CHAPTER 3

MATERIALS AND METHOD

3.1 Study site survey

The study area was located in northern, northeastern of Thailand and central of Lao People's Democratic Republic (Lao PDR). This study was carried out in May 2007 to April 2008 along Mekong River pass through Thailand and its five tributaries. Ten study sites were selected. Within these sites, 5 study sites were located in Mekong River and another 5 study sites located in Mae Kok River, Hurng River, Nam Ngum River, Songkram River and Mae Mun River (tributaries of Mekong River). Only Nam Ngum River was located in Lao PDR. Samples were collected 4 times covering 3 seasons from each study site and there was start collected sample from the first site at Mae Kok River; in the northern Thailand to the last site at Khongchiam in the northeastern Thailand. In each sampling site, was different of the location characteristic, substrate types, size of the river and land used. The description of each sampling site was showed in content 3.1.1and map of study sites in Mekong River and its tributaries is shown in figure 1.

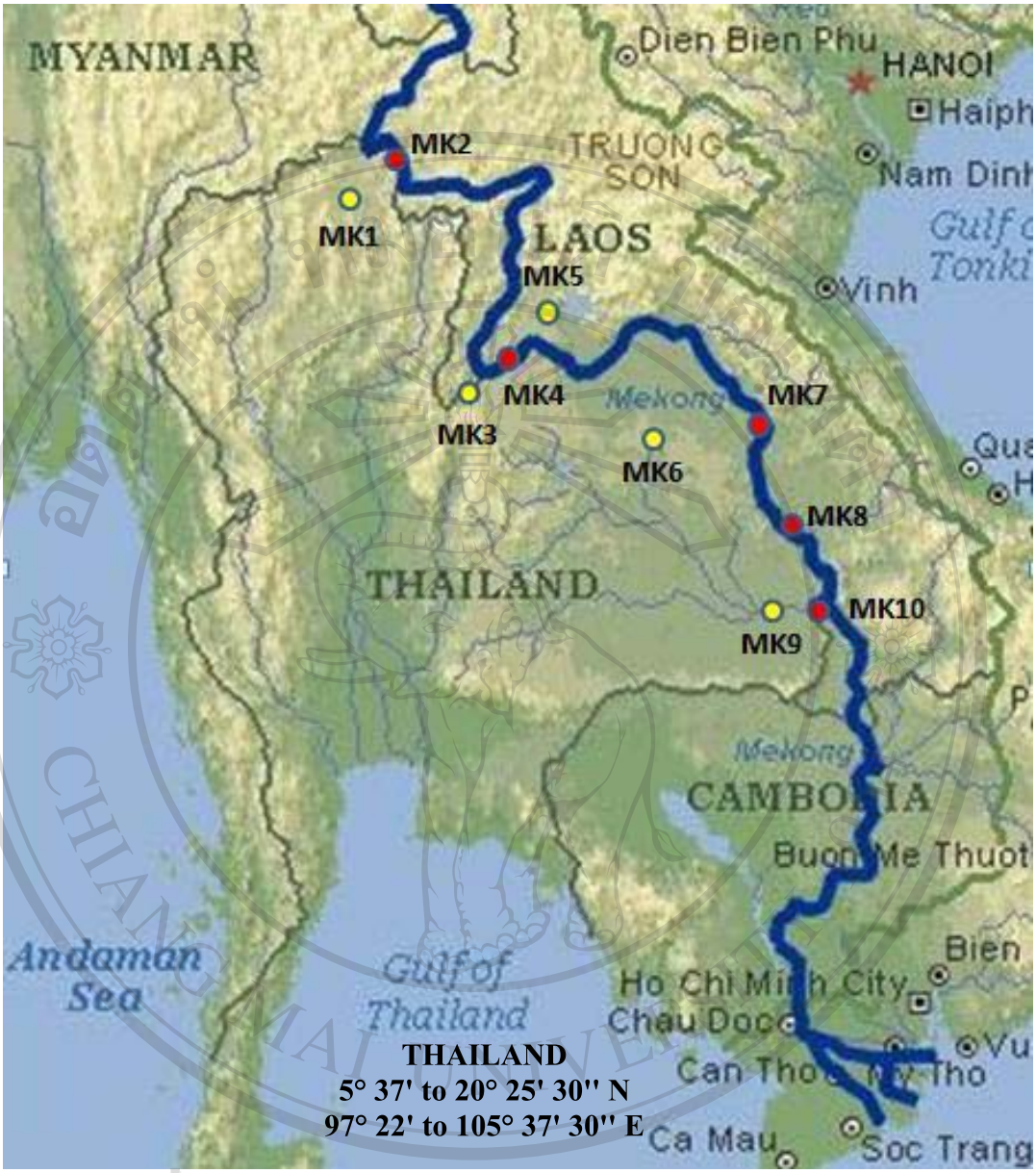

Figure 1 Map of Mekong River and its tributaries with 10 study sites.

(http://www.wm.hq.unu.edu)

MK1 (Mae Kok River), MK2 (Chiangkhong), MK3 (Hurng River), MK4 (Kaeng Kudku), MK5 (Nam Ngum River), MK6 (Songkram River), MK7 (Songkram River Delta), MK8 (Kaeng Hin Khan), MK9 (Mae Mun River), MK10 (Khong Chiam).

3.1.1 Sampling sites description

Site 1; Mae Kok River (MK1)

Kok River is located in Meuang District, Chiang Rai Province (northern Thailand) at approximately 19° 54' 714"N, 99° 47' 108" E. The river is tributary of Mekong River and it is important river of Chiang Rai Province. Water from this river is used in many activities such as agricultural, traveling and public utility and receives impact from those activities. The substrates in this area are sand, gravel cobble and some aquatic plant (Figure 2).

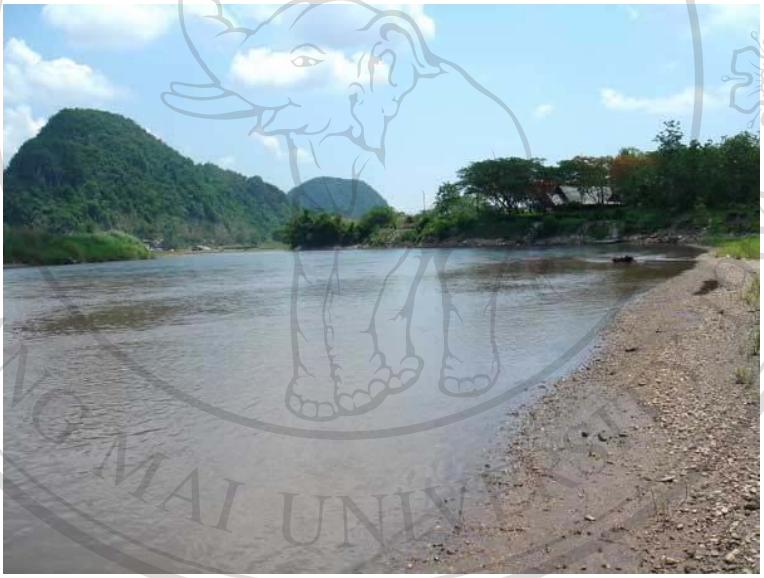

Figure 2 Mae Kok River; Meuang District, Chiang Rai Province (northern Thailand).

Site 2; Chiangkhong (MK2)

This study site is located in Hadkrai Village, Chiangkhong District, Chiang Rai Province (northern Thailand) at approximately 20° 15' 125"N, 100° 24' 832" E. In the past, this area had Giant Catfish (*Pangasianodon gigas*) catch festival from Mekong River pass through of Thailand. At present this location is Giant Catfish conservation are. The Giant Catfish is the largest freshwater fish of the world and its symbol of the ecological integrity of Mekong River. At present, the plight of Giant

Catfish was decline cause by the rapid environmental change in the Mekong river basin have all had an impacts (IUCN, 2005). The substrates in this area are sand, mud (in rainy season), gravel, cobble and some semi-aquatic plants (Figure 3).

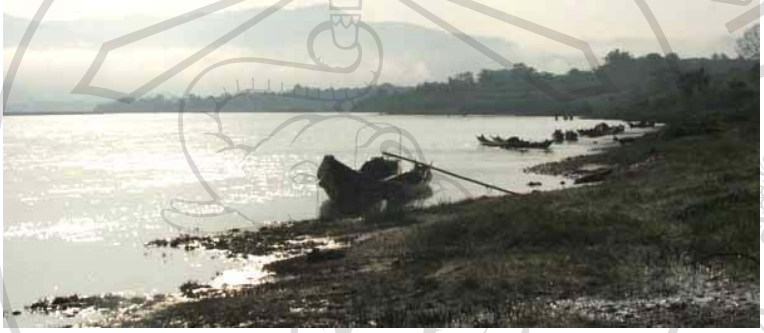

Figure 3 Hadkrai village; Chiangkhong District, Chiang Rai Province (northern Thailand).

Site 3; Hurng River

This site is located in Ta Li District, Loei Province (northeastern Thailand) at approximately 17° 46' 622"N, 101° 32' 450" E. The river is tributary of Mekong River and its boundary line between Thailand and Lao PDR. This study site is surrounding by small crop (corn and bean) area. The substrates in this area are gravel, cobble, rock and some aquatic plants (Figure 4).

Site 4; Kaeng Kudku (Mekong River)

Kaeng Kudku is located in Chiangkhan District, Loei Province at approximately 17° 54' 439"N, 101° 41' 116" E. This site affected from human activity, because it is tourism area and used to boat trip for traveling. The substrates in this area are gravel and cobble in dry season and mud in wet season (Figure 5).

Figure 4 Hurng River; Ta Li District, Loei Province (northeastern Thailand).

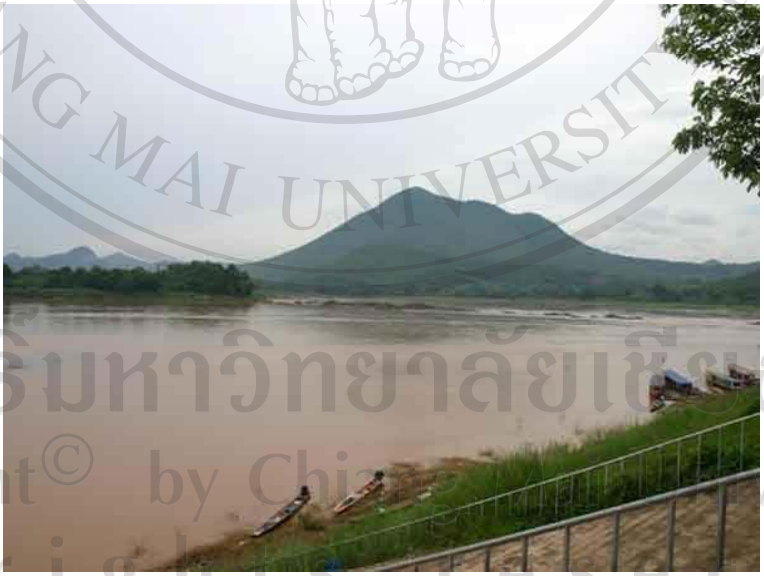

Figure 5 Kaeng Kudku; Chiangkhan District, Loei Province (northeastern Thailand).

Site 5; Nam Ngum River

This site is located in Vientiane Province, Lao PDR at approximately 18° 31' 457"N, 101° 38' 781" E. The river is of vital importance to Lao PDR. Its resources provide a subsistence livelihood to about 80,000 lowland and upland cultivators. Fishing is primary source of food for villagers and more than 55 indigenous fish species found in the Nam Ngum reservoir (MRCS, 2001). This study site is situated behind Nam Ngum dam. The water level of this site is depended on Nam Ngum dam water release. This dam has provided about one-quarter of the country's electricity requirement (Hirsch *et al.,* 1997). The substrates in this area are gravel, cobble, rock and aquatic plant (Figure 6).

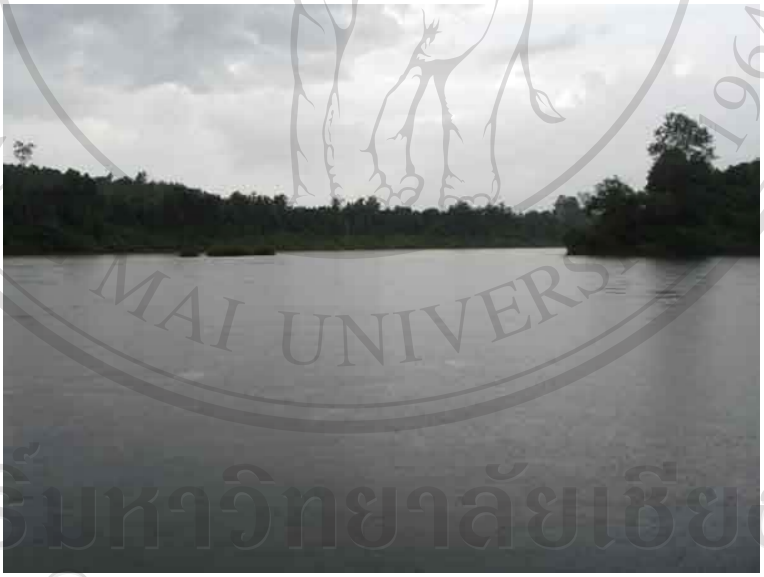

Figure 6 Nam Ngum River; Vientiane Province, central of Lao PDR.

Site 6; Songkram River

Songkram River is tributary of Mekong River and located in Kham Takla District, Nakhonphanom Province at approximately 17° 52' 016"N, 104° 46' 445" E. The basin of this river is used to produce rock-salt and its a important fisheries area.

The slope of this river is rather few, in rainy season its have influence water feed back from Mekong River and received a lot of sediment from border of the river. The substrates in this area are clay, aquatic plants and corpse of plant (Figure 7).

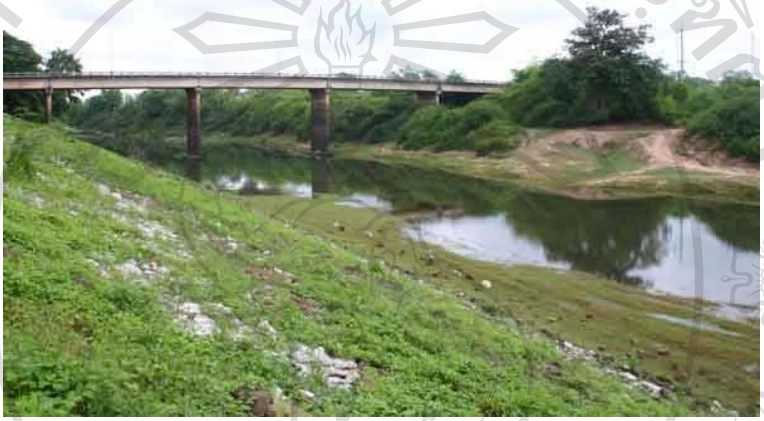

Figure 7 Songkram River; Kham Takla District, Nakhonphanom Province (northeastern Thailand).

Site 7; Songkram River Delta (Mekong River)

Songkram river delta is located in Tha Uthen District, Nakhonphanom Province at approximately 17° 39' 292"N, 104° 27' 945" E. Samples are collected from island in the middle of Mekong River. Around the sampling site is the mini fishery community. The substrates in this area are clay and sand (Figure 8).

Site 8; Kaeng Hin Khan (Mekong River)

Kaeng Hin Khan is located in Chanuman District, Amnatcharoen Province at approximately 16° 07' 697"N, 105° 01' 982" E. In this site have large rapid rocks distribution widely in the Mekong River, in riparian zone included of some aquatic plant. The area around sampling point is used to crop field and some fisheries from

local people. The substrates in this study site are aquatic plants, clay and sand (Figure 9).

Figure 8 Songkram River Delta; Tha Uthen District, Nakhonphanom Province (northeastern Thailand).

Site 9; Mae Mun River

Kaeng Sapheu situated in Mae Mun River (tributary of Mekong River) and located in Phibunmungsahan District, Ubon Ratchatani Province at approximately 15° 14' 732"N, 105° 14' 628" E. This site is traveling zone and mini-fisheries from local people. Substrate characteristic are rapid rocks to impede water flow in the river. The water level in this river is not stable because its received influence by open-close of water gate of Pakmun dam and affected to organism which live in the water (Figure 10).

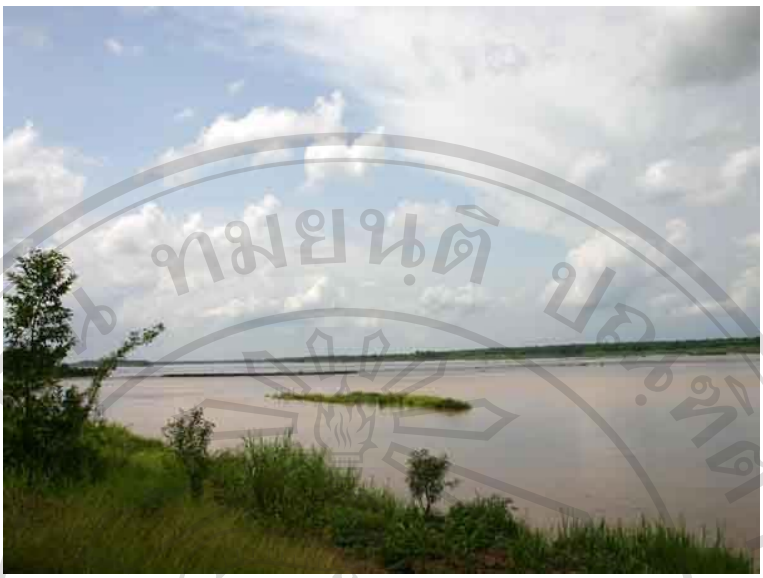

Figure 9 Kaeng Hin Khan; Chanuman District, Amnatcharoen Province (northeastern Thailand).

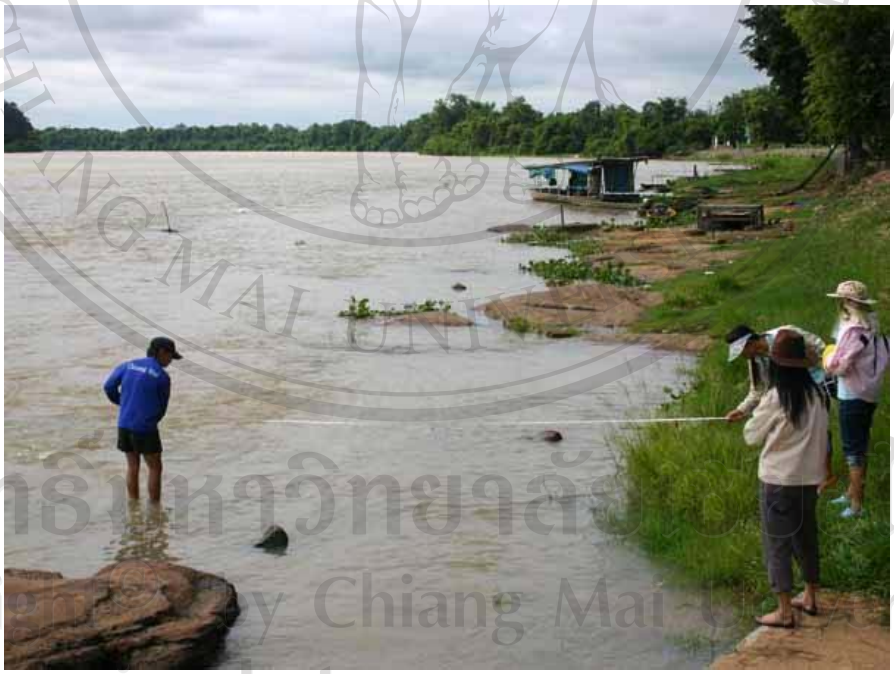

Figure 10 Mae Mun River; Phibunmungsahan District, Ubon Ratchatani Province (northeastern Thailand).

Site 10; Khong Chiam (Mekong River)

This site is located in Khongchiam District, Ubon Ratchatani Province at approximately 15° 19' 332"N, 105° 30' 314" E. This site is the last point of Mekong river pass through of Thailand and its the place of Mae Mun River to join with Mekong River. Sample point is situated on island in the middle of Mekong River. Substrates in this sampling point are bed rock, mud and aquatic plants (Figure 11).

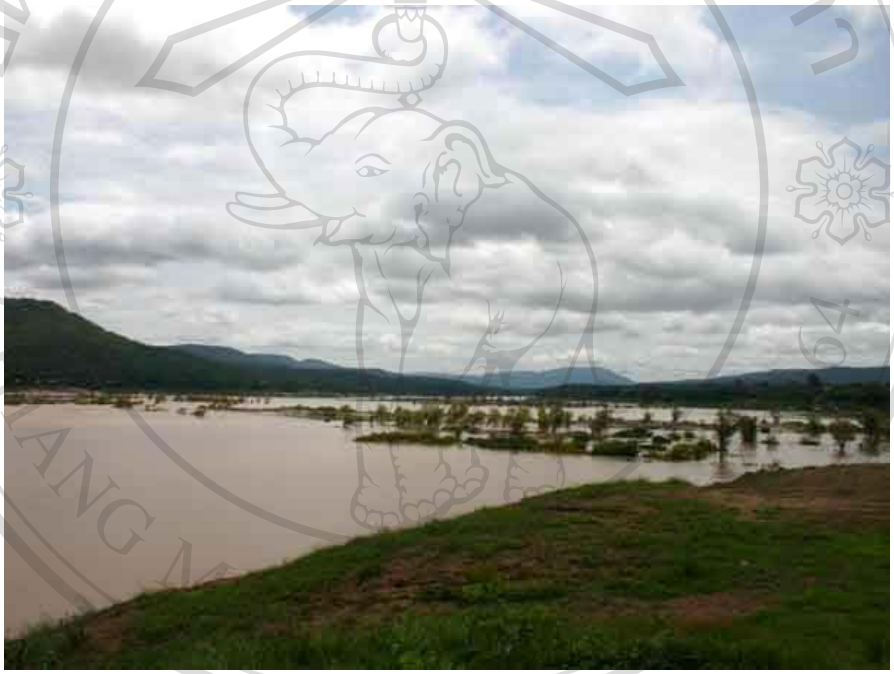

Figure 11 Khong Chiam; Khong Chiam District, Ubon Ratchatani Province (northeastern Thailand).

3.2 Water sampling and physicochemical parameters

The physicochemical parameters of water quality at each site will be measured in the field and in the laboratory are as follows;

1. Stream depth and width using measuring tape.
2. Current velocity using velocity meters.
3. Air and water temperature using a mercury thermometer.

4. pH using the pH meter.

5. Conductivity and total dissolved solid using Conductivity/TDS meter.

6. Turbidity using HACH DR2400 spectrophotometer.

7. Salinity using salinity meters.

8. Alkalinity using the Phenolthalene Methyl Orange Indicator Method (Greenberg *et al*., 1992).

9. Dissolved oxygen (DO) using the Azide Modification Method (Greenberg *et al*., 1992).

10. Biochemical oxygen demand (BOD_5) using the Azide Modification Method (Greenberg *et al*., 1992).

11. Orthophosphate ($O\text{-}PO_4^{3-}$) was determined by Ascorbic Acid Method using Phos Ver 3 reagent and measured by HACH DR2400 spectrophotometer.

12. Nitrate nitrogen (NO_3^--N) was determined by the Cadmium Reduction Method using Nitra Ver 5 Nitrate reagent and measured by HACH DR2400 spectrophotometer.

13. Ammonium nitrogen (NH_4^+-N) was determined by the Nesslerization technique, using Nessler reagent, Mineral stabilizer and Polyvinyl alcohol and measured by HACH DR2400 spectrophotometer.

3.3 Biological properties

3.3.1 Macroinvertebrates sampling

Macroinvertebrate fauna were sampled four times from 10 study sites. Pond net (0.5 mm^2 mesh), kick sampling and hand pick method (Furse *et al.,* 1981; Mustow, 2002) were used for collecting macroinvertebrates from mud, sand, gravel, cobble, rock, bed rock and aquatic plant covering 60 m^2 area for 15 minute. There were three replications in each sampling sites. The pond net was put in to the bottom against the stream current then the area in front of the net was disturbed by foot. The macroinvertebrates which live in gravel and sand drifted into the sieve bag. All

samples were preserved in 4% formalin for sorting and identification in laboratory. Then, the macroinvertebrates were sieved by using a 0.5-mm stainless steel sieve, sorted into a petri-dish and identified by using a taxonomical key to at least the family level or to the lowest possible level. The numbers of individuals in each family were counted. Identification was done by using, Brandt (1974), McCafferty (1998), Dudgeon (1999), Sangpradub and Boonsong (2006) and Merritt *et al*. (2008).

3.3.2 Biological Indices

1 Diversity Index

The result of aquatic insect communities were calculated to the diversity index by using the Shannon-Wiener Index (H') formula. Moreover, the diversity index can be used to assess water quality by comparing the table 1.

$$-\sum_{i=1}^{s}(Pi)(\log 2Pi)$$

H' = Diversity Index

P_i = Proportion of individuals of the total sample belonging to the *i*th species

Table 1 Description of water quality assessed by using diversity index value (Shannon and Weaver, 1963; Sripongpun 2003).

Shannon-Weiner Index	water quality
>4	clean water
3-4	slightly pollution
2-3	moderately pollution
<2	very polluted

2 BMWP^{Thai} score and ASPT

The Biomonitoring Working Party Score (BMWP score) is a score from family of macroinvertebrate families assigned to its tolerance to pollution and varies from 1 to 10 (Friedrich *et al*., 1996; Hynes, 1998; Mackie, 2001). The tolerance groups of pollution are assigned to low score and least tolerance assigned to high score (appendix A). Moreover, the BMWP score was adapted by Mustow (2002) for using assessment the water quality of rivers in Thailand. The total score of BMWP^{Thai} from each family was used to calculate the Average Score Per Taxa (ASPT) for assessed water quality. The description of water quality with the value of BMWP^{Thai} and ASPT were showed in table 1 and 2. ASPT value was calculated as:

$$ASPT = \frac{BMWP}{\text{Number of family with score}}$$

Table 2 Description of water quality as define by numerical value of BMWP index (Alba-Tercedor, 1996).

BMWP	Water quality
>120	excellent quality
101-120	good quality, no pollution or obvious distortions
61-100	regular quality, eutrophic, medium pollution
36-60	bad quality, polluted
16-35	bad quality, very polluted
<15	very bad quality, extremely polluted

Table 3 Description of water quality as define by ASPT value (Mackie, 2001; Mandaville, 2002).

ASPT	Water quality
>6	good
5-6	doubtful quality
4-5	probable moderate pollution
<4	probable severe pollution

3 Family Biotic Index (FBI)

This biotic index was originally developed by Hilsenhoff (1982) to assign a tolerance value of aquatic arthropod. The FBI was modified to the family level with the tolerance values ranging from 0 (very intolerance) to 10 (very tolerance) base on their tolerance to organic pollution. Subsequently, the FBI was modified by The State of New York to include other macroinvertebrates to use for assessment the water quality. FBI was calculated as:

$$FBI = \frac{\sum X_i T_i}{N}$$

X_i = the number of individual in the i^{th} taxon.

T_i = the tolerance value of the i^{th} taxon.

N = the total number of the organism in sample.

Table 4 Description of water quality assessed by using Family Biotic Index (Adapted from Hilsenhoff, 1977).

Family Biotic Index	Water quality	Degree of organic pollution
0.00-3.77	Excellent	Organic pollution unlikely
3.76-4.25	Very good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very poor	Severs organic pollution unlikely

3.3.3 Chemical Index (CI)

An index is a number without dimension. The index is often combined with mathematic. Many parameters including Saturated Oxygen, BOD_5, Water temperature, NH_4^+-N, NO3-N, O-PO_4^{3-}, pH and Conductivity will be calculated. The water quality assessed by this index will be used to compare the water quality assessed by using biological indices (Jurgen *et al*., 2002).

The chemical index is a multiplicative index and has a following from:

$$CI = \P q_i^{w_i} = q_1^{w_1} . q_2^{w_2} \ldots\ldots q_n^{w_n}$$

When CI = Chemical index

q_i = Subindex (potential value) for the measurement of parameters on a scale with definition zero point and interval (0, 100)

n = Number of parameter

w_i = relative weight of n parameter

Table 5 Classification of water quality assessed by chemical index.

Class	Range of chemical index	Site classification
I	>83	Very clean
I-II	73 - 83	Clean
II	56 - 73	Fairy clean
II-III	44 – 56	Moderated
III	27 – 44	Polluted
III-IV	17 – 27	Heavily polluted
IV	<17	Very heavily polluted

3.4 Research duration

Samples were collected from 3 seasons during from May 2007 to April 2008.

3.5 Statistical analysis

The results of physicochemical and biological parameters were analyzed by using program Multivariate Statistical Package (MVSP) version 3.1 and Statistical Package for The Social Science (SPSS) version 14.

Cluster analysis (CA) was used to grouping the study sites based on physicochemical parameters and biological properties. There have been many application of cluster analysis to practical problem. The cluster analysis has been use to find group of data in similar function.

Principal Component Analysis (PCA) is a useful statistical technique that has found application in fields such as face recognition and image compression, and is a

common technique for finding patterns in data of high dimension. There have been used to explain and discussed the result from cluster analysis in this study.

Canonical Correspondence Analysis (CCA), Ter Braak (1986) presented the CCA within an ecological context having the situation where the whole dataset consists of two sets: data on the occurrence or abundance of a number of species, and data on a number of environmental variables measured which may help to explain the interpretation of the scaled solution. In other words, they are incorporated as effects in the correspondence analysis computation in order to examine their influence on the scores. In this study, it's used to study relationship between water qualities with the macroinvertebrates fauna.

Pearson's correlation Its a correlation is a number between -1 and +1 that measures the degree of association between two variables**.** Positive value for the correlation implies a positive association and negative value for the correlation implies a negative or inverse association. It's has been used to analyze the relationship of biotic indices with the physicochemical parameters in this study.