CHAPTER 3
EXPERIMENTS AND METHODOLOGY

3.1 Materials and Apparatus

3.1.1 Sediment and Stream Water Samples

Sediment and stream water samples were collected from several natural canals. Six areas were selected around Samuth Prakarn Province of Thailand including Hua Lum Poo Canal (H3 and H6), Bang Pla Kod Canal (B1), a canal nearby small material recovery facilities (M1), and a canal nearby the South-Bangkok Power Plant (PP1). Four other sites were sampled along the Erh-Jen River in Tainan, Southern Taiwan. All sampling sites are shown in Figures 3.1 to 3.3.

3.1.2 Experimental Vessels

The 100 mL serum bottles were used as the experimental vessels. Each sample bottle was sealed with butyl rubber stopper and alumina-cap.

3.1.3 Analytical Procedures

3.1.3.1 Extraction Methods

Two mL of sediment slurry sample was injected into an extraction tube. After that, 0.2 mL of 6N sodium hydroxide solution was added together with 2 mL of n-hexane. The sample was then shaken by hand 100 times followed by ultra-sonicating for 10 minutes and centrifuged at 3000-4000 rpm for 10 minutes. After that, the upper-layer n-hexane was withdrawn as much as possible and injected into a new tube. The remaining mixture was re-extracted for other two times following the same procedure. At the third extraction, the upper-layer hexane was pulled out and filled the tube up to 5 mL mark. A small amount of sodium sulfate was added to remove remaining water before injecting to the gas chromatograph.

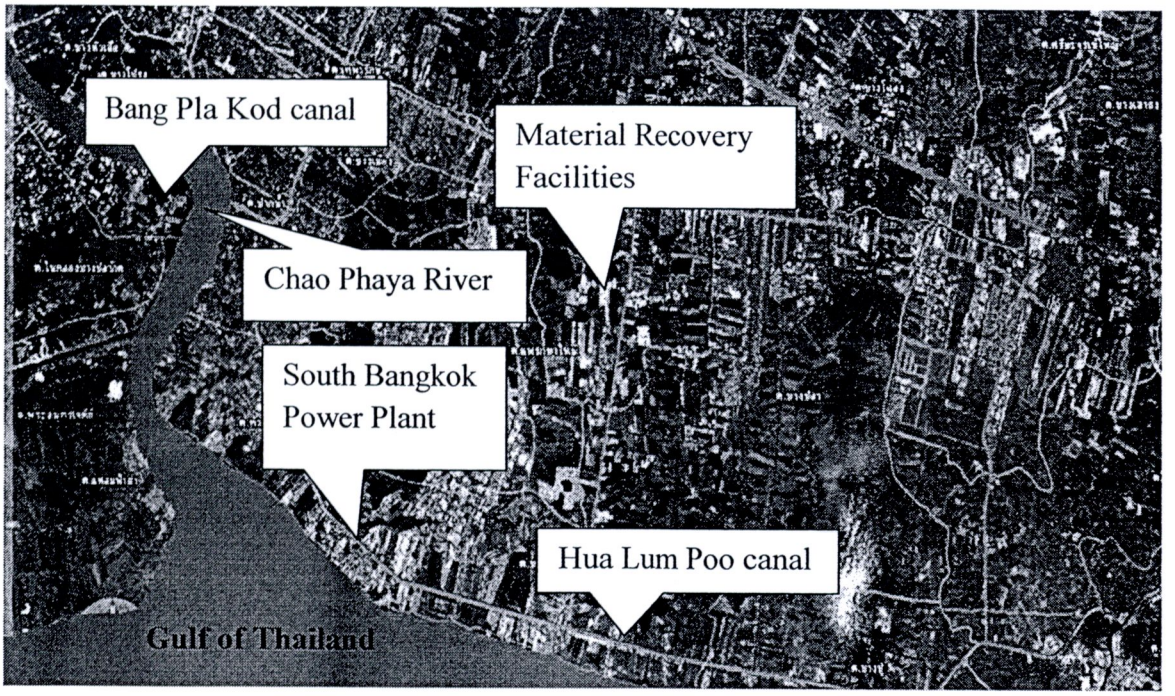


Figure 3.1 Sampling sites around Samuth Prakarn Province of Thailand

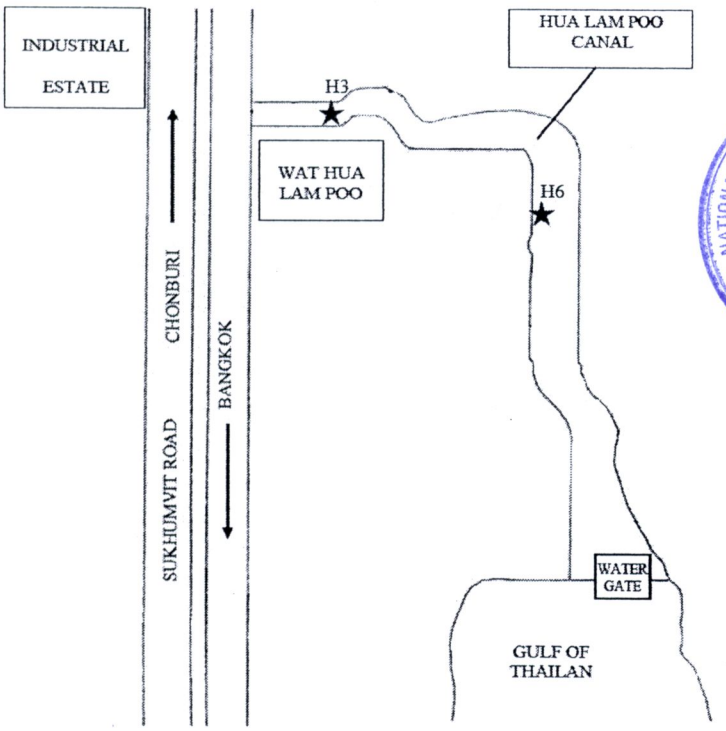
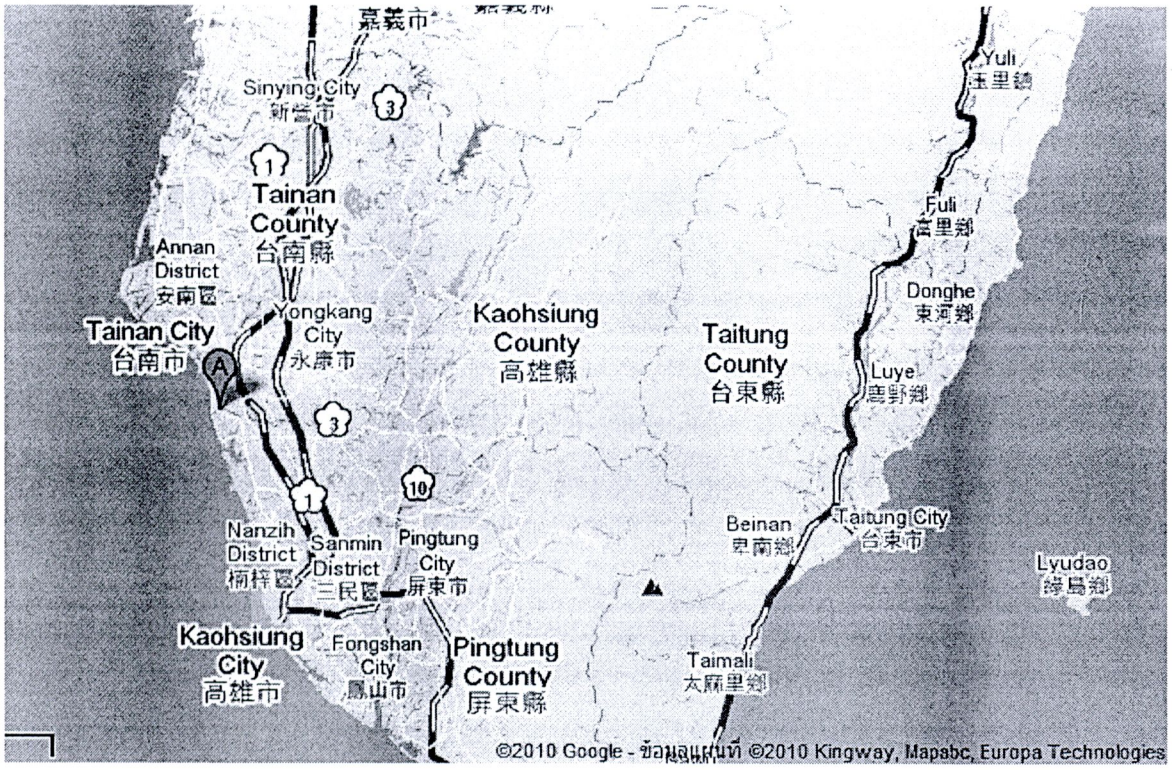
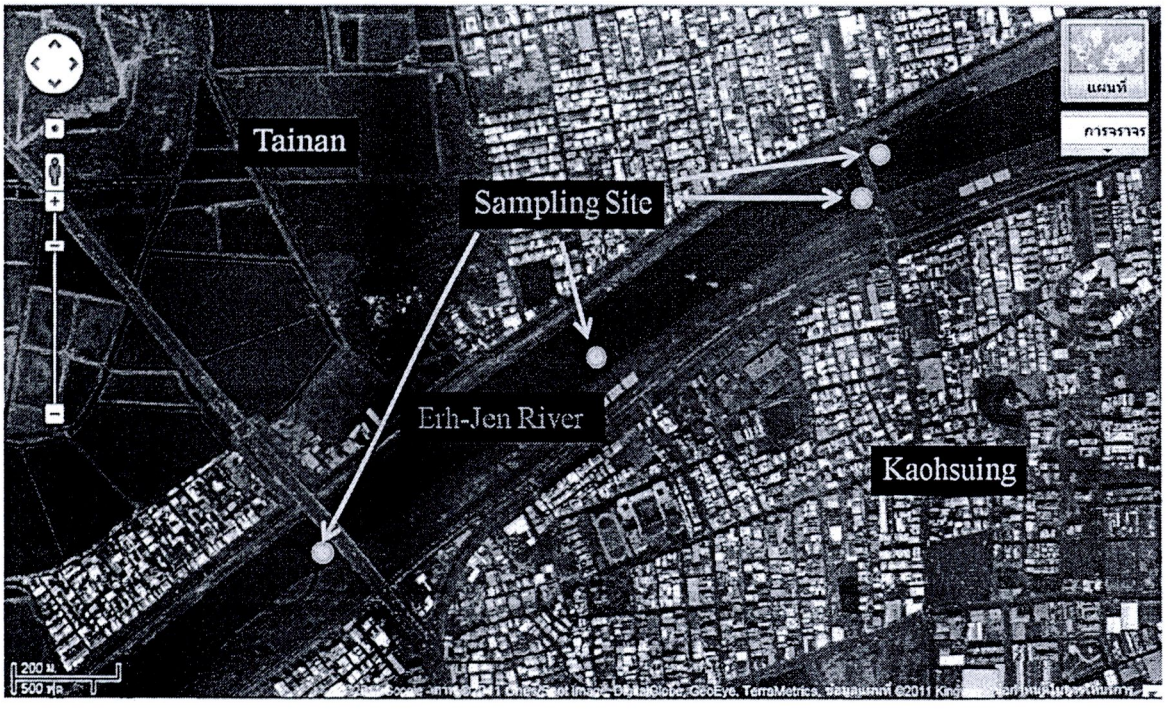


Figure 3.2 Sampling points at the Hua Lum Poo Canal in Samuth Prakarn Province



(a) Location of the river



(b) Sampling point in the river

Figure 3.3 Sampling sites of the Erh-Jen River in Taiwan

3.1.3.2 HCB and Its Intermediates Analysis

HCB and its dechlorination intermediates were analyzed by the Gas Chromatograph, Agilent 6890 N, which was equipped with a capillary column DB-5 (inner diameter 0.25 mm, length 30 m, film thickness 0.25 μm and maximum temperature of 325°C) and an electron capture detector (ECD) (Figure 3.5). The oven temperature was maintained at 80°C for 5 min, raised to 140°C at 3°C/min and then raised again at 10°C/min to the final temperature of 240°C, and held for 8 min. The temperature of the injector and the detector were set at 240°C and 280°C, respectively. Helium gas and nitrogen gas was employed as the carrier and the make-up gases, respectively. The average linear volume of carrier gas was 20 mL/min and the average linear volume of make-up gas was 60 mL/min. Inlets mode was set at splitless mode.

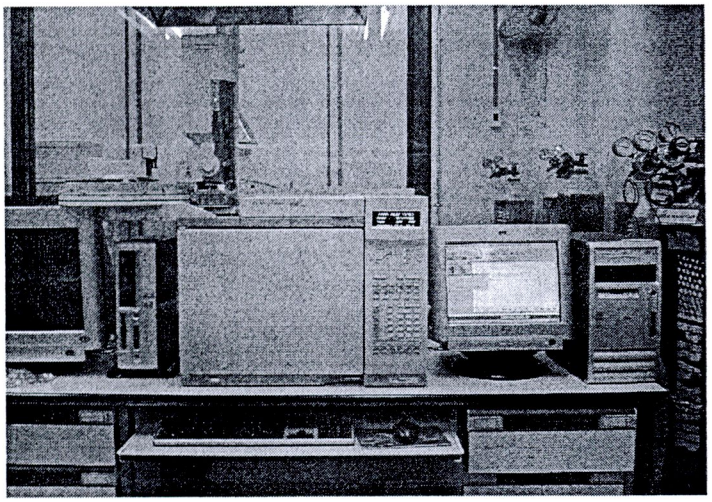


Figure 3.4 GC 6890N Gas Chromatograph

3.2 Solution and Medium Preparation

3.2.1 Sediment Slurry Preparation

Sediment slurries were freshly prepared prior to each experiment by mixing sediment and stream water at the ratio of 1:1 (v/v), removing the particles larger than 0.7 mm and keeping in an alumina-capped serum bottle until used.

3.2.2 HCB Stock Solution

Stock solution of 1000 mg/L HCB was prepared by dissolving an appropriate amount of HCB in acetone in a 100 mL serum bottle, then sealed with butyl rubber stoppers and alumina-caps, and kept refrigerated until used.

3.3 Experimental Design

3.3.1 Phase 1: Bioaugmentation of Active Consortia onto Historical Less-Potential Dechlorinating Sediment Slurries

A. Sediment slurries from Sites H3, B1, PP1, and M1 were prepared by using old sediments stored at 6°C for 10 months whereas those of E1 to E4 used freshly collected sediment as shown in Table 3.1.

B. Mixed sediment slurries between those collected in Thailand and in Taiwan were also prepared with the ratio as shown in Tables 3.2 and 3.3.

C. For each set of the sediment slurry, a series of yeast extract supplement was also prepared by dissolving 2.5 g/L of yeast extract to the sediment slurry.

D. Spike HCB into each bottle to make an initial concentration of 2 mg/L.

E. Store in the dark area, extract every 2 weeks, and analyze of HCB and its dechlorination by products.

Table 3.1 Preparation of various sediment slurries with supplement

Sediment Slurry	Supplement	Sediment Slurry	Supplement
$E1^f$ (50 mL)	YE	$H3^s$ (50 mL)	YE
	None		None
$E2^f$ (50 mL)	YE	$B1^s$ (50 mL)	YE
	None		None
$E3^f$ (50 mL)	YE	$PP1^s$ (50 mL)	YE
	None		None
$E4^f$ (50 mL)	YE	$M1^s$ (50 mL)	YE
	None		None

Note: ^{f}: Fresh sediment slurry

^{s}: Stored sediment slurry at 6 °C for 10 months

E1 - 4: Erh-Jen River site 1-4

H3: Hua Lum Poo canal site 3

B1: Bang Pla Kod canal

PP1: canal nearby South-Bangkok Power Plant

M1: canal nearby material recovery facilities

Table 3.2 Mixed sediment slurries from different sites at 45:5 ratio

Sediment Slurry		Supplement
$E1^f$ (45mL)	$H3^s$ (5 mL)	YE
		None
	$B1^s$ (5 mL)	YE
		None
	$PP1^s$ (5 mL)	YE
		None
	$M1^s$ (5 mL)	YE
		None
$E2^f$ (45mL)	$H3^s$ (5 mL)	YE
		None
	$B1^s$ (5 mL)	YE
		None
	$PP1^s$ (5 mL)	YE
		None
	$M1^s$ (5 mL)	YE
		None

Sediment Slurry		Supplement
$E3^f$ (45mL)	$H3^s$ (5 mL)	YE
		None
	$B1^s$ (5 mL)	YE
		None
	$PP1^s$ (5 mL)	YE
		None
	$M1^s$ (5 mL)	YE
		None
$E4^f$ (45mL)	$H3^s$ (5 mL)	YE
		None
	$B1^s$ (5 mL)	YE
		None
	$PP1^s$ (5 mL)	YE
		None
	$M1^s$ (5 mL)	YE
		None

Table 3.3 Mixed sediment slurries from different sites at 25:25 ratio

Sediment Slurry		Supplement
$E1^f$ (25mL)	$H3^s$ (25 mL)	YE
		None
	$B1^s$ (25 mL)	YE
		None
	$PP1^s$ (25 mL)	YE
		None
	$M1^s$ (25 mL)	YE
		None
$E2^f$ (25mL)	$H3^s$ (25 mL)	YE
		None
	$B1^s$ (25 mL)	YE
		None
	$PP1^s$ (25 mL)	YE
		None
	$M1^s$ (25 mL)	YE
		None

Sediment Slurry		Supplement
$E3^f$ (25mL)	$H3^s$ (25 mL)	YE
		None
	$B1^s$ (25 mL)	YE
		None
	$PP1^s$ (25 mL)	YE
		None
	$M1^s$ (25 mL)	YE
		None
$E4^f$ (25mL)	$H3^s$ (25 mL)	YE
		None
	$B1^s$ (25 mL)	YE
		None
	$PP1^s$ (25 mL)	YE
		None
	$M1^s$ (25 mL)	YE
		None

3.3.2 Phase 2: Biostimulation of Historical Less-Potential Dechlorinating Consortia by Using Sterilized Active Sediment Slurries as Cultural Media

A. Sediment slurries from various sites were prepared, some were supplemented with 2.5 g/L yeast extract and sterilized as shown in Table 3.4.

B. Mix the sediment slurries from the sampling sites in Thailand with the sediment slurries from the sampling sites in Taiwan with the ratio as shown in Tables 3.5 and 3.6.

C. Spike HCB into each bottle to make an initial concentration of 2 mg/L.

D. Store in the dark area, extract every 2 weeks, and analyze of HCB and its dechlorination by products.

Table 3.4 Preparation of sediment slurries for Part 3.3.2

Sediment Slurry	Supplement
$H3^f$ (50 mL)	YE
sterilized	None
$H6^f$ (50 mL)	YE
sterilized	None
$B1^f$ (50 mL)	YE
sterilized	None
$E1^s$ (50 mL)	YE
non-sterilized	None
$E2^s$ (50 mL)	YE
non-sterilized	None
$E3^s$ (50 mL)	YE
non-sterilized	None
$E4^s$ (50 mL)	YE
non-sterilized	None

Sediment Slurry	Supplement
$H3^f$ (50 mL)	YE
non-sterilized	None
$H6^f$ (50 mL)	YE
non-sterilized	None
$B1^f$ (50 mL)	YE
non-sterilized	None
$E1^f$ (50 mL)	YE
non-sterilized	None
$E2^f$ (50 mL)	YE
non-sterilized	None
$E3^f$ (50 mL)	YE
non-sterilized	None

Note: ^{f}: Fresh sediment slurry

^{s}: Stored sediment slurry at 6 °C for 2 months

H3 = Hua Lum Poo canal site 3

H6 = Hua Lum Poo canal site 6

B1 = Bang Pla Kod canal

E1-4: Erh-Jen River sites 1-4

Table 3.5 Bioaugmentation of non-sterilized sediment slurry to sterilized sediment slurry at 48:2 ratio

Sediment Slurry		Supplement
$H3^f$ (48 mL) sterilized	$E1^s$ (2 mL) non-sterilized	YE
		None
	$E2^s$ (2 mL) non-sterilized	YE
		None
	$E3^s$ (2 mL) non-sterilized	YE
		None
	$E4^s$ (2 mL) non-sterilized	YE
		None
	$E1^f$ (2 mL) non-sterilized	YE
		None
	$E2^f$ (2 mL) non-sterilized	YE
		None
	$E3^f$ (2 mL) non-sterilized	YE
		None
$H6^f$ (48 mL) sterilized	$E1^s$ (2 mL) non-sterilized	YE
		None
	$E2^s$ (2 mL) non-sterilized	YE
		None
	$E3^s$ (2 mL) non-sterilized	YE
		None
	$E4^s$ (2 mL) non-sterilized	YE
		None
	$E1^f$ (2 mL) non-sterilized	YE
		None
	$E2^f$ (2 mL) non-sterilized	YE
		None
	$E3^f$ (2 mL) non-sterilized	YE
		None
$B1^f$ (48 mL) sterilized	$E1^s$ (2 mL) non-sterilized	YE
		None
	$E2^s$ (2 mL) non-sterilized	YE
		None
	$E3^s$ (2 mL) non-sterilized	YE
		None
	$E4^s$ (2 mL) non-sterilized	YE
		None
	$E1^f$ (2 mL) non-sterilized	YE
		None
	$E2^f$ (2 mL) non-sterilized	YE
		None
	$E3^f$ (2 mL) non-sterilized	YE
		None

Table 3.6 Bioaugmentation of non-sterilized sediment slurry to sterilized sediment slurry at 40:10 ratio

Sediment Slurry		Supplement
$H3^f$ (40 mL) sterilized	$E1^S$ (10 mL) non-sterilized	YE
		None
	$E2^S$ (10 mL) non-sterilized	YE
		None
	$E3^S$ (10 mL) non-sterilized	YE
		None
	$E4^S$ (10 mL) non-sterilized	YE
		None
	$E1^F$ (10 mL) non-sterilized	YE
		None
	$E2^F$ (10 mL) non-sterilized	YE
		None
	$E3^F$ (10 mL) non-sterilized	YE
		None
$H6^f$ (40 mL) sterilized	$E1^S$ (10 mL) non-sterilized	YE
		None
	$E2^S$ (10 mL) non-sterilized	YE
		None
	$E3^S$ (10 mL) non-sterilized	YE
		None
	$E4^S$ (10 mL) non-sterilized	YE
		None
	$E1^F$ (10 mL) non-sterilized	YE
		None
	$E2^F$ (10 mL) non-sterilized	YE
		None
	$E3^F$ (10 mL) non-sterilized	YE
		None
$B1^f$ (40 mL) sterilized	$E1^S$ (10 mL) non-sterilized	YE
		None
	$E2^S$ (10 mL) non-sterilized	YE
		None
	$E3^S$ (10 mL) non-sterilized	YE
		None
	$E4^S$ (10 mL) non-sterilized	YE
		None
	$E1^F$ (10 mL) non-sterilized	YE
		None
	$E2^F$ (10 mL) non-sterilized	YE
		None
	$E3^F$ (10 mL) non-sterilized	YE
		None

3.3.3 Phase 3: Comprehensive Survey of HCB Dechlorination by Fusion of Various Active Sediment Slurries

Part 1. Indigenous Microbial Activity Test

A. Sediment slurries from various sites were prepared as shown in Table 3.7.

B. Mixed sediment slurries between those collected in Thailand and in Taiwan were also prepared with the ratio as shown in Table 3.7.

C. Spike HCB into each bottle to make an initial concentration of 2 mg/L.

D. Store in the dark area, extract every 2 weeks, and analyze of HCB and its dechlorination by products.

Part 2. Bioaugmentation Test

A. Transfer 10 mL of the sediment slurries from the serum bottles in Section 3.3.1. in which the HCB has not been completely dechlorinated to new serum bottles.

B. Add 20 mL non-sterilized sediment slurry of Site H3 into all transferred serum bottles.

C. Spike HCB into every bottle to the initial concentration to 2 mg/L.

D. Store in the dark area, extract every 2 weeks, and analyze of HCB and its dechlorination by products.

Table 3.7 Preparation of various sediment slurries

Sediment Slurry	Supplement	Sediment Slurry		Supplement
$H3^f$ (50 mL)	None	$H3^f$ (25 mL)	$E2^f$ (25 mL)	None
$H6^f$ (50 mL)	None	$H6^f$ (25 mL)	$E2^f$ (25 mL)	None
$B1^f$ (50 mL)	None	$B1^f$ (25 mL)	$E2^f$ (25 mL)	None
$E2^f$ (50 mL)	None			

Note: ^{f}: Fresh sediment slurry

H3: Hua Lum Poo canal site 3

H6: Hua Lum Poo canal site 6

B1: Bang Pla Kod canal

E2: Erh-Jen River site 2

3.4 Experimental Plan

This research study was divided into 6 stages, literature reviews, preparation, experiment, summary, thesis writing, and defense. Timeframe for the overall project is shown in Table 3.8.

Table 3.8 Experimental timeframe

Activity	2011									2012		
	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
1. Literature Reviews	←	—	—	→								
2. Preparation of materials and apparatus	←	—	—	→								
3. Experiments ▪ Part 3.3.1 ▪ Part 3.3.2 ▪ Part 3.3.3	←	—	—	—	—	→						
			←	—	—	—	—	—	→			
					←	—	—	—	—	—	→	
4. Summary of results and discussion								←	—	—	→	
5. Thesis writing										←	—	→
6. Thesis defense											←	→