CHAPTER 2

METHODOLOGY

2.1   Chemicals and materials

All chemicals were of analytical-reagent grade and nanopure water (>18 MΩ cm), obtained from Barnstead Systems (Germany) was used throughout. The mineral acids, (HNO_3 and HCl) were purified by sub-boiling distillation. Stock solutions (1000 mg L^{-1}) of Cd, Cu, Pb, Fe and Mn were purchased from J.T. Baker standards (USA) and working standard solutions were prepared fresh daily by appropriately diluting the stock solutions. The detail of preparation for extraction reagent (a mixture of APDC and DDDC), 2 M acetate buffer and mixing standard solution are given in Appendix B.

All plastic wares were acid-cleaned by soaking in 6% (v/v) of HNO_3 for one week, rinsed thoroughly with nanopure water and stored in plastic bags. Filter cartridges (Sartorius, Germany) and cellulose acetate membrane (0.45 μm pore size, 25 mm diameter) were cleaned by soaking in 0.2% HCl for a few days and rinsed thoroughly with nanopure water. Comprehensive list of reagents and materials are shown in Table B-1 and B-2 in Appendix B. The cleaning of sampling bottles, bagging, and treatment of water sample such as pre-concentration step were carried out in a clean laboratory with HEPA filtered unit. The critical step such as dispensing acid was performed in a Class 100 laminar flow clean bench.

2.2   Instruments and Apparatus

2.2.1 Graphite-Furnace Atomic Absorption Spectrometer (GFAAS)

Trace metal measurements were performed by Graphite-Furnace Atomic Absorption Spectrometer (Perkin-Elmer Model AAnalyst 800) with Zeeman background correction and an autosampler Model AS-800. The operating conditions were summarized in Table B-3 in Appendix B.

2.2.2   Flame Atomic Absorption Spectrometer (FAAS)

Metals concentrations in overlying water in the study of the effect of oxygen and salinity were performed by Flame Atomic Absorption Spectrometer (Shimadzu Model AA-680, Japan). The operating conditions were summarized in Table B-4 in Appendix B.

Strontium solution was used as a dilution marker in the benthic chamber. It was determined by Flame Atomic Absorption Spectrometer (Varian Model Spectra 220, Australia). The operating conditions are listed in Table B-4 in Appendix B.

2.2.3   Core sampler

Push corer was made of Plexi-glass tubes (4 inch diameter and 20 inch lengthy). The tubes were sealed at both ends with superlene stoppers as showed in Figure 2.1.
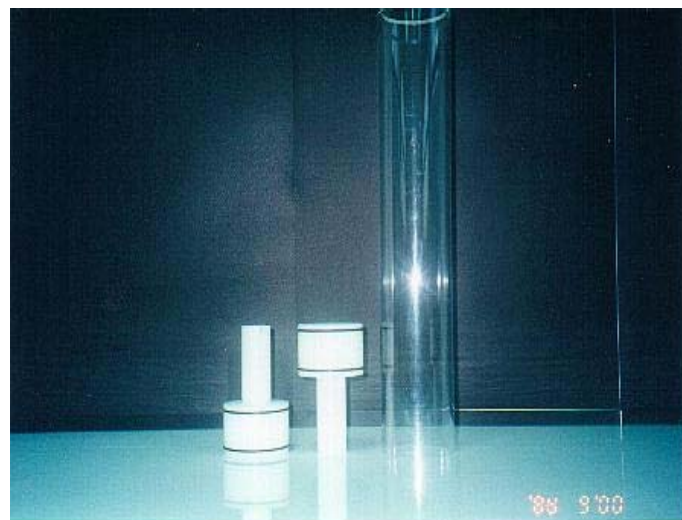

Figure 2- 1   Pushed core sampler

2.3 Construction the benthic chamber

Benthic chambers were assembled from a 40-L domestic food grade polyethylene bucket of cylindrical shape with internal diameter 15 cm and height of 32 cm. Two types of chamber were designed; a translucent bucket was named "light chamber" and a bucket painted outside with flint coat to exclude light was named "dark chamber". The bottom of the bucket was cut and its rim sharpened in order to easily insert into the sediment. A submersible aquarium pump (ORSUN model SP850) was modified by inserting L-shape glass tube to the outlet of the pump. A modified submersible aquarium pump was used to circulate the water in the chamber. The pump was installed inside the chamber, 15 cm. from the top of the bucket. Two holes were drilled at the lid and fitted with a rubber septum. The electrical wire for the pump and the plastic tubing for water sampling were inserted through these holes. A commercial refrigerator rubber seal was fixed around the edge of lid. The assembled chambers are illustrated in Figure 2-2. Leaching of materials from the polyethylene buckets and pumps was checked extensively before use by filling with nanopure water (>18 MΩ cm) and subsequent analysis of trace metals in the water after one week (Table C-28).

Figure2- 2   Lab-built light and dark benthic chamber (Left) and a submersible pump installed on the wall inside the bucket (Right)

2.4 Effect of oxygen and salinity on metal concentrations in overlying water under controlled conditions in laboratory

2.4.1 Effect of aerobic and anaerobic conditions on metal concentrations

Estuarine water from Kor Yor (salinity 5 psu) was spiked with appropriated volume of stock solution (1000 mg L^{-1}) of Cd, Cu and Pb to make the final concentration of metals at 1, 2 and 2 mg L^{-1}), respectively. A series of amber bottles were filled with 500 g of sediment. A bottle without sediment was used as a control. Two liters of spiked water was transferred into an amber bottle slowly to prevent the surface sediment disturbances. For oxic condition, the aeration was performed at all time. For anoxic condition, the nitrogen was purged gently into the water of the bottle for half an hour and sealed with polyethylene sheet which a tube was inserted through a hole for water sampling. Water samples (30 mL) from each bottle were taken for a period of about 30 hours in approximately every 0-3 hours from the initial time. Cadmium, Cu and Pb concentrations were determined using FAAS

2.4.2 Effect of salinity on metal concentrations

Water of various salinity (0, 10, 20 and 30 psu) was prepared by dilution of seawater (salinity 30 psu) with deionized (DI) water. Spiking stock solution (1000 mg L^{-1}) of Cd, Cu and Pb to make the final concentration of metals at 1, 2 and 2 mg L^{-1}, respectively. Two litters of water were transferred into a series of bottles as described above. Water samples (30 mL) from each bottle were taken for a period of about 48 hours in approximately every 0-3 hours from the initial time. Cd, Cu and Pb concentrations were determined using FAAS

Figure 2- 3   Effect of aerobic and anaerobic condition (Left) and salinity on metal concentrations in overlying water (Right)

2.5   Benthic fluxes of metals in the Outer Songkhla Lake

Benthic chamber experiments were performed at Khao-Daeng closed to the outlet of the Outer Songkhla Lake in 11-12 April 2005 (Dry season) and 25-27 January 2006 (Wet season).   Preliminary flux study was carrier out during 22-23 January 2005 at Kor-Yor station (Figure 2-4).   A set of two light chambers and two dark chambers were deployed.
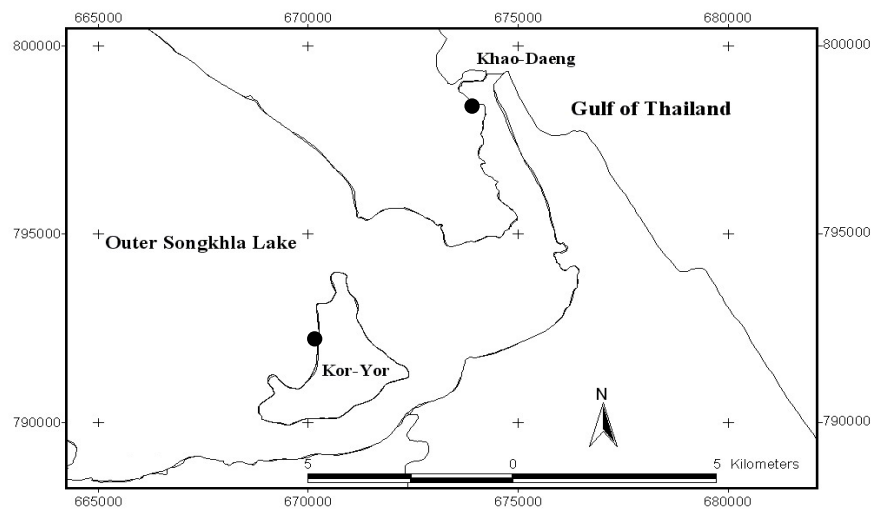

Figure 2- 4   Location of 2 sampling stations (●), Kor-Yor and Khao-Daeng

Prior deployment, these chambers were thoroughly cleaned with 10% HCl and DI water and then soaked in ambient seawater for half an hour before use to ensure that there is no chemical exchange between the chamber walls and the water enclosed. Two light and dark benthic chambers were carefully pushed in the sediment using all possible precautions not to disturb the superficial level of the sediments. The distance between each chamber was approximately 50 cm and a wooden pole was inserted next to the chamber to mark the chamber position. Closed the benthic chambers with the lids. Then put a heavy stone on the lid in order to secure the chamber. Turned on the pump to circulate water for half an hour and added 50 mL of 100 mg L^{-1} of strontium chloride as an inert maker into the chamber, equilibrated for half an hour.

Water samples from benthic chamber were taken approximately every 1–6 h for a period of about 30 h and 55 h in dry and wet season respectively. The 40 mL of sample was collected through the plastic tube by using syringe and the same volume of surrounding water was replaced simultaneously to avoid hydrostatic pressure forcing pore water from sediment released into the overlying water in the chamber (Figure 2-5).
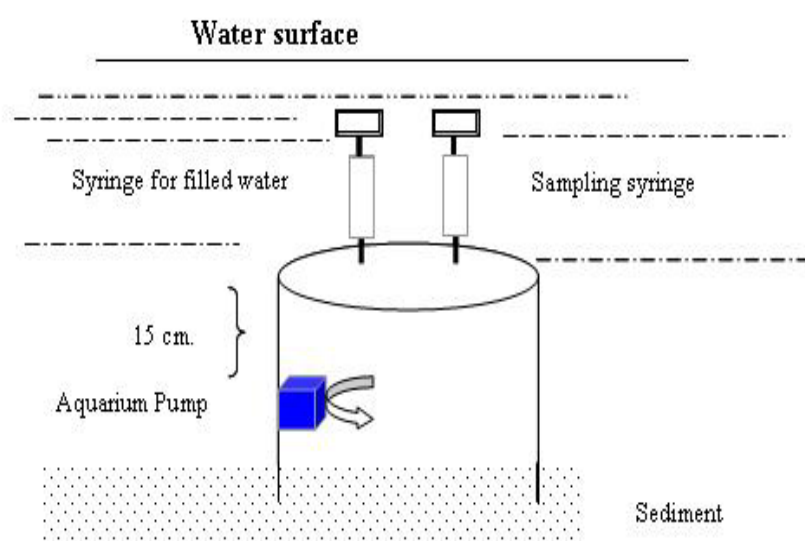

Figure 2- 5   Experimental set up showing a benthic chamber in position

The water samples were directly filtered in the field using the filter cartridge Sartorius (Germany) equipped with 0.45-µm cellulose membrane into 60 mL of polyethylene bottle (Figure 2-6), immediately acidified by using distilled nitric acid (40 µL acid to 40 mL sample) and stored in a portable refrigerator for further analyses. Water quality parameters (pH, Eh, dissolved oxygen, conductivity, salinity and temperature) were performed during each sampling by multi-parametric probe (Mettler-Toledo, Gmbh, Model 681, Switzerland). Dissolved oxygen (D.O) was determined by modified Winkler Method (Grasshoff, 1983). The concentration changes of metals in the enclosed overlying water as a function of time are used to calculate fluxes of metals.

Figure 2- 6   Filter water samples

2.6   Dissolved metals determination

Trace metals in filter solution (dissolved phase) were pre-concentrated 20 folds by using ammoniumpyrolidindithiocarbamate (APDC) and diethylammo-niumdithiocarbamate (DDDC) based on the procedure of Magnusson and Westerlund (1981). In brief, water samples were complexed by a mixture of APDC and DDDC, 1% each, prior to chloroform extraction. The metals in organic phase were back-extracted into 0.3 M HNO_3. The aqueous solution was then analyzed using either a Graphite-Furnace Atomic Absorption Spectrometer (GFAAS) for Cd, Cu, Pb, Fe and

Mn or a Flame Atomic Absorption Spectrometer (FAAS) for Mn in wet season. Blank was prepared in the same method as the samples. Percent recovery was used to check the analytical procedure. The detail of metal extraction is shown in Appendix F.

2.7   Calculation of Flux

2.7.1   Benthic Flux

The benthic flux obtained from the benthic chamber experiment is reported as a function of time by using followed equation (2-1):

$$F_b = R/S \qquad (2\text{-}1)$$

Where

F_b is the mean benthic flux during the experiment,

R   is the slope of the regression line in pmol/h and

S   is the sediment surface covered by the chamber.

Positive flux result from an increase in concentration in the water with time, on the contrary, negative flux result when concentrations in water decrease with time.

2.7.2   Diffusive Flux

Diffusive flux was computed by applying Fick's first law in equation (2-2)

$$J = -\phi D_s \frac{dC}{dz} \text{z=0} \qquad (2\text{-}2)$$

where

J is the diffusive flux (mol cm^{-2} h^{-1})

ϕ is the porosity of the surface sediments

D_s is the sediment diffusion coefficient (cm^2 s^{-1})

$\dfrac{dC}{dz}$ is the concentration gradient occurring over a distance

2.8   Sediment

After the benthic experiments were ended, sediment samples in the chamber were collected by using Plexiglas push corer 4 inch in diameter and 20 inch in length (Fig 2-7). The cores were pushed into the sediments and carefully pulled out. Both ends of the cores were closed with superlene caps and wrapped tightly prior to being transported (vertically) back to the laboratory within 2 h. immediately after collection. The cores were sub-sampled in a nitrogen chamber (Jungsrirutanakun, 2001). This operation was completed within 24 h after core recovery.

Figure 2- 7   Sediment in Core and overlying water

2.9   Sub sampling of sediment

The upper part of each core was inserted into the nitrogen chamber through a lock gate in order to obtain pore water and sediment at different depths. The sediment was carefully pressed out and sliced as follows: 0–1 and 1–2 cm, and then every 3 cm along the full length of the core with an acid-cleaned plastic knife. The pore water from each sub sample layer was immediately extracted in the nitrogen chamber.   The sediment sections were centrifuged by Automatic refrigeration (SORVALL, USA, Model T21) at 15000 rpm at 5°C for 20 minutes, in order to separate pore waters from the solid phase.   The sediments retained in the vial were freeze-dried.

Figure 2-8   The sediment was pressed out and sliced in the nitrogen chamber

2.10   Sediment digestions

After centrifuge, the sediment samples were distributed, which the thick of sediment sample was less than 1 centimeter, in the plastic dish.   Then the plastic film was covered the plastic dish to protect the contaminant from external sources and a slightly cut the plastic film for release the liquid from evaporating.   The dried sediments were ground with an agate mortar until fine particles were obtained.

The samples were analyzed for total metal concentrations using a strong acid digestion method of Loring and Rantala (1995). Samples were prepared by accurately weighing around 150 mg of dried sediment (<58 μm) into a clean 40-mL LORRAN$^{®}$ Teflon bomb (Lorran International, Porters Lake, N.S., Canada, BOJ 2S0) followed by addition of 6 mL of hydrofluoric acid (HF) and 1 ml of aqua regia (HNO_3 + HCl) and placed on a hotplate at a temperature of 120°C for 2 day. After cooling, the digested solution was transferred into 100 mL polyethylene volumetric flask containing 5.6 g of boric acid in 20 mL of deionized water for the removal of the excess of HF. The solution was then made up to 100 mL with deionized water and transfer into an acid-cleaned polyethylene bottle for storage. Allowing gelatinous precipitate of borosilicate to settle for several days before instrumental determination was performed. The resulting solution was then analysed for Cd, Cu and Pb by GFAAS. Concentrations of Fe and Mn in the solutions were determined by FAAS. A standard reference material for trace metals from the National Research Council of Canada (MESS-l) was used to check the analytical procedure.

2.11 Optimization the temperature program of GFAAS

The ashing and atomizing temperature for Cd, Cu, Pb, Fe and Mn GFAAS should be optimized according to the sample composition. The temperature program is illustrated in Table 2-1. The matrix modifier ($LaNO_3$) was used for Cu and Pb determination. The optimization was performed by changing one parameter and keeping other parameters constant and then the optimum value was selected for all experiments. The optimization tests were carried out by using 4, 25, 50, 20 and 25 ug L^{-1} for Cd, Cu, Pb, Fe and Mn standard solution, respectively.

Table 2- 1   Temperature Program for the Determination of Cd, Cu, Pb, Fe and Mn

Step of temperature Program	Temperature (c)	Ramp time (s)	Hold time (s)	Internal flow (mL/min)
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Ashing	Variable	10	20	250
Atomization	Variable	0	5	0
Cleanout	2250	1	3	250

2.12   Quantification of Metals

Trace metal analysis from both extracted water samples and digested sediment samples were performed with GFAAS and FAAS. The extractants for the natural samples always contain different matrix, compare to standard solution. Standard addition method is usually used for checking the differences of matrix in the real sample. Therefore, the matrix effect was studied by comparing the slope of standard addition curve and calibration curve. Three replicates were performed at each concentration.

2.13   Method of validation

The analytical performance characteristics were evaluated including detection limit, recovery and accuracy and precision of measurements.

2.13.1 Detection limit (DL)

The detection limit (IUPAC definition) is expressed as the smallest concentration that can be detected with a certainty of more than 95%. It is defined as the analyte concentration yielding a response k folds higher than the standard deviation of the blank (s_b) (k is defined as the confidence factor), the calculation of detection limit is given in equation (2-3) and (2-4) (Skoog $et\ al.$, 2004).

$$DL = \frac{ks_b}{m} \tag{2-3}$$

$$s = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{(n-1)}} \tag{2-4}$$

where

$\quad\quad s$ = standard deviation

$\quad\quad n$ = total number of values

$\quad\quad x_i$ = each individual value used to calculate mean

$\quad\quad \bar{x}$ = mean of n values

Where m is the slope of calibration curve, and factor k is chosen to be at 3 in order to gain a 98.3 % confidence level. It is normal to assume that the results of a blank and the sample will follow a normal distribution. It is also assumed that the standard deviation (s) from the blank and sample are the same. In this work, DL of AAnalyst 800-GFAAS was calculated by using Equation (2-3) (when s_b value was obtained from 10 measurements of reagent blank signal)

2.13.2 Precision

The precision is the measure of the degree of an analytical method under the same conditions. Normally it is expressed as a percentage of the relative standard deviation (%RSD) for a statically significant number of samples. The calculation of %RSD is given equation (2-5) (Skoog $et\ al.$, 2004).

$$\% RSD = \frac{s}{\bar{x}} \times 100\% \tag{2-5}$$

In this research, the precision were investigated for measurement of the degree of repeatability. Sediment samples from Khao-Deang were repeated 10 times.

2.13.3 Recovery

The terms recovery (R) is used to indicate the yield of an analyst in a pre-concentration or extraction stage in an analytical method. The percent recoveries were performed by using estuarine water from Kor-Yor spiked with the studied elements and treated as the same procedure as samples. Three replicates were performed at each concentration. Actually, the recovery value is presented as a percent recovery (%R) and it can be calculated from the equation given in equation (2-6) (Skoog $et\ al.$, 2004).

$$\% R = \frac{Measured\ value}{Real\ value} X\ 100 \tag{2-6}$$

2.13.4 Analytical accuracy

The accuracy term is the measurement of exactness value of the analytical concentration or agreement between measured value and certified value or an accepted reference value. The relative accuracy for the determination of metals in sediment was evaluated comparing to the certified values for the MESS-1. All blanks and the certified reference material were prepared in the same manure as the samples. The difference in values between the measured value and certified value was compared and the relative percent error was also calculated. The following calculation of relative percent error is given equation (2-7) (Skoog *et al*., 2004).

$$\% \text{ Error} = \frac{\text{Measured value} - \text{Real value}}{\text{Real value}} \times 100\% \qquad (2\text{-}7)$$