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Effect of EDTA and NTA on Arsenic Bioaccumulation and Translocation Using Phytoremediation by *Mimosa pudica* L. from Contaminated Soils

Pantawat Sampanpanish[1,2,3] · **Khamla Nanthavong**[4]

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Abstract

This study aimed to investigate the effects of Nitrilotriacetic acid (NTA) and Elthylenediaminetetraacetic acid (EDTA) on the bioaccumulation and translocation of arsenic (As) by *Mimosa pudica* L. using soils with 5 mg/kg of added As and NTA and EDTA concentrations of 50, 100, and 200 mg/kg. Soil and plant samples were collected every 30–120 days to analyze the As concentrations in the soil, underground part of the plants (root), and aboveground parts of the plants (shoots and leaves). The results showed that the plants with EDTA concentrations of 100 mg/kg had the highest As accumulation. At 120 days, *M. pudica* L. had a higher accumulation in the underground parts (29.71 mg/kg) than in the aboveground parts (6.32 mg/kg), with statistical significance ($p < 0.05$). The As translocation factor in the aboveground parts was less than 1, indicating As accumulation in the underground part only. With EDTA concentrations of 50 and 100 mg/kg, *M. pudica* L. had the highest bioaccumulation potential of As of 8.00 and 8.44, respectively. However, this research did not examine the reaction between As and any growth promoters. Further research should investigate the details of such a reaction at the molecular level, as well as explore how fertilizer factors might affect the As absorption of *M. pudica* L.

Keywords Phytoextraction · *Mimosa pudica* L. · Accumulation · Chelating agent · Arsenic

The expansion of the large-scale industrial sector has a direct and indirect impact on the quality of the environment; in particular, the release of toxic heavy metals (HMs) into the environment can be problematic. Thailand faces problems concerning contamination of HMs in various areas; As contamination has been found in Nakhon Si Thammarat, Loei, Pichit, Petchabun, and Pitsanulok provinces. Contamination can be caused by many factors, including the development of mining and industry as well as stripping for cultivation, which results in the dispersion of HMs into soil, surface water, underground water, sediments, and plants and animals used for consumption. In addition, HM contamination may arise naturally from weathering of underlying geological formations. These problems affect human health and the environment because As is a highly toxic heavy metal that is biologically non-degradable; consequently, As persists and accumulates in the environment (Surriya et al. 2015). For this reason, the guidelines for appropriately reducing As in the environment are worth noting, especially practical methods that are applicable for actual situations involving wide areas of land, such as those surrounding mining sites. There are diverse methods for As and heavy metal remediation, including biological, chemical and physical methods. At present, a low-cost method of particular interest is phytoremediation, or the absorption and accumulation of As or other heavy metals in plant parts (Sampanpanish et al. 2015). Although phytoremediation has many benefits, including the potentialto improve natural scenery, it also has limitations, especially concerning the toxin treatment period (Aisien et al. 2012).

Therefore, researchers have proposed the use of chelating agents, such as EDTA and NTA, which have been suggested for phytoremediation, to investigate the bioaccumulation

✉ Pantawat Sampanpanish
pantawat.s@chula.ac.th

[1] Environmental Research Institute, Chulalongkorn University (ERIC), Bangkok 10330, Thailand

[2] Research Program of Toxic Substance Management in the Mining Industry, Center of Excellence on Hazardous Substance Management (HSM), Bangkok 10330, Thailand

[3] Research Unit of Green Mining Management (GMM), Chulalongkorn University, Bangkok 10330, Thailand

[4] International Postgraduate Program in Environmental Management, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand

and translocation of As in plants. This is consistent with the research of Evangelou et al. (2007) and Chui et al. (2005), who reported that the plant absorption rate of heavy metals was higher when EDTA and NTA were added. Hence, this research aimed to examine the effects of NTA and EDTA on As bioaccumulation and translocation by *M. pudica* L. from synthetic contaminated soils.

Materials and Methods

1. Soil preparation: Uncontaminated soil was used in this experiment. The soil was collected from Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province. The soil was excavated from the upper layer (0–30 cm) of the surface soil. The soil was placed into pots (5 kg of soil per pot) and then amended by using a solution of disodium hydrogen arsenate ($Na_2HAsO_4{\cdot}7H_2O$) at a concentration of 5 mg As/kg soil. This concentration of As was selected because previous studies have shown that plants can grow healthily at concentrations up to 5 mg As/kg soil. Next, the prepared soil was left for three months, allowing the As and soil to mix to simulate the As-contaminated soil found in the natural environment.
2. Plant preparation: *M. pudica* L. was excavated from uncontaminated soils in Prawet District, Bangkok, Thailand. All plants were preliminarily grown at an equal size for two weeks before being transferred into the experimental pots and maintained in a nursery. After the preliminary growth stage, three plant samples were selected and prepared for analysis of As accumulation in different parts of the plants. The USEPA method 3052 (USEPA 1996) and the atomic absorption spectrometry with hydride (AAS hydride) analysis were used to prepare and analyze As in the plants. The detection limit of As in AAS is less than 0.01 ppm (Yamamoto et al. 1985). As was not detected after the analysis; therefore, these plants represent the background condition used in this experiment, and it was assumed that these plants were uncontaminated with As.

   Moreover, *M. pudica* L. is a native plant found in As contaminated soil areas. Plants found in contaminated areas exhibit the following traits: the ability to uptake metals at high concentrations; good growth and a high growth rate; resistance to high heavy metal contamination in the area; lack of phytotoxicity; ease of cultivation and maintenance; short life cycles and life spans; good ability to propagate; not edible; and the capability to absorb heavy metals and transport them into plant cells (Yang et al. 2017; Sampanpanish 2015; Tananonchai and Sampanpanish 2014).
3. Experimental design: the experiment was separated into a control set and a treatment set. The treatment set was divided into three groups using NTA and EDTA concentrations of 50, 100 and 200 mg/kg soil. The control groups contained As without NTA and EDTA. One seedling per pot of *M. pudica* L. was grown in the As-contaminated soil. The plants were grown and maintained in a nursery for 30 days before the experiments began. All plants were planted in plastic bags (12 × 20 cm) containing 5 kg of soil and watered by tap water daily. Larger external plastic bags were used to prevent the leached water from leaking. This leached water was used to water the plant with plastic rotary hand pumps to prevent As from leaching into the outside environment.
4. Sample collection and analysis: Soil and plant samples were collected 0, 30, 60, 90 and 120 days after planting. The plant samples were separated into two parts: the underground sample (root) and the aboveground sample (stem and leaves). Each part of the plant and the soil samples were prepared and analyzed regarding the As concentration using the USEPA method 3052 (USEPA 1996) and Atomic Absorption Spectrometry (AAS hydride) analysis. Then, the concentration of As in the various parts of the plants and soil was calculated in terms of the bioaccumulation factor (BCF) (Goabas and Morrison 2000; Lu et al. 2004) using Eq. 1 and the translocation factor (TF) (Bu-Olayan and Thomas 2002; Zacchini et al. 2009) using Eq. 2.

$$\mathrm{BCF} = \frac{\text{Total conc. of As in plant}}{\text{Total conc. of As in soil}} \quad (1)$$

$$\mathrm{TF} = \frac{\text{Total conc. of As in shoot}}{\text{Total conc. of As in root}} \quad (2)$$

5. Distribution of As: The distribution of As and other elements inside the plants was assessed using a Synchrotron Radiation analysis. The second plant samples or one sample per set were separately collected to determine the distribution of As 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).
6. Statistical analyses: Statistical Package for the Social Sciences (SPSS) version 13 was used to analyze significant differences in the effects of different types of plants on the accumulation and distribution of As in the plant samples. In addition, Duncan's new multiple range test (DMRT) was applied to compare the significant difference in the mean concentrations of As accumulated in

the plant tissues among different cultivation times. A p-value of <0.05 was accepted as significantly different.

Results and Discussion

Arsenic (As) occurs naturally in all soils. It is possible that As is an essential element for plant growth, although this has not been proven. There are no well documented beneficial effects of As on plants. However, As compounds cause short-term and long-term effects on individual plants and animals, as well as within populations and communities of organisms. The adaptation of plants to As compounds is of great practical interest because plants can be used in phytoremediation. Moreover, a plant's tolerance to As depends on the nature of the contaminated soil. Therefore, the chelates EDTA and NTA, which are recommended for phytoremediation processes, were used to promote bioaccumulation and translocation of As in plants.

As accumulation in both the root and the stem and leaves showed a higher uptake by *M. pudica* L. at 120 days. Therefore, As accumulation in the underground part (root) and aboveground parts (stem and leaves) of the plant at 120 days was chosen for comparison among treatments. For all treatments, the concentrations of As accumulation in the underground part were significantly greater than those in the aboveground parts of the plant (Fig. 1). Smith et al. (2002) also reported that plants normally accumulate more As in their roots than in other parts. As, which is taken up by plants, is rarely transported to the upper parts (stem and leaves) of plants. As enters into plant bodies after being absorbed by the plant roots (Schmoger et al. 2000; Pickering et al. 2000). The concentration of As in the aboveground parts was never greater than 6.32 mg As/kg of plant, while the concentration in the underground parts reached 29.71 mg/kg (set EDTA 100 mg/kg). Plaque formation on the plant roots can affect the uptake of As and heavy metals in different ways (Otte et al. 1991; Chiu et al. 2005).

Moreover, the plant sets with the same chelating agents but different applied doses also showed differences in the As accumulation ability. The ability of *M. pudica* L. to accumulate As increased when the applied dose of EDTA increased from 50 to 100 mg/kg, but the accumulation capacity decreased when the concentration of EDTA reached 200 mg/kg. Too high a dose of EDTA might cause phytotoxicity in the plant and lower its ability to stimulate the mobilization of As in soil. In contrast, As accumulation in the underground part of the plant in the NTA plants sets decreased, while the applied dose of NTA was increased for sets with NTA concentrations of 21.17, 18.20 and 14.62 mg/kg (sets NTA 50, 100 and 200 mg/kg, respectively).

The study of As bioaccumulation in *M. pudica* L. indicated that bioaccumulation was higher when the experiment period increased. There was a statistically significant difference ($p \leq 0.05$) among all experimental sets of plants, with a confidence level of 95%. This result was consistent with that of the study by Katagi (2010), who stated that heavy metal bioaccumulation in plants under experimentation was likely to increase as the experiment time increased. When comparing the As bioaccumulation in *M. pudica* L. in all the experimental sets, it was shown that, at the end of the experimental period, the experimental set with EDTA added, particularly those sets with added EDTA at 50 and 100 mg/kg concentrations, had higher As bioaccumulation than the other sets. The different bioaccumulation values of $8.00 + 0.16$ and $8.44 + 0.33$ mg/kg were statistically significant ($p < 0.05$) at a

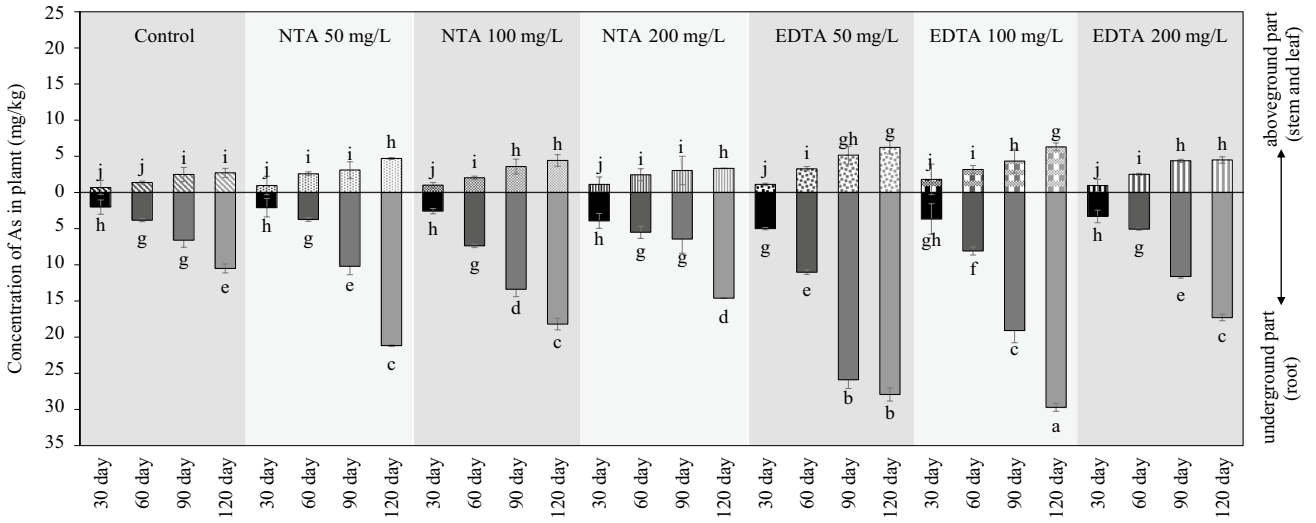

Fig. 1 Average As accumulations in the underground part (root) and aboveground parts (stem and leaf) of the plant (mg/kg). Note: The same alphabet above the bars means there is no significant difference ($p \leq 0.05$) when compared between the mean values of different treatments

confidence level of 95% (Fig. 2a). This result was consistent with that of the study by Wang et al. (2008), who found that EDTA addition had a positive impact on cadmium bioaccumulation in plants. In addition, the comparison between the control set and the set with added EDTA and NTA showed that As bioaccumulation in the set with added EDTA and NTA was significantly higher than that in the control set ($p < 0.05$). Additionally, the result was consistent with the research of Hsiao et al. (2007), who studied the effects of four chelates, DTPA, EDTA, CA, and oxalic acid, on bioaccumulation in *Brassica juncea*. The results indicated that these four chelates created a higher accumulation rate of heavy metals in *Brassica juncea*.

In addition to considering the effects of EDTA and NTA on As accumulation in the underground part (root) and aboveground parts (stem and leaves) of plants, the As translocation from root to stem and leaf was also considered. The As translocation factor in the aboveground parts was lower than 1, which indicated that As accumulation in the aboveground parts was lower than that in the underground parts (Fig. 2b). There was no change in the ratio of As accumulation in the underground and aboveground parts when considering the effect of time. This result explains why the increasing As accumulation in the underground part (root) caused higher As translocation and accumulation in the aboveground parts. The different concentrations of EDTA and NTA had no effect on the translocation of As by *M. pudica* L. This finding was consistent with the study by Sun et al. (2009) on the absorption of cadmium by *Solanum nigrum* L. with added EDTA. This study showed that bioaccumulation was higher throughout the duration of the experiment, while the translocation of As was not significantly different than the control set.

This analysis aimed to determine the distribution of As and other related elements inside plants. A plant sample was collected from each treatment at 30 and 120 days to analyze the distribution of the target elements using the Synchrotron Radiation method BL6b (Synchrotron Light Research Institute 2011).

Previous results illustrated that the highest As accumulation in plants at 30 and 120 days occurred in sets where EDTA was added at concentrations of 50 and 100 mg/kg, respectively; therefore, the plants from these plots were used to determine the distribution of As and other elements present inside the plants. Every element has a unique value of X-ray emission energy. When As absorbs X-ray radiation, approximately 10.543 kiloelectron volts (keV) are emitted from the As compound (Synchrotron Light Research Institute 2011). Figure 3a, b illustrate that As cannot be detected in these plant samples in all plant parts (root, stem and leaves). There was no peak found at 10.543 keV of the axis energy emission value as shown in Fig. 3a, b. However, atomic absorption spectrometry (AAS) analysis at 30 days found that the As concentrations in the root and in a combination of stem and leaves of plant sets with 50 mg/kg EDTA were 5 and 1.12 mg As/kg plant, respectively. At 120 days, AAS analysis showed As concentrations of 29.71 mg As/kg plant in the root and 6.32 mg As/kg plant in a combination of stem and leaves for the plant set with 100 mg/kg EDTA. This outcome causes a limitation of the beamline because a Si (111) crystal can be used to extract a monochromatic X-ray beam covering an energy scale from 2 to 12 keV, and the pixel size in the fluorescence maps can detect only 1×1 mm. In contrast, As accumulation in willow roots was previously detected using synchrotron μ-X-ray fluorescence spectroscopy (Zimmer et al. 2011). In this study, the micro XAS beamline was a dedicated, hard X-ray microprobe beamline that used a fixed-exit Si (111) double-crystal monochromator, covered an energy scale from 4 to 23 keV, and had a pixel size in all fluorescence maps of $1\ \mu m \times 1\ \mu m$. Moreover, the concentration of As inside the plant was

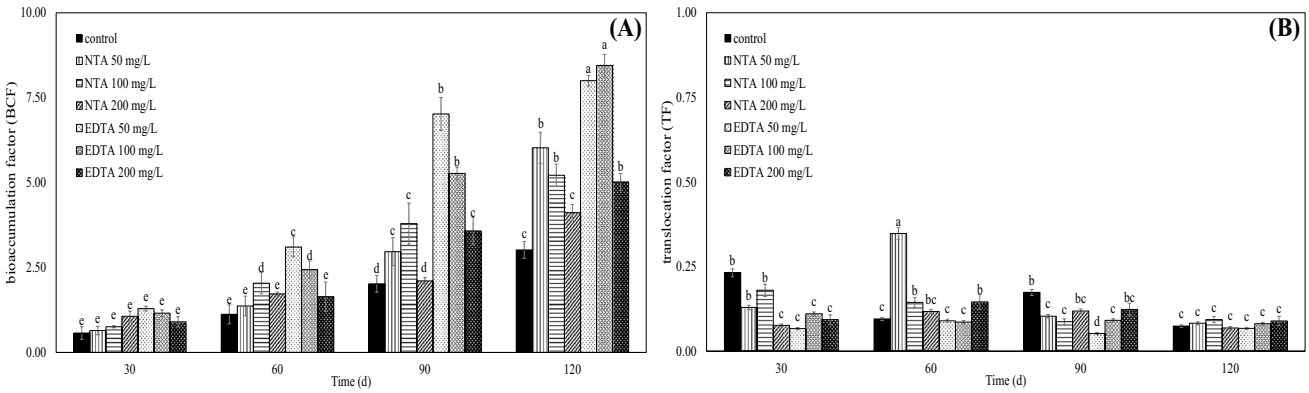

Fig. 2 **a** Effect of EDTA and NTA on the As bioconcentration factor (BCF) and **b** effect of EDTA and NTA on the As translocation factor (TF). Note: The same alphabet above the bars means there is no significant difference ($p \leq 0.05$) when compared between the mean values of different treatments

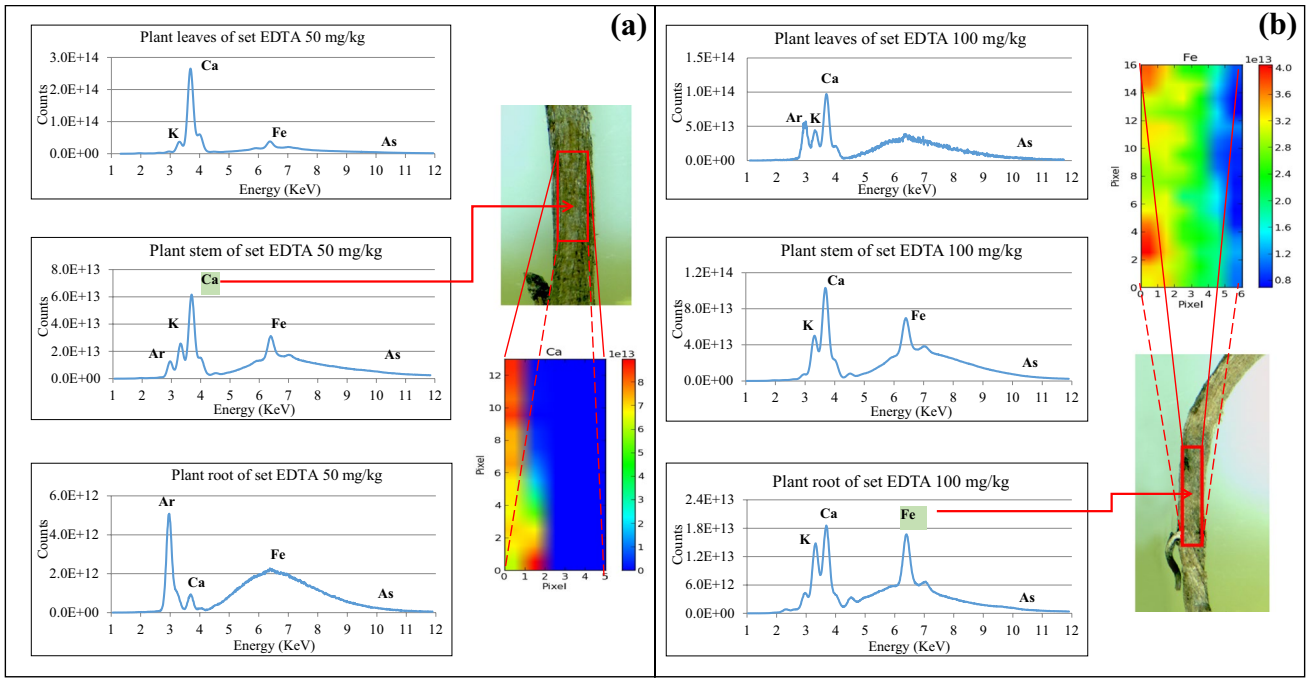

Fig. 3 **a** Distribution of elements inside the *M. pudica* L. at 30 days and **b** distribution of elements inside the *M. pudica* L. at 120 days. Note: Red to blue means the concentrations from high to low

very low, and the beamline of the synchrotron μ-X-ray fluorescence spectroscopy could not detect the As in the plant sample.

However, other elements (Ar, K, Ca and Fe) were detected in these plant samples using the synchrotron μ-X-ray fluorescence spectroscopy analysis. This finding indicates that the concentrations of these compounds were higher than those of As in the plant samples. The detected Ar might come from the surrounding air during measuring. An example of calcium (Ca) distribution inside the stem of the plant at 30 days is shown in Fig. 3a, and an example of iron (Fe) distribution inside the root of the plant at 120 days is presented in Fig. 3b. 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 could not detect As in the plant samples, this method would be ideal for the initial analysis of heavy metals because the synchrotron radiation is a new method for analysis in Thailand, and the detection limit for As was not prepared or tested before by the concerned material controllers.

The results of NTA and EDTA effects on the bioaccumulation and translocation of As by *M. pudica* L. grown in contaminated soil (5 mg/kg of soil) indicate that adding EDTA and NTA increases the bioaccumulation and translocation of As in the aboveground parts (stem and leave) and the underground part (root) of *M. pudica* L. The accumulation of As was higher in the root than in the stem and leaf of the plant. As was transported to all parts of the plants, it grew well, and it was a mechanism for phytoextraction or hyperaccumulator plants. Therefore, this result can be applied for the on-site remediation of As-contaminated soil. Likewise, the As translocation factor for *M. pudica* L. was lower than 1, which implies that the accumulation of As was high and concentrated in the root. The calculation of the bioaccumulation of As showed that EDTA concentrations of 50 and 100 mg/kg increased the bioaccumulation of As by *M. pudica* L., with a statistically significant difference ($p < 0.05$). Analysis of the results was conducted using Synchrotron μ-X-ray fluorescence spectroscopy (Beamline 6b), which could not specify the dispersion of As in the plant parts due to detection limitations of the tool and low As concentrations. Furthermore, this research did not investigate the reaction between As and any growth promoter, nor did it provide the details of such reactions at a molecular level.

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Author's Contributions Authors participated in all experiments, coordinated the data-analysis and contributed to the written text of this manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

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