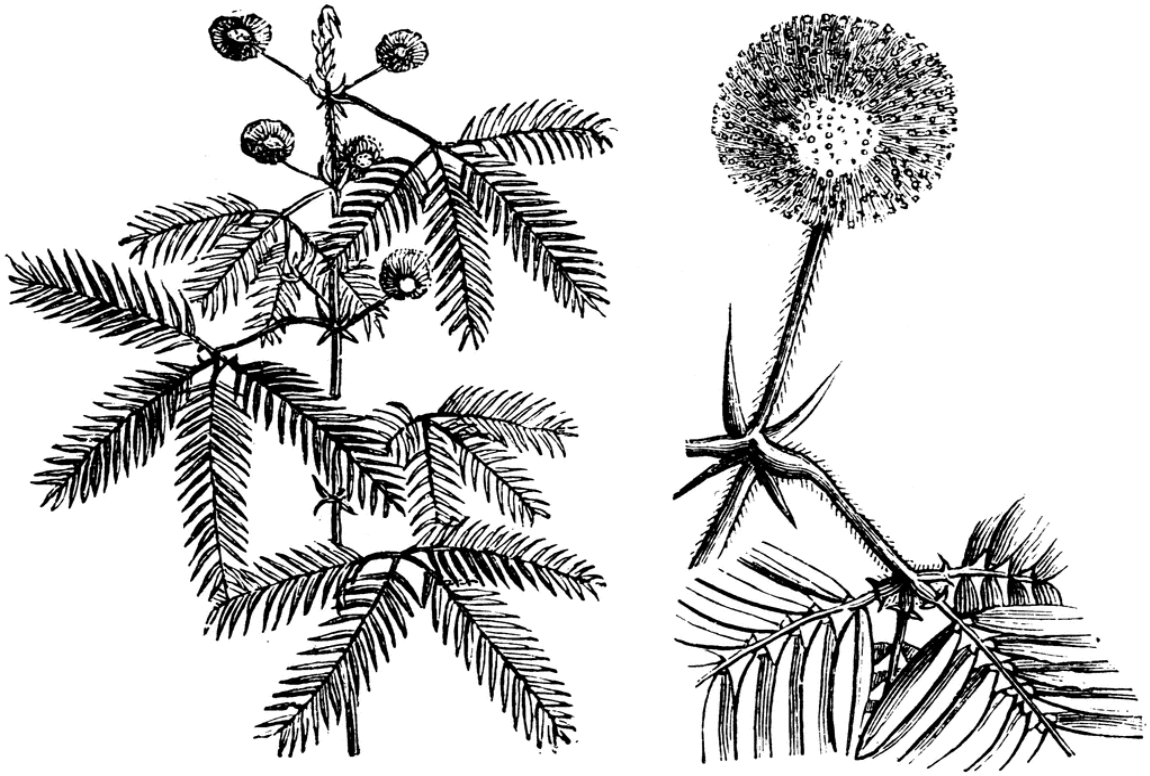


Figure 2.5 Leaves and flower structures

Source: etc.usf.edu.

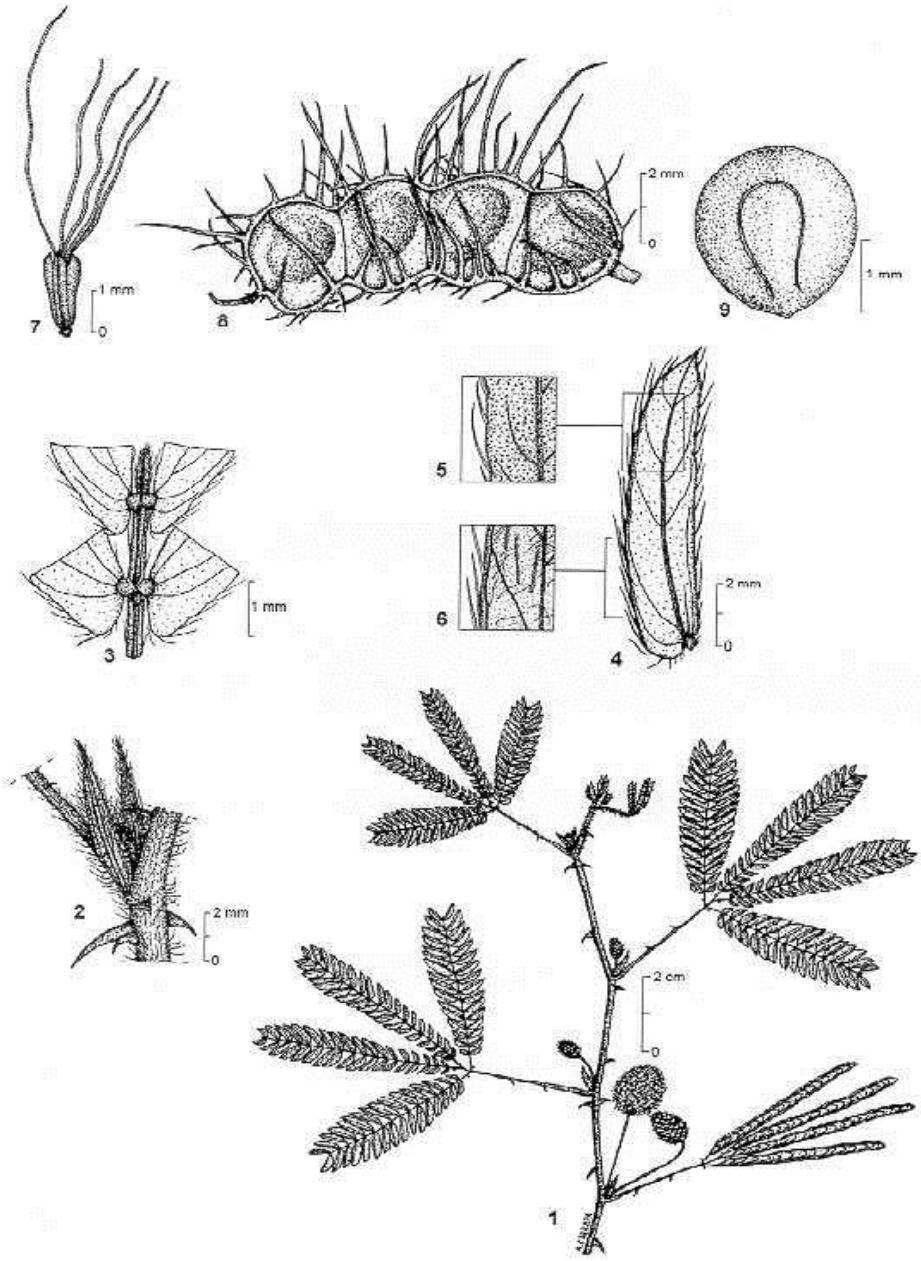


Figure 2.6 Aboveground structures of *Mimosa pudica L.*

Source: malherbologie.cirad.fr.

2.4 Chelating agents

Chelating agents are substances whose molecules can be compiler (?????) with several bonds to a single heavy metal ion. A chelate is composed of a metal ion and a chelating agent. Chelates can be many essential biological chemicals which play important roles in oxygen transport and in photosynthesis. Moreover, chelates are also biological catalysts (enzymes) and are significant for living organisms. The chemicals used to enhance phytoremediation are known as chelating agents (Salt et al., 1998). Only natural phytoremediation results in low quantity removal of contaminant and would take a long time to reach the expected level of remediation. Therefore, chelating agents have been suggested for enhancing ability of cleaning up the contaminated sites.

A combination of metal and a chelating agent is called a chelation that a ring of its compound is consisted metal. Chelating agents are the organic ligands which can react with multiple compounds of heavy metal. If the size of ring in a metal atom is greater than in a chelating agent atom; the reaction between metal and chelating agent would not be occurred or the metal compound tend to be stable. The stability of chelate depends on number of atom in the chelate ring. Mono-dentate ligands have only one coordination atom and they are broken easily from metal compound. But poly-dentate ligands can dominate multiple bonds to the metal ions; therefore, they are more stable than mono-dentate (Tananonchai et al., 2012). Chelating agents can be joined in a central magnesium atom which includes in chlorophyll or plant pigment. Moreover, the application of chelating agents can be also used in chemotherapeutic treatments of metal poisoning by offering a wide range of sequestrates for controlling metal ions in aqueous system.

There are several chelating agents that can enhance contaminant uptake by plants namely nitrilotriacetic acid (NTA), ethylenediaminetriacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), *trans*-1,2cyclohexyleneditrilotetraacetic acid (CDTA), ethylenebis (oxyethylenetrinitrilo) tetraacetic acid (EGTA), citric acid, N-hydroxyethylenediaminetriacetic acid (HEDTA), N-(2-hydroxyethyl) iminodiacetic acid

(HEIDA) and malic acid (Chiu et al., 2005). But these chelating agents have different abilities for enhancing in each specific contaminant and plant.

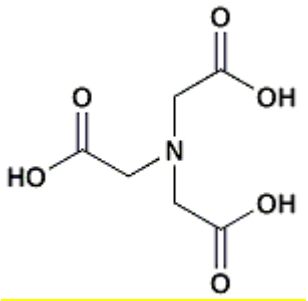
2.4.1 Nitrilotriacetic acid (NTA)

2.4.1.1 The uses and chemical properties of NTA

NTA is a tertiary amino-polycarboxylic acid chelating agent that exists as a white prism body crystalloid powder at room temperature (HSDB, 2009 and NCI, 1977). NTA is soluble in water, deuterated dimethyl sulfoxide and ethanol. It is insoluble in most other organic solvents (National Toxicology Program, 2011). IARC (1990) reported that NTA can react with strong oxidizing compounds of multiple heavy metals. Nitrilotriacetic acid has many commercial applications, but is used primarily as a metal ion chelating agent and as a laundry detergent builder (IARC 1990). NTA is used as a component in detergent instead of phosphate (NCL, 1977). It is generally used as an eluting agent in the purification of rare-earth element, as a boiler feed water additive, in water and textile treatment, in metal plating and cleaning, and in pulp and paper processing (NCL, 1977 and IARC, 1990).

It has also been evaluated as a soil additive in the phytoremediation of heavy-metal-contaminated soil (Evangelou et al., 2007); chelation of the metals with nitrilotriacetic acid mobilizes them for more rapid uptake by plants. The market price of solid NTA in Thailand is around 2,500 Baht per 100 gram bottle. Physical and chemical properties of nitrilotriacetic acid are listed in the following table.

Table 2.4 Physical and chemical properties of NTA

Property	**Information**
Product name	Nitrilitriacetic acid
CAS Registry Number	139-13-9
Molecular formula	$C_6H_9NO_6$
Molecular structure	
Molecular weight	191.14
Water soluble	59 g/L at 25 ºC
Melting point	230 - 235℃

Source: HSDB, 2009 and ChemIDplus, 2009.

2.4.1.2 Decomposition of NTA

Tabatabai (1975) studied the decomposition of NTA in soil by performing analyses for this element and inorganic nitrogen after incubation of NTA-treated soil under aerobic, aerobic and waterlogged conditions at 30˚C for various periods of time. The results illustrated that NTA is readily decomposed by soil micro-organisms. Under aerobic conditions, NTA-nitrogen was converted to nitrate. In additionally, 95-100% of the NTA-nitrogen was changed to the forms of (NO_3^-) after incubation under aerobic conditions. Under aerobic conditions, NTA was estimated to decompose in a timeframe of between 60 - 98 days.

This degradation was encouraged by soil micro-organisms. This study concluded that use of detergents containing NTA may lead to nitrate enrichment of water resources (Tabatabai, 1975). Tiedje and Mason (1971) reported that decomposition of NTA rarely occurred if the soil had an absence of oxygen (O_2) and the degradation rate of NTA in soils should be much slower under waterlogged conditions.

Nitrilotriacetic acid (NTA) and Fe(III)–NTA were determined to biodegrade under the microbial granules. Free NTA was degraded at a specific rate of 0.7 mM $(g\ MLSS)^{-1}\ h^{-1}$, while Fe(III)–NTA degraded at a specific rate of 0.37 mM $(g\ MLSS)^{-1}\ h^{-1}$ (Nancharaiah et al, 2006). The degradation rates of NTA and ferric-NTA were achieved when the microbial metabolism is not constrained by lack of essential elements.

2.4.1.3 Toxicity of NTA

Greenblatt et al. (1974) studied the carcinogenesis and chronic toxicity of NTA in Swiss mice. A carcinogenicity study carried out in mice examined the possible formation of a nitroso compound in rate treated simultaneously with NTA and sodium nitrite ($NaNO_2$). In their study, groups of mice were randomly examined in a drinking water test. NTA was given to 40 male and 40 female mice in a concentration of 5 g/L, no male mice developed lung tumors and the incidence in females was 12%. The incidence of lung tumors in untreated male and female control was 19 and 11% respectively. Addition of $NaNO_2$ (1g/L) to the same level of NTA in the drinking-water produced a tumor incidence of 33% in males and 16% in females.

The increased incidence in animals treated with the NTA/$NaNO_2$ mixture was not significant when the sexes were considered separately. The results of this experiment showed that NTA itself reported no evidence of carcinogenicity, but when it was combined with $NaNO_2$, a little incidence of lung tumor occurred. This was caused by the interaction between these two elements. However, there was no increase of tumor on addition of $NaNO_2$ alone (Greenblatt et al., 1974).

2.4.2 Ethylenediaminetetraacetic acid (EDTA)

2.4.2.1 The uses and chemical properties of EDTA

The abbreviation symbol of Ethylenediaminetetraacetic acid is EDTA. It is a synthetic chelating agent that consists of a poly amino carboxylic acid; it is a colorless and water-soluble solid. EDTA is commercially used in industrial activities such as paper manufacturing, photography, pharmaceutical production and cloth productions. In terms of environmental remediation, EDTA has been used as a chelating agent for heavy metal removal from contaminated soil, water and sediment (Oviedo and Rodriguez, 2003). EDTA is widely used the world over, mainly for industrial and household activities. The market price of solid EDTA in Thailand is around 7,00(7,000??) Baht per 100 gram bottle. The commercial use of EDTA is as shown in the following table.

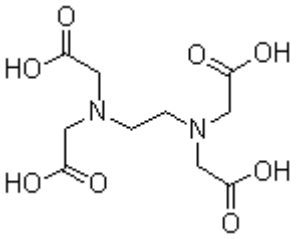
Table 2.5 Industrial and household uses of EDTA and its ligands (as percentages of the world market)

Use	% of world market
Detergents	33
Water treatment	18
Pulp and Paper Industry	13
Photography	5
Metal Cleaning	5
Cosmetics, foodstuffs, pharmaceuticals	5
Agrochemicals	4
Textile Industry	4
Printing inks	3
Concrete admixtures	2
Miscellaneous	12

Source: Oviedo, 2003.

In this case EDTA works as a chelating agent which refers to the process for enhancing the elimination of various trace metals. In addition, EDTA is particularly efficient in dealing with lead, iron, arsenic and mercury poison. Physical and chemical properties of Ethylenediaminetetraacetic acid are listed in the following table.

Table 2.6 Physical and chemical properties of EDTA

Property	Information
Product name	Ethylenediaminetetraacetic acid
CAS Registry Number	60-00-4
Molecular formula	$C_{10}H_{16}N_2O_8$
Molecular structure	
Molecular weight	292.24
Water soluble	0.5 g/L at 25 ºC
Melting point	237-245 ºC

Source: Chemical, 2003 and Maryadele et al., 2001.

2.4.2.2 Decomposition of EDTA

Efficiency of EDDS and EDTA has been studied by Meers et al. (2005). Several concentrations have been determined (0.8, 1.6 and 4 mmol/kg soil). The results from this study illustrated that at a concentration of 0.8 mmol/kg soil did not show a significant difference at 40 days after adding EDTA solution and the half-life of EDTA in soil has been reported at around 36 days (Meer et al., 2005). However, the decomposition of EDTA would be different between whether it is in soil or in water. Ginkel et al. (1999) studied the decomposition of EDTA in water. Na2EDTA at 8 mg/L of water was added in

a closed system with separating pH 6.5 and pH8. The results showed that at 28 days pH 6.5 did not show any change in decomposition of EDTA while pH 8 showed the decomposition of EDTA from 53 – 72%. Then at 49 days the decomposition of EDTA for pH 6.5 was ranged from 60 – 83% and at 35 days pH 8 increased the maximum decomposition to 75 - 89%.

2.4.2.3 Toxicity of EDTA

The Scientific Committee on Toxicity, Ecotoxicity, and the Environment or SCTEE (2003) reported that both EDTA and tetrasodium EDTA are mild skin irritants, but comparatively potent eye irritants. Some EDTA can form a solution that contains a sufficient amount of alkaline which can be hazardous to eyes.

The developmental effect from EDTA occurs if the human body is not properly supplemented with necessary trace metals (Schardein et al, 1981). Many studies reported that in several weeks there was no adverse effect on rats from administering doses up to 5 % of EDTA. Only diarrhea and loss of appetite were reported in animals given 5% disodium EDTA. However, abnormal effects were seen in animals that were fed mineral deficient diets. Abnormal symptoms were observed in male and female rats fed a low mineral diet (0.54% Ca and 0.013% Fe) with the addition of 0%, 0.5%, or 1% disodium EDTA for 205 days.

Rats fed a low percent of disodium EDTA in the diet for short term studies with adequate minerals showed no signs of toxicity. Rats fed 0.5% disodium EDTA for 44-52 weeks did not suffer deleterious effects on weight gain, appetite, activity and appearance. Rats fed 1% disodium EDTA with adequate mineral diet for 220 days showed no evidence of dental erosion. The agency reviewed data of the United State Environmental Protection Agency or USEPA (1979) reported that female rats were administered disodium EDTA in the diet ranging from 2% to 3%, or 3% EDTA plus 1,000 ppm zinc during pregnancy.

2.5 The Synchrotron radiation source

The Siam Photon Source (SPS) at Nakhon Ratchasima, Thailand, consists of three main parts: a 40 MeV linear accelerator, a 1 GeV booster synchrotron and a 1.2 GeV electron storage ring. The X-ray storage ring utilizes a double bend achromatic lattice consisting of four periods with four straight sections for insertion devices. Each period has two bending magnets, four focusing quadruple magnets and four defocusing quadruple magnets. Therefore, at least eight beamlines can be accommodated (Tancharakorn et al., 2012).

The beam size of the focused white beam has been measured to be 100 mm in the horizontal direction using a copper wire of diameter 10 mm. The wire is placed at the sample position where the distance between the poly capillary lens and sample is 22 mm (focal length of the poly capillary lens). The beam size measurement is carried out by measuring the intensity of Cu K_α X-ray fluorescence from the copper wire as a function of its position when scanned horizontally. The scanning step used here is 10 mm $step^{-1}$ and the exposure time is 15 s $step^{-1}$ (Tancharakorn et al., 2012).

The m-SXRF end-station consists of four main components including an X-ray optical system, a visible-light microscope, a motorized sample holder with three degrees of freedom, and an energy-dispersive detection system, as shown in Figure 2.7 below.

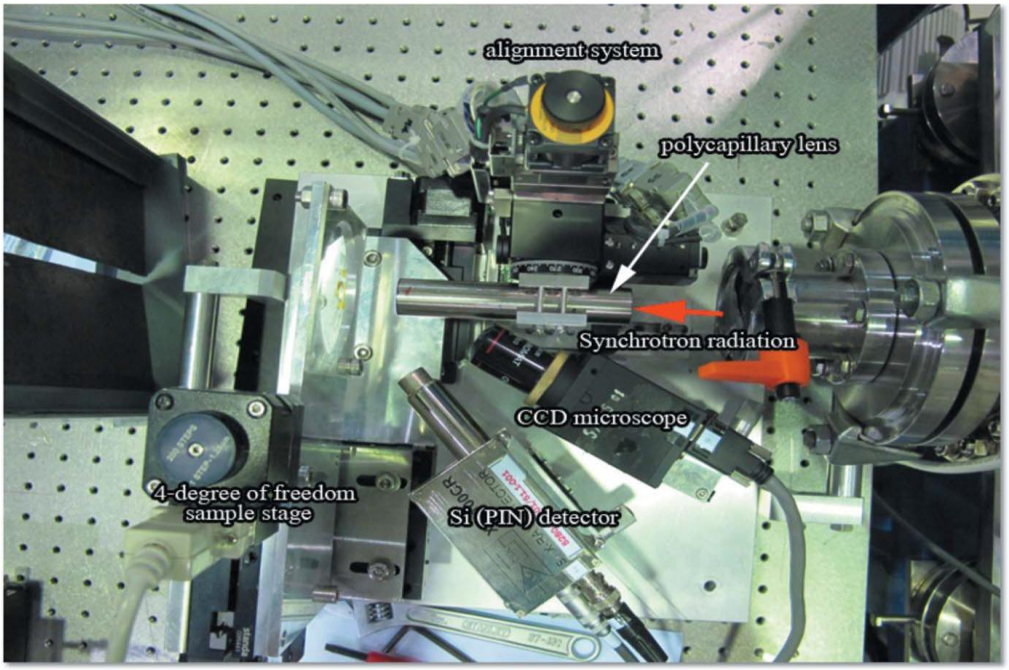


Figure 2.7 The μ-SXRF end-station

Source: Tancharakorn et al., 2012.

2.6 Review of previous studies

Visoottiviseth et al. (2002) studied the arsenic accumulation by local plants at Ron Phibun District, Nakon Si Thammarat Province where arsenic contamination occurs in soil and surface water. They found that *Mimosa pudica* L. is able to tolerate high arsenic soil concentrations and is the most common species on the toxic site. However, its ability to accumulate As was significantly lower than three other plant species (*Pityrogrammacalomelanos, Pterisvittata and Melastomamalabathricum).*

From the 36 plant species investigated, only four species could be considered as possible phytoremediators of arsenic contaminated soils. These were *Pityrogrammacalomelanos* (Silver fern), *Pterisvittata* (Chinese brake fern), *Melastomamalabathricum* (Blackmouth plant) and *Mimosa pudica* L. (Bashful mimosa). Although *Mimosa pudica* L. is ranked fourth as an effective plant for arsenic accumulation, it was selected for this research because it has a short life cycle which was

appropriate for the research period. Their results also showed that the arsenic accumulation in all parts of *Mimosa pudica* L. ranged from 41 – 55 mg kg^{-1}. The arsenic concentrations in top soil at 20-25 cm depth were found at 20-30 mg kg^{-1} and 52-62 mg kg^{-1} soil, respectively in their study.

Chiu et al. (2005) studied nine chelating agents for enhancing the arsenic uptake by *Vetiveriazizanioides* and *Zea mays*. One kilogram of air-dried soil was placed into each pot and the metal solution containing 100 mg/l As ($NaAsO_2$) was also added into each pot for amending the arsenic concentration in the soil. The results showed the efficiency of these chelating agents were in the order of: NTA > HEIDA > HEDTA > Citric acid > EDTA > EGTA > CDTA > DTPA > Malic acid. They also found that NTA mobilizes arsenic in soil more efficiently when compared to the other chelating agents but the application rate of NTA must be over 10 mmol/kg soil or over 95.57 mg/kg soil and it was noted that 20 mmol NTA would maximize arsenic bioavailability.

However, an existence of these chelating agents from phytoremediation doesn't significantly damage the environment, because they can increase in metal bioavailability in soil, surface water and ground water.

Another study conducted by Jirawan (2000) looked at the level of arsenic uptake from $Na_2HAsO_4.7H_2O$ contaminated soil by *Colocasiaesculenta*L. The target plant was grown in 6 different concentrations of contaminated soils (control, 50, 75, 100, 125 and 150 mg kg^{-1} soil) and the results showed that the plant could grow well in all concentrations. The maximum arsenic removal rate was 0.07% or 100 mg/kg soil.

Tambamroong (2002) studied the phytoextraction of arsenic from contaminated soil using *Colocasiaescuentta* L. and Schott (Taro and wild taro). He applied 4 concentrations of disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) as an amendment to arsenic contaminated soil (0, 100, 200 and 400 mg As/kg soil). A chelating agent (EDTA) was also applied to the surface soil (5 mmol kg^{-1}) for 2 weeks. The results showed that both plant species can grow well in concentrations of arsenic at 100 and 200 mg/kg soil. He also found that the arsenic accumulation in the root was more than in other parts of

the plant and that EDTA was an effective chelating agent for enhancing arsenic uptake by plants.

Arsenic accumulation in food crops such as rice is a major concern. Ye investigated phytoremediation of arsenic uptake by rice. *Pterisvittata* was gown in five contaminated paddy soils in pots in an experiment for a 9 month period. The results showed that 3.5 – 11.4% of the total soil arsenic was removed. Rice grown following *P.vittata* had significantly lower arsenic concentrations in straw and grain. This research showed that phytoremediation of arsenic contaminated soils can reduce arsenic uptake by rice (Wen-Ling Ye et al., 2011).

The effect of EDTA and Citric acid (CA) on cadmium uptake by water hyacinth was studied by Kunpapuek et al. (2010). They applied three doses of EDTA and CA (0.5, 1 and 2 mg/L) and grew water hyacinth in cadmium contaminated water for a period of 90 days. The plant samples were collected every 15 days for determining the cadmium level in the plant samples which were separated into two parts of plant samples (stem and leaves) and root. Their results showed that cadmium accumulated in this plant with added EDTA and CA was greater than in control samples; this may indicate that the applications of EDTA and CA have been effected on cadmium uptake by water hyacinth.

They also found that EDTA has more effluence than CA for enhancing cadmium uptake by this plant during the study period of 90 days. The cadmium accumulated in shoots for all treatments were significantly higher than in plant roots ($P \leq 0.05$).

Pojjanaporn et al. (2009) also studied the effect of EDTA and EDDS on phytoextraction of lead from contaminated soil by *Ananas comosus* (L.) Merr (Pineapples). They grew pineapples in a greenhouse for 120 days; after growing the plant for 30 days lead nitrate was added at a concentration 500 millimole per kilogram of soil. Treatments with and without chelates at a concentration of 2 millimole per kilogram soil were also conducted. Plant samples were harvested every 30 days for a 120 day period. From the results it was discovered that at 60 days EDTA affected lead absorption in both the above ground and below ground parts of pineapples at 288.14 and 796.66 milligram

per kilogram of plant respectively. They also found that EDTA has more efficiency than EDDS in the phytoextection of lead after 60 days.

Zimmer et al. (2011) studied the spatial distribution of arsenic and heavy metals in willow roots from contaminated floodplain soil measured by X-ray fluorescence spectroscopy. The willow roots samples were taken from a phytoremediation and cross section and were also mapped for the distribution of As, Ca, Cu, Fe, K, Mn, Ni, S and Zn by synchrotron based X-ray fluorescence spectroscopy. The As was detected by the X-ray fluorescence spectroscopy as shown on Figure 2.8 below and the observed association pattern between As and Fe was explained by the different sorption/desorption properties of As(III) and As(V).

Moreover, As(V) is less desorbed from Fe hydroxides at decreasing redox potentials than As(III) (Kocar et al., 2006; Burnol et al., 2007). Accordingly, As(V) dominated over As(III) (As(V): 71 to 82%) in root plaques. Zimmer et al. (2011) also suggested that willows are especially suited to stabilize low-phytoextractable elements like Cu and As in their roots and rhizosphere. Thus, short rotation coppicing of willows may be a practical approach to mitigate the adverse effects of floodplain soil contamination.

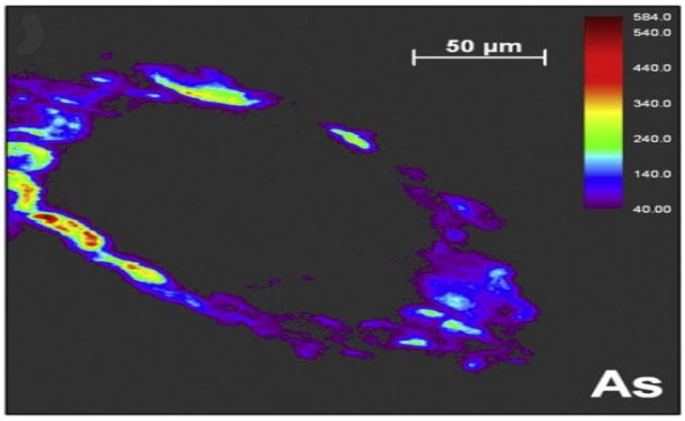


Figure 2.8 Scanning electron micrographs of a cross section of a willow root and X-ray fluorescence maps showing the distribution of As; higher fluorescence intensities (corresponding to higher concentrations) are nearer to red and lower intensities are nearer to lilac according to the color bars.

Source: Zimmer et al., 2011.

CHAPTER III

METHODOLOGY

3.1 Research location

This experimental procedure took place in a nursery on the 2nd floor of building 2, Environmental Research Institute Chulalongkorn University (ERIC). All samples were analyzed in the laboratory on the 3rd floor of ERIC building. This research was conducted from April, 2012 to April, 2013 (Figure C-2 in the appendix C).

3.2 Soil preparation

Uncontaminated soil was used in this experiment. This soil was collected from Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province. It was excavated from the upper layer (0 – 30 cm) of the surface soil. All soil was crushed and dried in the open air before analyzing the soil background as showing in Table 3.1 below:

Table 3.1 Physical and chemical properties of soil used in the experiment

Soil properties	Unit	Methods
pH		1:1 soil/water ratio
Conductivity	µS/cm	1:1 soil/water ratio
ORP	mV	1:1 soil/water ratio
Soil moisture	%	% moisture $= \frac{(\text{wet weight} - \text{dry weight}) \text{x} 100}{\text{wet weight}}$
Organic matter	%	$K_2Cr_2O_7$ digestion
Nitrogen	%	Kjedahl
Phosphorus	mg/kg	Molybdenum blue
Potassium	mg/kg	Digested with Na_2CO_3
CEC	meq/100g	Ammonium acetate

Soil properties	Unit	Methods
Soil texture:		Hydrometer and Synchronic methods
- Sand	%	
- Silt	%	
- Clay	%	
Arsenic (As)	mg/kg	US EPA-3052 and AAS

3.3 Plant preparation

Mimosa pudica L. was excavated from uncontaminated soil in Prawet District, Bangkok, Thailand. All plants were preliminary grown at an equal size for two weeks before transferring them into the real experiment pots. After the preliminary growth, three plant samples were selected and prepared for analyzing the arsenic accumulation in plants. The United States Environment Protection Agency (USEPA), method 3052 (USEPA, 1996) and Atomic Absorption Spectrometry (AAS) with hydride analysis were used to prepare and analyze arsenic in plants. These plants are represented to the background of the plants used in this experiment. After analysis, arsenic was not detected; the detection limit of AAS with hydride analysis for arsenic is less than 0.01 ppm (AAS hydride, 1987). Therefore, we can surmise that these plants were uncontaminated with arsenic.

3.4 Experimental design

This experiment was divided into two stages: preliminary study and experimental procedure. All the pots were randomly placed in a nursery by using the Completely Randomized Design (CRD) method. This aims to ensure that each plant could have exposure to available sunlight, air flow, temperature fluctuations, and other environmental factors equally.

3.4.1 Preliminary studies

The purpose of this study is to investigate the tolerance of *Mimosa pudica* L. growing in different concentrations of arsenic. Moreover, the phytotoxicity from an addition of two chelating agents (NTA and EDTA) was determined too.

The tolerance of plant study, *Mimosa pudica* L. was separately grown in ten soil pots (5 kg of soil per pot) during one month. The prepared plants were grown in each pot (one seedling per pot) with added disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) at different concentrations of arsenic (5, 10, 20, 40, 80, 120, 160, 200, 300 and 400 mg/kg soil). All the pots were watered daily by tap water. The growth properties of the plants were also recorded. Then a dose of arsenic which has no negative impact on the growth of the plant was selected for further study.

Other preliminary study is the phytotoxicities from an addition of chelating agents. In this study, twelve pots (5 kg of soil per pot) were prepared for growing *Mimosa pudica* L. All pots were taken care of and the phytotoxicities were recorded during one month period. The doses of arsenic and chelating agents were varied as follows:

- 6 pots: with adding a dose of arsenic (selected from the beginning of the study) and 3 doses (50, 100 and 200 mg/kg soil) of NTA or EDTA, separately.
- Other 6 pots: without arsenic, only add 3 doses (50, 100 and 200 mg/kg soil) of NTA or EDTA, separately.

3.4.2 Experimental procedure

For the main experiment, uncontaminated soil of 5 kg soil per pot was amended by the solution of disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) at a concentration of arsenic at 5 mg As/kg soil. This concentration of arsenic was selected because the preliminary studies showed that plants can grow healthily and there was no negative effect in plant growth in the concentrations of arsenic up to 10 mg As/kg soil. In order to save the cost, at a concentration of 5 mg As/kg soil was used in this experiment.

Then this prepared soil was left for three months; this aims to mix the arsenic and soil together in order to create similar conditions as contaminated soil in nature. The experiment was separated into 3 sets: Control, NTA, and EDTA sets as follows:

- Experimental set 1: Control (12 pots), without adding NTA or EDTA.
- Experimental set 2: NTA (36 pots), adding 3 doses of Nitrilotriacetic acid (NTA), [$C_6H_9NO_6$] at 50, 100 and 200 mg/kg soil.
- Experimental set 3: EDTA (36 pots), adding 3 doses of Ethylenediaminetetraacetic acid (EDTA), [$C_{10}H_{16}N_2O_8$] at 50, 100 and 200 mg/kg soil.

After that one seedling of *Mimosa pudica* L. per pot was grown in this arsenic contaminated soil. One week later three doses of NTA and EDTA (50, 100 and 200 mg/kg soil) were also added into each soil pot separately. All plants were planted in plastic bags (12 x 20 cm); each bag contained 5 kg of soil and plants were watered by tap water daily. External plastic bags which were bigger in size were used in order to prevent the leached water from leaking. This leached water was returned to water the plant using plastic rotary hand pumps (Figure C-4 in the appendix C). This aims to control the leaching of arsenic to the outside environment.

Additionally, the pH of NTA and EDTA were measured before adding to the soil. In total, 84 pots were prepared for this experiment allowing for 3 replications per one indicator sample collection. These plants were grown for 4 months.

3.5 Sample collection and analysis

Soil and plant samples were collected at 0, 30, 60, 90 and 120 days after planting (Table 4). The plant samples were separated into 2 parts of the plant: underground sample (root) and aboveground sample (stem and leaves). Each part of the plant and soil samples were prepared and analyzed for arsenic concentration using USEPA method 3052 (USEPA, 1996) and Atomic Absorption Spectrometry (ASS) analysis. The properties of

soil like pH, Conductivity and Oxidation Reduction Potential (ORP) were also measured each time of sample collection.

Second plant samples or, one sample per set, were separately collected for determining the distribution of arsenic and other related elements inside the plant using Synchrotron Radiation method BL6b [Micro–X–ray Fluorescence (μ-XRF) and X-ray Powder Diffraction (XPRD) (Synchrotron Light Research Institute, 2011).

Table 3.2 Samples collection time

Experimental sets	Time (day)				
	00	30	60	90	120
Control	22 Sep 2012	21 Oct 2012	20 Nov 2012	20 Dec 2012	19 Jan 2013
NTA (50, 100 and 200 mg/kg)	22 Sep 2012	21 Oct 2012	20 Nov 2012	20 Dec 2012	19 Jan 2013
EDTA (50, 100 and 200 mg/kg)	22 Sep 2012	21 Oct 2012	20 Nov 2012	20 Dec 2012	19 Jan 2013

3.6 Soil samples preparation and analysis

After the plant was taken out from the pot, the soil was mixed together and the soil sample was collected from different points of the sample around 4 - 6 points; this aims to make sure that the soil sample can represent that soil property. Around 100 g of soil was collected from each pot and stored in zip lock bags. Then every sample was separated into 2 partitions for analyzing (1) the concentration of arsenic in soil and (2) the soil properties. The soil samples were analyzed arsenic concentration using USEPA method 3052 (USEPA, 1996). The soil preparation was oven dried at 103˚C for 2 – 3 days to obtain a constant condition and measured for dry weight of soil.

After that all soil samples were crushed and passed through a 2 mm sieve. After that 0.5 g of each soil sample was taken and added with HCl (Hydrochloric acid) and HNO_3 (Nitric acid) at 9 ml and 3 ml, respectively. Then deionized water was added into

each prepared sample to achieve 50 ml. These samples were preserved at 4˚C and the arsenic concentration was determined using Atomic Absorption Spectrometry (AAS). The second half of each sample was dried in open air conditions for 2 -3 days and analyzed for pH, ORP and conductivity in soil.

3.7 Plant samples preparation and analysis

All plant samples were also collected at 0, 30, 60, 90 and 120 days the same as the soil collection. These plant samples were separated into three analyses as follows:

3.7.1 Relative growth rate (RGR) analysis

The relative growth rate was calculated for quantifying the speed of plant growth. It was measured as the mass increase per aboveground biomass per day. RGR was calculated using the following equation:

$$\mathbf{RGR = [Ln\ (W_2) - Ln\ (W_1)] / (t_2 - t_1)} \qquad \mathbf{(3.1)}$$

- Relative growth rate [RGR], (gram per day)
- Natural logarithm [Ln]
- Dry weight of plant at time one [W_1], (in grams)
- Dry weight of plant at time two [W_2], (in grams)
- Time one [t_1], (in days)
- Time two [t_2], (in days)

3.7.2 Arsenic concentration analysis

For the first plant samples, after the plants were taken out from each pot, they were washed clean by tap water twice to remove soil particles and ensure that outside plant samples were not contaminated with heavy metal from soil. All plant symptoms or phytotoxicities (if any) were recorded before transfer to next step of analysis. The samples were rinsed by deionized water and separated into 2 parts namely the

underground sample (root) and aboveground sample (stem and leaves). The plant samples were prepared using the USEPA method 3052 (USEPA, 1996).

All these samples were air dried at room temperature for 2 – 3 hours before measuring the wet weight. Then all plant samples were oven dried at 70˚C for 2 – 3 days and weighed for dry weight. After that each plant sample was digested by adding HNO_3 (Nitric acid) 9 ml and H_2O_2 (Hydrogen peroxide) 1 ml, respectively. Then deionized water was added to achieve the solution sample of 25 ml. These prepared samples were stored at 4˚C until analysis. Finally, the arsenic concentration in the samples was analyzed using Atomic Absorption Spectrometry (AAS) analysis.

3.7.3 The distribution of arsenic and other elements inside the plant using the Synchrotron Radiation analysis

For the second plant samples, a plant sample was collected from each set two times (after 30 and 120 days). These samples were washed by tap water twice followed by deionized water once. Then each sample was dried in the open air and packed into white papers. The plant samples were put in a plant press and tied (Herbarium); these prepared samples were preserved in this Herbarium (Figure C-7 in the appendix C) and placed in room temperature until analysis. Finally, the distribution of arsenic and other related elements inside the plant samples were analyzed using the Synchrotron Radiation method BL6b (Synchrotron Light Research Institute, 2011).

3.8 Statistical analysis

The data from 3 replications and 4 harvesting times was analyzed using the Statistical Package for the Social Sciences (SPSS). The arsenic concentration in soil and arsenic accumulation in each part of the plant were compared by analysis of variance (One-way ANOVA) under Duncan's new Multiple Range Test (DMRT) pathway. All of the statistical analysis was calculated using the 95% confidence level ($P \leq 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Soil used in the experiment

The soil used in this experiment is silt clay; it has organic matter around 2.50 % and soil pH at 6.71. Generally, these soil properties are appropriate for plant growth (David et al., 2011). The concentration of arsenic in soil was detected under 0.01 mg/kg soil (Table 4.1).

Table 4.1 Physical and chemical properties of soil before using in the experiment

Soil properties	**Unit**	**Value**
pH		6.71
Conductivity	µS/cm	583
ORP	mV	195.60
Soil moisture	%	2.97
Organic matter	%	2.50
Nitrogen	%	0.13
Phosphorus	mg/kg	93.00
Potassium	mg/kg	430.00
CEC	meq/100g	19.71
Soil texture:		Silt clay
- Sand	%	16.3
- Silt	%	46.5
- Clay	%	37.2
Arsenic (As)	mg/kg	< 0.01

4.2 Tolerance of *Mimosa pudica* L. on difference concentrations of arsenic and two chelating agents (NTA and EDTA)

These studies aimed to investigate the tolerance and phytotoxicity of *Mimosa pudica* L. to differently applied concentrations of arsenic and the two chelating agents (NTA and EDTA). First plants were grown on arsenic contaminated soils which were amended by Disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) at ten different concentrations of arsenic (5, 10, 20, 40, 80, 120, 160, 200, 300 and 400 mg/kg soil).

After growing plants for one month, the result of this preliminary study showed that plants can grow healthily in arsenic concentrations up to 10 mg/kg soil while other upper concentrations showed phytotoxicities in plant growth. The symptoms of the plants occurred differently such as dry and curly leaves and stem; finally they died from getting too high concentrations of arsenic (Figure 4.1). Holm et al. (1977) also reported that *Mimosa pudica* L. is a plant that can survive in conditions with low levels of sunlight; therefore, *Mimosa pudica* L. can be alternative arsenic tolerate plant for phytoremediation and is also able to clean up the low arsenic contamination in agricultural soil. The standard of arsenic for residential and agricultural soil in Thailand is only 3.9 mg As/kg soil (PCD of Thailand, 2004).

Although *Mimosa pudica* L. can grow well in soil arsenic up to 10 mg As/kg soil under conditions of no application any fertilizer and grow in the control nursery; in order to save cost only 5 mg As/kg soil was selected for future study. Then the NTA and EDTA were also studied by growing plants on the prepared soils with 5 mg As/kg soil and without arsenic by amending three doses of NTA and EDTA at 50, 100 and 200 mg/kg soil separately. After monitoring for one month, we found that three doses NTA or EDTA did not show any phytotoxicity in plant growth and that plants still grow well in these amended soils. Moreover, an existence of these chelating agents in soil doesn't significantly damage the environment of the surrounding soil habitat, because they can increase metal bioavailability in soil (Chiu et al., 2005). NTA is readily decomposed by soil micro-organisms (Tabatabai, 1975). Meer et al. (2005) also reported that the decomposition of EDTA would be different between the soil and in water phase.

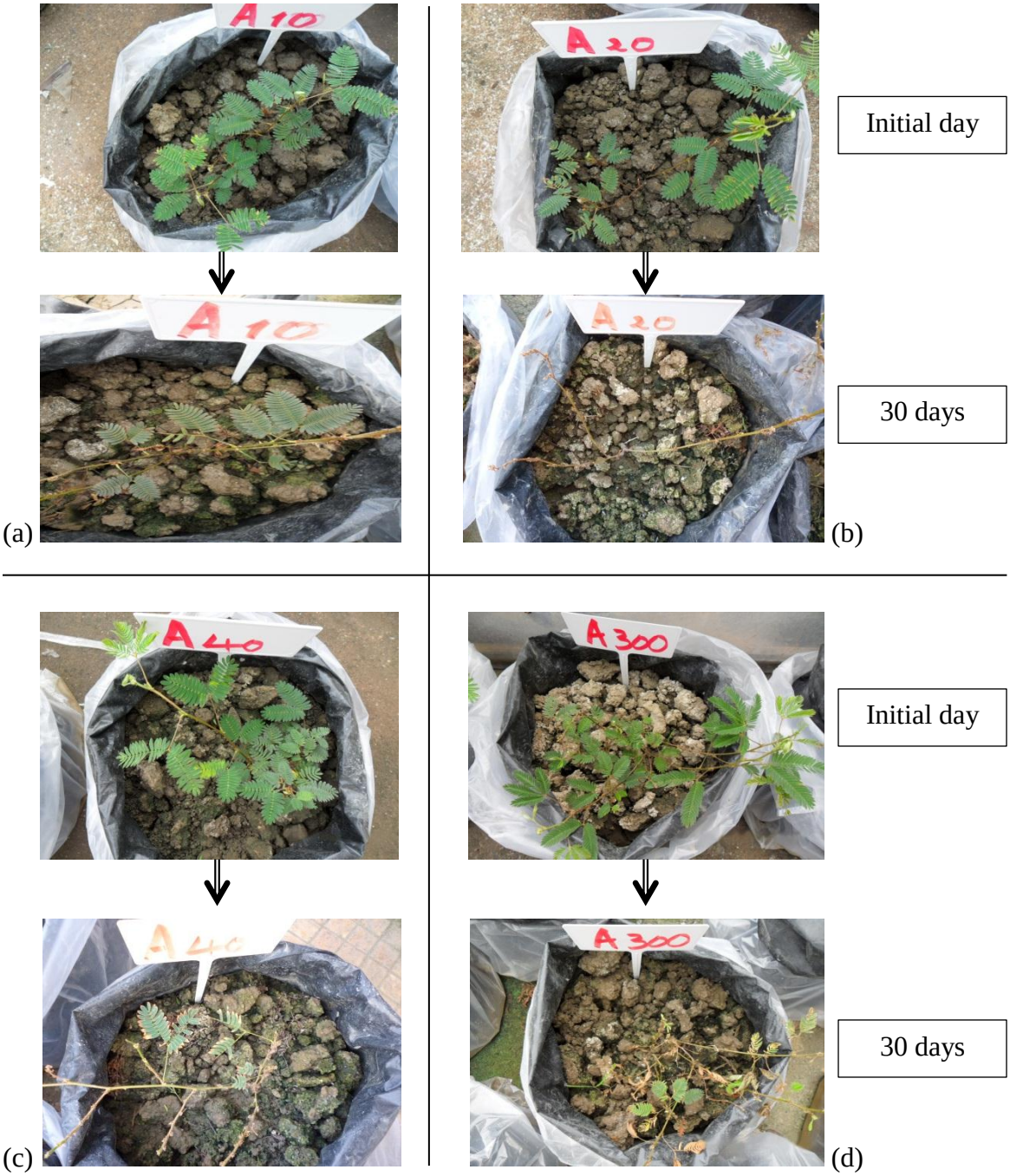


Figure 4.1 The symptoms of *Mimosa pudica* L. from getting too high concentrations of amended soil arsenic during one month period (a = 10 mg As/kg soil, b = 20 mg As/kg soil, c = 40 mg As/kg soil and d = 300 mg As/kg soil)

4.3 The growth rate of *Mimosa pudica* L. on different application doses of NTA and EDTA in arsenic contaminated soil.

The relative growth rates of plants for all treatments during four months ranged from 0.020 - 0.051 gram/day (Figure 4.3). Overall, the growth rates of plants decreased while the numbers of days or times increased. The growth rates in the initial period (30 days) showed the highest amount for all treatments, but the values are not so different. The highest growth rate was occurred in a pot with EDTA 50 mg/kg with the value at 0.051 gram/day; whereas the lowest growth rate appeared in the treatments at 120 days with the value around 0.020 gram/day. Some plant samples from each harvesting time are shown in Figure 4.2 below:

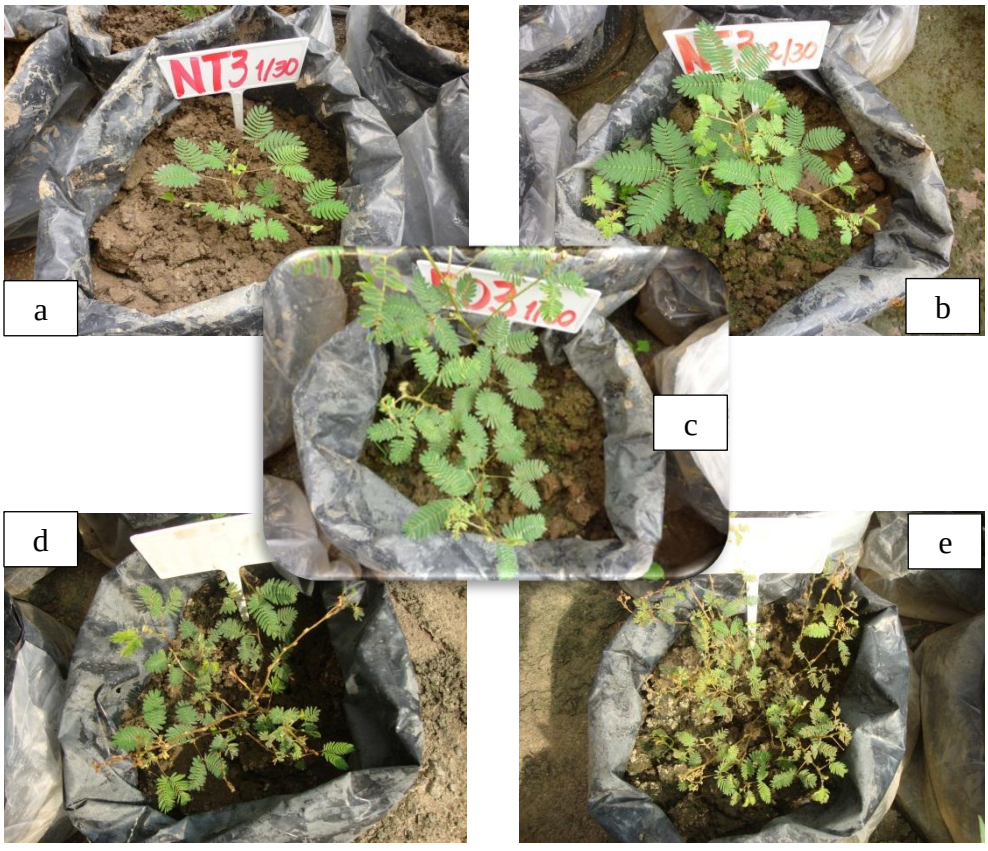


Figure 4.2 The difference of plant growth at each harvesting time (a = 00 day, b = 30 days, c = 60 days, d = 90 days and e = 120 days)

Moreover, the highest growth rates in 60, 90 and 120 days are 0.031 gram/days (Pots EDTA 50 mg/kg and EDTA 200 mg/kg), 0.025 gram/day (Pot control) and 0.021 gram/day (Pots control and NTA 50 mg/kg) respectively (Figure 4.3). After 120 days, plants showed the lowest growth rates when compared to other harvesting times; this might be caused by lack of nutrients in the plants or a lack of organic matter for growing because there was no addition of any fertilizer in this experiment. Therefore when the harvesting time increased; the organic matter in soil decreased.

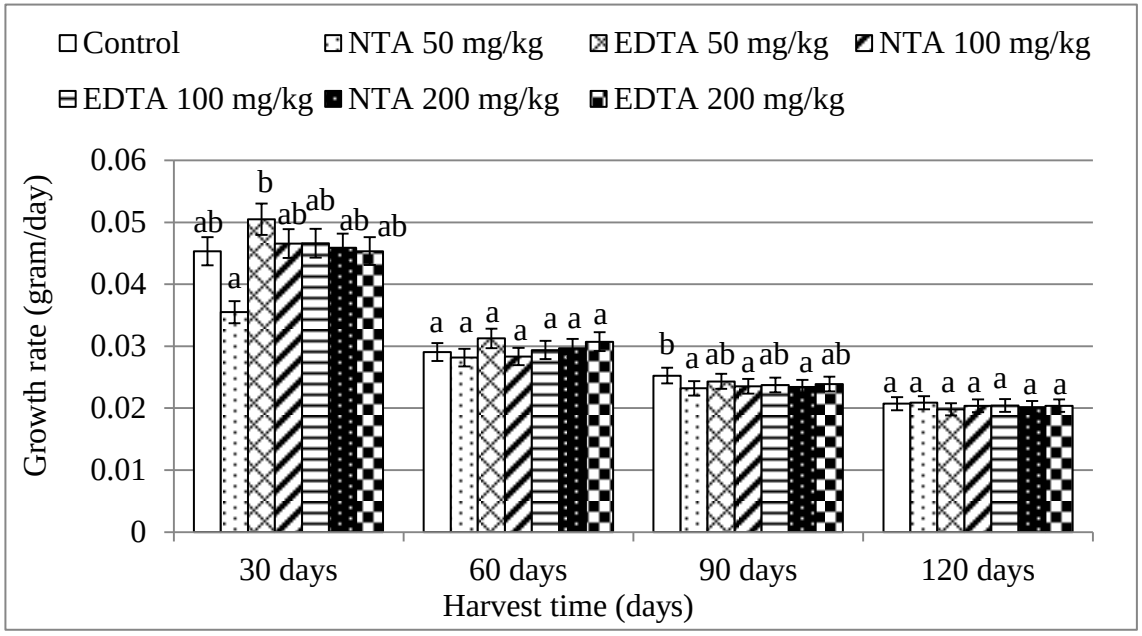


Figure 4.3 Average relative growth rates of plants on different application doses of NTA and EDTA over period of time.

(Note: The same letter next to the bars means there is no significant difference ($P \leq 0.05$) when compared to the mean values of different treatments at the same harvesting time)

4.4 The pH, ORP and electrical conductivity in soil

The pH values in soil of all treatments during four months did not change much; they were in the range of 7.09 - 7.36 (Table 4.2). Overall, the soil pH values of all treatments increased when the time increased and the pH values of the control treatments are higher than the values of other treatments, this might be because the control treatments did not contain any added chelating agent which could low the pH in soil. The pH values in the initial period (30 days) are lower than the pH values in the later periods (60, 90 and 120 days, respectively) because the applied chelating agents would be well dissolved in soil and make the pH increase. The different varieties of arsenic occur more in the soil pH range from 2 to 7 (Nriagu, 1994). In general, the pH values of the treatments with added NTA is a little more acidic than EDTA. The pH values increased when increasing the concentrations of both NTA and EDTA in soil (Table 4.2).

The oxidation-reduction potential (ORP) values in soil for each harvesting time from 30 – 120 days were not significant different between application of NTA and EDTA. Table 4.3 showed the ORP values in soil were in the range of 157.6 – 216.73 mV. The highest ORP values was found in the set EDTA 200 mg/kg in initial harvesting time (30 days) while the lowest ORP values appeared in the set control at 90 days. The decomposition of organic matter in soil oxidation reactions is most important to produce electrons and then oxygen in soil works as an electron accepter (Wiwatwongwana, 2003).

Within the same chelating agent, the ORP values in soil at 30 days for sets NTA 50, 100 and 200 mg/kg were at 182, 190.43 and 215.8 mV respectively and then at 120 days these ORP values changed to 181.5, 168.33 and 167.47 mV respectively. Whereas, the ORP values for sets EDTA 50, 100 and 200 mg/kg showed the values in initial time (30 days) at 216.73, 197.9 and 195.37 mV respectively; at the end at 120 days the ORP values became at 184.7, 185.6 and 171.07 mV respectively. In comparison with control sets, the ORP values with application of NTA and with EDTA were not significantly different on the change of ORP values in soil for along four harvesting times (30, 60, 90 and 120 days). The ORP values of control sets were also lower than other adding chelating agent sets.

Table 4.2 The soil pH during the experimental period

Treatments	pH in soil			
	30 days	60 days	90 days	120 days
Control	7.27 ± 0.07	7.25 ± 0.05	7.36 ± 0.04	7.35 ± 0.09
NTA 50 mg/kg	7.14 ± 0.18	7.19 ± 0.06	7.29 ± 0.03	7.28 ± 0.07
NTA 100 mg/kg	7.14 ± 0.01	7.15 ± 0.08	7.22 ± 0.03	7.27 ± 0.12
NTA 200 mg/kg	7.09 ± 0.05	7.2 ± 0.10	7.17 ± 0.14	7.26 ± 0.10
EDTA 50 mg/kg	7.22 ± 0.09	7.22 ± 0.11	7.33 ± 0.12	7.35 ± 0.10
EDTA 100 mg/kg	7.21 ± 0.10	7.21 ± 0.17	7.3 ± 0.16	7.32 ± 0.04
EDTA 200 mg/kg	7.17 ± 0.13	7.19 ± 0.02	7.31 ± 0.03	7.3 ± 0.10

Table 4.3 Oxidation-reduction potential in soil

Treatments	Oxidation-reduction potential in soil (mV)			
	30 days	60 days	90 days	120 days
Control	181.87 ± 0.46	173.93 ± 0.76	157.6 ± 0.26	166 ± 1.05
NTA 50 mg/kg	182 ± 1.14	174.67 ± 0.23	167.13 ± 0.60	181.53 ± 0.91
NTA 100 mg/kg	190.43 ± 1.10	179.4 ± 0.29	170.1 ± 1.49	168.33 ± 1.43
NTA 200 mg/kg	215.8 ± 1.28	177.77 ± 1.19	161.63 ± 1.29	167.47 ± 0.72
EDTA 50 mg/kg	216.73 ± 0.65	183.97 ± 0.61	175.3 ± 0.52	184.7 ± 0.97
EDTA 100 mg/kg	197.9 ± 0.36	178.97 ± 1.80	175.37 ± 0.85	185.6 ± 0.61
EDTA 200 mg/kg	195.37 ± 0.71	176.4 ± 1.31	169.83 ± 0.20	171.07 ± 0.87

Table 4.4 illustrated that the electrical conductivity (EC) values in soil for all treatments ranged from 793.00 – 879.33 µS/cm. The electrical conductivity value of the control set at 90 days dropped to 809.00 µS/cm while the other control sets had harvesting times of 30, 60 and 120 days and showed the values at 836.67, 845.33 and 837.67 µS/cm. In addition, the EC values of set NTA 50 mg/kg increased from 800.33 – 879.33 µS/cm at harvesting times of 30 to 90 days respectively while at the EC value at

120 days was dropped to 835.33 µS/cm (See table 4.4). Likewise, the EC values of NTA 100 mg/kg also increased from 793.00 – 872.33 µS/cm at times 60 – 90 days. The EC values of other treatments were not changed much. In comparison with the control sets, the electrical conductivity values in soil during four months of our experimental period were not significant affected by NTA and EDTA.

Table 4.4 Electrical conductivity in soil

Treatments	Electrical conductivity in soil (µS/cm)			
	30 days	60 days	90 days	120 days
Control	836.67 ± 1.53	845.33 ± 2.08	809.00 ± 2.00	837.67 ± 2.89
NTA 50 mg/kg	800.33 ± 3.21	870.67 ± 6.66	879.33 ± 4.51	835.33 ± 3.06
NTA 100 mg/kg	855.33 ± 1.53	793.00 ± 4.58	872.33 ± 6.81	819.67 ± 2.08
NTA 200 mg/kg	810.00 ± 3.00	875.67 ± 4.93	828.67 ± 3.79	808.33 ± 3.51
EDTA 50 mg/kg	846.67 ± 1.53	847.67 ± 5.13	827.67 ± 2.08	862.33 ± 6.11
EDTA 100 mg/kg	839.67 ± 5.13	829.33 ± 5.13	795.33 ± 6.51	823.67 ± 1.53
EDTA 200 mg/kg	819.33 ± 4.51	795.33 ± 3.51	821.67 ± 4.93	803.33 ± 5.51

4.5 Arsenic accumulation in underground part (root) and aboveground parts (Stem and leaves) of *Mimosa pudica* L.

Figure 4.4 showed that the average arsenic accumulations in the underground part (root) of *Mimosa pudica* L. were in the range of 2.01 – 29.71 mg As/kg plant. Overall, the concentrations of arsenic accumulation in the plant root increased depending on the time. When the numbers of days increased from 30 to 60, 90 or 120 days, the concentrations of arsenic in root of *Mimosa pudica* L. also increased. At 120 days, the experimental sets EDTA 100 mg/kg showed the highest arsenic accumulation in the root with the concentration at 29.71 mg As/kg plant and followed by sets EDTA 50 mg/kg with the concentration at 27.93 mg/kg plant; but from the statistical analysis the As accumulations of these two sets are not significantly different ($P \leq 0.05$). The EDTA 50

mg /kg at 90 days also showed the second highest As accumulation in the plant root with concentrations at 25.88 mg As/kg plant. In addition, the lowest arsenic accumulation in the root of the plant during our experimental period of four months occurred in the control set at 30 days at the value of 2.01 mg As/kg plant.

In comparison among these two chelating agents with the same doses added, EDTA showed to be more effective than NTA for enhancing arsenic accumulation in root of *Mimosa pudica* L. except at the dose 200 mg/kg. EDTA was an effective chelating agent for enhancing arsenic uptake by plants (Tambamroong, 2002). The highest arsenic accumulation in plant roots of NTA application sets was only 21.17 mg As/kg plant (Set NTA 50 mg/kg at 120 days) and followed by set NTA 200 mg/kg plant at 90 days with the concentration of 13.37 mg/kg plant (Figure 4.4). Moreover, the sets with added chelating agents also reported significantly higher concentrations of arsenic accumulation in the root than the sets without chelating agents (Control sets).

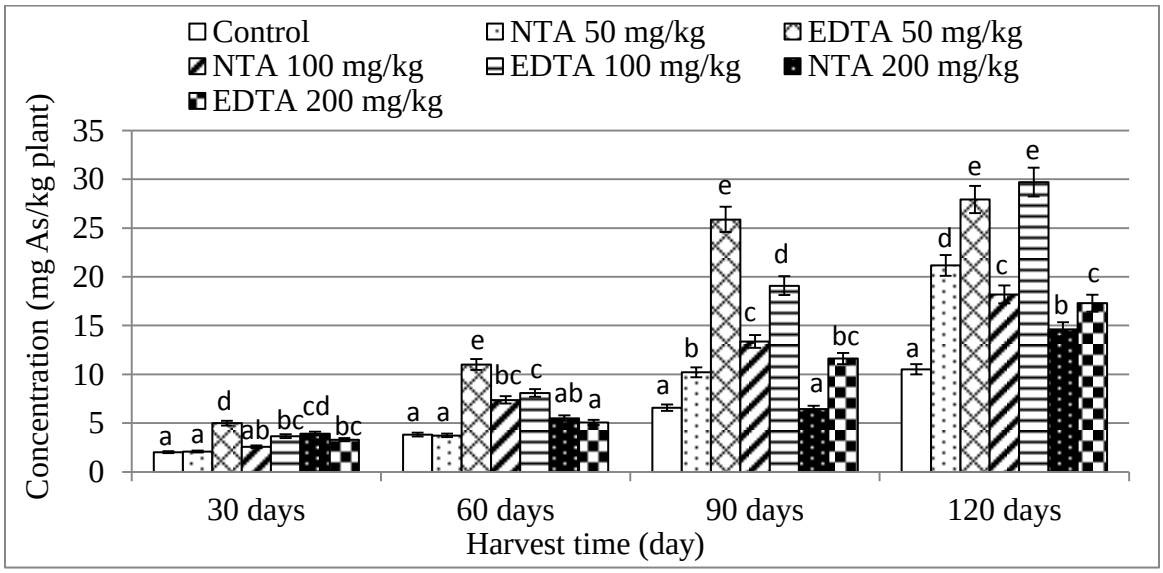


Figure 4.4 Average arsenic accumulations in underground part (root) of plant (mg As/kg plant)

(Note: The same letter above the bars means there is no significant difference ($P \leq 0.05$) when the mean values of different treatments at the same harvesting time are compared)

The average arsenic accumulations in the aboveground parts (stem and leaves) of *Mimosa pudica* L. reported very small concentrations. There is no significant difference ($P \leq 0.05$) between arsenic accumulations in the combination of stem and leaves of the sets EDTA 50 mg/kg and EDTA 100 mg/kg at 120 days; these two sets also showed that the highest As accumulation in the aboveground part of the plant with the concentration at 6.24 and 6.32 mg As/kg plant respectively (See Figure 4.5). The lowest arsenic accumulation in the stem and leaves was in the set that did not contain NTA and EDTA (control set at 30 days) with the concentration at 0.70 mg As/kg.

Overall, the arsenic accumulations in stem and leaves for the sets with added EDTA were higher than with the sets that had NTA added. The sets EDTA 100 mg/kg, EDTA 200 mg/kg (At 90 days), NTA 100 mg/kg and EDTA 200 mg/kg (At 120 days) have a similar ability to stimulate arsenic accumulation in the stem and leaves of plants with the concentrations at 4.35, 4.38, 4.43 and 4.50 mg As/kg plant respectively. Moreover, at 30 and 120 days the set with EDTA 100 mg/kg reported the highest arsenic accumulation in aboveground parts of plant with the concentrations at 1.82 and 6.32 mg As/kg respectively while at 60 and 90 days the highest ability to remove arsenic from soil occurred in the set with EDTA 50 mg/kg with concentrations at 3.23 and 5.16 mg As/kg plant respectively.

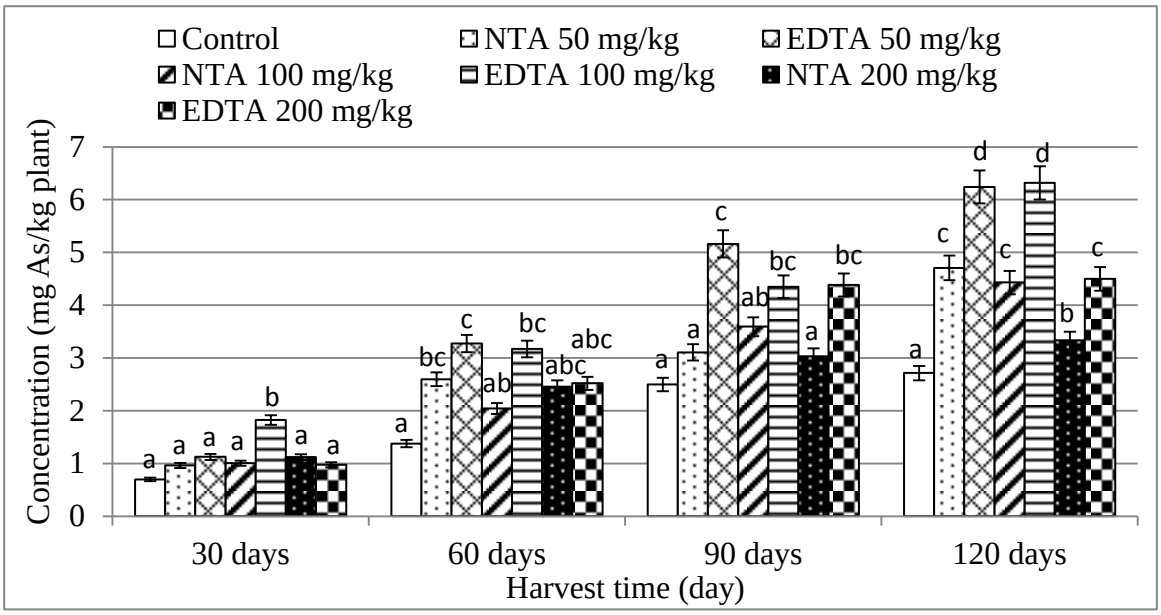


Figure 4.5 Average combinations of arsenic accumulation in aboveground parts (stem and leaves) of plants (mg As/kg plant)

(Note: The same letter above the bars means there is no significant difference ($P \leq 0.05$) when compare between the mean values of different treatments at the same harvesting time)

4.5.1 The comparison for capacity of arsenic accumulation in underground part and aboveground parts of *Mimosa pudica* L. at 120 days

From previous results, the arsenic accumulations in both root and in a combination of stem and leaves showed higher arsenic uptake by *Mimosa pudica* L. at 120 days. Therefore, arsenic accumulations in the underground part (root) and aboveground parts (stem and leaves) of plant at 120 days were picked to compare. For all treatments, the concentrations of arsenic accumulation in the underground part were significant greater than in the aboveground parts of the plant (Figure 4.6).

Smith et al. (2002) also reported that plants normally accumulate arsenic in plant roots more than other parts. Arsenic which is taken up by plants rarely moves to the upper parts of plants (i.e. stem and leaves). It enters into the plant bodies through the absorption of plant roots (Schmoger et al., 2000; Pickering et al., 2000). The

concentrations of arsenic in aboveground parts were not greater than 6.32 mg As/kg plan for all sets while the concentration in the underground part can reach 29.71 mg As/kg (set EDTA 100 mg/kg). Generally, plaque formation on plant roots can affect the uptake of arsenic and heavy metals by the plants in different ways (Otte et al., 1991).

In addition, the sets with the same chelating agents but different applied doses also presented different properties for arsenic accumulation in plants. The ability of arsenic accumulation in *Mimosa pudica* L. increased when the application doses of EDTA increased from 50 - 100 mg/kg, but the accumulation capacity dropped when the concentration of EDTA reached 200 mg/kg; too high an application dose of EDTA might cause phytotoxicity in plants and they may have less ability to stimulate the mobilization of arsenic in soil.

In contrast, the arsenic accumulations in the underground part of the plant of NTA sets decreased while increasing the applied doses of NTA with the concentrations at 21.17, 18.20 and 14.62 mg As/kg plant (sets NTA 50, 100 and 200 mg/kg respectively).

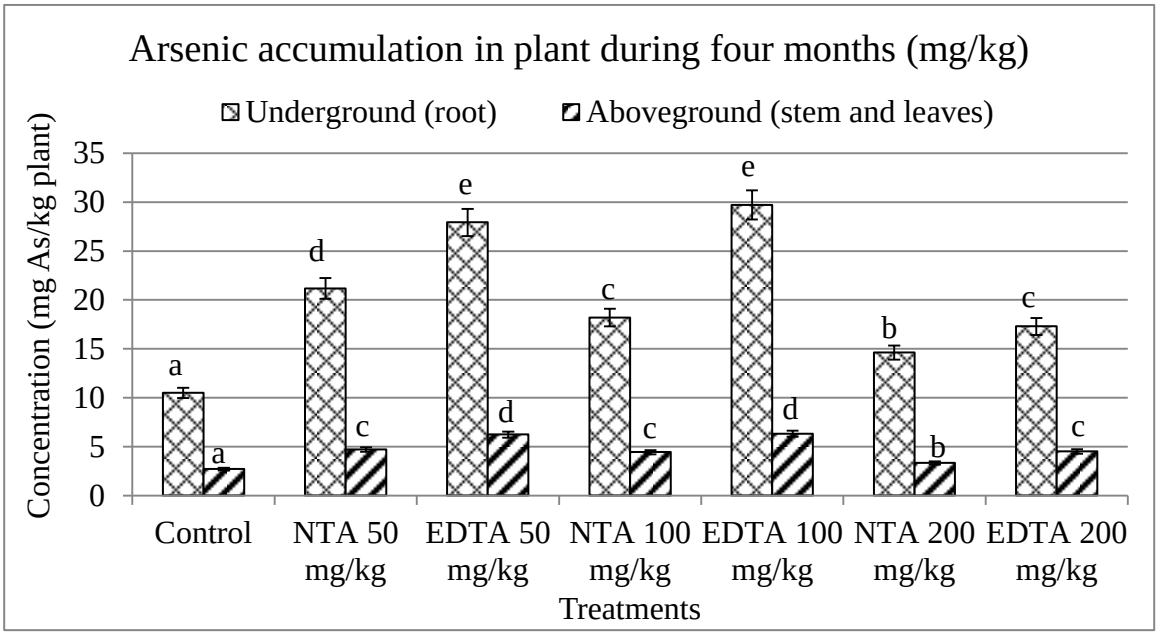


Figure 4.6 The comparison between average arsenic accumulation in the underground and aboveground parts of plants during a four months period (mg As/kg plant)

(Note: The same letter above the bars means there is no significant difference (P≤0.05) when comparing between the mean values of different treatments at the same harvesting time)

4.5.2 The dry weight ratio of underground part (Root) and aboveground parts (Stem and leaves) of *Mimosa pudica* L. at 120 days (grams)

The previous results showed very low arsenic accumulation in the aboveground parts when compared to the underground parts. In contrast, when the dry weights of these plant portions are compared, the dry weight of the underground plant part was significantly lower than the dry weight of the aboveground plant parts. This is a typical in the characterization growth of plants. The dry weight of underground plant parts ranged from 0.31 – 0.38 grams while the dry weight of aboveground plant parts ranged from 4.00 – 4.53 grams. , Furthermore the similar maximum dry weights of underground plant parts were 0.38, 0.36, 0.35, 0.33 and 0.32 gram (Set control, EDTA 100, NTA 50, EDTA 50 and NTA 200 mg/kg respectively).

The maximum dry weights of aboveground plant parts reached 4.53, 4.46, 4.34, 3.32 and 4.27 grams (Sets control, EDTA 100, EDTA 50, NTA 100 and NTA 50 mg/kg respectively). However, the dry weights of each plant parts were not significantly different (P≤0.05). When these dry weights are combined, the highest dry weight of this plant was 4.91 grams which occurred in the set control and were followed by sets EDTA 50 and NTA 50 mg/kg with the dry weights at 4.83 and 4.67 grams respectively.

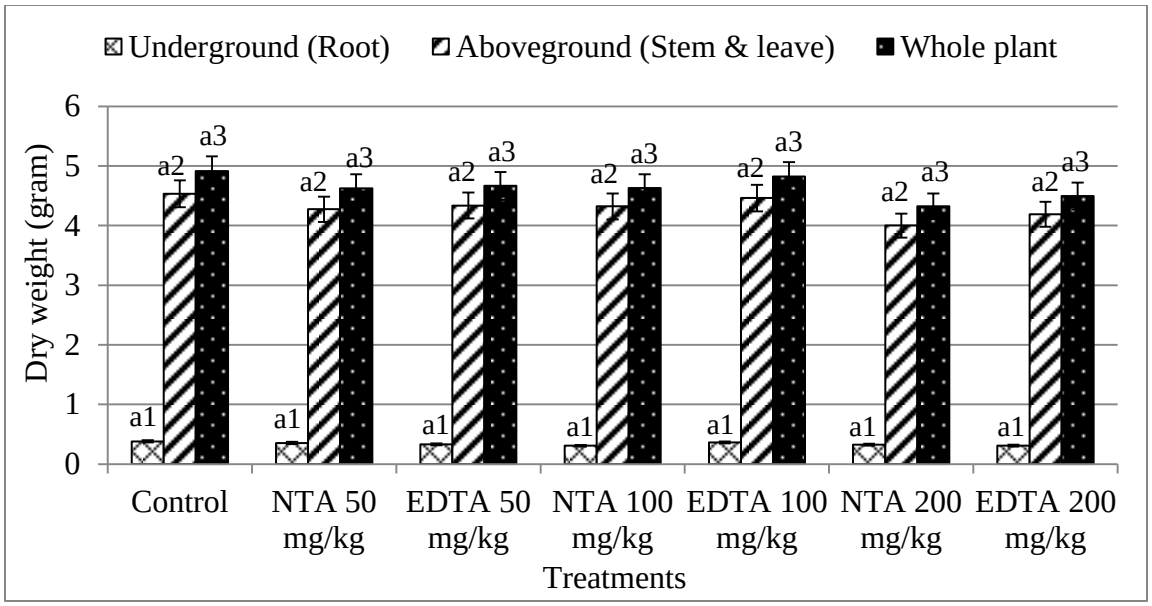


Figure 4.7 The dry weight ratio of the underground part (Root) and aboveground parts (Stem and leaves) of *Mimosa pudica* L. at 120 days (grams)

(Note: The same letter above the bars means there is no significant difference (P≤0.05) when comparing between the mean values of different treatments in the portions of plants)

4.6 Efficiency of arsenic accumulation in all parts of *Mimosa pudica* L.

Table 4.5 showed the arsenic accumulations in all parts of *Mimosa pudica* L. (Bashful mimosa) were in the range of 2.71 to 36.03 mg As/kg plant. An experimental set EDTA 100 mg/kg at 120 days showed the highest arsenic accumulation in all parts *of Mimosa pudica* L. with the concentration at 36.03 mg As/kg plant and followed by the set EDTA 50 mg/kg with the concentration at 34.17 mg As/kg plant; but in statistical analysis these two numbers are not significantly different (P≤0.05). Although, the *Mimosa pudica* L. ranks fourth in 36 plant species that have high tolerance to highly arsenic contaminated soil (Visoottiviseth et al., 2002), the removal ratios compared to total arsenic in soil was very low (Jirawan, 2000). The same applied doses of two chelating agents (NTA and EDTA) for all harvesting times, EDTA sets showed higher

efficiency for enhancing arsenic uptake by *Mimosa pudica* L. than NTA except at the dose 200 mg/kg.

In contrast, Chiu et al. (2005) claimed that NTA has more efficiency than EDTA for enhancing arsenic uptake by using *Vetiveriazizanoides* (Vetiver and Wen-Ling Ye et al. (2011) also reported that around 3.5 - 11.4% of the total soil arsenic was removed using *Pterisvittata* (Chinese brake fern). The difference of these chelating agents to stimulate the movement of arsenic in soil might be caused by using different plants varieties for phytoremediation. The differences of arsenic uptake by plants also depend on plant varieties (Bieleski and Ferguson, 1983; Nriagu, 1994). Beside this when the harvest time increased the capacity for accumulating arsenic in *Mimosa pudica* L. also increased too. However when we increased the application doses of NTA at 90 and 120 days, the ability to accumulate arsenic in plants decreased because too high concentrations of NTA might cause phytotoxicity in plants, and has an effect on the mobilization of arsenic in soil.

Overall, both chelating agents acted as very important substances for enhancing arsenic uptake by *Mimosa pudica* L. but the ability to uptake arsenic from applications of EDTA was significantly better than NTA because EDTA might have more efficiency for stimulating the arsenic in soil from immobile form to mobile form than NTA. Tambamroong et al. (2002) also reported that EDTA was an effective chelating agent for enhancing arsenic uptake by taro. EDTA is widely used for increasing the heavy metals uptake by various plants. EDTA has more effluence than Citric acid for enhancing cadmium uptake by water hyacinth during the study period of 90 days (Kunpapuek et al., 2010). Pojjanaporn et al. (2009) also found that EDTA has more efficiency than EDDS in the phytoextection of lead by pineapples after 60 days.

The maximum arsenic accumulation in plants of all NTA sets was only 25.88 mg As/kg plant (Set NTA 50 mg/kg at 120 days) as shown in Table 4.5. In contrast, the arsenic accumulations in plants of control sets were very low; the maximum amount at 120 days was only 13.22 mg As/kg plant while Visoottiviseth et al. (2002) reported the arsenic accumulation in this plant was in the range of 41 – 55 mg As/kg plant. This might

be influenced by the growing conditions of *Mimosa pudica* L. because we grew them in a control nursery and without adding any fertilizer while Visoottiviseth et al. (2002) collected the plant from the wild which would have a greater age and the plants have been also grown in more fertile soil.

Table 4.5 Total arsenic accumulation in all parts of *Mimosa pudica* L.

Experimental sets	Average arsenic accumulation in *Mimosa pudica* L. at harvest time (mg As/kg plant)			
	30 days	60 days	90 days	120 days
Control	2.71 [a]	5.20 [a]	9.09 [a]	13.22 [a]
NTA 50 mg/kg soil	3.06 [a]	6.33 [ab]	13.31 [b]	25.88 [d]
EDTA 50 mg/kg soil	6.12 [d]	14.29 [e]	31.04 [e]	34.17 [e]
NTA 100 mg/kg soil	3.58 [ab]	9.42 [cd]	16.97 [c]	22.63 [c]
EDTA 100 mg/kg soil	5.48 [d]	11.26 [d]	23.45 [d]	36.03 [e]
NTA 200 mg/kg soil	5.04 [cd]	7.96 [bc]	9.47 [a]	17.95 [b]
EDTA 200 mg/kg soil	4.28 [bc]	7.58 [abc]	16.01 [bc]	21.79 [c]

(Note: The same letter on the top right corner means there is no significant difference ($P \leq 0.05$) when comparing between the mean values of different treatments at the same harvesting time)

4.6.1 The capacity of arsenic accumulation in all parts of plants on different application doses of NTA

The difference application doses of NTA showed difference capacity to accumulate arsenic in *Mimosa pudica* L. for each harvesting time. Set NTA 50 mg/kg at 120 days presented the maximum arsenic accumulation in this plant with a concentration at 25.88 mg As/kg plant. Whereas the highest arsenic accumulations at 30 and 60 or 90

days occurred in sets NTA 200 mg/kg and NTA 100 mg/kg with the concentrations at 5.04 and 3.58, 16.97 mg As/kg plant respectively.

In comparison between arsenic accumulations in the plant among harvesting time at 120 days; when we increased the applied doses of NTA to 100 and 200 mg/kg soil, the arsenic accumulations in all parts of the plant decreased. This might be caused by the applied concentrations of NTA were too high and it might reduce the capacity for arsenic uptake by the plant. In contrast, the application rate of NTA must be over 10 mmol/kg soil and at 20 mmol of NTA would maximize arsenic bioavailability in soil (Chiu et al., 2005). However, after 60 days NTA 200 mg/kg reported a lower ability to accumulate arsenic in plants compared to other sets. At 90 days, set NTA 200 mg/kg showed similar amounts compared to the control set for removing arsenic from soil (with concentration at 9.49 and 9.47 mg As/kg plant respectively).

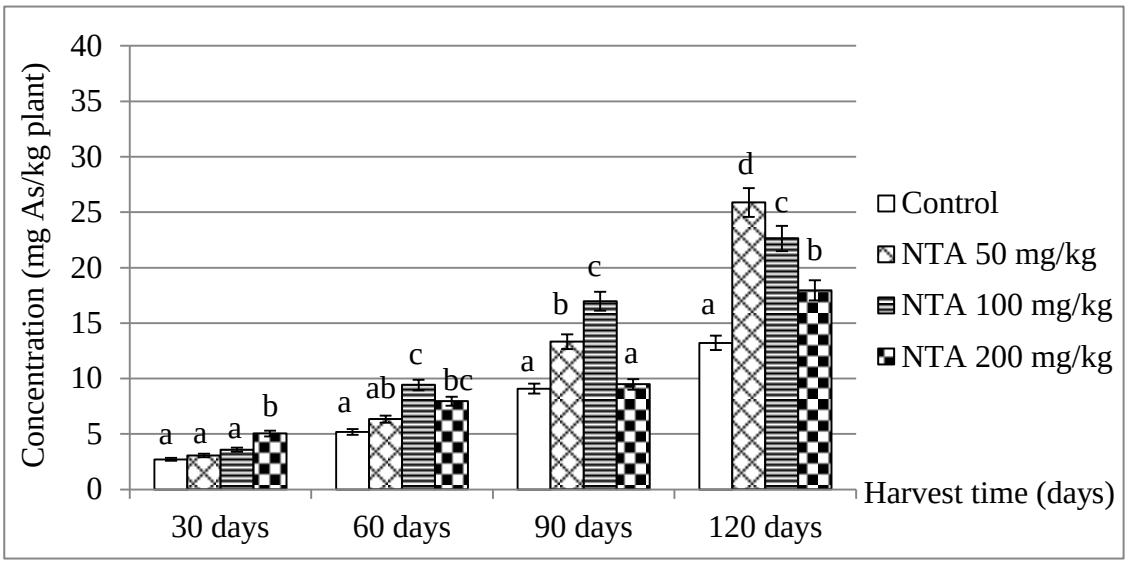


Figure 4.8 Effect of NTA for enhancing arsenic accumulation in *Mimosa pudica* L.

(Note: The same letter above the bars means there is no significant difference (P≤0.05) when comparing between the mean values of different treatments at the same harvesting time)

4.6.2 The capacity of arsenic accumulation in all parts of plants on different application doses of EDTA

Figure 4.9 illustrated that the arsenic accumulations in plants on different applied concentrations of EDTA over four months were in the range of 2.71 – 36.03 mg As/kg plant). The As accumulation in plant of sets EDTA 50 mg/kg and EDTA 100 mg/kg at 120 days showed no significant difference ($P \leq 0.05$) with the concentration at 34.17 and 36.03 mg As/kg plant); these numbers also showed the highest As accumulation in the plant and were followed by set EDTA 50 mg/kg at 90 days with the amount at 31.04 mg As/kg plant. In comparison with the control sets, EDTA was an effective chelating agent for stimulating the bioavailability of arsenic contaminated soil.

Overall, when we increased the application doses of EDTA the ability to accumulate arsenic in plant was decreased except in a harvesting time of 120 days. At a harvesting time of 120 days, set EDTA 200 mg/kg soil showed lower arsenic accumulation in plants than in other EDTA sets and the amount of arsenic in the plant was significantly different when compared to the set EDTA 100 mg/kg soil; this might be caused by the applied concentration of EDTA being too high and it can reduce the uptake capacity by plant or too high a concentration of EDTA might produce phytotoxicity in the plant.

Furthermore, the control sets showed the lowest arsenic accumulation in plants for each harvesting time. From these results, we recommend that an optimum concentration of EDTA for enhancing arsenic uptake by *Mimosa pudica* L. should not be greater than 100 mg EDTA/kg soil.

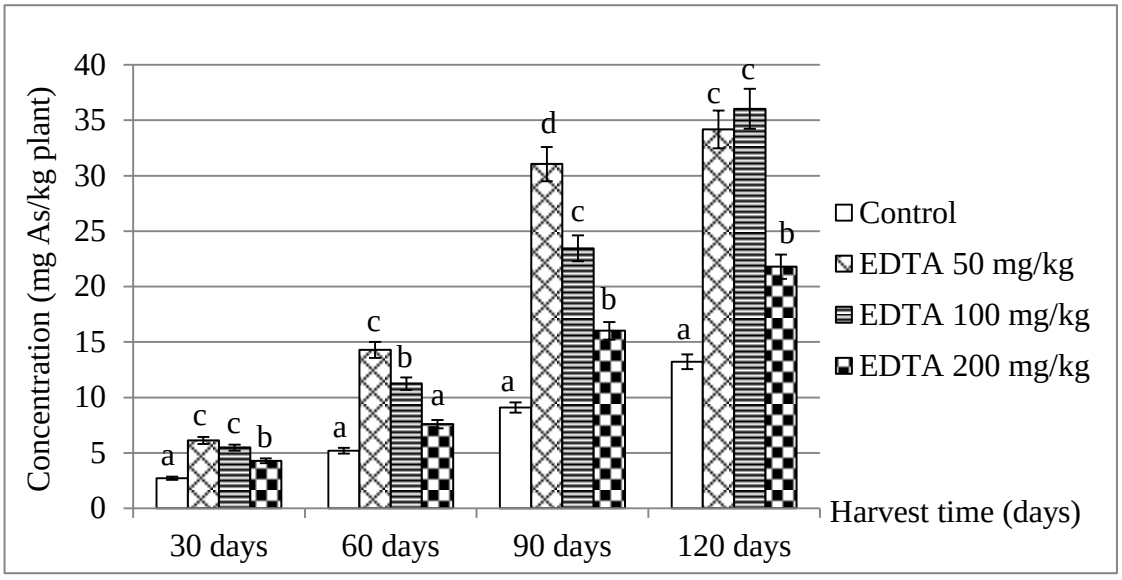


Figure 4.9 Effect of EDTA for enhancing arsenic accumulations in *Mimosa pudica* L.

(Note: The same letter above the bars means there is no significant difference (P≤0.05) when comparing between the mean values of different treatments at the same harvesting time)

4.7 The percentage of arsenic uptake by plants and arsenic remaining in soil during four month study period

This study focuses on the effect of NTA and EDTA for stimulation arsenic uptake by *Mimosa pudica* L. However, if the removal ratio was compared to total arsenic in soil; the removal amount was very small because this experiment used only one seedling of plant per pot. The maximum arsenic that was removed by plant is only 0.17 mg (0.67 % of total arsenic in soil) with appearing in set EDTA 100 mg/kg soil and set EDTA 50 mg/kg reported the second highest removal with a quantity of 0.15 mg or equal to 0.59 % of total arsenic in soil. Without application of any chelating agent or control set showed very small removal capacity (At 0.06 mg or 0.26 % of total arsenic in soil) as shown in Table 4.6.

Moreover, at every harvesting time the concentrations of arsenic in soil were also measured. Overall, the concentration of arsenic in soil was decreased when increasing the harvesting time. The lowest arsenic remains in soil for all treatments at 30, 60, 90 and 120 days were 4.73, 4.60, 4.41 mg/kg soil (Set EDTA 50 mg/kg) and 4.26 mg As/kg soil (Set EDTA 100 mg/kg) respectively (Figure 4.10). The total arsenic in soil at the initial day is 5 mg As/kg soil or equal 25 mg in total because 5 kg of soil was used per pot. When the removal rates and the remaining arsenic in soil for each harvesting time are compared; some missing amount of arsenic occurred. This may be caused of the volatilization of arsenic during experimental period or the arsenic might be degraded by other mechanisms in soil. Liu et al. (2006) also reported that arsenic can be volatile in outdoor temperatures especially when it is exposed to sunlight but the volatilization of arsenic depends largely on the properties, occurrence, reactivity, etc.

Table 4.6 Percentage arsenic up taken by plants during four months of the experiment

Treatments	Concentration of arsenic at starting time	Total arsenic accumulation in all parts of plant	Percentage of arsenic removal from soil
	Mg	mg	%
Control	25	0.06	0.26
NTA 50 mg/kg	25	0.13	0.51
EDTA 50 mg/kg	25	0.15	0.59
NTA 100 mg/kg	25	0.10	0.42
EDTA 100 mg/kg	25	0.17	0.67
NTA 200 mg/kg	25	0.08	0.32
EDTA 200 mg/kg	25	0.10	0.40

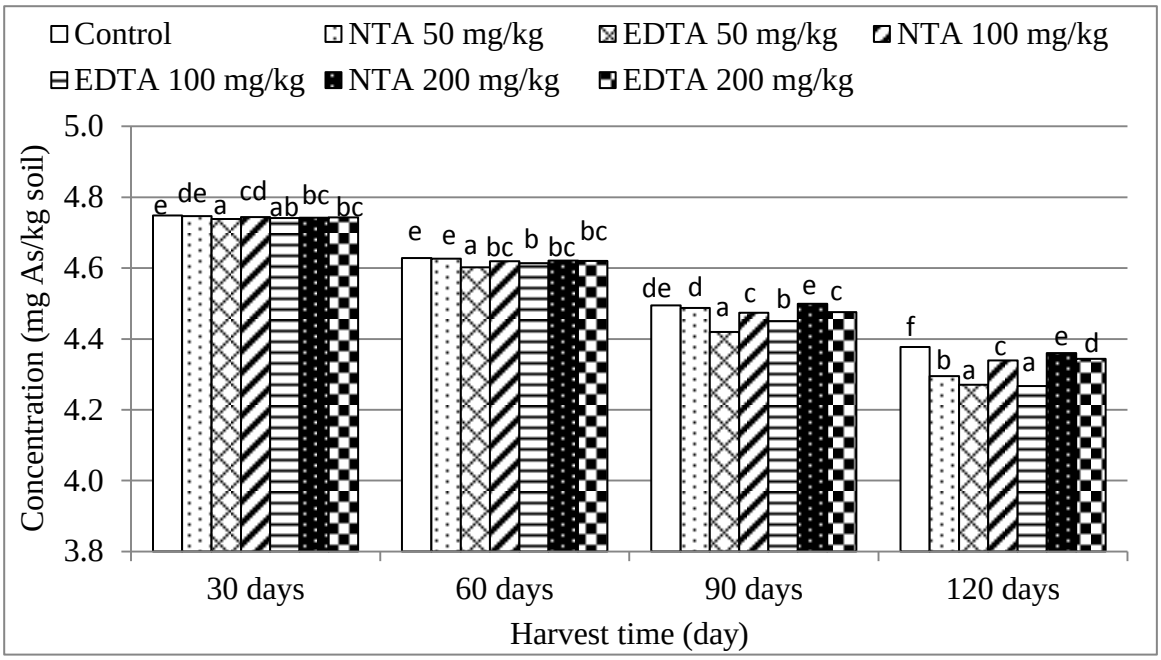


Figure 4.10 Concentrations of arsenic in soil at each time of sample collection (mg As/kg soil)

(Note: The same letter above the bars means there is no significant difference ($P \leq 0.05$) when comparing between the mean values of different treatments at the same harvesting time)

4.8 The distribution of arsenic and other elements inside the *Mimosa pudica* L.

This analysis aims to determine the distribution of arsenic and other related elements inside the plants. A plant sample was collected from each treatment at 30 and 120 days for analyzing the distribution of concerned element using the Synchrotron Radiation method BL6b (Synchrotron Light Research Institute, 2011).

The previous results illustrated that the highest arsenic accumulations in plants at 30 and 120 days occurred in set EDTA 50 mg/kg and EDTA 100 mg/kg respectively, therefore the plants from these plots were used to determine the distribution of arsenic and other elements when they were present inside the plant. Every element has a value for X-Ray emission energy itself. When arsenic absorbs X-Ray radiation, around 10.543 Kilo electron volts (KeV) would be emitted from the arsenic compound (Synchrotron

Light Research Institute, 2011). Figures 4.11 and 4.12 below show that arsenic cannot be detected in plant samples for all in the root, stem and leaves (There is no peak found at 10.543 KeV of axis energy emission value as shown on Figure 4.11 and 4.12); while Atomic Absorption Spectrometry (AAS) analysis at 30 days found the arsenic concentrations in root and in a combination of stem and leaves of set EDTA 50 mg/kg at 5 and 1.12 mg As/kg plant respectively.

At 120 days, AAS analysis also found the arsenic concentrations in the root and in a combination of stem and leaves of set EDTA 100 mg/kg at 29.71 and 6.32 mg As/kg plant respectively. This could be caused by the limitation of this beamline because a Si(111) crystal is allowed to be used for extracting a monochromatic X-ray beam covering an energy scale 2 - 12 keV and the pixel size in the fluorescence maps can detect only 1x1 mm.

In contrast, arsenic accumulation in willow roots was detected using synchrotron μ-X-ray fluorescence spectroscopy (Zimmer et al., 2011). In their research, the micro XAS beamline is a dedicated hard X-ray microprobe beamline using a fixed-exit Si (111) double-crystal monochromator and covering an energy scale from 4 to 23 keV and the pixel size in all fluorescence maps was 1 μm × 1 μm. Moreover, the concentration of arsenic inside the plant is very low and the beamline of the synchrotron μ-X-ray fluorescence spectroscopy cannot detect the arsenic in the plant sample.

However, other elements (Ar, K, Ca and Fe) were detected in these plant samples under the synchrotron μ-X-ray fluorescence spectroscopy analysis; we can estimate that the concentrations of these compounds are higher than arsenic in these plant samples. The detected Ar might come from the surrounding air during measuring the samples greater than from the samples. An example of Calcium (Ca) distribution inside the stem of plant at 30 days was shown in Figure 4.11-d and an example of iron (Fe) distribution inside the root of plant at 120 days was also presented in Figure 4.12-d. The colors represent the concentrations of the elements in the samples (Red to blue means the concentrations from high to low). Although, the beamline 6b of the Synchrotron Radiation cannot detect arsenic in the plant samples these would be a good beginning to use the beamline 6b into the

heavy metals analysis fields. However the Synchrotron Radiation is a new method for analysis in Thailand and the detection limit for arsenic was not prepared and tested before by the concerned material controllers.

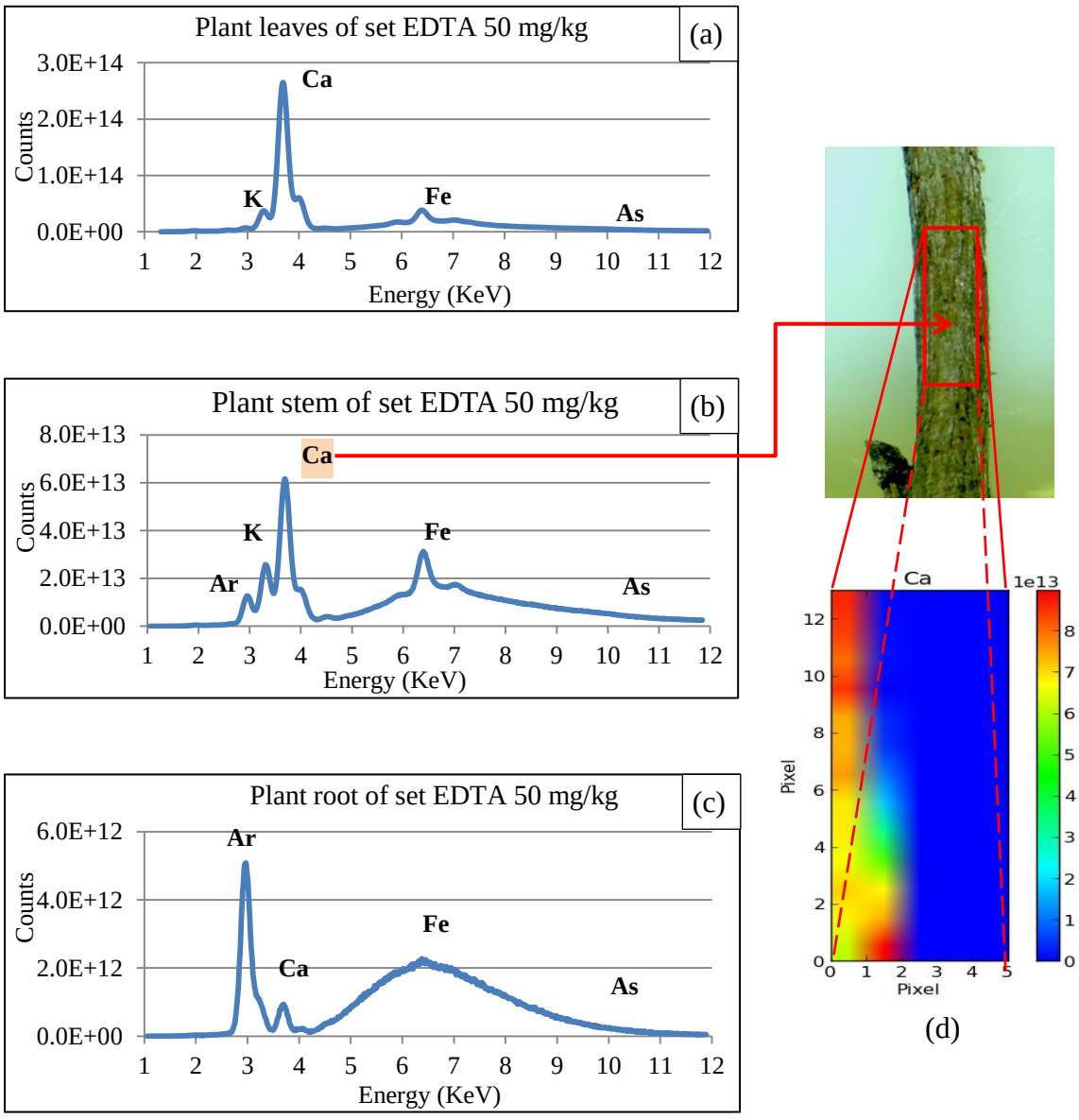


Figure 4.11 Distribution of elements inside the *Mimosa pudica* L. at 30 days (a = in leave, b = in stem, c = in root and d = distribution of Ca in plant stem) (Note: Red to blue means the concentrations from high to low)

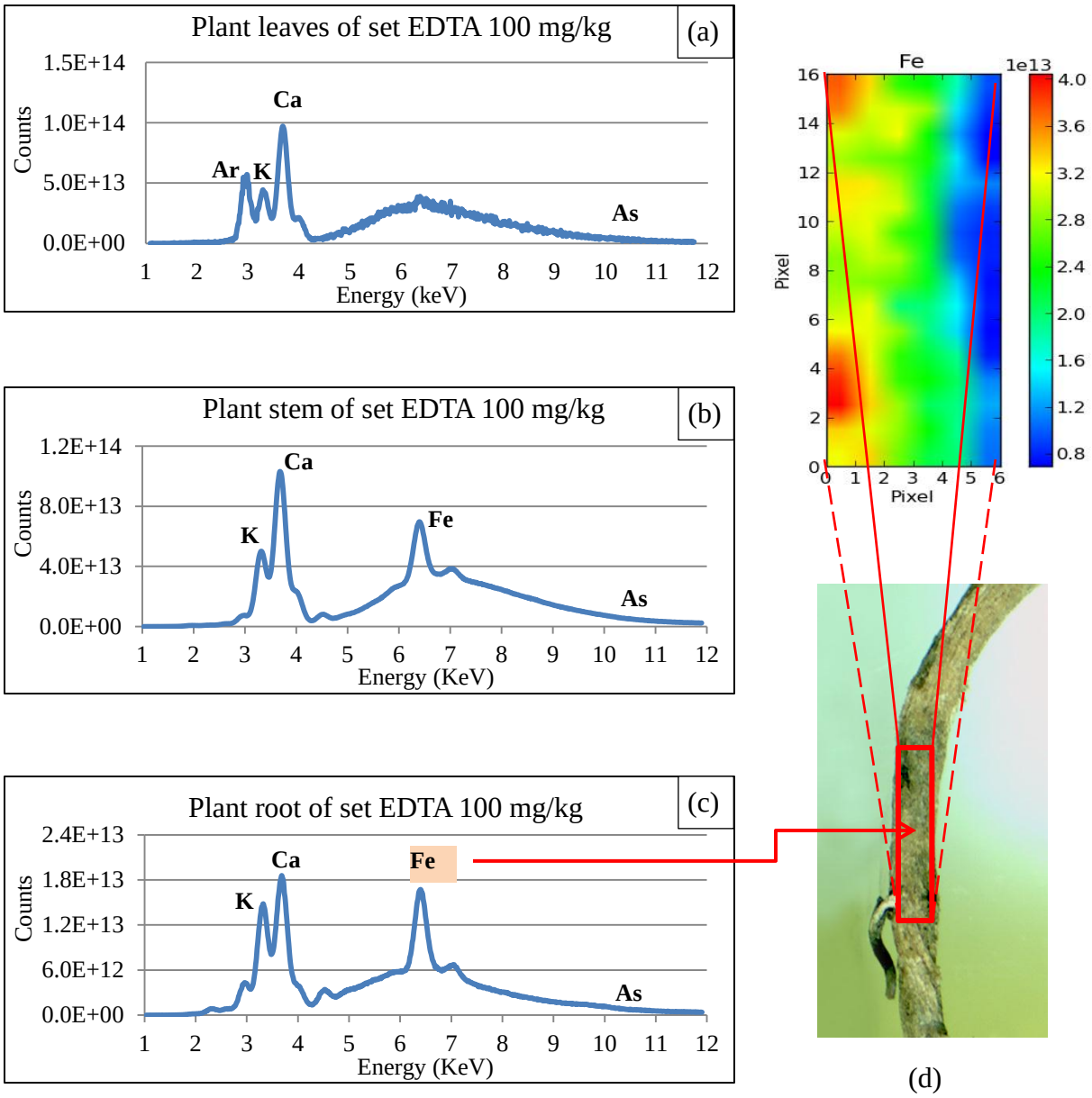


Figure 4.12 Distribution of elements inside the *Mimosa pudica* L. at 120 days (a = in leave, b = in stem, c = in root and d = distribution of Fe in plant root) (Note: Red to blue means the concentrations from high to low)

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the experimental results, *Mimosa pudica* L. can survive in arsenic contaminated soil up to 10 mg As/kg soil under conditions of no application of any fertilizer and growing in a control nursery. Therefore, *Mimosa pudica* L. can be an alternative arsenic tolerant plant variety for phytoremediation and be able to manage and clean up the low arsenic contamination in agricultural soil. All three applied doses of NTA and EDTA at 50, 100 and 200 mg/kg did not show phytotoxicity in plant growth during a month of preliminary studies. The average values of arsenic accumulation in all parts of *Mimosa pudica* L. during four months were in the range of 2.71 - 36.03 mg As/kg plant. Experimental sets of 50 and 100 mg EDTA/kg soil at 120 days reported the highest arsenic accumulation in *Mimosa pudica* L. with the concentrations at 34.17 and 36.03 mg As/kg plant. In addition, the capacity for arsenic accumulation in *Mimosa pudica* L. decreased when we increased the applied doses of EDTA to 200 mg/kg soil and NTA to 100 and 200 mg/kg soil. This might be caused by too high concentrations of EDTA and NTA that reduce the ability of arsenic into mobile form.

Overall, with the same applied doses of two chelating agents, (NTA and EDTA), EDTA sets showed higher efficiency than NTA for enhancing arsenic uptake by *Mimosa pudica* L.

The arsenic accumulation in the underground part of the plant (root) was significantly higher than in the aboveground parts (stem and leaves). Moreover, when the time increased from 30 to 60, 90 and 120 days, the ability for accumulating arsenic in the *Mimosa pudica* L. increased too. Generally, both chelating agents (NTA and EDTA) acted as important substances for enhancing arsenic uptake by *Mimosa pudica* L. but the capacities to uptake arsenic from adding EDTA was better than NTA.

In terms of the synchrotron μ-X-ray fluorescence spectroscopy analysis, the concentrations of arsenic in all parts of *Mimosa pudica* L. at 6.12 mg As/kg plant for an initial time (30 days) and at 36.03 mg/kg plant for the final period (120 days) were not detected in the plant samples; this might be caused by the limitation of the beamline and/or the methods for preparing the samples are not appropriate.

In contrast, other elements like K, Ca and Fe were significantly detected in these plant samples under the synchrotron μ-X-ray fluorescence spectroscopy analysis because the concentrations of these compounds would be higher than arsenic in these plant samples.

5.2 Recommendations for future research

The *Mimosa pudica* L. is an alternative plant variety for cleaning up arsenic contamination in agricultural soil. It is a weed that can tolerate arsenic contaminated soil and commonly found in all parts of Thailand especially in grass fields, in gardens, beside roads, besides the railway, in young forests and other places. The *Mimosa pudica* L. is not only a weed but it is also a useful plant for dealing with a heavy metal like arsenic and helps to manage and decrease the concentration of arsenic in agriculture soil. Therefore, we can grow this plant during the non-crop season and take them out before starting the new short rotation cropping. *Mimosa pudica* L. grows easily and has a short life cycle.

The *Mimosa pudica* L. can uptake arsenic from soil naturally but we can increase the ability by adding chelating agents. From the results of this study, EDTA at 50 and 100 mg/kg soil can maximize arsenic accumulation in this plant during four months; but in order to save money, EDTA 50 mg/kg is recommended for using to enhance the As uptake by *Mimosa pudica* L. Moreover, EDTA also has a higher efficiency than NTA for enhancing arsenic uptake by this plant.

However, the synchrotron μ-X-ray fluorescence spectroscopy analysis on beamline 6b method provided an unexpected result in this study. Therefore, future research is recommended to use combined with other beamlines which can detect also the

species of elements in the samples as well. Moreover, the sample preparation should be also considered as an important factor by using this analysis method because the x-ray can determine only a very small area of the sample and we have to prepare the sample little by little until finish all parts of the samples. However, the study using the synchrotron μ-X-ray fluorescence spectroscopy analysis would be a good starting method for analyzing the distribution of heavy metals in green plants.

This research did not study the chemical reactions between arsenic and chelating agents. Therefore, future studies are also recommended to investigate the detail in molecular level and study more between the difference of application of fertilizers and without fertilizers because other factors that can enhance arsenic uptake by plant might also effect soil properties or organic matters in soil; these would help us to clarify which factor is more effective for simulating the movement of arsenic from the soil up to the plant between fertilizers and chelates.

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APPENDICES

APPENDIX A

1. The growth rate of *Mimosa pudica* L. on difference application doses of NTA and EDTA

Table A-1 Relative growth rates over period of times

Treatments	The growth rates of *Mimisa pudica* L. (gram/day)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	0.0416	0.0453	0.0292	0.0291	0.0266	0.0253	0.0217	0.0207
	0.0381		0.0273		0.0249		0.0200	
	0.0564		0.0307		0.0243		0.0205	
NTA 50 mg/kg	0.0355	0.0355	0.0279	0.0282	0.0229	0.0232	0.0218	0.0209
	0.0340		0.0286		0.0239		0.0201	
	0.0371		0.0280		0.0228		0.0208	
NTA 100 mg/kg	0.0473	0.0466	0.0279	0.0283	0.0234	0.0235	0.0208	0.0204
	0.0532		0.0284		0.0233		0.0197	
	0.0391		0.0287		0.0239		0.0206	
NTA 200 mg/kg	0.0476	0.0459	0.0316	0.0297	0.0238	0.0234	0.0206	0.0202
	0.0460		0.0288		0.0235		0.0201	
	0.0441		0.0288		0.0230		0.0197	
EDTA 50 mg/kg	0.0552	0.0505	0.0307	0.0313	0.0249	0.0243	0.0194	0.0198
	0.0440		0.0340		0.0230		0.0205	
	0.0523		0.0291		0.0250		0.0196	
EDA 100 mg/kg	0.0480	0.0466	0.0286	0.0294	0.0233	0.0237	0.0195	0.0204
	0.0426		0.0308		0.0228		0.0216	
	0.0492		0.0288		0.0251		0.0202	
EDA 200 mg/kg	0.0507	0.0454	0.0297	0.0307	0.0244	0.0239	0.0199	0.0204
	0.0344		0.0331		0.0231		0.0206	
	0.0509		0.0294		0.0242		0.0208	

2. The pH, ORP and electrical conductivity in soil

Table A-2 Soil pH during the experimental period

Treatments	Soil pH							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	7.24	7.27	7.28	7.25	7.32	7.36	7.39	7.35
	7.35		7.28		7.36		7.25	
	7.21		7.19		7.39		7.42	
NTA 50 mg/kg	7.34	7.14	7.14	7.19	7.32	7.29	7.31	7.28
	7.08		7.25		7.26		7.20	
	7.00		7.19		7.28		7.34	
NTA 100 mg/kg	7.13	7.14	7.11	7.15	7.23	7.22	7.40	7.27
	7.15		7.09		7.24		7.26	
	7.14		7.24		7.18		7.16	
NTA 200 mg/kg	7.07	7.09	7.30	7.20	7.32	7.17	7.18	7.26
	7.05		7.18		7.12		7.22	
	7.15		7.11		7.06		7.37	
EDTA 50 mg/kg	7.32	7.22	7.12	7.22	7.21	7.33	7.24	7.36
	7.14		7.33		7.45		7.42	
	7.20		7.20		7.32		7.41	
EDA 100 mg/kg	7.21	7.21	7.39	7.21	7.46	7.30	7.28	7.32
	7.12		7.06		7.15		7.36	
	7.31		7.19		7.30		7.32	
EDA 200 mg/kg	7.31	7.16	7.21	7.20	7.34	7.31	7.19	7.30
	7.11		7.21		7.28		7.34	
	7.07		7.17		7.30		7.38	

Table A-3 Oxidation-reduction potential in soil

Treatments	Oxidation-reduction potential (ORP) in soil (mV)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	182.11	181.87	173.10	173.93	157.40	173.93	167.00	166.00
	182.15		174.60		157.50		164.90	
	181.34		174.10		157.90		166.10	
NTA 50 mg/kg	180.70	182.00	174.80	174.67	167.20	174.67	181.90	181.53
	182.80		174.80		166.50		182.20	
	182.50		174.40		167.70		180.50	
NTA 100 mg/kg	191.70	190.43	179.50	179.17	171.80	179.17	172.00	172.33
	189.70		179.00		169.00		173.90	
	189.90		179.00		169.50		171.10	
NTA 200 mg/kg	216.60	215.47	178.60	177.77	162.00	177.77	167.09	167.47
	214.08		178.30		160.20		167.02	
	215.73		176.40		162.70		168.30	
EDTA 50 mg/kg	216.57	216.73	183.67	183.97	175.00	183.97	184.66	184.70
	216.18		183.58		175.90		185.68	
	217.45		184.67		175.00		183.75	
EDA 100 mg/kg	198.00	197.90	181.00	178.97	176.00	178.97	185.20	185.60
	197.50		177.60		175.70		185.30	
	198.20		178.30		174.40		186.30	
EDA 200 mg/kg	195.72	195.37	175.80	176.40	169.30	176.40	170.10	171.07
	195.83		177.90		169.70		171.80	
	194.55		175.50		169.50		171.30	

Table A-4 Electrical conductivity in soil

Treatments	Electrical conductivity in soil (µS/cm)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	837	836.67	846	845.33	809	809.00	836	837.67
	835		847		811		836	
	838		843		807		841	
NTA 50 mg/kg	799	800.33	875	870.67	884	879.33	836	835.33
	804		863		875		832	
	798		874		879		838	
NTA 100 mg/kg	855	855.33	792	793.00	870	872.33	819	819.67
	857		798		880		822	
	854		789		867		818	
NTA 200 mg/kg	807	810.00	879	875.67	833	828.67	808	808.33
	810		870		827		812	
	813		878		826		805	
EDTA 50 mg/kg	848	846.67	842	847.67	830	827.67	857	862.33
	847		852		827		861	
	845		849		826		869	
EDA 100 mg/kg	841	839.67	828	829.33	795	795.33	825	823.67
	834		835		802		822	
	844		825		789		824	
EDA 200 mg/kg	815	819.33	795	795.33	825	821.67	806	803.33
	819		792		816		797	
	824		799		824		807	

3. Arsenic accumulation in *Mimosa pudica* L.

Table A-5 Arsenic accumulations in underground part (Root) of *Mimosa pudica* L.

Treatments	Arsenic accumulations in underground part (Root) of plant (mg As/kg plant)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	3.04	2.01	4.86	3.82	7.54	6.59	10.72	10.50
	1.05		2.89		5.14		11.54	
	1.95		3.71		7.09		9.25	
NTA 50 mg/kg	1.87	2.09	4.45	3.73	11.37	10.21	22.87	21.17
	1.89		2.55		9.80		19.02	
	2.52		4.21		9.45		21.62	
NTA 100 mg/kg	2.73	2.58	7.41	7.38	11.05	13.37	19.68	18.20
	2.42		6.14		15.18		16.39	
	2.58		8.59		13.89		18.52	
NTA 200 mg/kg	2.92	3.92	5.41	5.51	6.29	6.44	14.47	14.62
	4.36		5.76		7.01		15.83	
	4.48		5.35		6.02		13.55	
EDTA 50 mg/kg	5.17	5.00	11.64	11.01	24.76	25.88	28.24	27.93
	4.97		11.89		28.95		28.71	
	4.85		9.51		23.93		26.83	
EDA 100 mg/kg	3.01	3.66	7.22	8.09	20.61	19.10	31.14	29.71
	3.88		9.90		18.45		27.74	
	4.09		7.14		18.23		30.25	
EDA 200 mg/kg	3.85	3.31	4.28	5.07	10.51	11.62	15.81	17.29
	3.56		6.86		13.06		17.43	
	2.51		4.06		11.30		18.64	

Table A-6 Combinations of arsenic accumulation aboveground parts (Stem and leaves) of *Mimosa pudica* L.

Treatments	Arsenic accumulations in Aboveground parts (Stem and Leaves) of plant (mg As/kg plant)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	0.47	0.70	1.25	1.38	2.20	2.50	2.81	2.71
	0.73		0.83		2.68		2.57	
	0.89		2.05		2.61		2.76	
NTA 50 mg/kg	0.96	0.96	3.36	2.59	3.96	3.10	4.66	4.71
	0.73		2.66		3.05		4.72	
	1.20		1.77		2.30		4.74	
NTA 100 mg/kg	0.70	1.00	1.00	2.04	2.95	3.59	3.82	4.43
	1.31		2.66		3.97		4.60	
	1.00		2.46		3.86		4.87	
NTA 200 mg/kg	1.26	1.12	2.42	2.45	3.59	3.03	3.22	3.33
	1.00		2.03		2.50		3.27	
	1.10		2.92		3.01		3.51	
EDTA 50 mg/kg	1.33	1.12	3.37	3.28	4.75	5.16	6.33	6.24
	1.00		3.21		4.56		5.82	
	1.04		3.24		6.17		6.57	
EDA 100 mg/kg	2.23	1.82	3.92	3.17	3.69	4.35	6.22	6.32
	1.29		2.56		5.06		6.76	
	1.95		3.03		4.30		5.98	
EDA 200 mg/kg	0.97	0.97	2.37	2.52	3.89	4.38	4.75	4.50
	1.00		3.00		4.84		4.69	
	0.95		2.18		4.42		4.05	

Table A-7 Total arsenic accumulations in all parts of *Mimosa pudica* L.

Treatments	Total arsenic accumulations in all parts of plant at harvest time (mg As/kg plant)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	3.51	2.71	6.11	5.20	9.74	9.09	13.53	13.22
	1.78		3.72		7.82		14.11	
	2.84		5.76		9.70		12.01	
NTA 50 mg/kg	2.83	3.06	7.81	6.33	15.33	13.31	27.53	25.88
	2.62		5.20		12.85		23.74	
	3.72		5.98		11.75		26.36	
NTA 100 mg/kg	3.43	3.58	8.41	9.42	14.00	16.97	23.50	22.63
	3.73		8.80		19.15		20.99	
	3.58		11.05		17.75		23.39	
NTA 200 mg/kg	4.18	5.04	7.83	7.96	9.88	9.47	17.69	17.95
	5.36		7.79		9.51		19.10	
	5.58		8.27		9.03		17.06	
EDTA 50 mg/kg	6.50	6.12	15.01	14.29	29.51	31.04	34.57	34.17
	5.97		15.10		33.51		34.53	
	5.89		12.75		30.10		33.40	
EDA 100 mg/kg	5.24	5.48	11.13	11.26	24.30	23.45	37.36	36.03
	5.17		12.46		23.51		34.50	
	6.04		10.17		22.54		36.23	
EDA 200 mg/kg	4.82	4.28	6.65	7.58	14.40	16.01	20.56	21.79
	4.56		9.86		17.90		22.12	
	3.46		6.24		15.72		22.69	

4. The percentage of arsenic uptake by plants and arsenic remain in soil during four months of studying period

Table A-8 Concentration of arsenic remain at each harvesting time

Treatments	Concentration of arsenic in each time of sample collection (mg As/kg soil)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	4.75	4.75	4.63	4.63	4.49	4.49	4.38	4.38
	4.75		4.63		4.50		4.38	
	4.75		4.63		4.50		4.38	
NTA 50 mg/kg	4.75	4.75	4.62	4.63	4.48	4.49	4.29	4.29
	4.75		4.63		4.49		4.30	
	4.75		4.63		4.49		4.29	
NTA 100 mg/kg	4.74	4.74	4.62	4.62	4.48	4.47	4.34	4.34
	4.74		4.62		4.47		4.34	
	4.75		4.62		4.47		4.34	
NTA 200 mg/kg	4.74	4.74	4.62	4.62	4.50	4.50	4.36	4.36
	4.74		4.62		4.50		4.36	
	4.74		4.62		4.50		4.36	
EDTA 50 mg/kg	4.74	4.74	4.60	4.60	4.42	4.42	4.27	4.27
	4.74		4.59		4.42		4.27	
	4.74		4.61		4.42		4.27	
EDA 100 mg/kg	4.74	4.74	4.62	4.61	4.45	4.45	4.27	4.27
	4.74		4.61		4.46		4.26	
	4.74		4.62		4.44		4.27	
EDA 200 mg/kg	4.74	4.74	4.62	4.62	4.48	4.48	4.34	4.34
	4.74		4.61		4.47		4.34	
	4.74		4.63		4.47		4.35	

Table A-9 Percentage arsenic uptake by plants during four months of the experiment

Treatments	Percentage arsenic uptake by plants during four months of the experiment (%)							
	30 days	Average	60 days	Average	90 days	Average	120 days	Average
Control	0.02	0.02	0.06	0.05	0.17	0.14	0.29	0.26
	0.01		0.03		0.12		0.25	
	0.02		0.06		0.14		0.22	
NTA 50 mg/kg	0.01	0.01	0.07	0.05	0.19	0.17	0.60	0.51
	0.01		0.05		0.18		0.42	
	0.02		0.05		0.15		0.51	
NTA 100 mg/kg	0.02	0.02	0.07	0.08	0.18	0.23	0.46	0.42
	0.03		0.08		0.25		0.36	
	0.02		0.10		0.24		0.44	
NTA 200 mg/kg	0.03	0.03	0.08	0.08	0.13	0.12	0.34	0.32
	0.03		0.07		0.13		0.34	
	0.03		0.07		0.11		0.29	
EDTA 50 mg/kg	0.05	0.05	0.15	0.15	0.44	0.44	0.56	0.59
	0.04		0.19		0.43		0.65	
	0.05		0.12		0.46		0.56	
EDA 100 mg/kg	0.04	0.04	0.10	0.11	0.32	0.32	0.62	0.67
	0.03		0.13		0.29		0.74	
	0.04		0.09		0.35		0.65	
EDA 200 mg/kg	0.04	0.03	0.06	0.08	0.21	0.22	0.36	0.40
	0.02		0.12		0.23		0.42	
	0.03		0.06		0.22		0.44	

APPENDIX B

STATISTICS ANALYSIS

Table B-1 Relative growth rates of *Mimosa pudica* L. over period of times (gram/day)

At harvest time of 30 days

Treatments		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	NTA 50 mg/kg	3	.0355	
	EDTA 200 mg/kg	3	.0453	.0453
	Control	3	.0454	.0454
	NTA 200 mg/kg	3	.0459	.0459
	NTA 100 mg/kg	3	.0465	.0465
	EDTA 100 mg/kg	3	.0466	.0466
	EDTA 50 mg/kg	3		.0505
	Sig.		.077	.387

(B-1a)

At harvest time of 60 days

Treatments		N	Subset for alpha = 0.05
			1
Duncan[a]	NTA 50 mg/kg	3	.0282
	NTA 100 mg/kg	3	.0283
	Control	3	.0291
	EDTA 100 mg/kg	3	.0294
	NTA 200 mg/kg	3	.0297
	EDTA 200 mg/kg	3	.0307
	EDTA 50 mg/kg	3	.0313
	Sig.		.052

(B-1b)

At harvest time of 90 days

Treatments		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	NTA 50 mg/kg	3	.0232	
	NTA 200 mg/kg	3	.0234	
	NTA 100 mg/kg	3	.0235	
	EDTA 100 mg/kg	3	.0237	.0237
	EDTA 200 mg/kg	3	.0239	.0239
	EDTA 50 mg/kg	3	.0243	.0243
	Control	3		.0253
	Sig.		.185	.065

(B-1c)

At harvest time of 120 days

Treatments		N	Subset for alpha = 0.05
			1
Duncan[a]	EDTA 50 mg/kg	3	.0198
	NTA 200 mg/kg	3	.0201
	NTA 100 mg/kg	3	.0204
	EDTA 100 mg/kg	3	.0204
	EDTA 200 mg/kg	3	.0204
	Control	3	.0207
	NTA 50 mg/kg	3	.0209
	Sig.		.134

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

(B-1d)

Table B-2 Arsenic accumulations in underground part (Root) of *Mimosa pudica* L. during four months period

At harvest time of 30 days

Treatments		N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Control	3	2.0133			
	NTA 50 mg/kg	3	2.0933			
	NTA100 mg/kg	3	2.5767	2.5767		
	EDTA 200 mg/kg	3		3.3067	3.3067	
	EDTA 100 mg/kg	3		3.6600	3.6600	
	NTA 200 mg/kg	3			3.9200	3.9200
	EDTA 50 mg/kg	3				4.9967
	Sig.		.315	.063	.275	.054

(B-2a)

At harvest time of 60 days

Treatments		N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	NTA 50 mg/kg	3	3.7367			
	Control	3	3.8200			
	EDTA 200 mg/kg	3	5.0667			
	NTA 200 mg/kg	3	5.5067	5.5067		
	NTA100 mg/kg	3		7.3800	7.3800	
	EDTA 100 mg/kg	3			8.0867	
	EDTA 50 mg/kg	3				11.0133
	Sig.		.119	.078	.485	1.000

(B-2b)

At harvest time of 90 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	NTA 200 mg/kg	3	6.4400				
	Control	3	6.5900				
	NTA 50 mg/kg	3		10.2067			
	EDTA 200 mg/kg	3		11.6233	11.6233		
	NTA100 mg/kg	3			13.3733		
	EDTA 100 mg/kg	3				19.0967	
	EDTA 50 mg/kg	3					25.8800
	Sig.		.911	.298	.204	1.000	1.000

(B-2c)

At harvest time of 120 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Control	3	10.5033				
	NTA 200 mg/kg	3		14.6167			
	EDTA 200 mg/kg	3			17.2933		
	NTA100 mg/kg	3			18.1967		
	NTA 50 mg/kg	3				21.1700	
	EDTA 50 mg/kg	3					27.9267
	EDTA 100 mg/kg	3					29.7100
	Sig.		1.000	1.000	.468	1.000	.163

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

(B-2d)

Table B-3 Arsenic accumulations in aboveground parts (Stem and leaves) of *Mimosa pudica* L. during four months period

At harvest time of 30 days

Treatments		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Control	3	.6967	
	NTA 50 mg/kg	3	.9633	
	EDTA 200 mg/kg	3	.9733	
	NTA100 mg/kg	3	1.0033	
	NTA 200 mg/kg	3	1.1200	
	EDTA 50 mg/kg	3	1.1233	
	EDTA 100 mg/kg	3		1.8233
	Sig.		.093	1.000

(B-3a)

At harvest time of 60 days

Treatments		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Control	3	1.3767		
	NTA100	3	2.0400	2.0400	
	NTA 200	3	2.4567	2.4567	2.4567
	EDTA 200	3	2.5167	2.5167	2.5167
	NTA 50	3		2.5967	2.5967
	EDTA 100	3		3.1700	3.1700
	EDTA 50	3			3.2733
	Sig.		.056	.062	.166

(B-3b)

At harvest time of 90 days

Treatments		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Control	3	2.4967		
	NTA 200	3	3.0333		
	NTA 50	3	3.1033		
	NTA100	3	3.5933	3.5933	
	EDTA 100	3		4.3500	4.3500
	EDTA 200	3		4.3833	4.3833
	EDTA 50	3			5.1600
	Sig.		.071	.171	.161

(B-3c)

At harvest time of 120 days

Treatments		N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Control	3	2.7133			
	NTA 200	3		3.3333		
	NTA100	3			4.4300	
	EDTA 200	3			4.4967	
	NTA 50	3			4.7067	
	EDTA 50	3				6.2400
	EDTA 100	3				6.3200
	Sig.		1.000	1.000	.357	.776

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

(B-3d)

Table B-4 Arsenic accumulations in underground part (Root) and aboveground parts (Stem and leaves) of *Mimosa pudica* L. during four months period

Arsenic accumulations in underground part of plants (mg As/kg plant)

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Control	3	10.5033				
	NTA 200 mg/kg	3		14.6167			
	EDTA 200 mg/kg	3			17.2933		
	NTA 100 mg/kg	3			18.1967		
	NTA 50 mg/kg	3				21.1700	
	EDTA 50 mg/kg	3					27.9267
	EDTA 100 mg/kg	3					29.7100
	Sig.		1.000	1.000	.468	1.000	.163

(B-4a)

Arsenic accumulations in aboveground parts of plants (mg As/kg plant)

Treatments		N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Control	3	2.7133			
	NTA 200 mg/kg	3		3.3333		
	NTA 100 mg/kg	3			4.4300	
	EDTA 200 mg/kg	3			4.4967	
	NTA 50 mg/kg	3			4.7067	
	EDTA 50 mg/kg	3				6.2400
	EDTA 100 mg/kg	3				6.3200
	Sig.		1.000	1.000	.357	.776

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

(B-4b)

Table B-5 Total arsenic accumulations in all parts of *Mimosa pudica* L. (mg/kg plant)

At harvest time of 30 days

Treatments		N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Control	3	2.7100			
	NTA 50 mg/kg	3	3.0567			
	NTA 100 mg/kg	3	3.5800	3.5800		
	EDTA 200 mg/kg	3		4.2800	4.2800	
	NTA 200 mg/kg	3			5.0400	5.0400
	EDTA 100 mg/kg	3				5.4833
	EDTA 50 mg/kg	3				6.1200
	Sig.		.115	.178	.146	.056

(B-5a)

At harvest time of 60 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Control	3	5.1967				
	NTA 50 mg/kg	3	6.3300	6.3300			
	EDTA 200 mg/kg	3	7.5833	7.5833	7.5833		
	NTA 200 mg/kg	3		7.9633	7.9633		
	NTA 100 mg/kg	3			9.4200	9.4200	
	EDTA 100 mg/kg	3				11.2533	
	EDTA 50 mg/kg	3					14.2867
	Sig.		.056	.178	.133	.116	1.000

(B-5b)

At harvest time of 90 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Control	3	9.0867				
	NTA 200 mg/kg	3	9.4733				
	NTA 50 mg/kg	3		13.3100			
	EDTA 200 mg/kg	3		16.0067	16.0067		
	NTA 100 mg/kg	3			16.9667		
	EDTA 100 mg/kg	3				23.4500	
	EDTA 50 mg/kg	3					31.0400
	Sig.		.786	.074	.502	1.000	1.000

(B-5c)

At harvest time of 120 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Control	3	13.2167				
	NTA 200 mg/kg	3		17.9500			
	EDTA 200 mg/kg	3			21.7900		
	NTA 100 mg/kg	3			22.6267		
	NTA 50 mg/kg	3				25.8767	
	EDTA 50 mg/kg	3					34.1667
	EDTA 100 mg/kg	3					36.0300
	Sig.		1.000	1.000	.443	1.000	.100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

(B-5d)

Table B-6 Concentration of As in soil at each time of sample collection (mg As/kg soil)

At harvest time of 30 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	EDTA 50 mg/kg	3	4.7387				
	EDTA 100 mg/kg	3	4.7410	4.7410			
	NTA 200 mg/kg	3		4.7420	4.7420		
	EDTA 200 mg/kg	3		4.7432	4.7432		
	NTA 100 mg/kg	3			4.7441	4.7441	
	NTA 50 mg/kg	3				4.7464	4.7464
	Control	3					4.7482
	Sig.		.094	.125	.148	.090	.182

(B-6a)

At harvest time of 60 days

Treatments		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	EDTA 50 mg/kg	3	4.6021		
	EDTA 100 mg/kg	3		4.6136	
	NTA 100 mg/kg	3		4.6193	4.6193
	EDTA 200 mg/kg	3		4.6203	4.6203
	NTA 200 mg/kg	3		4.6210	4.6210
	NTA 50 mg/kg	3			4.6263
	Control	3			4.6279
	Sig.		1.000	.131	.090

(B-6b)

At harvest time of 90 days

Treatments		N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	EDTA 50 mg/kg	3	4.4194				
	EDTA 100 mg/kg	3		4.4504			
	NTA 100 mg/kg	3			4.4736		
	EDTA 200 mg/kg	3			4.4752		
	NTA 50 mg/kg	3				4.4870	
	Control	3				4.4945	4.4945
	NTA 200 mg/kg	3					4.4988
	Sig.		1.000	1.000	.729	.133	.382

(B-6c)

At harvest time of 120 days

Treatments		N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	EDTA 100 mg/kg	3	4.2665					
	EDTA 50 mg/kg	3	4.2705					
	NTA 50 mg/kg	3		4.2944				
	NTA 100 mg/kg	3			4.3390			
	EDTA 200 mg/kg	3				4.3438		
	NTA 200 mg/kg	3					4.3598	
	Control	3						4.3773
	Sig.		.091	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

(B-6d)

APPENDIX C

Figure C-1 Investigation the arsenic contamination site

Figure C-2 Nursery and plant preparations

Figure C-3 Application of chelating agents

Figure C-4 Pots experiment and plastic rotary hand pumps

Figure C-5 Soil and plant samples

Figure C-6 Soil properties measurement and Atomic absorption spectrometer equipment

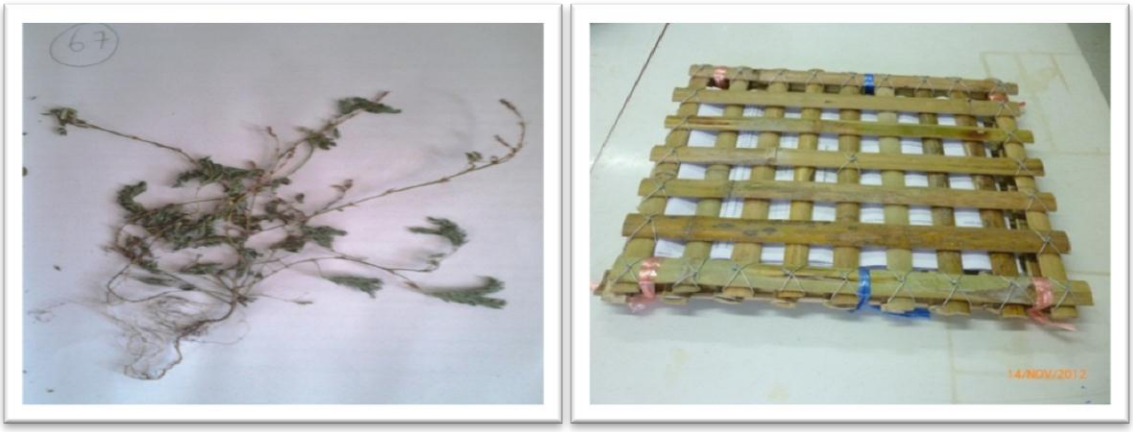

Figure C-7 Dry plant sample and Herbarium type

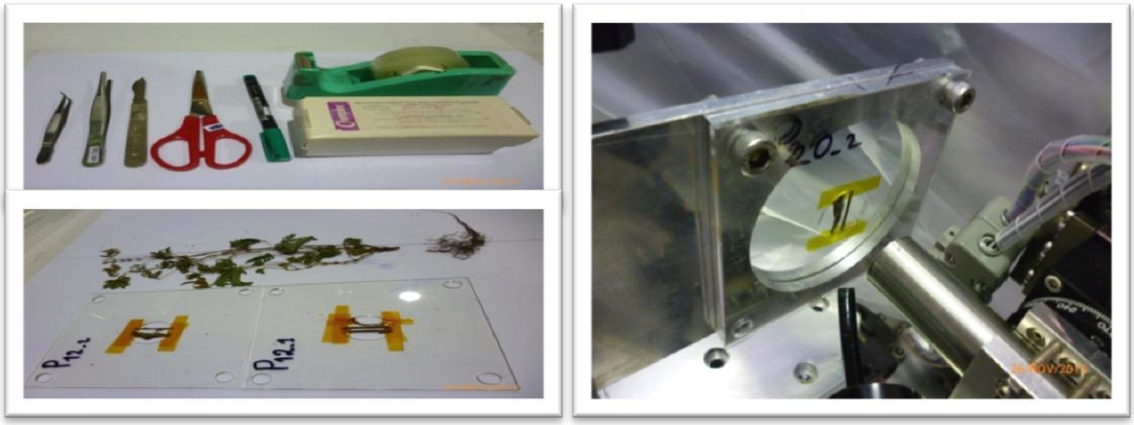

Figure C-8 Sample preparation and analysis by the microbeam synchrotron X-ray fluorescence beamline

Figure C-9 The microbeam synchrotron X-ray fluorescence beamline 6b equipment

BIOGRAPHY

Mr. Khamla Nanthavong was born on May 07, 1985 in Phiawat Village, Khoun District, Xiengkhouang Province, Lao PDR. He attended Chomphet High School in Xiengkhouang and graduated in 2003. In 2008, he graduated with a Bachelor degree in Plant Science from the Faculty of Agriculture and Natural Resources, Souphanouvong University, Luangprabang, Lao PDR. Then he pursued his master's degree in the International Postgraduate Program in Environmental Management, National Center of Excellence for Environmental and Hazardous Waste Management (NCE-EHWM), Graduate School, Chulalongkorn University from 2011-2013.

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