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Effect of NTA and EDTA on Arsenic Uptake from Contaminated Soil by *Mimosa Pudica*

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Abstract

The purposes of this study were to determine arsenic accumulation in the root, stem and leaves of *Mimosa Pudica* L. and compare the efficiency of two chelating agents, in enhancing arsenic uptake by the plant. This study also investigated the distribution of arsenic in the plant. The results showed that arsenic accumulation in root was significantly higher than in stem and leaves ($P \leq 0.05$). The maximum arsenic accumulated in roots, stem and leaves were 29.71 and 6.32 mg arsenic/kg plant, after 120 days, respectively. The average arsenic accumulation in all parts of the plant over four months was in the range of 2.71 - 36.03 mg arsenic /kg plant and set ethylenediaminetetraacetic acid 100 mg/kg soil showed the highest arsenic accumulation in *Mimosa Pudica* L. Overall, with the same harvesting times and application doses of chelating agents, ethylenediaminetetraacetic acid has a greater efficiency for enhancing arsenic uptake in this plant than nitrilotriacetic acid. Moreover, the synchrotron μ-X-ray fluorescence spectroscopy (Beamline 6b) analysis provided an unexpected result on the distribution of arsenic in the plant caused by the limitation of the radiation beam line. However, this research did not study the chemical reactions between arsenic and the chelating agents. Therefore, for future studies it is recommended that more detail at molecular level be investigated and more study be done on the influence between the applications of fertilizers and without fertilizers which might help us to clarify the factors that stimulate the movement of arsenic from the soil up to the plants.

Keywords: nitrilotriacetic acid, ethylenediaminetetraacetic acid, arsenic, *Mimosa Pudica*, soil

1. Introduction

Contamination with arsenic (As) in soil is a widespread problem due to human activities such as mining, the past use of al agrochemicals (pesticides/ insecticides) and smelter activities. These activities have many negative effects on the environment and ultimately on human health. Hazardous substances which are released into the soil, water and ground water are numerous and As is one of the most common. Plants absorb As easily so high concentrations may be present in food. A concentration of dangerous inorganic As is currently present in surface water and can enhance changes in fish genetics. This is mainly caused by accumulation of As in the bodies of plant-eating freshwater organisms. This poison can move to humans and other animals through the food chain. In addition, As 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.

The case of Southern Thailand is an example of an As contaminated area producing many health problems to humans. In 1992 the department of mineral resources investigated and measured the As concentration on site and they found that the As concentration in the soil ranged from 0-1,770 mg As/kg soil. An analysis for species showed that As(V) was found in more than 90% of all deposits at this site (Department of Mineral Resources [DMR], 1992). Moreover, the DMR also reported that the As contamination in soil and sediment was higher than the background concentration of 50 mg As/kg soil. This As poison came from Arsenopyrite mineral (FeAsS) which is dissolved by reacting with air and water and transformed to another form as in the following equation:

$$4FeAsS + 13O_2 + 6H_2O \rightarrow 4FeSO_4 + 4H_3AsO_4 \quad (1)$$

At present, those mining sites are closed, but the As contamination is still spreading 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. The people have become sick and many of them are infected with skin diseases including alternate pigmentation, small corns on the palms and soles, purplish-red flush and skin cancer (Pollution Control Department [PCD], 1998; Ranjan, *et al*., 2012). The concentration of As in surface soil (0-25 cm depth) on site represented in soil has been found from 20 - 62 mg As/kg soil (Visoottisethet *et al*., 2002), while Jankong (2007) has found 136 – 269 mg As/kg in the soil samples (0-15 cm). However, in Ferneziu area and Săsar district of the North West region of Romania has found the concentration of arsenic in soil ranged between 0.25-255 mg/kg and 5.5-295 mg/kg, respectively (Oprea, *et al*., 2010).

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 of cleaning 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 such as Thailand. In addition, phytoremediation is an environmentally friendly technology that aims to reduce heavy metal contamination in soil (Akegacha, *et al*., 2014). 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 as a component of cement.

The plant species used to remediate toxic metal contaminated soil should satisfy several criteria including: wide distribution, high above ground biomass, high bioaccumulation factors, short life cycle and high propagation rate. *Mimosa Pudica* L. (Bashful mimosa) is a plant species that tolerates high As contamination and has a short life cycle. This plant is also found commonly in As contaminated sites and it is ranked as the fourth most suitable plant species for phytoremediation found in As contaminated areas. Visoottiviseth *et al*. (2002), reported that other plants such as *Pityrogramma calomelanos* (Silver fern), *Pterisvittata* (Chinese brake fern), and *Melastomamalaba-thricum* (Blackmouth plant) can be used in phytoremediation as well. However, the time needed to remediate the toxic site is quite long. Thus this research will study the use of chelating agents (Nitrilotriacetic acid; NTA and Ethylenediaminetetraacetic acid; EDTA) to enhance the heavy metal uptake by *Mimosa Pudica* L. This will help to clean up the toxic areas faster. NTA and EDTA have been previously studied to improve the phytoremediation efficiency of plants on other heavy metal contaminants and the results have shown that NTA and EDTA significantly enhanced the heavy metal uptake by various other plants (Chiu *et al*., 2005). Therefore, the aims of this research were to determine As accumulation in root, stem and leaves of *Mimosa Pudica* L. and compare the efficiency of different doses of NTA and EDTA for enhancing uptake by *Mimosa Pudica* L. This study also investigated the distribution of As with other elements when present inside the plant. The results aim to establish the optimum concentrations of required chelating agents to promote optimal As accumulation in the underground part (root) and aboveground parts (stem and leaves) of the target plant.

2. Materials and Methods

2.1 Soil and Plant Preparation

Uncontaminated soil was used in this experiment; this soil was excavated from Nakhon Pathom Province, Thailand. It was excavated from the upper layer (0 - 30 cm) of the surface soil. All soil was crushed and dried in an open air temperature and analyzed for soil background (pH, soil texture, moisture, conductivity, oxidation reduction potential (ORP), cat-ion exchange capacity (CEC), organic matter (OM), nitrogen, phosphorus, potassium and As concentration). *Mimosa Pudica* L. was also collected from uncontaminated soil in Bangkok, Thailand. All plants were grown for two weeks before transferring them into the experiment pots. After preliminary growth, three plant samples were selected and prepared for analyzing the As accumulation in plants. The United States Environment Protection Agency (USEPA), method 3052 and A Perkin Elmer Atomic Absorption Spectrometer Model AAnalyst 800 (Perkin Elmer Instruments LLC, Unberlingen, Germany) with hydride analysis were used to prepare and analyze As in the soil and plants.

2.2 Experimental Procedures

This experiment was separated into two stages: preliminary study and experimental procedure. All the pots were randomly placed in a nursery using the Completely Randomized Design (CRD) method. This aims to ensure that each plant will have exposure to available sunlight, air flow, temperature fluctuations and other environmental factors equally. The preliminary study purposed to investigate the tolerance of *Mimosa Pudica* L. growing in different concentrations of As and determine the phytotoxicities from an addition of two chelating agents (NTA

and EDTA).

The tolerance of plant study, *Mimosa Pudica* L. was separately grown in ten soil pots (5 kg of soil per pot) over one month. The two layer of plastic bags were used as soil container pots; planting plastic bags (with hole) were use as inside layer and general plastic bags (without hole) were use as outside layer to contain and keep the water that is released from giving water. 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 (5, 10, 20, 40, 80, 120, 160, 200, 300 and 400 mg/kg soil). All the pots were watered daily with tap water and the water which is released from watering will be used to recover water the particular plant again by using plastic rotary hand pumps (such released water was contained and kept in the outside plastic bags layer separately for each pot), this aims to make sure that the contaminant is not distributed to other area. The growth properties of the plants were also recorded. Then a dose of which had no negative impact on the growth of the plant was selected for further study. Another preliminary study was of the phytotoxicities from the 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 over a one month period. The doses of As and chelating agents were varied as follows:

- 6 pots: with an added dose of As (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 As and only 3 added doses (50, 100 and 200 mg/kg soil) of NTA or EDTA, separately.

For the main experiment, the 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 As at 5 mg As/kg soil. This concentration of As was selected because preliminary studies showed that plants can grow healthily and there was no negative effect on plant growth in the concentrations of As up to 10 mg As/kg soil. In order to save costs, a concentration of 5 mg As/kg soil was used in this experiment. Then this prepared soil was left for three months with the aim on mixing the As 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 NTA, [$C_6H_9NO_6$] at 50, 100 and 200 mg/kg soil.
- Experimental set 3: EDTA (36 pots), adding 3 doses of EDTA, [$C_{10}H_{16}N_2O_8$] at 50, 100 and 200 mg/kg soil.

After this, one seedling of *Mimosa Pudica* L. per pot was grown in the As contaminated soil. One week later three doses of NTA and EDTA (50, 100 and 200 mg/kg soil) were also added to each soil pot separately. All plants were planted in plastic bags (12 x 20 cm); each bag contained 5 kg of soil and the plants were watered with 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. This aimed to control the leaching of As 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.

2.3 Soil Samples Preparation and Analysis

Soil samples were collected at 0, 30, 60, 90 and 120 days. After the plant was taken out of the pot, the soil was mixed together and the soil sample was collected from different points of the sample at 4 - 6 points. Around 100 g of soil was collected from each pot and stored in zip lock bags. Then each sample was separated into 2 partitions for analyzing (1) the concentration of As in the soil and (2) the soil properties. The soil samples were analyzed for As concentration using the USEPA method 3052. The soil preparation was oven dried at 103˚C for 2 – 3 days to obtain a constant condition and measured for the dry weight of the soil.

After that all the soil samples were crushed and passed through a 2 mm sieve. After that 0.5 g of each soil sample was taken and HCl (Hydrochloric acid) and HNO_3 (Nitric acid) added 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 As concentration was determined using Atomic Absorption Spectrometry (AAS hydride). 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.

2.4 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:

2.4.1 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:

$$RGR = [Ln (W_2) – Ln (W_1)] / (t_2-t_1) \quad (2)$$

Where:

- 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)

2.4.2 As Concentration Analysis

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

All these samples were air dried at room temperature for 2 – 3 hours before measuring the wet weight. Then all the 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 As concentration in the samples was analyzed using Atomic Absorption Spectrometry (AAS hydride) analysis.

2.4.3 The Distribution of As and Other Elements Inside the Plant Using 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 with 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 and placed at room temperature until analysis. Finally, the distribution of As and other related elements inside the plant samples were analyzed using the Synchrotron Radiation method BL6b (Synchrotron Light Research Institute, 2011).

2.5 Statistical Analysis

The data from 3 replications and 4 harvesting times was analyzed using the Statistical Package for the Social Sciences (SPSS). The concentration in soil and 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 ($P \leq 0.05$).

3. Results and Discussion

3.1 Soil Properties

The soil used in this experiment was silt clay; it has organic matter of 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 As in soil was detected at under 0.01 mg/kg soil **(Table 1)**.

The result of this preliminary study showed that plants can grow healthily in As concentrations up to 10 mg/kg soil while other upper concentrations showed phytotoxicities in plant growth. The symptoms of the plants presented differently such as dry and curly leaves and stem; finally they died from getting too high a concentration of As. 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 an alternatively tolerant plant for phytoremediation and is also able to clean up low contamination in agricultural soil. The standard of As 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 with As up to 10 mg As/kg soil under the condition of no application of any fertilizer and growth in a control nursery, in order to save costs only 5 mg As/kg soil was selected for future study. NTA and EDTA were also studied by growing plants on the prepared soils with 5 mg As/kg soil and without As by amending the 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 the 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 soils and in the water phase.

Table 1. Physical and chemical properties of soil before use in the experiment

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

3.2 The Growth Rate of Plants

The relative growth rates of plants for all treatments over four months ranged from 0.020 - 0.051 gram/day (**Figure 1**). 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 were not so different. The highest growth rate occurred in a pot with EDTA 50 mg/kg with a value of 0.051 gram/day; whereas the lowest growth rate appeared in the treatments at 120 days with a value around 0.020 gram/day.

Moreover, the highest growth rates in 60, 90 and 120 days were 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 1**). After 120 days, plants showed the lowest growth rates when compared to other harvesting times; this might have been caused by a 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.

The pH values in soil of all treatments over four months did not change much; they were in the range of 7.09 - 7.36. Overall, the soil pH values of all treatments increased when the time increased and the pH values of the control treatments were higher than the values of other treatments and the pH values in the initial period (30 days) were lower than the pH values in the later periods (60, 90 and 120 days, respectively); these changes of pH values in soil might have been influenced by natural processes in soil and other concerned factors after phytoremediation. The different varieties of As occurred more in the soil pH range from 2 to 7 (Nriagu, 1994). In general, the pH values of the treatments with added NTA were a little more acidic than EDTA. The pH values increased when increasing the concentrations of both NTA and EDTA in soil.

3.3 As Accumulation in Plants

Figure 2 shows that the average As accumulation 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 As accumulation in the plant roots increased depending on the time. When the numbers of days increased from 30 to 60, 90 or 120 days, the concentrations of As in the roots of *Mimosa Pudica* L. also increased. At 120 days, the experimental sets EDTA 100 mg/kg showed the highest As accumulation in the roots with a concentration at 29.71 mg As/kg plant and followed by sets EDTA 50 mg/kg with a 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 As accumulation in the root of the plant during our experimental period of four months occurred in the control set at 30 days at a value of 2.01 mg As/kg plant.

In comparison, among these two chelating agents with the same doses added, EDTA was shown to be more effective than NTA for enhancing As accumulation in the roots of *Mimosa Pudica* L. except at a dose of 200 mg/kg. EDTA was an effective chelating agent for enhancing As uptake by plants (Tambamroong, 2002). The highest As accumulation in the 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 2**). Moreover, the sets with added chelating agents also reported significantly higher concentrations of accumulation in the roots than the sets without chelating agents (Control sets).

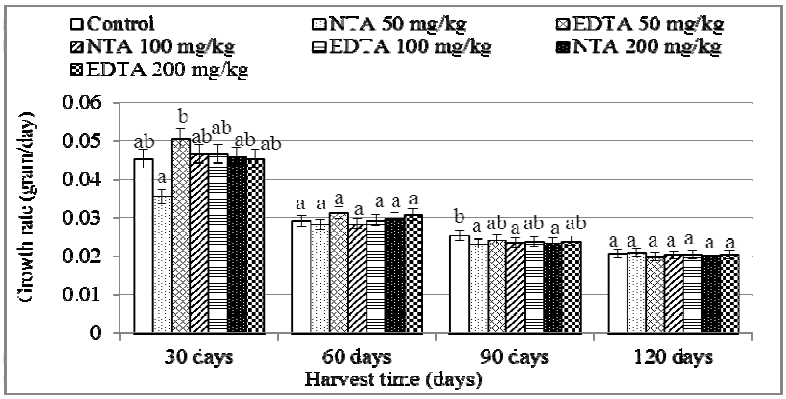

Figure 1. Average relative growth rates of plants on different application doses of NTA and EDTA over a period of time

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

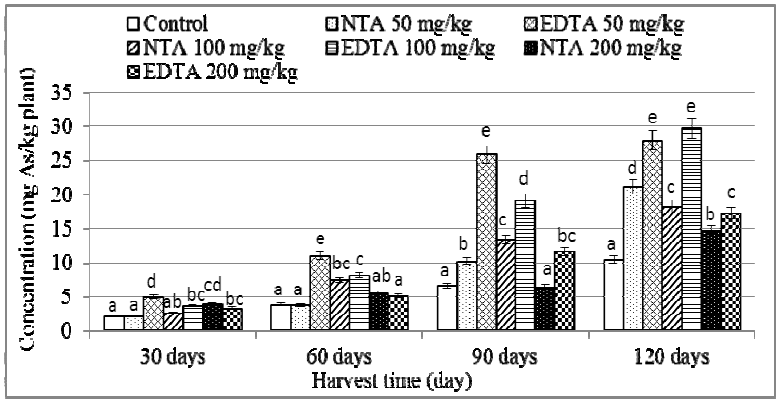

Figure 2. Average As accumulation in the underground parts (roots) of plants

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

The average As accumulation in the aboveground parts (stem and leaves) of *Mimosa Pudica* L. reported very small concentrations. There is no significant difference (P≤0.05) between As 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 parts of the plant with concentrations at 6.24 and 6.32 mg As/kg plant, respectively (**Figure 3**). The lowest 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 As 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) had a similar ability to stimulate As accumulation in the stem and leaves of plants with 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 As accumulation in the aboveground parts of plants with concentrations at 1.82 and 6.32 mg As/kg, respectively while at 60 and 90 days the highest ability to remove As from soil occurred in the set with EDTA 50 mg/kg with concentrations at 3.23 and 5.16 mg As/kg plant, respectively.

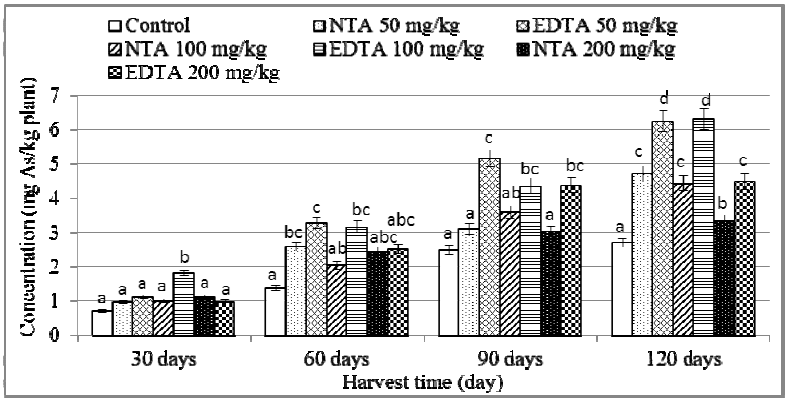

Figure 3. Average combinations of As accumulation in the aboveground parts (stem and leaves) of plants

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

3.4 Comparison of As Accumulation in Plants at 120 Days

From previous results, the As accumulations in both the roots and in a combination of stem and leaves showed a higher As uptake by *Mimosa Pudica* L. at 120 days. Therefore, As 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 As accumulation in the underground part were significant greater than in the aboveground parts of the plant (**Figure 4**).

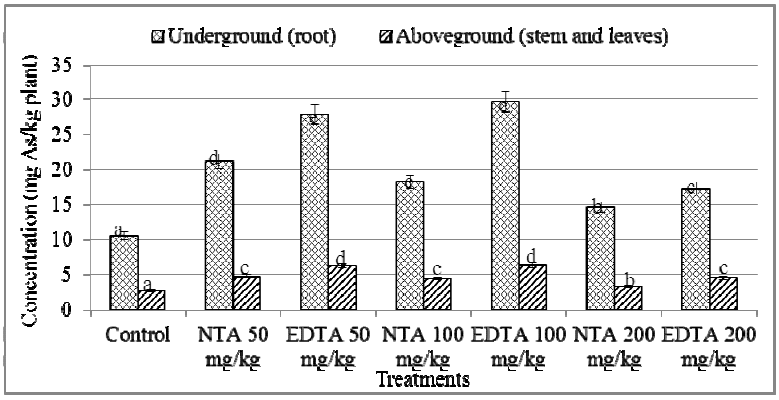

Figure 4. The comparison between average As accumulation in the underground and aboveground parts of plants over a four month period

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

Smith *et al*. (2002) also reported that plants normally accumulate As in plant roots more than other parts. As 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 absorption by the plant roots (Schmoger *et al*., 2000; Pickering *et al*., 2000). The concentrations of As in the aboveground parts were no greater than 6.32 mg As/kg plan for all sets while the concentration in the underground parts was able to reach 29.71 mg As/kg (set EDTA 100 mg/kg). Generally, plaque formation on plant roots can affect the uptake of As and heavy metals by the plants in different ways (Otte *et al*., 1991).

In addition, sets with the same chelating agents but different applied doses also presented different properties for As accumulation in plants. The ability of As accumulation in *Mimosa Pudica* L. increased when the application doses of EDTA increased from 50 - 100 mg/kg 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

will have less ability to stimulate the mobilization of As in soil.

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

3.5 Efficiency of as Accumulation in Plants

Table 2 shows the As accumulations in all parts of *Mimosa Pudica* L. (Bashful mimosa) was 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 As accumulation in all parts *of Mimosa Pudica* L. with concentrations at 36.03 mg As/kg plant, followed by the set EDTA 50 mg/kg with concentrations at 34.17 mg As/kg plant; but in statistical analysis terms 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 high As contaminated soil (Visoottiviseth *et al*., 2002), the removal ratios compared to total As in soil was very low (Jirawan, 2000 ; Luc, *et al*., 2012). With the same applied doses of two chelating agents (NTA and EDTA) for all harvesting times, EDTA sets showed a higher efficiency for enhancing uptake by *Mimosa Pudica* L. than NTA except at a dose of 200 mg/kg.

Table 2. Total accumulation in all parts of *Mimosa Pudica* L

Experimental sets	Average 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 in the top right corner means there is no significant difference (p≤0.05) when comparing the mean values of different treatments at the same harvesting time.

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

Overall, both chelating agents acted as very important substances for enhancing As uptake by *Mimosa Pudica* L. but the ability to uptake As from applications of EDTA was significantly better than NTA because EDTA may have greater efficiency in stimulating the As in soil from an immobile form to a mobile form than NTA. Tambamroong *et al*. (2002), also reported that EDTA was an effective chelating agent for enhancing As uptake by taro. EDTA is widely used for increasing the heavy metal uptake of various plants. EDTA had more effluence than citric acid (CA) for enhancing cadmium uptake by water hyacinths 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 As 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 2**. In contrast, the As accumulations in plants of the 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 As accumulation in this plant was in the range of 41 – 55 mg As/kg plant. This might have been influenced by the growing conditions of *Mimosa Pudica* L. because we grew them in a control nursery, without adding any fertilizer while, Visoottiviseth *et al*. (2002), collected the plants from the wild so that then would had a greater age and the plants would have also been grown in more fertile soil.

3.6 The Distribution of as and Other Elements inside the Plants

This analysis aims to determine the distribution of As 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 elements using the Synchrotron Radiation method BL6b (Synchrotron Light Research Institute, 2011).

The previous results illustrated that the highest As 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 As and other elements when they presented inside the plant. Every element has a value for X-Ray emission energy itself. When As absorbs X-Ray radiation, around 10.543 Kilo electron volts (KeV) will be emitted by the As compound (Synchrotron Light Research Institute, 2011). The results show that As could not be detected in all plant samples which was collected from both harvesting times (At 30 and 120 days); while Atomic Absorption Spectrometry (AAS hydride) analysis found the As concentrations in the roots 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 harvesting time 30 days) and at 120 days, AAS hydride analysis also found the concentration of As in 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 have been caused by the limitation of this beamline because a Si (111) crystal was 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 could detect only 1x1 mm.

In contrast, As 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 As inside the plant is very low and the beamline of the synchrotron μ-X-ray fluorescence spectroscopy cannot detect the As in the plant sample.

Figure 5 shows the distribution of elements in all parts of the plant samples (In the root, stem and leaves) at harvesting time 120 days. This figure shows that As can be detected under synchrotron μ-X-ray fluorescence spectroscopy analysis (There is no peak to be found at 10.543 keV of axis energy emission value as shown on **Figure 5a, 5b and 5c**). However, other elements (K, Ca and Fe) were detected in these plant samples. Therefore, we can estimate that the concentrations of these compounds are higher than As in these plant samples. The detected argon (Ar) might come from the surrounding air during the sample measurement (Not in the sample). An example of iron (Fe) distribution inside the plant root at 120 days is shown on **Figure 5d**. and the colors represent a concentration of Fe in the sample (Blue to red means the concentration from low to high). Although, the beamline 6b of the Synchrotron Radiation cannot detect As in the plant samples this would be a good starting point for using the beamline 6b to detect the heavy metals in green plant. However the Synchrotron Radiation is a technology new to analysis in Thailand and so the detection limit for As in green plants has not been tested and published in the database yet but the concerned organizations are preparing the analyzing evidence for supporting the use of this beamline.

4. Conclusions

From the experimental results, *Mimosa Pudica* L. can survive in As contaminated soil up to 10 mg As/kg soil under conditions of no application of any fertilizer and growth in a control nursery. Therefore, *Mimosa Pudica* L. can be an alternative As tolerant plant variety for phytoremediation and is able to manage and clean up low As contamination in agricultural soil. All three applied doses of NTA and EDTA at 50, 100 and 200 mg/kg showed no phytotoxicity in plant growth during a month of preliminary studies. The average values of As accumulation in all parts of *Mimosa Pudica* L. over 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 As accumulation in *Mimosa Pudica* L. with concentrations at 34.17 and 36.03 mg As/kg plant, respectively. In addition, the capacity for As 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 have been caused by too high concentrations of EDTA and NTA that reduce the ability of As into mobile form.

Overall, with the same applied doses of two chelating agents, (NTA and EDTA), EDTA sets showed a greater efficiency than NTA for enhancing As uptake by *Mimosa Pudica* L. The As 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 As in the *Mimosa Pudica* L. increased too. Generally, both chelating agents (NTA and EDTA) acted as important substances for enhancing As uptake by *Mimosa Pudica* L. but the capacity to uptake As from adding EDTA was better than NTA.

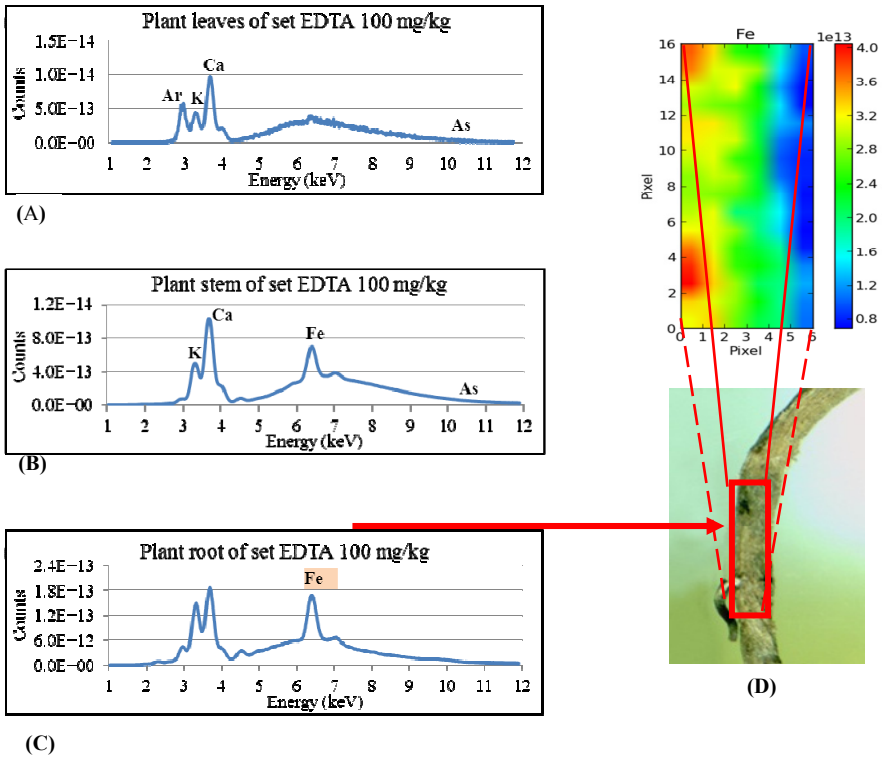

Figure 5. The distribution of elements inside the *Mimosa Pudica* L. at 120 days (A = in leave, B = in stem, C = in root and D = distribution of Fe in plant root).

Note. Blue to red means the concentration from low to high.

In terms of the synchrotron μ-X-ray fluorescence spectroscopy analysis, the concentrations of As 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 have been caused by the limitation of the Beamline and/or the mistake might occur during the samples preparation. In contrast, other elements such as 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 were higher than As in these plant samples.

This research did not study the chemical reactions between As and chelating agents however the As contamination is achieved by supplying arsenate solution in soil, which means the primary speciation of As is in anionic form, and would be competing for sorption onto soil or into plant's roots with chelating agents. Therefore, for more explanations in future studies are recommended to investigate the detail in molecular level and study more between the differences of plants application. This will help to clarify which factor is more effective for simulating the movement of As from the soil up to the plant between fertilizers and chelates.

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