

CHAPTER 2

METHODOLOGY

2.1 Chemicals and apparatus

All preparation steps were performed in a HEPA filtered, dedicated trace metal analysis laboratory at Department of Chemistry, Faculty of Science, Prince of Songkla University. Critical steps such as drying, double bagging, and acid dispensing were carried out under class-100 laminar flow cabinet. All plastic wares and Teflon bombs were cleaned by soaking in 6% (v/v) nitric acid (HNO₃) for a minimum 3 days and then were rinsed with nanopure water and kept in plastic bags before use. The polysulfone filters were soaked in 2N HCl for 24 hours while the polycarbonate holders were soaked in 6N HCl. Then they were rinsed with nanopure water and kept in plastic bags. Polyethylene gloves were used at all time when handling sample bottles and filtration apparatus in laboratory and field.

All chemicals used in this work were analytical grade and prepared in nanopure water (>18 MΩ) obtained from Barnstead (Germany) purification system. Nitric acid and hydrochloric acid were purified by sub-boiling distillation. The standard 1,000 mg/L Cd, Cu, Pb, Zn, Al, Fe and Mn stock solution (MERCK, Germany). Lists of reagents are given in Table A-1 and lists of apparatus are given in Table A-2 in Appendix A.

2.2 Instrument

2.2.1 GFAAS (Perkin-Elmer Zeeman 800 AAS)

A Perkin-Elmer Zeeman 800 atomic absorption spectrophotometer equipped with a computer controlled HGA-800 graphite furnace, Zeeman background correction and AS-800 autosampler was used for the determination of Cd, Cu, Pb in dissolved and particulate phases and Zn in particulate phase in water samples. The optimum conditions were given in Table A-3 in Appendix A.

2.2.2 FAAS (Perkin-Elmer Analyst 300)

A Perkin-Elmer Analyst 300 flame atomic absorption spectrophotometer was used for determination Zn in dissolved phase and Al, Fe and Mn in particulate phase in water samples. The operating parameters used in this instrument were shown in Table A-4 in Appendix A.

2.2.3 ICP-AES (Perkin Elmer Model Optima 4300 DV)

A Perkin-Elmer instrument Model Optima 4300 DV was used for direct analysis of Al, Fe and Mn in dissolved phases for both the wet and dry season. The operating conditions of the ICP-AES instrument were summarized in Table A-5 in Appendix A.

2.3 Optimization of GFAAS

The optimum conditions of GFAAS system were studied for pyrolysis temperature, atomization temperature, to compare the use of with matrix modifier and without matrix modifier and types of matrix modifier.

2.3.1 Pyrolysis and atomization temperature

The standard solutions 2.0 µg/L for Cd and Zn, 25.0 µg/L for Cu and 50.0 µg/L for Pb were determined by GFAAS. Other parameters were set as the recommended conditions by AAnalyst 800 instrument manual. Optimum pyrolysis temperature was investigated by varied temperature at 300 - 700 °C, 700 - 1300 °C, 450-1050 °C and 400- 900 °C for Cd, Cu, Pb and Zn respectively. The integrated absorbance of these metals was obtained from the peak areas. Pyrolysis temperature was obtained from at least 100 °C below the temperature where the first analyte losses are measured (Schlemmer and Radziuk, 1999).

The optimum atomization temperature were investigated by varied temperature at 1000-1600 °C, 1500-2300 °C, 1000-1800 °C and 1300-2000 °C for Cd, Cu, Pb and Zn, respectively. In investigated of optimum atomization temperature, pyrolysis temperature was set at optimum condition and other parameters were set as

the recommended conditions by AAnalyst 800 instrument manual. The integrated absorbance of these metals was obtained from the peak areas. The atomization temperature was obtained from good peak shape i. e. minimizing peak tailing and keeping integrated time to a reasonable length (Schlemmer and Radziuk, 1999).

2.3.2 Effect of matrix modifier

2.3.2.1 The effect of using matrix modifier and without matrix modifier

The standard solutions 2.0 µg/L for Cd and Zn, 25.0 µg/L for Cu and 50.0 µg/L for Pb were determined by GFAAS between with and without matrix modifier. Pyrolysis and atomization temperature were set at optimum condition obtained from 2.3.1. The integrated absorbance of these metals was obtained from the peak areas. The result was evaluated from good peak shape and good peak area.

2.3.2.2 Type of matrix modifiers

The standard solutions 2.0 µg/L for Cd and Zn, 25.0 µg/L for Cu and 50.0 µg/L for Pb were determined by GFAAS with modifiers. Pyrolysis temperature and atomization temperature were set at optimum condition obtained from 2.3.1. The optimum type of modifier were investigated by varied type of modifiers as follow: Mg(NO₃)₂, NH₄H₂PO₄ and La(NO₃). The optimum type of matrix modifier was obtained from good peak area and peak shape.

2.4 Calibration and standard addition method

The calibration curve established by GFAAS was evaluated with matrix-matched standard solutions. The working concentrations of each metals were prepared by auto-diluting an appropriate aliquot of stock standard solution (8 µg/L for Cd and Zn, 100 µg/L for Cu; 200 µg/L for Pb) with an appropriate volume of its blank to a 20 µL of total volume under the command of computer-controlled GFAAS to final concentration following in series: 2, 4, 8 µg/L for Cd and Zn; 25, 50, 100 µg/L for Cu; 50, 100, 200 µg/L for Pb. In the determination, 20 µL of standard was introduced into the graphite tube under the command of computer-controlled GFAAS.

The calibration curve established by FAAS was evaluated with matrix-matched standard solutions. The working concentrations of each metals were prepared in 0.5, 1, 2, 4 mg/L for Fe and Mn and 100, 200, 400 mg/L for Al. In the determination, 1 mL of standard was introduced into the furnace under the recommend condition

The calibration curve established by ICP-AES was constructed in 0.3M HNO₃. The working concentrations of each metals were prepared in 50, 100, 200 µg/L for Al and Fe and 20, 40, 60 µg/L for Al. In the determination, about 1 mL of standard was introduced into the furnace under the recommend condition

The standard addition curve was evaluated by added stock standard solution into sample in the same range of calibration curve.

2.5 Sample collection

2.5.1 Sampling site

Surface water samples were collected in 74 stations from Songkhla Lake System (Thale Noi 15 stations, Inner and Middle Lakes 32 stations and Outer Lake 27 stations). The sampling period was performed in December 2003-January 2004 (wet season) and in April 2004 (dry season). The sampling sites are shown in Figure 2-1 and the positions at each station were presented in Table A-6 in Appendix A.

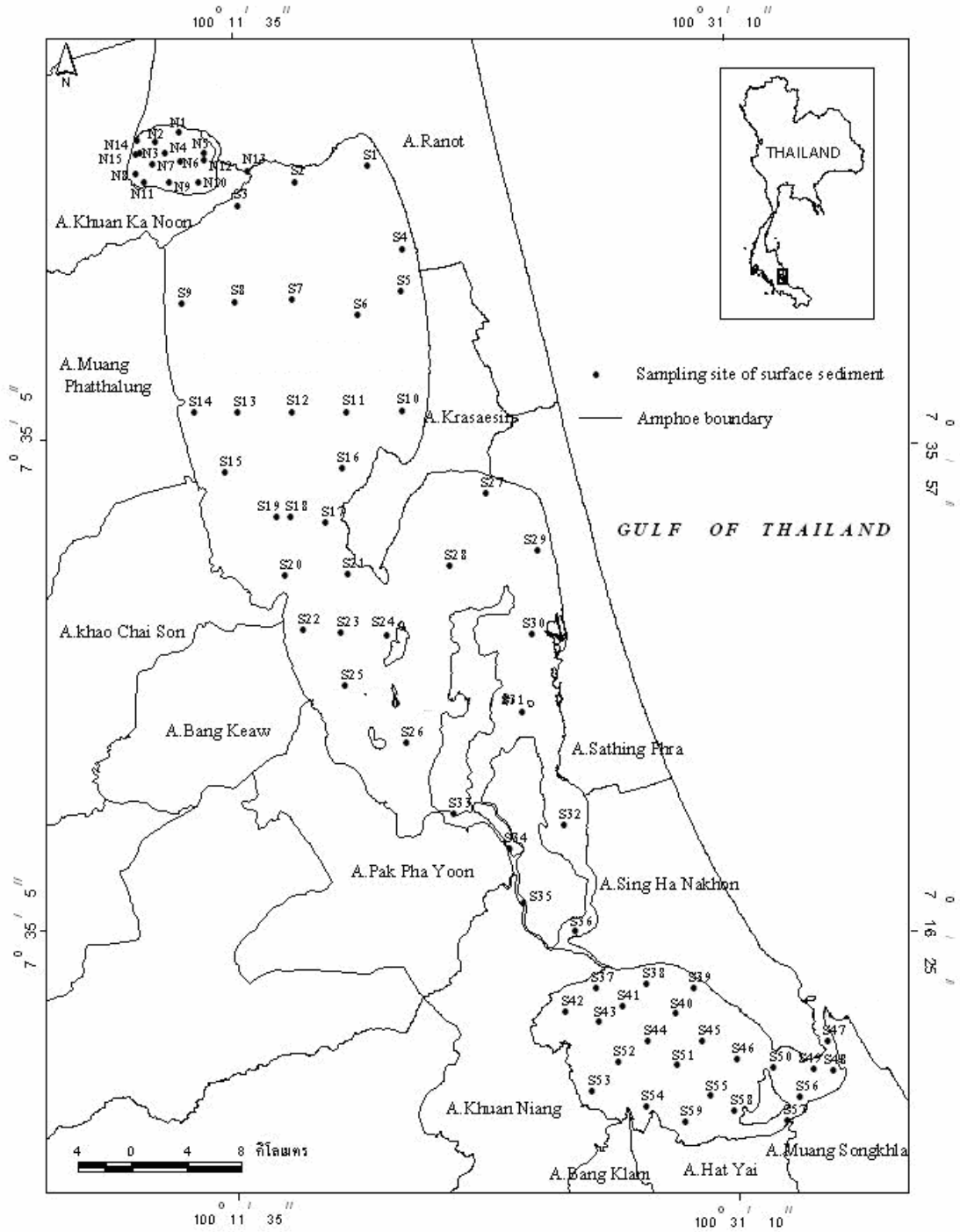


Figure 2-1 The station of water sampling in Songkhla Lake System. (The number indicated the location of sampling sites).

2.5.2 Sampling method

Surface water sample was collected by immersing a 500 mL acid-cleaned polyethylene bottle with the closed cap into the water, removing the cap, allowing the bottle fill up then replacing the cap under water. All these operations were performed by using long-arm plastic gloves. Immediately upon recovery, the sample bottle is placed in a polyethylene zip-lock bag, then double bagged, stored in ice box and brought back to the laboratory for further filtration.

2.5.3 Sample preservation

The water sample was filtered through acid washed 0.45 μm polysulfone filters (47 mm diameter) in an acid cleaned Nalgene polycarbonate pressure filter system using a vacuum pump with low pressure. To ensure a representative of sample, the unfiltered sample bottle was agitated by inverting and returning to its upright position for uniform distribution of particulate matter each time prior to taking a sub-sample for filtration. The process was continued until 350 mL of final solution was obtained. Sample bottles were rinsed with 20 mL of filtered sample before filling with the actual sample. The sample volume used was noted each time. Between the filtration of each sample, the Nalgene polycarbonate pressure filter was cleaned by flushing with nanopure water. The clogged filter was rinsed with nanopure water in order to remove salt before the filter was folded in acid cleaned Petri dish, and packed in plastic bag. The filter was then immediately stored frozen at 4°C for further analysis.

The filtrates were immediately acidified with sub-boiled distilled nitric acid (1mL/L). The acidification was performed under class-100 laminar flow cabinet. The filtration blanks were performed by using nanopure water in parallel with the sample.

2.6 Sample preparation

2.6.1 Solvent Extraction

The acidified filtered sample of 300 mL and a mixing reagent of 2 mL (preparation method was presented in Appendix B) were transferred to a 500 mL Teflon separating funnel. The funnel was shaken for 60 seconds and 20 mL of chloroform was then added. The funnel was shaken vigorously for 120 seconds and the phases allowed separating. The lower organic phase was collected in 60 mL polypropylene bottle and 10 mL of chloroform was added then the funnel was shaken for 30 seconds. The two organic phases were combined.

For back-extraction, 100 μ L of distilled nitric acid was added to the combined organic phases, the bottle was shaken for a few seconds and left overnight. The 4.9 mL of nanopure water was then added and mixed vigorously. The aqueous phase was collected in polypropylene bottles and trace metals were analyzed by GFAAS and FAAS techniques. Extraction blank was performed by using acidified nanopure water in parallel with the sample.

2.6.2 Particulate digestion: Total decomposition method

Trace metals in particulate were digested by using total decomposition method which was a combination of hydrofluoric acid (HF) and aqua regia (HNO_3 : HCl, 1:3 v/v). Particulate sample collected on polysulfone filters were subjected to microwave digestion in Teflon bomb with pressure relief (Lorrain International, Porters Lake, N.S., Canada). A set of four to five Teflon vessels was placed in a pressure cooker and it was then put in a domestic microwave oven. The procedure was followed method of Loring and Rantala (1992) and Jungsiruntanakun (2002).

Filters were placed in a Teflon bomb using plastic tweezers and squashed into the bottom of the vessel. A portion of 1 mL aqua regia and 1 mL of HF were added to cover the filter and the bomb was closed tightly. A set of vessels along with a beaker containing 50 mL of water were put in the microwave for 4 minutes on high power and 8 minutes on mid-high power. The bombs were cooled in water bath; the contents were decanted into a 30 mL polypropylene bottle containing 0.5 g of

boric acid and approximately 3 mL of nanopure water. The filter remaining in bomb was washed several times with small volumes of water, each time collecting the washing in 30 mL polypropylene bottle. Finally, the bottles were shaken to complete the dissolution and the volume made up to 15 mL with nanopure water. The solutions were then analyzed for Cd, Cu, Pb and Zn by GFAAS and Al, Fe and Mn by FAAS.

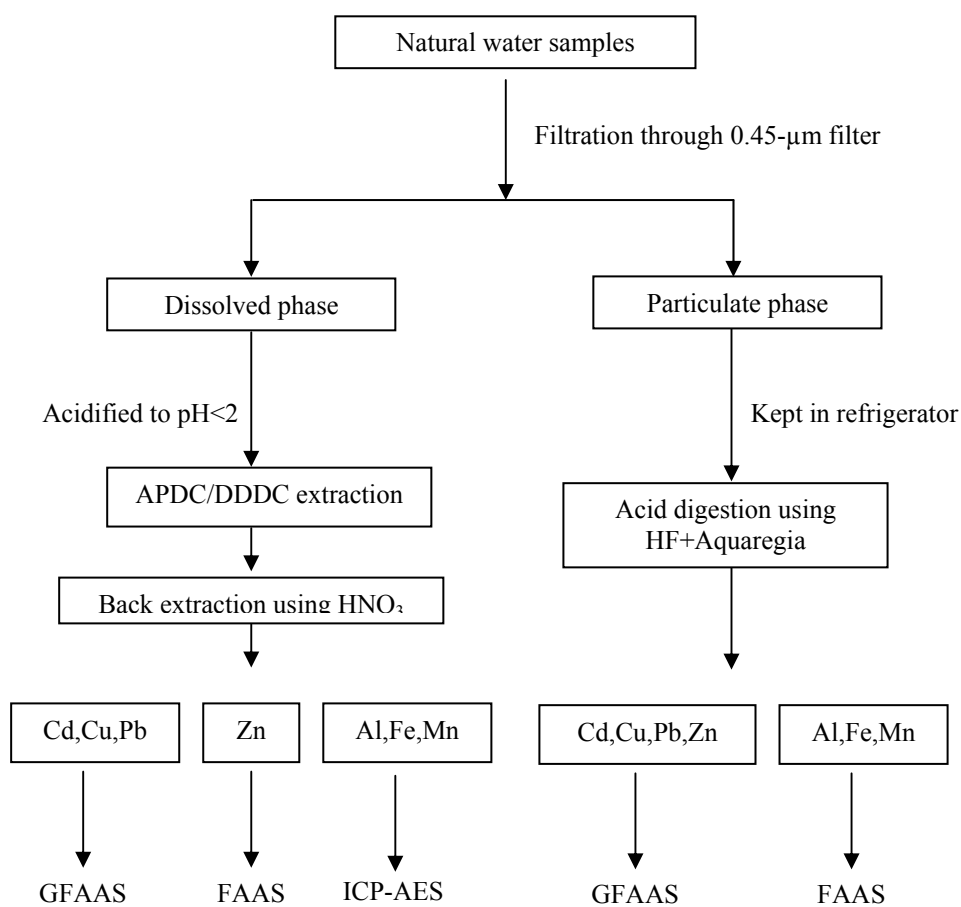


Figure 2 - 2 The schematic of sample preparation

2.7 Method of validation

In order to obtain the reliable results, the validation method should be used to assume the analyses quality of data. The analytical performance characteristic was evaluated in these terms of: limit of detection, precision, accuracy and percent recovery.

2.7.1 Limit of Detection (LOD)

The detection limit (IUPAC definition), is expressed as a concentration which will give an absorbance signal three times the magnitude of the baseline noise. The baseline noise may be statistically quantitated typically by making 10 or more replicate measurements of the baseline absorbance signal observed for an analytical blank, and determining the standard deviation of the measurements. The detection limit is then defined as the concentration which produces an absorbance signal three times the standard deviation of the blank (Beaty and Kerber, 1993). The limit of detection was then calculated by using the following equation.

$$\text{Detection limit} = (3\sigma) / m$$

σ = standard deviation of ten replicates of procedural blank

m = slope of calibration curve

In this work, the analysis of procedural blanks was achieved by using two types of blanks; extraction blank and particulate digestion blank. The procedural blanks were performed in the same manner as the sample described in section 2.6.1.-2.6.2. The detection limit of method was calculated by $3\sigma/m$ for each metal. The detection limit is reported as LOD for dissolved metals and LOD for particulate.

2.7.2 Accuracy and precision

The accuracy and precision of analytical method can be evaluated from percent recovery and % RSD, respectively (EPA method 7010). The percent recovery was performed by spiking standard solution 1 $\mu\text{g/L}$ of Cd and Zn, 20 $\mu\text{g/L}$ of Cu and Pb, 1 mg/L of Fe and Mn and 100 mg/L of Al for filter sample and spiking standard solution 1 $\mu\text{g/L}$ of Cd and Zn, 20 $\mu\text{g/L}$ of Cu and Pb, 100 $\mu\text{g/L}$ Fe and Al and 40 $\mu\text{g/L}$

of Mn for particulate sample. Three replicates were performed of each sample. The concentration of spiking solution was evaluated by using calibration graph and then % recovery and % RSD were calculated. The calculation of %RSD is given as: (Skoog *et al.*, 2004)

$$\%RSD = \frac{s}{\bar{x}} \times 100\%$$

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{(n-1)}}$$

Where :

s	=	standard deviation
N	=	total number of values
x _i	=	each individual value
\bar{x}	=	mean of n values

2.8 Statistical data analysis

Principle component analysis (PCA) is a multivariate statistical technique used to identify important component or factors that explain most of variances of a system. It is designed to reduce the number of variables to a small number of indices (i.e. principal components or factors) while attempting to preserve the relationships present in the origin data. The PCA allows ‘a factor score’ for each sample to be calculated. When plotted by factor scores, samples with similar analyte compositions (i.e. scores) will be closer than those with dissimilar compositions. In this study, the statistical data analysis was made using the MINITAB 14 for Windows software package. The PCA was applied to analyze the trace element patterns. In addition the spatial and seasonal distributions of metal in Songkhla Lake System were also performed.