CHAPTER 3
MATERIALS AND METHODS

3.1 Site description

The Mekong River is one of the most important rivers in Asia. Originating in the Tibetan mountains, it runs through China with approximately half the river's length in China. The river then forms the border between Myanmar and Lao PDR and border Myanmar, Lao PDR and Thailand at the Golden Triangle. This point also marks the division between the Upper and Lower Mekong Basin. The river then divides Lao PDR and Thailand, before stretch pass through Lao PDR. It veers back once again in the form of the border between Thailand and Lao PDR. It then flows across Cambodia and Southern Vietnam into a rich delta before emptying into the South China Sea. The length of the river is around 4,900 km, maker it the longest river in this area (www.irn.org/programs/mekong/). The river is the main source of livelihoods for approximately 60 million people who live in the Lower Mekong Basin.

In Thailand, the Mekong River flows through Chiang Rai Province, in the North. As the Mekong River continues along its path in Lao PDR, it once again flows back into Loei, Nong Khai, Nakhon Phanom, Mukdahan, Amna Charoen and Ubon Ratchathani Provinces in the Northeast of Thailand. A distance of the Mekong River passing Thailand is about 832 km, 85 km in the North and 747 km in the Northeast.

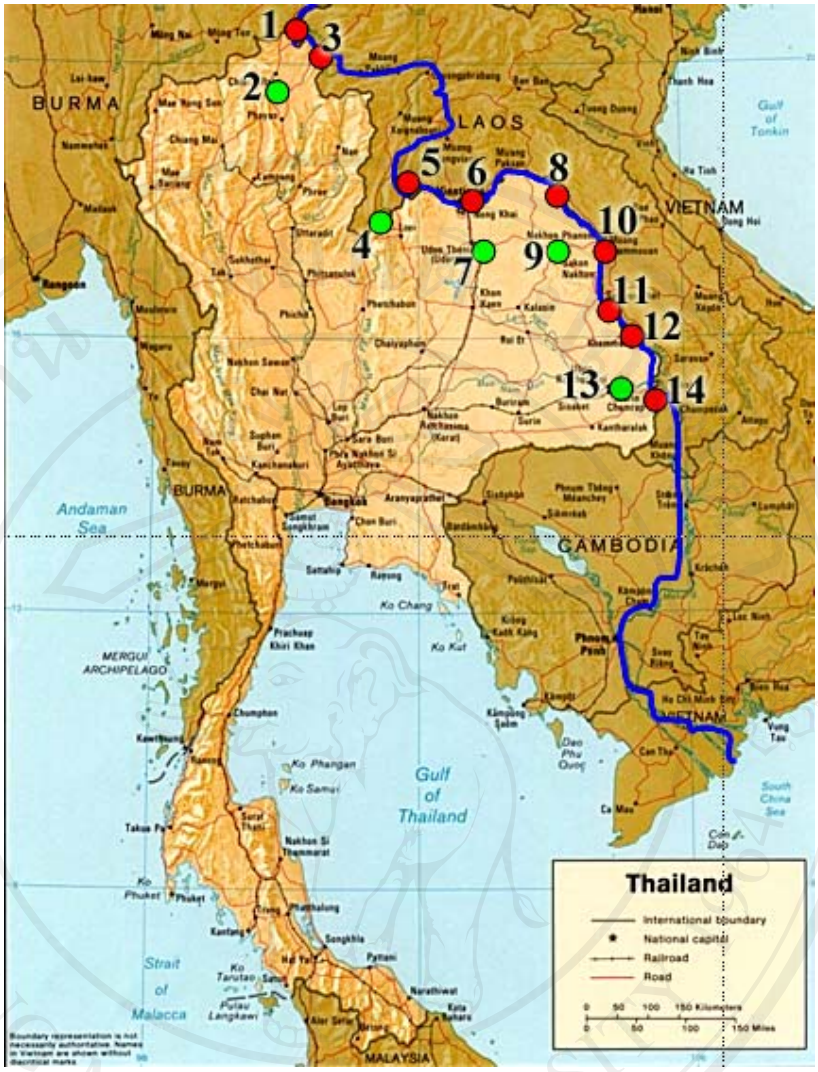
There are many rivers which could be tributaries and connected with the Mekong River. The water quality in these tributaries affects the Mekong River especially at Kok River in the North and Hueng River, Laung River, Songkram River and Mun River the the Northeast of Thailand. These are the most important tributaries of the Mekong River in Thailand.

The riparian zone in each part of the Mekong River and its tributaries surrounds by living communities, tourism activities and agricultural areas. Besides, the Mekong River and its tributaries have an influence on people’s methods of earning for a living along these rivers, in the form of irrigation, consumption, fisheries and transportation including a raw water source for water supplies.

Fourteen sampling sites in the Mekong River and its tributaries were selected, nine sites on the Mekong River and five sites from its tributaries. Each sampling site is considered for the distance and any environmental impacts. The details of the sampling sites were shown in Table 7 and Figures 2-3.

Table 7 The detail of sampling sites including site name, ordination, riparian zone and description in Mekong River and its tributaries.

Site Name	Ordinations		Altitudes	Environmental features	Situated area
1. Golden Triangle (GT)	20°22′07″ N	100°05′31″ E	360	Tourist attraction and tourist boat traffic	Mekong River
2. Kok River (KO)	19°55′13″ N	99°46′49″ E	394	Tourist attraction and tourist boat traffic	Tributary
3. Had Krai Temple (HK)	20°16′32″ N	100°24′23″ E	345	Agricultural and transportation	Mekong River
4. Hueng river (HG)	17°43′88″ N	100°33′07″ E	736	Agricultural	Tributary
5. Kaeng Khood Khoo (KK)	17°54′45″ N	100°42′09″ E	300	Tourist attraction and tourist boat traffic	Mekong River
6. Phon Phisai (PS)	18°01′46″ N	103°04′54″ E	501	Agricultural and residential area	Mekong River
7. Laung River (LG)	18°00′96″ N	103°04′88″E	521	Small dam and residential area	Tributary
8. Nakorn Panom (NP)	17°23′85″ N	104°47′49″ E	450	Transportation and residential area	Mekong River
9. Songkram River (SK)	17°52′03″ N	103°46′47″ E	150	Residential area	Tributary
10. Kaeng Ka Bao (KB)	16°43′11″ N	104°41′03″ E	135	Tourist attraction	Mekong River
11. Had Hin Win Chai (HW)	16°20′03″ N	104°55′03″ E	134	Tourist attraction	Mekong River
12. Kaeng Hin Kan (KH)	17°07′70″ N	105°01′98″ E	122	Agricultural and residential area	Mekong River
13. Kaeng Sapue (KP)	15°14′12″ N	105°14′63″ E	114	Tourist attraction	Tributary
14. Kong Jium (KJ)	15°17′00″ N	105°30′15″ E	94	Tourist attraction and tourist boat traffic	Mekong River



● Sampling site in Mekong River
● Sampling site in tributaries of Mekong River

Figure 2 Map of Thailand, shows the location of 14 sampling sites of Mekong River and its tributaries
(http://www.hoteleasy.com/Info/Thai%20Map/Thailand_Map.html)

site 1 (GT) Golden Triangle, Chiang Rai Province
site 2 (KO) Kok River, Chiang Rai Province
site 3 (HK) Ban Had Krai, Chiang Rai Province
site 4 (HG) Hueng river, Loei Province
site 5 (KK) Kaeng Khood Khoo, Loei Province
site 6 (PS) Phon Phisai, Nong Khai Province
site 7 (LG) Laung River, Nong Khai Province
site 8 (NP) Ferry Port, Nakhon Phanom Province
site 9 (SK) Songkram River, Sakon Nakhon Province
site 10 (KB) Kaeng Ka Bao, Nakhon Phanom Province
site 11 (HW) Had Hin Win Chai, Amnat Charoen Province
site 12 (KH) Kaeng Hin Kan, Mukdahan Province
site 13 (KP) Kaeng Sapue, Ubon Ratchathani Province
site 14 (KJ) Kong Jium, Ubon Ratchathani Province

Figure 3 Photographs of sampling sites of Mekong River and its tributaries

site 1 (GT) Golden Triangle, Chiang Rai Province

site 2 (KO) Kok River, Chiang Rai Province

site 3 (HK) Had Krai Temple, Chiang Rai Province

site 4 (HG) Hueng river, Loei Province

site 5 (KK) Kaeng Khood Khoo, Loei Province

Figure 3 Photographs of sampling sites of Mekong River and its tributaries (continued)

site 6 (PS) Ponpisai, Nong Khai Province
site 7 (LG) Laung River, Nong Khai Province
site 8 (NP) Ferry Port, Nakhon Phanom Province
site 9 (SK) Songkram River, Sakon Nakhon Province
site 10 (KB) Kaeng Ka Bao, Nakhon Phanom Province

Figure 3 Photographs of sampling sites of Mekong River and its tributaries (continued)

site 11 (HW)	Had Hin Win Chai, Amnat Charoen Province
site 12 (KH)	Kaeng Hin Kan, Mukdahan Province
site 13 (KP)	Kaeng Sapue, Ubon Ratchathani Province
site 14 (KJ)	Kong Jium, Ubon Ratchathani Province

Fourteen sites were sampling during July 2005 to April 2007. The particular geomorphological and environmental features of each site that may have influenced the results of biomonitoring are listed below.

Sampling site 1 (GT) Golden Triangle, Chiang Saen District, Chiang Rai Province

This sampling site is the first place that Mekong River runs through Thailand and located at the Thailand, Myanmar and Lao PDR border. This site is a tourist attraction and is disturbed by frequent tourist boat traffic. The samples were collected from the island shore in the middle of Mekong River that contains gravel and cobble except in rainy season that the water level was very high (Figure 3-1). The samples were collected from the left bank of the river that contains the substrate of stone.

Sampling site 2 (KO) Kok River, Mueang District, Chiang Rai Province

This site is situated between two tourist attractions, about 2 km above Had Chiang Rai at Kok River and a hilltribe village upstream from this site (Figure 3-2). It is disturbed by frequent tourist boat traffic and agriculture. Substrate was composed of gravel and cobble. The water level was lower in cool-dry season and summer but high in rainy seasons so the samples were collected from the bank of the river that contains the substrate of stone.

Sampling site 3 (HK) Had Krai Temple, Chiang Khong District, Chiang Rai Province

The giant catfish (*Pangasianodon gigas*), one of the world's largest freshwater fishes was found in this site at the Mekong River passing Thailand. This site contains gravel and cobble substrates that suitable for growing of macroalgae, *Cladophora* spp. and *Microspora* spp. Local residents use this algae for food. The water level was lower in cool-dry season and summer but high in rainy seasons so the samples were collected from aquatic plant on the river bank. The left bank was gently slope, with vegetable plots and a benthic substratum of sand (Figure 3-3).

Sampling site 4 (HG) Hueng River, Chiang Khan District, Loei Province

This river is located in Thailand and Lao PDR border. The riparian zone on the right bank of the site is bamboo, bananas and vegetables on the left bank. The water velocity was very high. The type of substrate in this site includes gravel and cobble. In rainy seasons, the samples were collected from aquatic plant on the river bank (Figure 3-4).

Sampling site 5 (KK) Kaeng Khood Khoo, Chiang Khan District, Loei Province

This sampling site is a tourist attraction which is disturbed by frequent tourist boat traffic and restaurants on the left bank of the river. The substratum at this site was formed by extensive beds of gravel and cobble (Figure 3-5). In rainy seasons which the water level was very high, the samples were collected from the bamboo pier and artificial substrate on the right bank of the river.

Sampling site 6 (PS) Thai Temple, Phon Phisai District, Nong Khai Province

The river bed was mainly mud and clay. The samples were collected from aquatic plants and floating bamboo sticks from the left bank of the river (Figure 3-6). Some diatom samples were collected from the existing structures such as metal piers.

Sampling site 7 (LG) Laung River, Phon Phisai District, Nong Khai Province

This site is located about 100 m downstream from a small dam in the inner city area. Most substrates were mud and clay. The flow rate was extremely low. The samples were collected from the stones on the right bank of the river (Figure 3-7).

Sampling site 8 (NP) Ferry Port, Mueang District, Nakhon Phanom Province

This site was impacted from ship transportation and waste from the ferry station. The type of substrates were mud and clay. The samples were collected from the stones on the left bank of the river (Figure 3-8).

Sampling site 9 (SK) Songkram River, Sakon Nakhon Province

Songkram River runs through the saline soil area, so the water was high in salinity. This sampling site was also impacted from floating restaurants and fish farms. The water level and velocity were slow in summer and cool dry season but high in rainy season (Figure 3-9). The type of substrate in this site includes mud and clay. The water level was lower in cool-dry season and summer but high in rainy seasons so the samples were collected from stones and wood sticks on the bank of the river.

Sampling site 10 (KB) Kaeng Ka Bao, Nakhon Phanom Province

This sampling site is a tourist attraction and affected by many restaurants. The water level was very high. This site was contains bedrock substrate at the left bank (Figure 3-10). The samples were collected from the bed rock and some from aquatic plants.

Sampling site 11 (HW) Had Hin Win Chai, Amnat Charoen Province

The river was extremely wide at this site. This site contains a bedrock substrate at the bank. The water level was very high all year round. The samples were collected from the bed rock on the left bank of the river (Figure 3-11).

Sampling site 12 (KH) Kaeng Hin Kan, Mukdahan Province

The left bank of the river was gently slop, with vegetable plots. The water level was very high. The samples were collected from the stones on the left bank of the river (Figure 3-12). Some diatom samples were collected from floating boats and bamboo sticks.

Sampling site 13 (KP) Kaeng Sapue, Ubon Ratchathani Province

This sampling site is a rapid that located in the Mun River. It is located downstream from Pak Mun Dam and used for tourism. This sampling site was impacted from many restaurants and people swimming. The water velocity was very high. The type of substrate in this site includes bed rock and sand (Figure 3-13). The samples were collected from the bed rock and aquatic plants on the left bank of the river.

Sampling site 14 (KJ) Mun River mount, Khong Jium District, Ubon Ratchathani Province

This is a last sampling site in Thailand before the Mekong River runs into Lao PDR. The samples were taken from 500 m on the island shore in the middle of Mekong River above the confluence of the Mun and Mekong Rivers (Figure 3-14). This site contains a bedrock substrate at the bank. This site is disturbed by frequent tourist boat traffic and restaurants on the left bank of the river.

3.2 Water sampling analysis

Water samples were collected for 3 replicates along the river at each sampling site. Water samples were collected by polyethylene bottles. The bottles were kept in a cool box at 5-7 ^{o}C.

3.2.1 Physico-chemical analysis

3.2.1.1 The limnological data of each sampling site, such as riparian zone, type of substrates and human activities were recorded.

3.2.1.2 The ordination was recorded with GPS Reciever (Batch Meridian XL).

3.2.1.3 Water and air temperature were recorded by thermometer.

3.2.1.4 Turbidity was measured with turbidity meter.

3.2.1.5 Conductivity was measured with conductivity meter (electrode kit of WTW Company).

3.2.1.6 pH level was recorded with pH meter (electrode kit of WTW company)

3.2.1 7 Alkalinity analysis was determined by phenolpthalein methyl orange indicator method (Greenberg *et al*., 2005).

3.2.1.8 Dissolved oxygen (DO) analysis was measured by azide modification method (Greenberg *et al*., 2005).

3.2.1.9 Nutrient analysis (Greenberg *et al*., 2005)

3.2.1.9.1 Nitrate nitrogen analysis was carried out by cadmium reduction method.

3.2.1.9.2 Ammonium nitrogen analysis was conducted using Nesslerization method.

3.2.1.9.3 Soluble Reactive Phosphorus (SRP) analysis was determined ascorbic acid method

3.2.2 Biological analysis

3.2.1.1 Coliform bacteria analysis was conducted using the method of Harrigan and Cance (1976)

3.3 Investigation of benthic diatoms

3.3.1 Collecting of benthic diatoms

Benthic diatoms were collected for 10 replicates along the river at each sampling site. A random number table was used to select 10 plots of 1 m^2 within the sampling area of 100 m x 5 m. A single stone was selected in each plot that contain high abundant of benthic diatoms, evident as a thin brownish film or by having a slippery feel. For plot with no stone, the nearest hard substratum was sampled. Diatom sampling was done using a plastic sheet with a 10-cm^2 cutout that was placed on the upper surface of the selected stone or other substratum and benthic diatoms was brushed and washed off into a plastic bowl until the cutout area will be completely clear. At sites that lacked stones but had predominantly muddy or sandy beds, suitable

substrata included bamboo sticks, aquatic plants, and artificial substrata. Each sample will be transferred to a plastic container, and labeled with the site name, location code, date, and replicate number.

3.3.2 Cleaning process for benthic diatoms

In the laboratory, the samples were cleaned by concentrated acid digestion method. The samples were centrifuged at 3,500 rpm for 15 minutes. The diatom cells (brown layer between supernatant and solid particles) were placed in an 18-cm core tube. Strong acid (HNO_3) was added and heated in a boiler (70-80 ^oC) for 30-45 minutes. The samples were rinsed 4-5 times with deionized water. The cleaned diatom samples were precipitated in the tube as a gray powder (Rott *et al.*, 1997).

3.3.3 Slide preparation

3.3.3.1 Light microscope investigation

Three drops (0.02 ml per drop) of sample were placed on a microscope slide and dried. A mounting agent solution such as diatom mountant was used to prepare a permanent diatom slide. The permanent slide was used for the counting and identifying processes.

3.3.3.1 Scanning electron microscope investigation

The cleaned diatom samples were dropped on coverslip and dried on a hotplate. Then, it was kept in desicator overnight and fixed on stub and coated with gold. LEO 1450 VT scanning electron microscope were used for observations.

3.3.4 Benthic diatoms identification

Frustule structure of diatom samples such as diameter, length, weight, striae, striae frequency per 10 μm and others features (raphe, puncta, areolae, fibulae, nodule, septa, costae, stigmata, rib, spine, wing and canals) were observed.

The diatom samples were identified by 100X light microscope. The specimens were photographed using an Olympus Normaski microscope. A scanning electron microscope was used for the species that unable to be identified by light microscope.

The samples were identified according to Ohno *et al.* (1971), Reichardt (1984), Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot (1993, 1995), Lee *et al*. (1994a and 1994b), Benavides (1996), Cox (1996), Mettzeltin and Lange-Bertalot (1998), Peerapornpisal *et al.* (2000), Rumrich *et al*. (2000), Lange-

Bertalot (2001), Tuji and Houki (2001), Pekthong (2002a, b), Kunpradid (2005), Watanabe *et al.*(2005), Peerapornpisal (2005) and Lange-Bertalot (2007).

3.3.4 Counting of benthic diatoms

Diatom counting was done under compound microscope. About 300 diatom cells were counted per slide and used to estimate total numbers per sample.

3.4 Data analysis

3.4.1 Evaluation of water quality

The water quality of Mekong River and its tributaries were evaluated by AARL PC Score (Peerapornpisal *et al*, 2007). The method was modified from Wetzel (1983), Lorraine and Vollenweider (1981) and Kelly (2000). The standard justified the water quality category using alkalinity, conductivity, nitrate nitrogen, ammonium nitrogen and soluble reactive phosphorus.

3.4.2 Statistical analysis

ANOVA single factor was performed on the datasets of water quality and benthic diatoms assemblages to determine any significant differences between groups. Pearson's correlation was also calculated to see if certain variables were significantly correlated.

Cluster analysis was performed on the log of transformed water quality data using Multivariate Statistical Package (MVSP) software with UPGMA cluster method to show similarity percentage between samples. The environmental variables of the clusters were then tested for significance with ANOVA single factor. Principal Component Analysis (PCA) was performed on the centred, log ratio transformed diatom data using MVSP, with axes extracted using Kaiser's rule. Canonical Correspondence Analysis (CCA) was performed water quality and diatom data to any relationship.

3.4.3 Diversity index

The Shannon-Wiener Diversity Index was calculated for all diatom sampling sites. The Shannon-Wiener Diversity Index (*H'*) is based on species richness and evenness (E) in abundance among species (Pinder, 1999; Stiling, 2002), and is calculated by the following formula:

$$H' = -\sum_{i=1}^{s} pi.\log(pi)$$

Where *pi* is the proportion of individuals in the sample that belong to the *i*th of *s* taxa.

$$E = H' / \ln S$$

Where S is number of species

3.4.3 Trophic Diatom Index

Saprobic Index of Rott *et al.* (1997) Mae Sa Index (Pektong, 2002a) and Ping and Nan Index (Kunpradid, 2005) were applied to diatom data of Mekong River and its tributaries indexes.