CHAPTER 3

METHODOLOGY

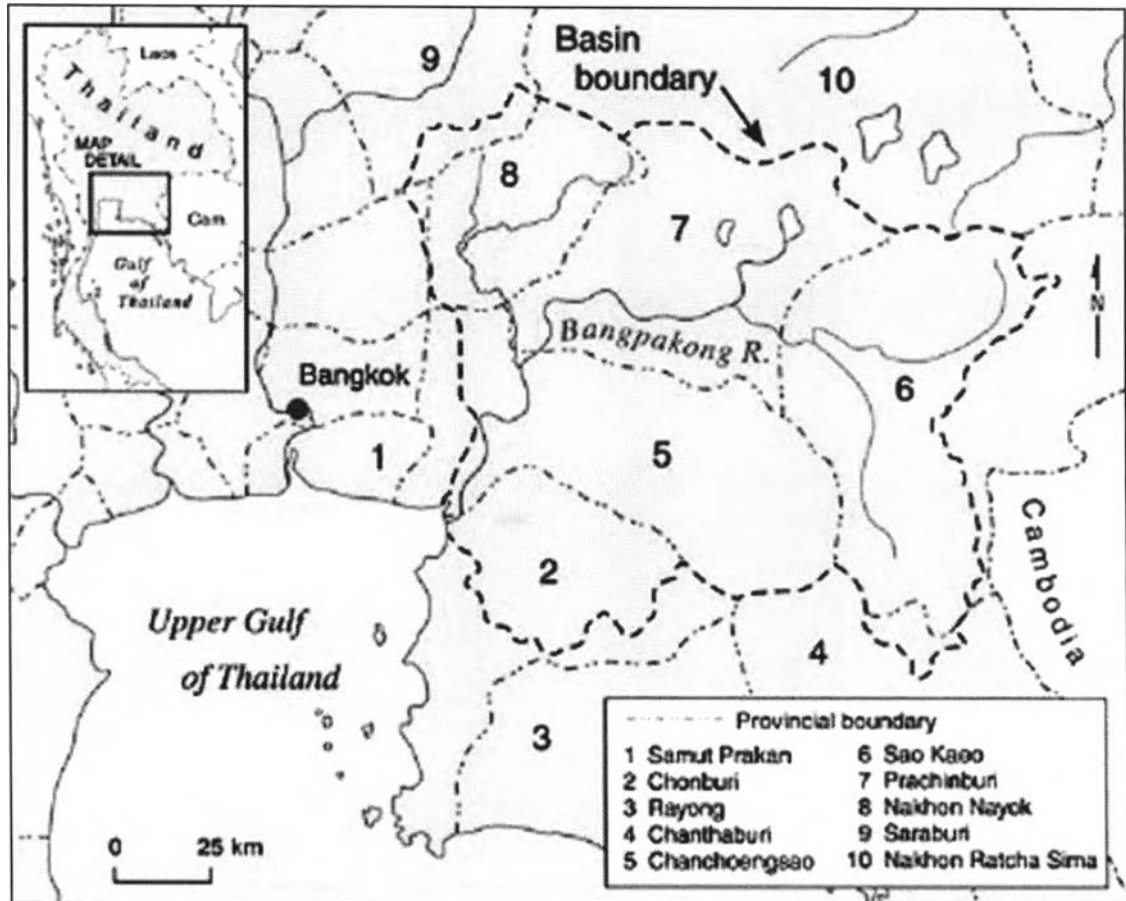## 3.1 Study area: Bangpakong River

Figure 3.1 Bangpakong River Basin

The Bangpakong River Basin is approximately 18,758 km^2 in area (Figure 3.1). Two major tributaries (Nakhon Nayok and Prachinburi Rivers) join in the northern part of Chachoengsao province to create the Lower Bangpakong River. This major watercourse then flows 122 kilometers through a flat alluvial plain before empting into the Upper Gulf of Thailand. Usually, the wet season lasts from June to November and the dry season from December to May. Air temperature ranged from 23.8 to 32.6 °C, with a yearly average of 27.9 °C. Railfall averaged 1315 mm for the period 1961 – 1991, and the number of rainy days covered one third of the year.

Concomitantly, about 96% of the annual river discharge occurs during the wet season (Bordalo et al., 2001).

Approximately 1.2 million people live within the river basin. The lower Bangpakong subbasin is a highly productive agricultural region with fertile clay soils and an extensive man-made irrigation network dating back over one hundred years (Szuster and Flaherty, 2002).

Pollutants discharged into the river are from point and non-point sources. Major point sources of pollutants to the Bangpakong River include domestic and industrial waste discharges as well as some agricultural point sources such as shrimp, duck, fish, pig and other farms (http://data.ecology.su.se/mnode/Asia/Thailand/Bangpakong/bpbud.htm).

Approximately 18,530 hectares of low salinity shrimp ponds have been identified in the Bangpakong River Basin as a part of this study, and could be responsible for as much as 40 percent of Thailand's total cultured shrimp production (Szuster and Flaherty, 2002).

3.2 Sampling method

Samples were taken directly from shrimp farms with salinity in the range of 0-30 part per thousand (ppt). The total number of sampling locations was 16. In addition, 6 samples from Bangpakong river were taken both upstream and downstream along the river to test for their trihalomethanes formation potential. These sampling locations are displayed in Figure 3.2.

Samples were directly collected, thermally stabilized by freezing them in ice and properly transported by packing them into closed boxes to ensure consistent quality control. Samples were refrigerated in the laboratory at 4°C throughout the 14-day holding time. Milli-Q water was used for all dilutions, solution preparation and the final glassware washing.
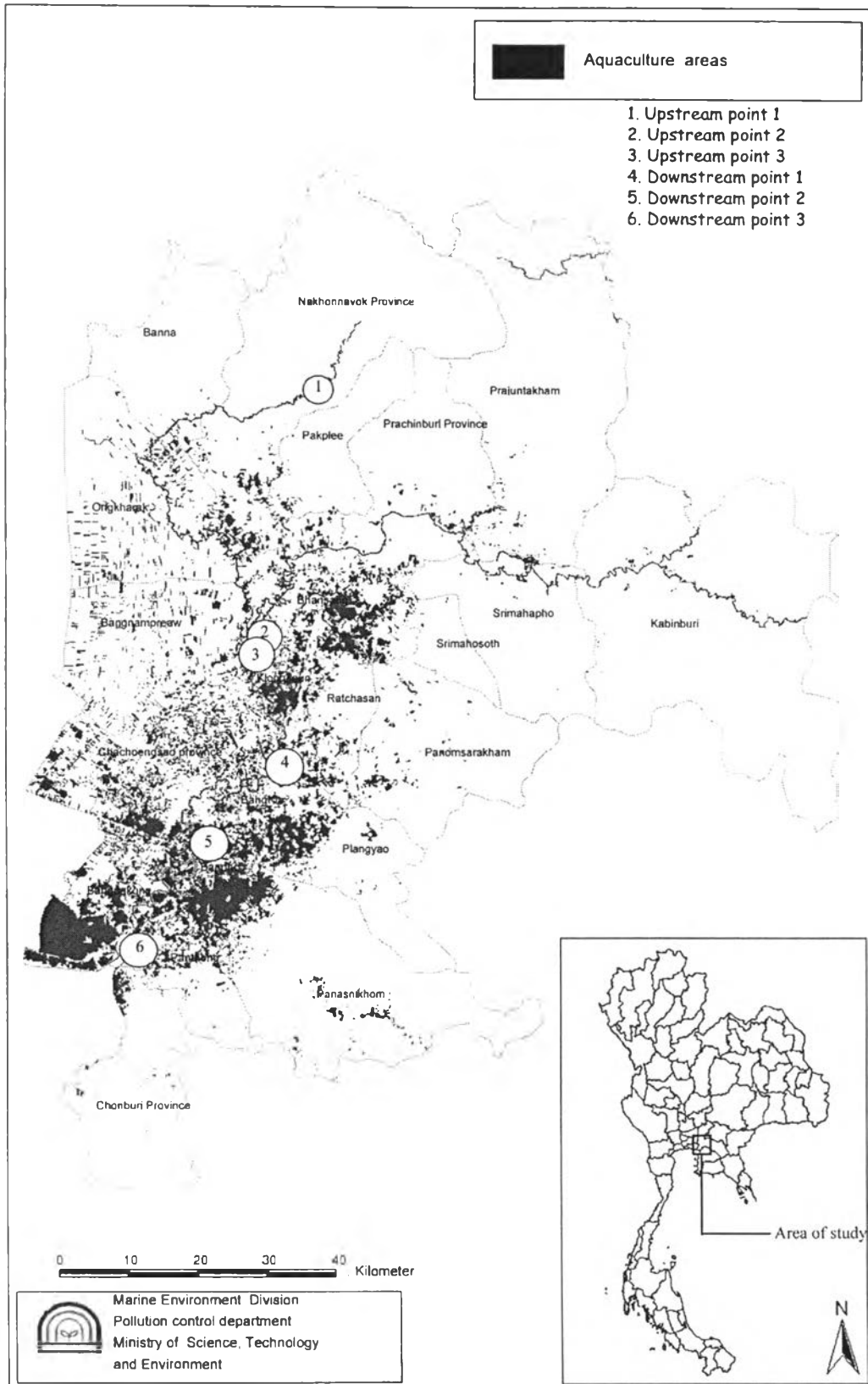
Figure 3.2 Locations of the six sampling points along the Bangpakong River

3.3 On-site measuring parameters

Several parameters were promptly measured at location points. These are summarized in Table 3.1.

Table 3.1 Analytical methods and instruments

Parameter	Analytical method	Analytical Instrument
Salinity	Direct measurement	HACH model sension 5
Conductivity	Direct measurement	HACH model sension 5
Turbidity	Direct measurement	Turbidity meter model WTW(Germany) series Turb 350 IR

3.4 Analysis of sample properties

3.4.1 Total organic carbon (TOC) and Dissolved organic carbon (DOC)

TOC was used as a measure of the organic concentration in the raw water. The TOC of all samples was measured by a Model 1010 O.I. Corp. total organic carbon analyzer equipped with a model 1051 vial autosampler using the Persulfate-Ultraviolet Oxidation method (Standard methods 5310-C., 1995, see Appendix A). All the samples were filtered through a 0.45 μm cellulose nitrate filter prior to DOC analysis to remove suspended particles. The analyzer was regularly calibrated with 1,000 ppm of standard potassium hydrogen phthalate (KHP) (CARLO ERBA brand). Each sample was prepared and diluted differently depending on whether the solvent contained 0.1 N of HCl or 0.1 N of NaOH. The analyzer was programmed accordingly with 200 μL of 5% (vol/vol) orthophosphoric acid (CARLO ERBA brand) as an acid, 1,000 μL of 100 g/L sodium peroxodisulphate (Fluka brand) as an oxidant and for a 10-minute reaction time. At least 3 blanks were analyzed prior to the analysis of each sample to establish and verify the appropriate background for quality assurance and control.

3.4.2 Total trihalomethanes (TTHMs)

In accordance with the EPA method 551.1 (see Appendix A), total trihalomethanes were analyzed using a Hewlett-Packard 6890N gas chromatography with a split/splitless injector equipped with HP-1 columns and micro electron capture

detector. Gas Chromatography was used to measure THMs under the condition as follows :

3.4.2.1 Inlet condition

Mode: split, initial temp: 225°C., pressure: 12.38 psi, split ratio: 10:1 split flow 30 mL/min, gas Type: helium, and total flow: 35.9 mL/min.

3.4.2.2 Oven condition

The conditions of the oven's temperature programs adjusted for analyzing THMs is shown in Table 3.2:

Table 3.2 Temperature program for analyzing THMs

Ramp	Rate (°C/min)	Final temperature (°C)	Holding time of final temperature (minute)	Remark
1	10	40	1.00	Initial temp.: 35°C, Initial temp. Holding Time 1.00 min
2	15	130	1.00	-
3	30	180	1.00	-

3.4.2.3 Detector condition

Temperature: 250°C, mode: constant make up flow, makeup flow: 60 mL/min, and makeup gas type: nitrogen

The THMs standard mixture contained the four THM species, i.e. chloroform, bromodichloromethane, dibromochloromethane, and bromoform, in the concentration range of 200 to 2000 μg/mL. It was prepared at a concentration range of 50- 1500 μg/L. Pentane was used as the extraction solvent. Bromofluorobenzene was employed as an internal standard where decafluorobiphenyl was used as a surrogate standard. All extracts were analyzed within 24h of the completion of the liquid–liquid

extraction procedure. GC signals were interpreted by using the ChemStation program, Agilent.

3.4.3 Trihalomethanes formation potential (THMFP)

The THM formation potential or THMFP was determined by exposing a raw (untreated) water sample to an excess of oxidizing disinfectant for 7 days at 25 °C. The change in the THM concentration relative to time zero is the THMFP. The total concentration of THMs at any time is expressed as

$$[CHX_3]T = [CHCl_3] + [CHBrCl_2] + [CHBr_2Cl] + [CHBr_3] \qquad (3.1)$$

where $[CHX_3]T$ = Total concentration of trihalomethanes

   $[CHCl_3]$ = Concentration of chloroform

   $[CHBrCl_2]$ = Concentration of bromodichloromethane

   $[CHBr_2Cl]$ = Concentration of dibromochloromethane

   $[CHBr_3]$ = Concentration of bromoform

Thus, the THMFP at time t = 7 is given by

$$THMFP(7) = [CHX_3]T(t = 7) - [CHX_3]T(t = 0) \qquad (3.2)$$
$$THMFP = TTHM_7 - TTHM_0 \qquad (3.3)$$

A 7-day Trihalomethanes formation potential test was carried out in accordance with Standard Method 5710-B (APHA, AWWA and WEF, 1995, see Appendix A). Free Chlorine Residual was measured in accordance with Standard method 4500-Cl G. DPD Colorimetric Method, see Appendix A.

3.4.4 Bromide and chloride ions

In accordance with the EPA method 300.0A (Appendix A), the analysis of bromide and chloride concentrations before and after the trihalomethanes formation potential test was done by using the Dionex ICS-2500 Reagent-Free Modular IC System connected with a EG 50 Eluent Generator. The KOH was used as an eluent for adjusted to 100 mM before the analysis. ASI- 100 Autosamplers were used to

inject the sample into the column for high precision and high linearity. Chloride and bromide standard concentrations were prepared by using 1,000 ppm standard of Merck. Chloride concentrations were prepared concentration in the range of 50 – 1000 ppm. Bromide concentrations were prepared concentration in the range of 0.005 – 100 ppm.

3.4.5 Chlorine demand

Actual chlorine demand in the THMFP experiment was rather difficult to determine as it was required that the final chlorine concentration must lie between 3-5 mg/L. Hence, additional experiment was performed to determine the approximate chlorine demand for the determination of THMFP. This was to ensure that the reaction could be moved towards completion as quickly as possible. The following procedure provides detail for this estimation.

Firstly, a 5 mL of chlorine dosing solution was pipetted into a 250-mL bottle which was filled completely with chlorine-demand-free water. This bottle was capped with a TFE-lined screw cap and was rigorously shaken ensure a complete mixing. Then 100 mL of this solution was titrated with 0.025 N sodium thiosulfate to determine the initial chlorine concentration (C_i). This concentration should be about 100 mg Cl_2/L. After that, a 5 mL of phosphate buffer and 5 mL chlorine dosing solution were pipetted into a second 250 mL bottle, and filled completely with the sample, sealed with a TFE-lined screw cap. The bottle was stored in the dark for at least 4h at 25 °C. The estimated chlorine demand can then be calculated as follows:

$$D_{Cl} = C_i - C_R$$

where:

D_{Cl} = chlorine demand, mg Cl_2/L,

C_R = chlorine residual of sample after at least 4 h storage, mg Cl_2/L, and

C_i = initial (dosed) chlorine concentration, mg Cl_2/L

Note that this is only a rough estimate of the actual chlorine demand needed for the determination of THMFP. In actual THMFP experiment, the chlorine doses used in the reaction must be varied around this estimate. For example, if the chlorine demand was estimated to be 60 mg/L, the THMFP experiment must be performed with the chlorine doses of around 50-70 mg/L.

3.4.6 Functional groups

FTIR spectrometry is used to determine the functional chemistry of unknown materials. The samples were freeze-dried before and after chlorination for the FTIR analysis. The functional groups of the organic fractions were detected by using Fourier transform infrared spectrometry (FTIR) Perkin Elmer 1760X. Infrared spectra were obtained using 0.2 mg of filtrated sampling isolates in 150 mg of potassium bromide pellets. FTIR was set to scan from 4,000 to 400 cm^{-1}, averaging 8 scans at 1.0 cm^{-1} intervals with a resolution of 8 cm^{-1}. All spectra were normalized after acquisition to a maximum absorbance of 1.0 for comparative purposes.

The summary of analytical methods and instruments used in this study is reported in Table 3.3. Figure 3.3 illustrates the hierarchical experimental concept for this work.

Table 3.3 Analytical methods and instruments

Parameter	Analytical method	Method	Analytical Instrument
Bromide ion	Ion chromatography	EPA 300.0A	Dionex ICS-2500
Chloride ion	Ion chromatography	EPA 300.0A	Dionex ICS-2500
TOC	Persulfate-Ultraviolet Oxidation Method	Standard method 5310C	O.I. analytical 1010 TOC Analyzer
DOC	Persulfate-Ultraviolet Oxidation Method	Standard method 5310C	O.I. analytical 1010 TOC Analyzer
Free chlorine residual	Colorimetric Method	Standard method 4500-Cl G	Perkin-Elmer Model Lambda 25, UV/VIS spectrometer
$TTHM_0$ and $TTHM_7$	Formation of Trihalomethane and Other Disinfection By-Products and Liquid-Liquid Extraction Gas Chromatography Method	Standard method5710B and EPA method 551.1	Agilent 6890 Series Gas Chromatography with ECD detector
Functional groups	-	-	Perkin Elmer 1760X

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┌─────────────────────────┐
│    Sample collection    │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│  Measure salinity and   │──────────────────────────────┐
│      turbidity          │                              │
└─────────────────────────┘                              │
             │                                            │
             ▼                                            │
┌─────────────────────────┐                              │
│   Adjust pH to 7.0 and  │                              │
│   temperature 25.0 °C   │                              │
└─────────────────────────┘                              │
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Determine functional groups and their concentrations by using FTIR

Determine Bromide and chloride concentrations by using IC

Determine $TTHM_0$ by using GC-ECD

Holding time for 7 days

Determine functional groups and their concentrations by using FTIR

Determine TOC and DOC by using TOC analyzer

Determine Bromide and Chloride concentrations by using IC

Determine $TTHM_7$ by using GC-ECD

Determine both of qualitative and quantitative of functional groups which react with chlorine

$THMFP = TTHM_7$
$TTHM_0$

Determine the relationship between salinity, organic contents, structure and THMFP
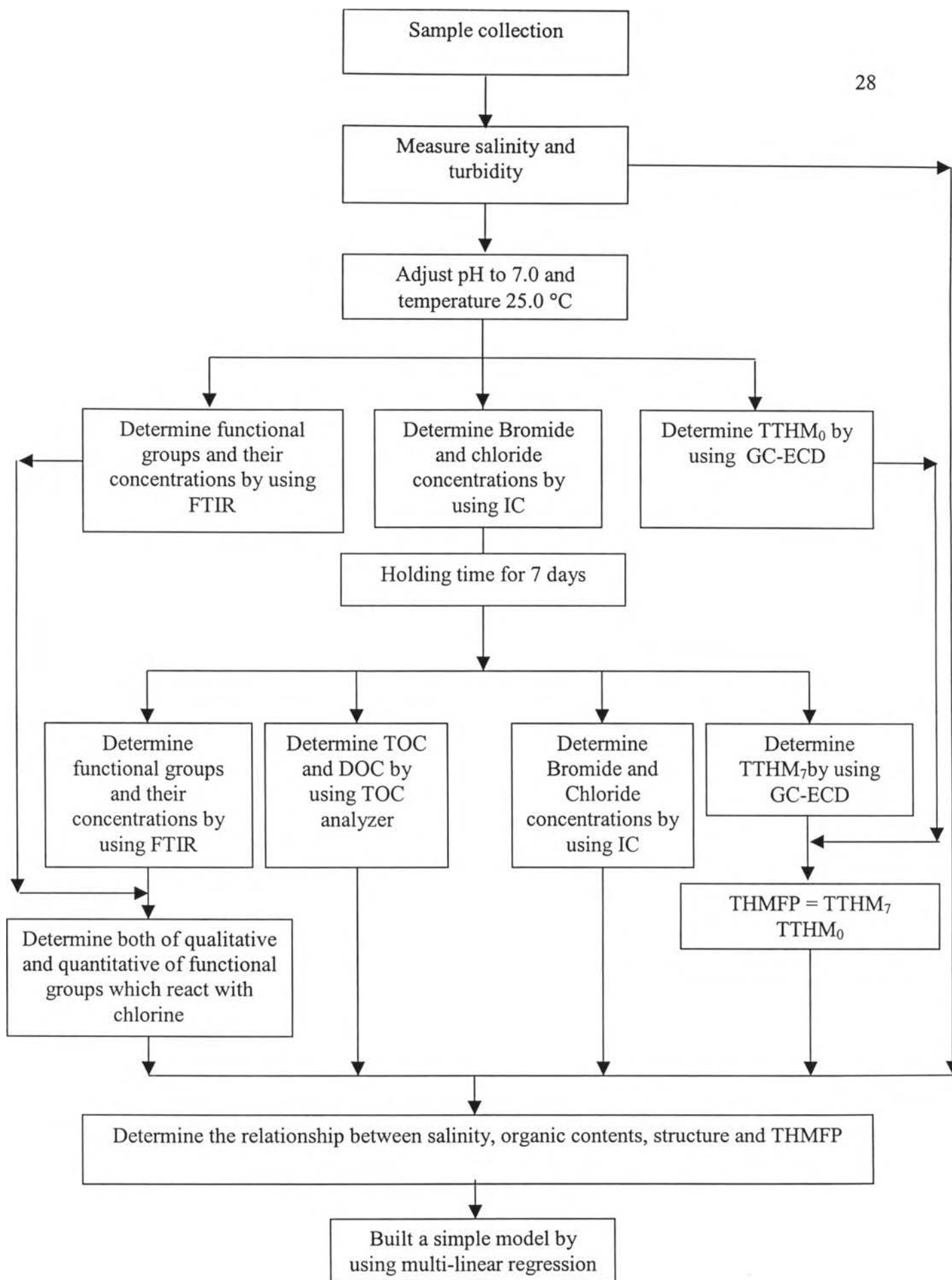
Built a simple model by using multi-linear regression

Figure 3.3 Schematic diagram of the overall experimental approach