



## CHAPTER II

### MATERIALS AND METHODS

The concentrations of four heavy metals; lead, copper, zinc and iron in the seawater both in particulate and dissolved forms were studied. Each seawater sample was also analyzed for dissolved organic carbon. Tissues of bivalves collected from the vicinity area of ship-breaking plants were also analyzed. The procedures of study can be separated into three activities which are field study, laboratory experiment and data analysis. These activities are described in details as follow.

#### Field Study.

##### 1. Sampling.

Seawater samples were collected off Mab Ta Phud coastal area by survey vessels provided by the Eastern Marine Fisheries Development Center, Division of Marine Fisheries, Department of Fisheries, Ban Phe, Muang, Rayong. Those vessels were Pramong Talay 15, Pramong Talay 12 and Ban Phe 14. Marine organism samples were bought from local fishermen at Ban Pla.

1.1 Sampling Station. Four groups of sampling stations had been designed for this study (Figure 1-1). The first group was located in the area where fresh water channels discharge to the nearshore area. Six natural channels and an artificial channel, constructed to drain a wastewater treatment plant effluent of the

Mab Ta Phud Industrial Estate were chosen as study stations. The second group was a group of stations located in a nearshore line, 2 km from the ship-breaking plants in the west-east direction from Ban Pla to Ban Ta Kuan. The third group, offshore stations located in line at a distances of 3 km away from the nearshore line and was in the same direction. The last group located at Rayong River mouth and was near the petrochemical plant. The reference station in this study refers to a location unaffected by ship-breaking activities, was about 3 km from the offshore stations line. The location and identification of stations are shown in Figure 1-1 and Table 2-1.

1.2 Sampling Frequency. The objective of this study focused on the quantity of four heavy metals released due to ship-breaking activity. As a consequence, sampling frequencies were design in correlation with the periods before, during and after the ship-breaking activity. Sampling frequencies are shown in Table 2-2 and can be summarized as follows :

1.2.1 October 20-25, 1988. This period was a period before ship-breaking activity started. The ship-breaking activity was halted since June 19, 1988 due to unfavorable national economic situation.

1.2.2 June 21 - 22, 1989 and July 11, 1989. Sampling was carried out during this period when ship-breaking activity was under way.

1.2.3 November 23-24, 1989. This sampling period was the same as the period described in 1.2.2



Table 2-1 Location of sampling stations off Ban Nong Faeb,  
Mab Ta Phud, Rayong Province.

Station	Latitude	Longitude
C-1	12° 40' 00" N	101° 02' 56" E
C-2	12° 40' 30" N	101° 04' 22" E
C-3	12° 40' 44" N	101° 06' 06" E
C-4	12° 40' 40" N	101° 06' 50" E
C-5	12° 40' 37" N	101° 07' 40" E
C-6	12° 40' 30" N	101° 08' 16" E
C-7	12° 40' 15" N	101° 10' 25" E
SB-01	12° 39' 25" N	101° 02' 50" E
SB-02	12° 39' 25" N	101° 04' 25" E
SB-03	12° 39' 25" N	101° 06' 00" E
SB-04	12° 39' 55" N	101° 06' 40" E
SB-05	12° 39' 25" N	101° 07' 23" E
SB-06	12° 39' 25" N	101° 08' 53" E
SB-07	12° 39' 25" N	101° 10' 33" E
SB-08	12° 37' 55" N	101° 02' 50" E
SB-09	12° 37' 55" N	101° 04' 25" E
SB-10	12° 37' 55" N	101° 06' 00" E
SB-11	12° 37' 55" N	101° 07' 23" E
SB-12	12° 37' 55" N	101° 08' 53" E
SB-13	12° 37' 55" N	101° 10' 33" E
SB-14	12° 36' 00" N	101° 06' 00" E
SB-15	12° 39' 00" N	101° 15' 00" E
SB-16	12° 40' 17" N	101° 10' 40" E
SB-17	12° 40' 30" N	101° 08' 49" E

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Table 2-2 Seawater sampling times at Ban Nong Faeb, Mab Ta Phud, Muang, Rayong province.

Station	Trip N° of Sampling							
	1		2		3		4	
	Date	Time	Date	Time	Date	Time	Date	Time
C-1			22/06/89	10:40	23/11/89	17:30	15/05/90	9:00
C-2			22/06/89	11:10	24/11/89	9:50	15/05/90	10:50
C-3			22/06/89	13:00	24/11/89	10:50		
C-4					23/11/89	16:00		
C-5								
C-6					23/11/89	15:00	15/05/90	15:15
C-7			21/06/89	17:30	23/11/89	11:50	15/05/90	16:30
SB-01	21/10/88	16:45	22/06/89	14:35	23/11/89	17:37	16/05/90	9:40
SB-02	21/10/88	15:30	22/06/89	13:40	23/11/89	17:10	16/05/90	10:12
SB-03	21/10/88	14:55	22/06/89	10:15	23/11/89	15:55	16/05/90	10:40
SB-04	21/10/88	12:55	22/06/89	12:20	23/11/89	15:20	16/05/90	11:15
SB-05	21/10/88	12:00	22/06/89	11:20	23/11/89	14:10	16/05/90	12:15
SB-06	20/10/88	15:05	11/07/89	13:05	23/11/89	13:30	16/05/90	12:50
SB-07	20/10/88	14:05	11/07/89	11:55	23/11/89	12:57	16/05/90	13:15
SB-08	25/10/88	14:55	11/07/89	15:30	24/11/89	12:35	22/05/90	11:15
SB-09	25/10/88	14:15	11/07/89	15:15	24/11/89	13:30	22/05/90	11:45
SB-10	25/10/88	13:25	11/07/89	14:30	24/11/89	9:30	22/05/90	12:10
SB-11	25/10/88	12:35	11/07/89	14:10	24/11/89	14:05	22/05/90	12:25
SB-12	25/10/88	11:35	11/07/89	13:45	24/11/89	14:35	22/05/90	12:45
SB-13	22/10/88	14:20	11/07/89	13:20	24/11/89	15:45	22/05/90	13:05
SB-14			11/07/89	16:20	24/11/89	11:45	22/05/90	10:30
SB-15	22/10/88	11:50	11/07/89	10:45	24/11/89	16:30	14/05/90	11:30
SB-16	22/10/88	11:25	11/07/89	10:10	24/11/89	16:50	14/05/90	12:30
SB-17	22/10/88	10:55	11/07/89	9:45	24/11/89	17:10	14/05/90	12:05

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1.2.4 May 14-22, 1990. This sampling period was a period after ship-breaking activity was finished.

### 1.3 Sampling Methods.

#### 1.3.1 Seawater Sampling.

1.3.1.1 Dissolved Heavy Metals. Seawater samples were collected at 1 meter depth with a GO-FLO water sampler. Samples were carefully released into a clean 1 liter polyethylene bottle which was later kept in a plastic bag. Samples were immediately preserved by cooling in ice box until they were taken ashore to the laboratory for analysis. Extreme care was made to avoid metal contamination. The four metals to be analyzed were copper, iron, lead and zinc, in dissolved form.

1.3.1.2 Particulate Heavy Metals. The samples were separated from the dissolved form samples in 1.3.1.1. Separation method is shown in Section 1.2.1.2 of laboratory experiment.

1.3.1.3 Dissolved Organic Carbon. Each seawater sample collected at 1 meter depth was transferred into a clean 100 ml glass bottle for dissolved organic carbon analysis. Samples were immediately preserved after collection in an ice box.

1.3.1.4 Variation due to Tidal Cycle. At an interval of about 4 hours at station SB-10 (shown in Figure 1-1.) seawater samples were collected for four heavy metals analysis. Sampling method has already been explained in 1.3.1.1 & 1.3.1.2.

1.3.2 Bivalves Sampling. The pen shells, Atrina vexillum, were collected from the sites near ship-breaking plants. They were preserved in a plastic bag and kept in an ice box.

1.3.3 Scrap-steels Sampling. During steel cutting process scrap-steels can release some heavy metals into the seawater. Pieces of scrap-steels were collected from the beach nearby and within the plants' boundary. They were also kept in a plastic bag. The quantity and the dissolved rate of heavy metals into the seawater from this scrap-steel were studied in the laboratory.

1.4 Cleaning of Containers. The sample bottles for seawater analysis were one liter polyethylene bottles. Brand new bottles were rinsed with a small amount of organic solvent such as chloroform in order to remove any oil or grease present. The bottles were then cleaned by detergent and rinsed with distilled water. The bottles, then, were filled with concentrated nitric acid, loosely capped and then maintained at 60°C on a hot plate. After three days, the acid solution was stored in a container for reuse. The bottles were rinsed and filled with double distilled water. They were placed on a hot plate at 60°C for at least 24 hours. Then, they were rerinsed with distilled water and dried on a clean cabinet. After during, they were kept covered in plastic bags until use.



## 2. Survey of Current Characteristics.

Tidal current was measured with a current meter , model CM-2S at all three depths ( 2, 8 & 18 meters) at station SB-10 (shown in Figure 1-1). The interval of tidal current recording was every one hour for 25 hours.

### Laboratory Experiment.

#### 1. Analysis of Samples.

1.1 Apparatus Cleaning. The sample containers were cleaned as specified in 1.4. The Millipore membrane filters were soaked in 10% nitric acid solution for 6 hours and later in distilled water in a covered teflon beaker until use.

#### 1.2 Sample Preparation.

##### 1.2.1 Heavy Metals in Seawater.

1.2.1.1 Particulate Heavy Metals. Seawater sample was filtered through a Millipore filtration system. A volume of sea water sample was about 1,000 ml (accurately noted). The particulate form was retained on a Millipore membrane filter, pore size 0.45  $\mu\text{m}$ . A filter was removed from a filter holder with plastic forceps and then transferred to a polyethylene vial, labelled, kept in a clean plastic bag and stored in a refrigerator.

1.2.1.2 Dissolved Heavy Metals. The seawater sample for dissolved metals analysis was filtered into a precleaned 200 ml Nalgene bottle by the method described above. The sub-boiling redistilled concentrated nitric acid was added

into the sample to adjust the pH of each filtered seawater sample to around 4. Then each bottle was placed into a plastic bag and labelled.

1.2.2 Dissolved Organic Carbon in Seawater. Glass-fibre filters (GF/C) were precombusted at 450°C. The seawater sample for dissolved organic carbon analysis was filtered through a precombusted GF/C filter paper. The filtered sample was stored in a glass ampoule which later was sealed and kept in a refrigerator.

1.2.3 Bivalves. The soft part of the bivalve was dried in a freeze dryer, ground, stored in a plastic bag and kept in a desiccator.

### 1.3 Sample Analysis.

#### 1.3.1 Seawater Analysis.

In this step, prepared sample was analyzed for heavy metals both in the forms of particulate and dissolved matter. In addition, analysis for dissolved organic carbon was also carried out.

##### 1.3.1.1 Particulate Heavy Metal Analysis.

The method was as described in the Inorganic Handbook for River Transport Workshop, GEMSI, 1986. The procedures were as follows :

- A Millipore filter paper was transferred from a polyethylene vial into a covered teflon beaker and 900 µl of concentrated nitric acid and 100 µl of



hydrofluoric acid (40 %) were added.

- The teflon beaker was placed on a hot plate and heated to 150°C until the particulate and millipore paper were gently digested in an acid solution.

- The acid solution was diluted to 5 ml with double-distilled water and stored in polyethylene vials.

The solution was kept in a refrigerator until measurement for lead, copper, zinc and iron by a polarized Zeeman atomic absorption spectrophotometer, HITACHI model Z-8100.

#### 1.3.1.2 Dissolved Heavy Metal Analysis.

The method, used in study, was an improved solvent extraction method investigated by Sukasem, 1989. The procedure was as follow :

- 100 ml of filtered seawater was transferred into a teflon beaker and the pH was adjusted to around 5 by diluted ammonium hydroxide or diluted nitric acid and later transferred to a 250 ml separatory funnel.

- 5 ml 2% (w/v) APDC was added and swiveled.

- 5 ml MIBK was added.

- The sample was shaken for 4 min and was let stand for clear phase separation and later the lower layer was drained off .

- 5 ml 4N nitric acid was added into

an organic phase in a separatory funnel. A funnel was shaken for 5 min and let stand for phase separation.

- The lower layer was collected in a precleaned 8 ml polyethylene vial and kept in a refrigerator until analysis with a polarized Zeeman atomic absorption spectrophotometer.

1.3.1.3 Dissolved Organic Carbon. The combustion-infrared analysis method was used for this study. A water sample was injected into a heated reaction chamber packed with an oxidative catalyst. The  $\text{CO}_2$ , a product of oxidation of organic and inorganic carbon, was transported in the carrier gas stream and measured by means of a nondispersive infrared analyzer. This value read was a total carbon concentration. The total organic carbon value was obtained by subtracting total carbon by inorganic carbon concentrations. Inorganic carbon was measured by injecting the water sample into a separate reaction chamber packed with phosphoric acid-coated quartz beads. Under these conditions organic carbon was not oxidized and only inorganic carbon was measured. The apparatus used to carry out these analyses was a Total Carbon Analyzer, SHIMADZU model TOC-10B.

1.3.2 Bivalves Analysis. The details of the analytical method is described below.

- 0.1 g of dried bivalve was transferred to a teflon beaker and 3 ml of sub-boiling redistilled concentrated nitric acid was added.

- The teflon beaker was heated at  $150^\circ\text{C}$  on a hot plate until the bivalve tissue was gently digested to clear



solution.

- The acid solution was diluted to 10 ml with double distilled water and stored in Nalgene bottles. The solution was kept in a refrigerator until analysis for lead, copper, zinc and iron with a polarized Zeeman atomic absorption spectrophotometer, HITACHI model Z-8100 .

## 2. Laboratory Experiment on Leaching of Heavy Metals.

Material samples were collected from the vicinity of the ship-breaking site in order to investigate the concentrations of copper, lead, iron and zinc leached. Material samples were:

Type I material, scrap-paints which came off the steel plate of a vessel, as shown in Figure 2-1,

Type II material, a piece of iron splashed from high temperature cutting, as shown in Figure 2-2,

Type III material, scrap-iron, a part of steel plate cut from the vessel present on the ground around the plant area, as shown in Figure 2-3, and

Type IV material , a scrap-iron piece occasionally submerged in the seawater and present on the sand beach, as shown in Figure 2-4.

Those materials were cleaned in a laboratory with distilled water and let dried.



Figure 2-1 Scrap-paints which came off the steel plate of a vessel.



Figure 2-2 Piece of iron splashed from high temperature cutting.





Figure 2-3 Scrap-iron, a part of steel plate cut from the vessel present on the ground around the plant area.



Figure 2-4 Scrap-iron piece occasionally submerged in the seawater and present on the sand beach.



## 2.1 Leaching Experiment.

2.1.1 A seawater sample collected off the Mab Ta Phud coast was filtered by a Millipore membrane filter (pore size 0.45  $\mu\text{m}$ ) and stored in a precleaned 5 liter polyethylene bottle. Filtered sample was analyzed for four heavy metals concentrations, i.e. : copper, lead, iron and zinc, particularly in the dissolved form.

2.1.2 Material samples were placed in a precleaned polyethylene containers. Each container was filled with 1.5 liter filtered seawater and covered. The containers were kept at room temperature for 7 days.

2.1.3 After 7 days, the material samples were taken out. Each seawater sample was carefully mixed and drawn for lead, copper, zinc and iron analysis both in dissolved and particulate forms. The analysis methods are already explained in 1.3.1.1 & 1.3.1.2.

The flow charts of leaching experiment in the laboratory are shown in Figure 2-5.

## 3. Recovery of Solvent Extraction Method

The solvent extraction method, used in this study, is the APDC-MIBK extraction system followed by back extraction with 4N nitric acid. This method is an improved solvent extraction method investigated by Sukasem, 1989. The salt matrix is a source of interference during the analysis by atomic absorption spectrophotometer. This improved method is able to remove salt



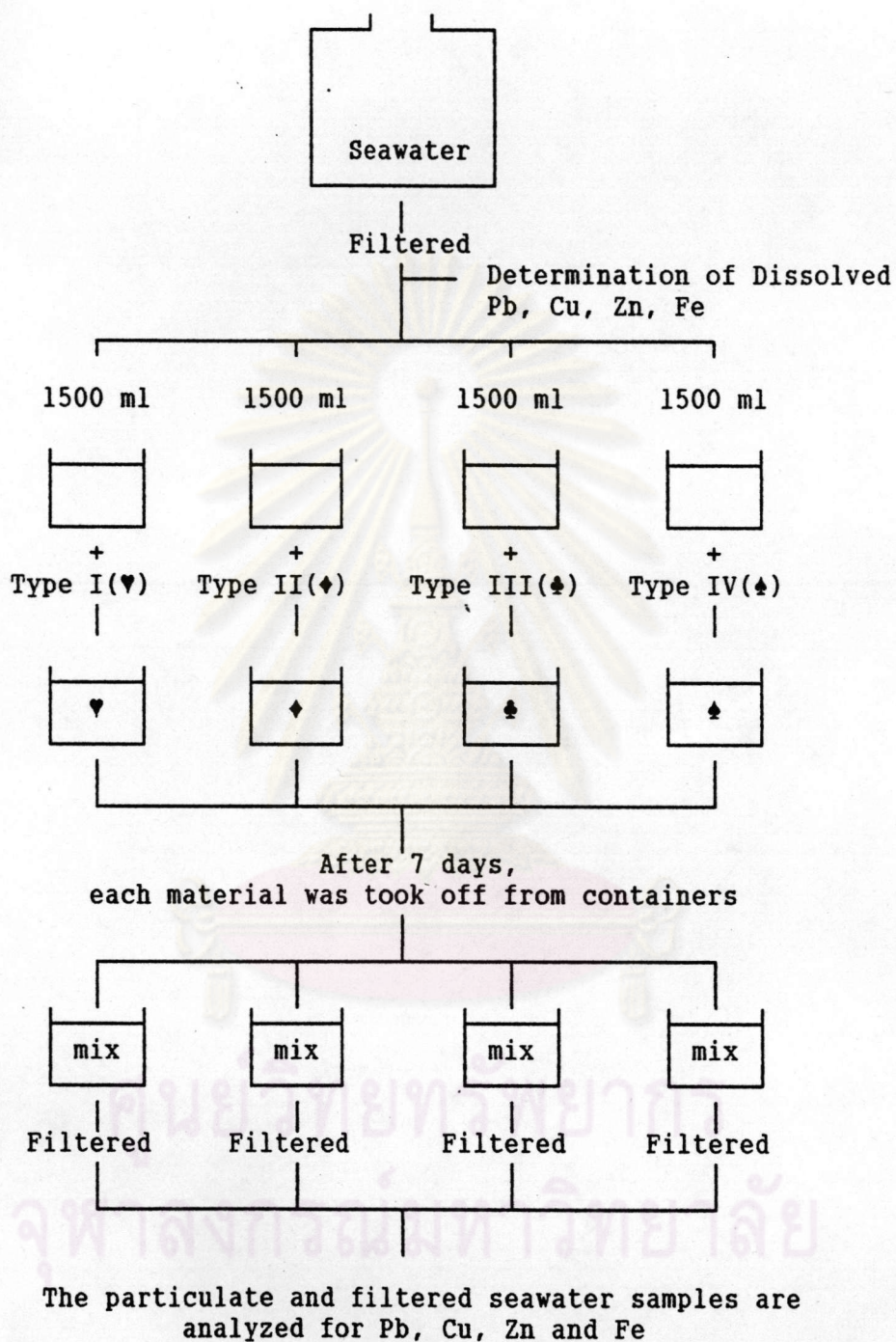


Figure 2-5 Flow charts of leaching experiments in the laboratory

matrix from some heavy metals. In addition, it can be efficiently used to determine low concentration of some heavy metals. The efficiency of the solvent extraction method is checked by comparing measured to known concentrations of standard solutions. Details of the procedure are as follows :

3.1 The standard solutions were prepared with concentration range covers expected metals concentrations of the seawater samples. They were prepared with seawater collected from certain sampling stations in the study area.

3.2 The standard solutions were analyzed for copper, lead, iron and zinc concentrations by a solvent extraction method.

3.3 Each measured concentration of copper, lead, iron and zinc was calculated and compared to a known standard concentration. The calculation is as follows :

$$\text{Percentage of Recovery (\%)} = ( Y \times 100 ) / X$$

Where X = concentration of standard solution from calculation

Y = concentration of standard solution from determination

The percentage of recovery is a factor used in the calculation of heavy metal concentration in seawater samples.

#### Data Analysis.

##### 1. Quantity and Distribution of Heavy Metals.

A comparison of heavy metal concentrations found in this study was carried out with different approaches to identify their distribution characteristics during different ship-breaking



activities and distances from the shoreline in relation to prevailing current. In addition, heavy metal and dissolved organic carbon concentrations were also analyzed to identify their relationship.

## 2. Impact of Heavy Metals on Bivalves.

Four heavy metal concentrations in pen shell, Atrina vexillum, were compared with the concentrations observed in the seawater in order to determine the concentration factor. The calculation formula is as follows :

$$\text{Concentration Factor} = \frac{\text{Concentration of heavy metal in } \underline{\text{Atrina vexillum}} \text{ (}\mu\text{g/g)}}{\text{Concentration of heavy metal in seawater (}\mu\text{g/g)}}$$

## 3. Leaching of Heavy Metals from Scrap-steels.

Four heavy metals concentrations were compared with their initial concentrations in the seawater in order to identify the quantity and their leaching rate from scrap-steels.

## 4. Calculation and Statistics Analysis.

The average and standard deviation were calculated by the following formula.

$$\text{Average (X)} = \frac{x_1 + x_2 + x_3 + \dots + x_N}{N}$$

and

$$\text{Standard deviation ( SD )} = \sqrt{\frac{\Sigma (x-X)^2}{N}}$$

Where  $x_1, \dots, x_N$  = concentration of metal in each sample  
N = number of sample



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