CHAPTER III

METHODOLOGY

3.1 Instruments

Atomic absorption spectrophotometer; 2380, Perkin-Elmer, USA

Atomic absorption spectrophotometer; 3100, Perkin-Elmer, USA

Conductivity bridge; pp-50, Sartorius professional meter, Germany

Mechanical shaker; SA-31, Yuamato, Japan

Nitrogen distillatory; 30, Gerhardt vatodest, Germany

pH Meter with combined electrode; pp-50, Sartorius professional meter, Germany

Spectrophotometer; U-1000, Hitachi, Japan

3.2 Chemicals

8-Hydroxyquinoline; GR. grade, Merck

Ammonium acetate (NH_4OAC); AR. grade, J.K Baker

Ammonium heptamolybdate ($(NH4)6Mo_7O_{24}.4H_2O$); AR. grade, Carlo ERBA

Ammonium hydroxide (NH_4OH); GR. grade, Merck

Ammonium sulfate [$(NH_2)_2SO_4$]; AR. grade, Fluka

Amnionium molybdate ($(NH4)6Mo_7O_{24}.4H_2O$); AR. grade, Mallinckrod

Antimony potassium tartrate ($KSbO.C_4H_4O_6$); AR. grade, Carlo ERBA

Ascorbic acid ($C_6H_8O_6$); AR. grade, Carlo ERBA

Boric acid (H_3BO_3); GR. grade, Merck

Butyl acetate ($C_6H_{12}O_2$); GR. grade, Merck

Calcium carbonate ($CaCO_3$); GR. grade, Merck

Calcium chloride ($CaCl_2$); GR. grade, Merck

Devarda's alloy; GR. grade, Merck

Dihydrogen phosphate (KH_2PO_4); GR. grade, Merck

Diphenylamine indicator [$(C_6H_5)_2NH$]; AR. grade, Riedel

Disodium hydrogen As(V) ($Na_2HAsO_4.7H_2O$); AR. grade, Fluka

Diethylene triamine pentaacetic acid (DTPA); GR. grade, Merck

Ethanol (C_2H_5OH); AR. grade, Lab scan

Glacial acetic acid ($C_2H_4O_2$); AR. grade, Labscan

Hexametaphosphate $(NaPO_3)_{13}$; GR. grade, Merck

Hydrochloric acid (HCl); AR. grade, J.K Baker

Hydroxylamine hydrochloride (NH_2OH+HCl); GR. grade, Merck

Lanthanum chloride; GR. grade, Merck

Lithium chloride; AR. grade, Fisher scientific

Magnesium oxide (MgO); GR. grade, Merck

Nitric acid (HNO_3); AR. grade J.K Baker,

Orthophosphoric acid (H_3PO_4); GR. grade, Merck

P-nitrophenol indicator; AR. grade, BDH

Potassium aluminum sulfate [$KAl(SO_4)_2.12H_2O$]; AR. grade, Carlo ERBA

Potassium antimonyl tartrate ($KSbO.C_4H_4O_6$); AR. grade, Carlo ERBA

Potassium chloride (KC1); GR. grade, Merck

Potassium dichromate ($K_2Cr_2O_7$); AR. grade, fisher chemicals

Potassium iodide (KI); AR. grade, UWR (prolab) NORMAPUR

Potassium nitrate (KNO_3); GR. grade, Merck

Sodium acetate (NaOAc); AR. grade, Fisher Scientific

Sodium bicarbonate ($NaHCO_3$); GR. grade, Merck

Sodium borohydride ($NaBH_4$); GR. grade, Merck

Sodium carbonate (Na_2CO_3); GR. grade, Merck

Sodium chloride (NaCl); GR. grade, Merck

Sodium diethyldithiocarbarmate trihydrate (NaDDC); AR. grade, BDH

Sodium hydroxide (NaOH); GR. grade, Merck

Sodium As(III) ($NaAsO_2$); AR. grade, Sigma

Sulfuric acid (H_2SO_4); AR. grade, J.K. Baker

Triethanolamine (TEA; $(HOCH_2CH_2)_3N$); GR. grade, Merck

3.3 Types of plants used

This experiment used 4 aquatic plants which were emerged plants. There were *Colocasia esculenta* (L.), *Canna sp*, *Cyperus papyrus* (L.), and *Typa angustifolia* (L.). The prepared plants are planted in a pot with 5 kilograms of submerged soil which composed of sodium As(III) ($NaAsO_2$) for As (III) and disodium hydrogen As(V)

($Na_2HAsO_4.7H_2O$) for As (V) with the concentrations of 175 mg $As.kg^{-1}$ soil. Twelve replicated pots are performed (Table 3-1 and 3-2).

3.4 Experimental design

A 4 x 3 Factorial plot experimental designed in RCB was conducted with four aquatic plants, three treatment (control, As(III) and As(V)) and four harvested time (15 days, 30 days, 45 days and 60 days). There were 3 replications.

Table 3.1 Experimental design

Types of Aquatic plants	As Speciations	
	As(III) 175 mg.$As.kg^{-1}$.soil	As(V) 175 mg.$As.kg^{-1}$.soil
Canna sp	P_1As(III)	P_1As(V)
C. papyrus	P_2As(III)	P_2As(V)
C. esculenta	P_3As(III)	P_3As(V)
T. angustifolia	P_4As(III)	P_4As(V)

Note; P_1: *Canna* sp., P_2: *C. papyrus*, P_3: *C. esculenta*, P4: *T. angustifolia*

R: replication, Harvest plants 4 times viz; 2, 4, 6, and 8 weeks (n = 4)

Matrix of unit experiments and control= (3 x 4 x 3 x 4) + (3 x 3 x 4) = 180 pots

(Formula of treatments = 3, Formula of control = 3, and Replication = 3)

Table 3.2 Control groups

Control group	Soil condition
Group 1: With plants	No Arsenic: 12 pots
Group 2: Without plants	No Arsenic: 12 pots
Group 3: Without plants	As (III): 12 pots
Group 4: Without plants	As (V): 12 pots

3.5 Experimental preparation

3.5.1 Soil preparation

The submerged soil was Mae Rim complex series that collected from Amphur Hangdong Chiang Mai province. The soil was dried by open air, grinded and glided by 2 mm sieve. Incorporated of arsenic in soil to the desired arsenic concentrations of 175 mg As.kg^{-1} was performed.

3.5.2 Plant preparation

C. esculenta., *Canna sp*, *C. papyrus.*, and *T. angustifolia* were collected from natural areas of Chiang Mai. They are acclimated for 2 months in submerged soil. Mature plants with the same sizes were selected for the experiment. In each experimental pot consisted of two plants which cultivate in arsenic contaminated submerged soil for eight weeks. The total arsenic in plants was analyzed before the experiment.

3.5.3 Water preparation

Tap water was stocked in 1,000 liters tank for using throughout the experiment. water was analyze for some chemical characteristics.

3.6 Experiment

This study was conducted at Mae Hea Agricultural Research Station and Training Center, Faculty of Agriculture, Chiang Mai University, where the plant nursery house. The plant nursery had clear plastic roof and the height of roof was 2.5 m. Plants was grown in 15 July 2006 to 12 September 2006 which temperature stayed between 22.7 – 36.5 °C and light intensity was 875 – 89200 lux.m^{-2} (see appendix table A.1-A2). The ground of nursery was concrete, 175 mg.kg^{-1} of arsenic treatment soil was taken to 12 × 12 inch pot and grew plants at middle of pots that was 5 inches under the soil surface. Then the water level was controlled to 2 cm above soil surface. Water was added and checked every day at 7.00 O'clock; moreover the temperature,

relative humidity, and light intensity were collected 3 times per day that was 8.00, 12.00, and 16.00 O'clock. The plant growth data was observed every day. At harvest time, soil was detected the pH, EC and redox potential *in situ*, and was collected 20 g at 5 inches under the soil surface by PE plastic bag for analysis in laboratory. Plants were cleaned by tap water and brought to weigh the wet weight that was collected plants weight for every part of plants. After that the plants were separated into different parts of plants and ground by liquid nitrogen immediately. The soil and plants were weighed 2 g. accurately wet weight and extracted by NaDDC 6 hr on shaker. The remained soil and plants were dried at 70°C until stable weight for experiment.

3.7 Sample collection

3.7.1 Soil samplings

Soil samples were collected from every pot at the beginning and the end of the experiment. Fifty gram of arsenic contaminated submerged soil and non arsenic contaminated submerged soil using as control were collected per pot, twelve replicated pots, three replications were collected biweekly. Soil samples were dried in hot air oven at 85°C for 72 hours. Dried soil was ground and sieved through 2mm, then kept in plastic bags to avoid contamination.

3.7.2 Plant sampling

The whole plants were harvested biweekly for eight weeks. Each plant was cleaned and separated into three parts; roots, stems, and leaves. All parts of plant samples were weighed for wet weight, cut into small pieces and then dried in the hot air oven at 85°C for 72 hours. Dried specimens were kept in plastic bag to avoid contamination.

3.8 Sample analysis

3.8.1 Soil texture; Pipette Method (Abdul *et al.*, 2001)

Procedure

Weigh 40 g air-dry soil (2-mm) accurately. Add 60-mL dispersing solution. Cover the beaker with a watch-glass, and leave overnight. Quantitatively transfer contents of the beaker to a soil-stirring cup, and fill the cup to about three-quarters with water. Stir suspension at high speed for 3 minutes. Rinse stirring paddle into a cup, and allow to stand for 1 minute. Transfer suspension quantitatively into a 1000 ml calibrated cylinder (hydrometer jar), and bring to volume with water.

A. Determination of blank

Dilute 60 ml dispersing solution to l000 ml hydrometer jar with water. Mix well, and insert hydrometer, and take hydrometer reading, Rb. The blank reading must be re-determined for temperature changes of more than 2°C from 20°C.

B. Determination of silt plus clay

Mix suspension in the hydrometer jar, using a special paddle carefully, withdraw the paddle, and immediately insert the hydrometer. Disperse any froth, if needed, with one drop of amyl alcohol, and take hydrometer reading 40 seconds after withdrawing the paddle. This gives reading, Rsc.

Calculations

Percentage silt plus clay in soil

$$\% \text{ [Silt + Clay] (w/w)} = \frac{(Rsc - Rb) \times 100}{\text{Dry soil (g)}}$$

C. Determination of clay

Mix suspension in the hydrometer jar with paddle, withdraw thepaddle, with great care, leaving the suspension undisturbed. After 4 hours, insert the hydrometer, and take hydrometer reading, Rc.

Percentage clay in soil:

$$\% \text{ Clay (w/w)} = \frac{(Rc - Rb) \times 100}{\text{Dry soil (g)}}$$

Percentage silt in soil:

$$\% \text{ Silt (w/w)} = [\% \text{ Silt + Clay (w/w)}] - [\% \text{ Clay (w/w)}]$$

D. Determination of sand

After taking readings required for clay and silt, pour suspension quantitatively through a 50 mm sieve. Wash sieve until water passing the sieve is clear. Transfer the sand quantitatively from sieve to a 50 ml beaker of known weight. Allow the sand in the beaker to settle, and decant excess water. Dry beaker with sand overnight at 105°C. Cool in a desiccator, and re-weigh beaker with sand.

Percentage sand in soil:

$$\% \text{ Sand (w/w)} = \frac{\text{Sand weigh} \times 100}{\text{Dry soil (g)}}$$

3.8.2 pH; pH meter with combined electrode (Abdul *et al.*, 2001)

Procedure

Weigh 50 g air-dry soil (<2-mm) into a 1 00-mL glass beaker. Add 50 ml DI water using a graduated cylinder or 50-mL volumetric flask. Mix well with a glass rod, and allow to standing for 30 minutes. Stir suspension every 10 minutes during this period. After 1 hour. Stir the suspension. Put the combined electrode in suspension (about 3-cm deep). Take the reading after 30 seconds. Remove the combined electrode from the suspension, and rinse thoroughly with DI water in a separate beaker, and carefully dry excess water with a tissue.

3.8.3 Electrical Conductivity; Conductivity bridge Method (Abdul *et al.*, 2001)

Procedure

Prepare a 1:1 (soil: water) suspension, as for pH determination. Filter the suspension using suction. First, put a round Whatman No. 42 filter paper in the Buchner funnel. Moisten the filter paper with DI water. Start the vacuum pump. Open the suction, and add suspension to Buchner funnel. Continue filtration until the soil on the Buchner funnel starts cracking. Transfer the clear filtrate into a 50 ml. bottle, immerse the conductivity cell in the solution, and take the reading.

3.8.4 Organic Matter; Black and Walkley Method (Abdul *et al.*, 2001)

Procedure

Weigh 1 g air-dry soil (0.15 mm) accurately into a 500 ml beaker. Add 10 ml 1 N potassium dichromate solution using a pipette, add 20 ml concentrated sulfuric acid using a dispenser, and swirl the beaker to mix the

suspension. Allow to stand for 30 minutes. Add about 200 ml DI water, then add 10 ml concentrated orthophosphoric acid using a dispenser, and allow the mixture to cool. Add 10- 15 drops diphenylamine indicator, add a teflon-coated magnetic stirring bar, and place the beaker on a magnetic stirrer. Titrate with 0.5M ferrous ammonium sulfate solution, until the color changes from violet-blue to green. Prepare two blanks, containing all reagents but no soil, and treat them in exactly the same way as the soil suspensions.

Calculations

Percentage Organic Matter in soil;

$$M = \frac{10}{V \text{ blank}}$$

$$\% \text{ Oxidizable Organic Carbon (w/w)} = \frac{[V \text{ blank} - V \text{ sample}] \times 0.3 \times M}{Wt}$$

% Total Organic Carbon (w/w) = 1.334 × % Oxidizable Organic Carbon

% Organic Matter (w/w) = 1.724 × % Total Organic Carbon

3.8.5 Cation Exchange Capacity (Abdul *et al.*, 2001)

Procedure

Weigh 4 g air-dry accurately soil into a 40 ml centrifuge tube, and add 33 ml 1 N sodium acetate trihydrate solution, stopper tube, and shake for 5 minutes. Remove stopper from tube and centrifuge at 3000 rpm until supernatant liquid is clear. Decant the supernatant as completely as possible and discard. Repeat with 33 ml portions 1 N sodium acetate trihydrate solution, a total of four times, discarding the supernatant liquid each time. Then add 33 ml 95% ethanol, stopper tube, and shake for 5 minutes, unstopper tube, and centrifuge until the supernatant is clear and decant. Wash the sample

with 33 ml portions 95% ethanol, a total of three times, discarding the supernatant liquid each time. The electrical conductivity (EC) of the supernatant liquid from the third washing should be less than 400 $\mu S.cm^{-1}$. Replace the adsorbed sodium ion from the sample by extraction with three 33 ml portions 1 N ammonium acetate solution. Each time shake for 5 minutes, and centrifuge until supernatant liquid is clear. Decant the three supernatant liquids as completely as possible into a 100 ml volumetric flask, bring to volume with 1 N ammonium acetate solution, and mix well. Run a series of suitable sodium ion standards, and draw a calibration curve. Measure the samples (soil extract) and take the emission readings by a flame photometer at 767 nm wavelength. Calculate sodium concentration according to the calibration curve.

Calculation

For Cation Exchange Capacity in soil:

$$CEC\ (meq.100g^{-1}) = \frac{meq.L^{-1}.Na\ (from\ calibration\ curve) \times A \times 100}{Wt \times 1000}$$

Where: A = Total volume of the extract (mL)

Wt = Weight of the air-dry soil (g)

3.8.6 Inorganic N (Abdul *et al.*, 2001)

Procedure

Weigh 30 g air-dry soil (2 mm) accurately and add 150 ml 2 M potassium chloride solution (1:5 soil: solution ratio). Stopper flasks, shake for 1 hour on an orbital shaker at 200 - 300 rpm, and filter suspensions using Whatman No. 42 filter paper. Calibrate pH-meter, and standardize the 0.01 N H_2SO_4 in the autotitrator, as done for Kjeldahl-N. Before starting distillation, the distillation unit should be steamed out for at least 10 minutes. Adjust steam rate to 7 - 8 ml distillate per minute. Water should flow through the condenser jacket at a rate sufficient to keep distillate temperature below 22°C.

Carry out distillations as follows: Dispense 1 ml saturated boric acid solution and 1 ml DI water into a 100 ml. Pyrex evaporating dish, placed underneath the condenser tip, with the tip touching the solution surface. Pipette 20 ml aliquot of the clear supernatant into a 100-ml distillation flask. To determine NH_4-N in solution, add 0.2 g heavy magnesium oxide with a calibrated spoon to the distillation flask. Immediately attach the flask to the distillation unit with a clamp, start distillation, and continue for 3 minutes. Lower the dish to allow distillate to drain freely into the dish. After 4 minutes, when about 35 ml distillated solution is collected, turn off the steam supply, and wash tip of the condenser into the evaporating dish with a small amount of DI water.

Titrate the distillate to pH 5.0 with standardized 0.01 N H_2SO_4 using the auto-titrator. After finishing titration, wash the Teflon-coated magnetic stirring bar, the burette tip, and the combined electrode into the dish. To determine NO_3-N (plus NO_2-N) in the same extract, add 0.2 g Devarda's alloy with a calibrated spoon to the same distillation flask. Attach flask to distillation unit with a clamp, and start distilling.

Further proceed as for ammonium-N. Between different samples, steam out the distillations. Disconnect distillation flasks containing the KCl extracts, and attach a 100 ml empty distillation flask to distillation unit, and place a 100 ml empty beaker underneath the condenser tip, turn off cooling water supply (drain the water from the condenser jacket), and steam out for 90 seconds. Steaming-out is done only between different samples, not between distillation for ammonium (MgO) and nitrate (Devaida's alloy) in the same sample. Each distillation should contain at least two standards and two blanks, i.e., 2 M KC1 extracts with no soil added (reagent blanks).

Calculation

For Ammonium-N in air-dry soil:

$$NH_4\text{-N (ppm)} = \frac{(V - B) \times N \times R \times 14.01 \times 1000}{Wt}$$

Where: V = Volume of 0.01 N H_2SO_4 titrated for the sample (ml)

B = Blank titration volume (ml)

N = Normality of H_2SO_4 solution

14.01 = Atomic weight of N

R = Ratio between total volume of the extract and the extract volume used for distillation

Wt = Weight of air-dry soil (30g)

3.8.7 Available P (Abdul *et al.*, 2001)

Procedure

Weigh 5 g air-dry soil (2-mm) accurately and add 100 ml 0.5 M sodium bicarbonate solution. Close the flask with a rubber stopper, and shake for 30 minutes on a shaker at 200 - 300 rpm. Include one flask containing all chemicals but no soil (Blank). Filter the solution through a Whatman No.5 filter paper, and pipette 10 ml clear filtrate into a 50 ml volumetric flask. Acidify with 5 N sulfuric acid to pH 5.0. This can be done by taking 10 ml 0.5 M $NaHCO_3$ solution and determining the amount of acid required to bring the solution pH to 5.0, using P-nitrophenol indicator (color change is from yellow to colorless). Then add the required acid to all the unknowns. Adding 1 ml 5 N H_2SO_4 is adequate to acidify each 10 ml $NaHCO_3$ extract. Add DI water to about 40 ml volume, add 8 ml Reagent B, and bring to 50-ml volume.

Prepare a standard curve as follows: Pipette 2 ml of each standard (1-5 ppm), and proceed as for the samples. Also make a blank with 10 ml 0.5 M $NaHCO_3$ solution, and proceed as for the samples. Read the absorbance of blank, standards, and samples after 10 minutes at 882 nm wavelength. Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations. Read P concentration in the unknown samples from the calibration curve.

Calculation

For Extractable Phosphorus in soil:

$$\text{Extractable P (ppm)} = \frac{\text{ppm P (from calibration curve)} \times A \times B}{Wt \times V}$$

Where: A = Total volume of the extract (ml)

B = Final volume of used for measurement (ml)

Wt = Weight of air-dry soil (g)

V = Volume of extract used for measurement (ml)

3.8.8 Exchangeable Ca and Mg (Abdul *et al.*, 2001)

Procedure

Weigh 4 g air-dry soil (<2-mm) accurately into a 100 ml centrifuge tube, add 40 ml ammonium acetate solution, and shake for 30 minutes on a shaker. Centrifuge until the supernatant liquid is clear and collect the extract in a 100 ml volumetric flask through a filter paper no 5 to exclude any soil particles. Repeat this process two more times and collect the extract each time. Dilute 5 ml of the combined ammonium acetate extracts to 25 ml with Lanthanum 0.2% Run a series of suitable Ca and Mg standards, and draw a calibration curve. Measure the samples (soil extracts), and take the emission readings on a flame photometer at 422.7 nm wavelength for Ca and 285.2 nm wavelength for Mg.

Calculation

For extractable Ca and Mg in soil:

$$\text{Extractable Ca and Mg (ppm)} = \frac{\text{ppm Ca or Mg (from calibration curve)} \times A \times B}{Wt \times V}$$

Where: A = Total volume of the extract (ml)

B = Final volume of used for measurement (ml)

Wt = Weight of air-dry soil (g)

V = Volume of extract used for measurement (ml)

3.8.9 Exchangeable Al (Paul and Paul, 1996),

Procedure

Weigh out 5 g of < 2mm soil into a 50 mL polyethylene or some other appropriate container. Add 25 mL of 1 M NH_4Cl and shake for 30 min and then filter the supernatant solution through either a prewashed Whatman no 42 filter. Acidify the filtrate to pH < 0.3 with a few drops of concentrated HNO_3. The filtrate should be stored in plastic containers. Add deionized water to the extraction tube so that this volume plus that ofthe sample containing Al equals 25 mL. Them add 5 mL each of Reagent 5 (four volumes of 1% 8 – hydroxyquinoline and one volume of hydroxylamine hydrochloride) followed by Reagent 2 (Sodium acetate (NaOAc), 1 M, containing 0.2% phenanthroline). Adding Reagent 2 first can result in excessively high pH with precipitation of Al. Add a sample containing 1 to 20 ug of Al, and briefly shake the tube. The final sample mixture should have a pH of 5.0, and it is advisable to test a few solutions for pH. Allow to stand 15 min for complexation of Fe by phenanthroline, then add 5 mL of butyl acetate. Shake the tubes vigorously for 15 s, then let them stand for 15 min for complete phase separation. Transfer the butyl acetate phase to a 1.00 cm borosilicate glass cuvette, and measure the absorbance at 395 nm against a butyl acetate blank solution. The complex is stable for at least 24 h.

Calculation

For extractable aluminium in soil:

$$\text{Extractable Al (ppm)} = \frac{\text{ppm Al (from calibration curve)} \times A \times B}{Wt \times V}$$

Where: A= Total volume of the extract (ml)

B = Final volume of used for measurement (ml)

Wt = Weight of air-dry soil (g)

V= Volume of extract used for measurement (ml)

3.8.10 Exchangeable Fe; DTPA method (Abdul *et al.*, 2001)

Procedure

Weigh 10 g accurately air-dry soil (2-mm). Add 20 ml extraction solution. Shake for 2 hours on a reciprocal shaker. Filter the suspension through a Whatman no. 42 filter paper. Measure Fe and Mn directly in the filtrate by an Atomic Absorption Spectrophotometer.

Calculation

For Extractable Micronutrient cations in soil:

$$\text{Fe and Mn (ppm)} = \frac{(\text{ppm from calibration curve}) \times A \times B}{Wt \times V}$$

Where: A= Total volume of the extract (ml)

B = Final volume of used for measurement (ml)

Wt = Weight of air-dry soil (g)

V= Volume of extract used for measurement (ml)

3.8.11 Total As (applied from American Water Work Association Water Environment Federation (1998))

Procedure

To 10 ml digest standard or sample added 6 ml HCl: H_2O (1:1) and mix. Add 1 ml of 10% KI analytical reagent grade, mix, and allow about one

hour for the reaction to be completed. Determine by atomic absorption spectrophotometer. The reaction can be speeded up by heating the solutions to about 80°C for 10 minutes.

Calculation

$$\text{Total As} = \frac{A \times \text{final volume(ml)} \times \text{dilute volume (ml)}}{10 \times \text{sample volume using (ml)} \times \text{dry weigh sample (g)}}$$

Where; A = Absorbance from AAS

10 = Change value from ng.10 ml^{-1} to $\mu g.L^{-1}$

3.8.12 As(III) and As(V)

Procedure (David *et al.*, 1972)

Pipette 40 ml of the sample into each of six 50-mi glass-stoppered test tubes (or 125-mi Erlenmeyer flasks). Two tubes will be for the "oxidized" aliquots, two for the "reduced", and two for the "untreated" aliquots. Add 1 ml of 1 M hydrochloric acid to each of the "oxidized' aliquots and then one drop of the 50% saturated potassium iodate solution. Mix well and allow to react for at least 2 mm. Now add 4 ml of mixed reagent to each of the "oxidized" and the "untreated" sample aliquots, and mix immediately. Then add one drop of the iodate solution to each of the "untreated" aliquots, and after mixing add 1 ml of the 1 M hydrochloric acid to these aliquots and mix again.

Allow color formation in the "oxidized" and "untreated" aliquots to take place for 4 h. Read the absorbances in a 10-cm cell at a wavelength of 865 nm. For clarity, the treatment of the "reduced" aliquols is described separately below, though in practice they are run concurrently with the "oxidized" aliquots.

Add 4 ml of reducing reagent to the "reduced" aliquots; mix and allow to: react for.3 h: Then add 4 ml of the mixed reagent. Wait 5 min for color formation to be complete and read the absorbance as above. To minimize the accuracy, these measurements should be made within an hour of the time the

mixed reagent added run deionized water blanks by the above procedures, along with the samples.

Calculation (David *et al.*, 1972)

Since the final volume of the "reduced" aliquots is greater than that of the other aliquots, a dilution correction must be made to obtain corrected absorbance would be applied to make the "reduced" absorbance comparable to those of the other aliquots.

$$\text{In this procedure, the correction} = \frac{\text{final volume "reduced"}}{\text{final volume oxidized or untreated}}$$

.After dilution correction, absorbances of the blanks are subtracted to give corrected absorbance (CA.). Absorbance factors for As(III), As(V), and phosphate are determined from standard additions. As(III), As(V), and phosphate concentrations are calculated as follows:

As(III) = (C.A. "oxidized" – C.A. "untreated") × abs factor As(III)
As(V) = (C.A. "untreated" – C.A. "reduced") × abs factor As(V)
Phosphate = (C.A. "reduce") × abs factor PO_4^{3-}

Note; Abs factor As(III) and As(V) = 0.99 μM

3.9 Data analysis

3.9.1 Plant dry weight decrease

Dry weight decrease was calculated from the ratio of total plant dry weight of arsenic treatments (DW_{As}) per control (DW_C) and minus from 1 as following equation.

Plant dry weight decrease (%) = $\{1 - [DW_{As}\ (g) / DW_C\ (g)]\} \times 100$

3.9.2 Total arsenic accumulation in plants

Arsenic accumulation in plants was calculated from total arsenic accumulation in plant per dry weight as followed equation (derive from Assoc. Prof. Dr. Somporn Choonluchanon)

$$\text{Arsenic concentration of plant} = \frac{\sum \text{Arsenic concentration in each organ}}{\sum \text{Dry weight in each organ}}$$

3.9.3 Arsenic accumulation in different organs of plants

Arsenic accumulation was calculated from amount of arsenic in which organ of plant per dry weight as followed equation (applied from American Water Work Association Water Environment Federation (1998)).

$$\text{Arsenic accumulation in organ} = \frac{\text{Amount of arsenic in organ (mg)}}{\text{Dry weight (kg)}}$$

3.9.4 Arsenic accumulation efficiency

The arsenic accumulation efficiency was considerate plants could be assessed from bioconcentration factor (BF) which was the ratio of arsenic concentration in plant and soil as followed equation (Zhang *et al.*, 2002; Tu *et al.*, 2004; Tu and Ma, 2002).

$$\text{Bioconcentration Factor (\%)} = \frac{\text{Amount of arsenic in plants (mg.kg}^{-1}\text{)} \times 100}{\text{Amount of arsenic in soil (mg.kg}^{-1}\text{)}}$$

3.9.5 Arsenic removal efficiency of plants

Arsenic removal efficiency was calculated from the ratio of arsenic content in plant (mg) per arsenic content in soil (mg) by calculation in percentage form as followed equation (Dhitivara, 2000; Jampanil, 2000).

$$\text{Arsenic removal efficiency (\%)} = \frac{\text{Total of arsenic in plants (mg)} \times 100}{\text{Total of arsenic in soil (mg)}}$$

3.9.6 Arsenic accumulated translocation factor (ATF)

Arsenic accumulated translocation factor (ATF) were calculated from arsenic accumulation in aboveground organs divided by underground organs (Tu and Ma, 2002; Wei *et al.*, 2006; Singh and Ma, 2006; Tu *et al.*, 2004).

$$\text{ATF} = \frac{\text{Arsenic accumulation in aboveground (mg.kg}^{-1}\text{)}}{\text{Arsenic accumulation in underground (mg.kg}^{-1}\text{)}}$$

3.9.7 Arsenic transformation efficiency in submerged soil

Transformation efficiency of As(III) to As(V) assessed from ratio of As(V) per summation of As(V) and As(III) content in soil, when taken As(III). Whereas, transformation efficiency of As(V) to As(III) assessed from ratio of As(III) per summation of As(V) and As(III) content in soil, when taken As(III) and As(V) as followed equation (Masscheleyn *et al.*, 1991a)..

$$\text{Transformation efficiency of As(III) to As(V)}^{1} = \frac{\text{Amount of As(V) (mg.kg}^{-1}\text{)} \times 100}{\sum \text{As(III) + As(V) (mg.kg}^{-1}\text{)}}$$

$$\text{Transformation efficiency of As(V) to As(III)}^{2} = \frac{\text{Amount of As(III) (mg.kg}^{-1}\text{)} \times 100}{\sum \text{As(III) + As(V) (mg.kg}^{-1}\text{)}}$$

When ^{1}: As(III) Treatment

^{2}: As(V) Treatment

3.9.8 Arsenic transformation efficiency in plants

Transformation efficiency of arsenic in plants accessed from ratio of As(III) or As(V) concentration in plant per summation of As(V) and As(III) in plant. It was calculated in percentage form as followed equation (Masscheleyn *et al.*, 1991a).

$$\text{Transformation efficiency of As(III) to As(V)}^{1} = \frac{\text{Amount of As(V) (mg.kg}^{-1}\text{)} \times 100}{\sum \text{As(III) + As(V) (mg.kg}^{-1}\text{)}}$$

$$\text{Transformation efficiency of As(V) to As(III)}^{2} = \frac{\text{Amount of As(III) (mg.kg}^{-1}\text{)} \times 100}{\sum \text{As(III) + As(V) (mg.kg}^{-1}\text{)}}$$

When ^{1}: As(III) Treatment

^{2}: As(V) Treatment

3.10 Statistics analysis (Fisher, 1966)

Arsenic accumulation in parts of *C. esculenta*, *Canna* sp., *C. papyrus*, and *T. angustifolia*, (root, stem, and leaves) were analyzed using SX version 8.0 by Multi-analysis of Variance (MANOVA) LSD with confidential level of 95%.