CHAPTER 2
LITERATURE REVIEWS

2.1 Characteristic of diatoms

Diatoms are microscopic unicellular, photosynthetic algae, sometime colonial or pseudofilamentous algae (Round *et al.*, 1990; Lee, 1999). They have silica skeleton called frustule and are found in soils and almost all aquatic environments including fresh water and marine water (Barber and Haworth, 1981). They are non-motile, or capable of only limited movement along a substrate by secretion of mucilaginous material along a slit-like groove or channel called a raphe. Being autotrophic they are restricted to the photic zone (water depths down to about 200m depending on clarity). Both benthic and planktic forms exist (Round *et al*., 1990).

Diatoms are usually between 5-500 µm in diameter or length, although sometimes they can be up to 2 ml. long (Barber and Haworth, 1981).

In the past, diatoms are formally classified to the Division Chrysophyta, Class Bacillariophyceae (Bold and Wynne, 1978). The Chrysophytes are algae which form endoplasmic cysts, store oils rather than starch, possess a bipartite cell wall and secrete silica at some stages of their life cycle.

Diatoms are divided into two orders. The Centrales (now called the Biddulphiales) which have valve striae arranged basically in relation to a point, an annulus or a central areola which tend to appear radially symmetrical, and the Pennales (now called the Bacillariales) which have valve striae arranged in relation to a line and tend to appear bilaterally symmetrical. The valve face of the diatom frustule is ornamented with pores (areolae), processes, spines, hyaline areas and other distinguishing features. These skeletal features are used to classify and describe diatoms, which is an advantage in terms of palaeontology since the same features are used to define extant species as extinct ones (Barber and Haworth, 1981; Round *et al*., 1990).

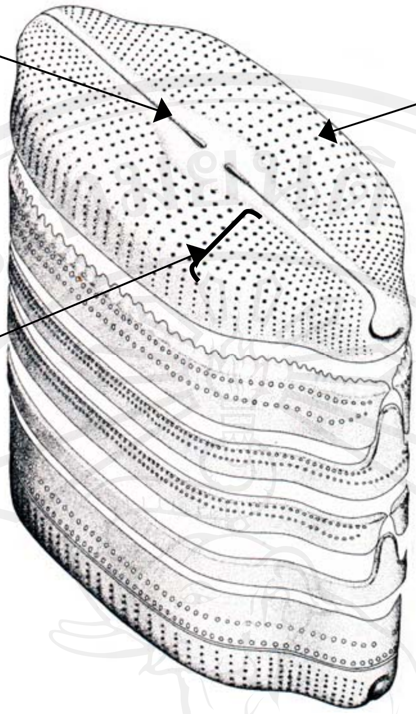

Figure 1 Diatom frustule showed the details that used in diatom identifications. (Round *et al*., 1990)

Diatom chloroplasts are characterized by possession of chlorophyll-*a* and –*c*, and the primary accessory pigment, -carotene and fucoxanthin (van Den Hoek *et al*., 1995). Chloroplast number and arrangement differ among taxa but are consistent within most taxa (Cox, 1996). Most centric diatoms and some araphid pennate diatoms possess numerous small, disc-shaped chloroplasts. In contrast, the chloroplasts of the majority of pennate taxa are large and fewer numbers per cell. Nevertheless, chloroplast number and shape may a in different stages of cell cycle (Wehr and Sheath, 2003). Cox (1996) provided a key to freshwater diatoms based on chloroplasts and other cytoplasmic features visible in the light microscope.

Some diatoms are free-living cells which may be found floating in the water, mobile or immobile on the bottom, or caught up in the detritus around other plants. Others are attached in various ways to other cells to form colonies, or to different vegetable or mineral substrata (Barber and Haworth, 1981).

2.2 Classification of diatoms

The classification system of Simonsen (1979) which further developed by Round *et al*. (1990) is currently the most commonly accepted. Diatoms commonly found in marine planktons may be divided into the centric diatoms including three sub-orders based primarily on the shape of the cells, the polarity and the arrangement of the processes. These are the Coscinodiscineae, with a marginal ring of processes and no polarity to the symmetry, the Rhizosoleniineae with no marginal ring of processes and unipolar symmetry, and the Biddulphiineae with no marginal ring of processes and bipolar symmetry. The pennate diatoms are divided into two sub-orders, the Fragilariineae which do not posses a raphe (araphid) and the Bacillariineae which posses a raphe. In this research, as shown in Table 1, diatoms are arranged following the classification of Round *et al*. (1990). All genera are listed below and represented only presented genera in this.

Table1 Classification of diatoms from Round *et al*. (1990)

Division	Class	Subclass	Order	Family	Genus
Bacillariophyta	Coscinodiscineae	Thalassiosirophycidae	Thalassiosirales	Stephanodiscaceae	*Cyclotella*
		Coscinodiscophycidae	Melosirales	Melosiraceae	*Melosira*
			Aulacoseirales	Aulacoseiraceae	*Aulacoseira*
		Biddulphiophycidae	Triceratiales	Triceratiaceae	*Pleurosira*
			Biddulphiales	Biddulphiaceae	*Hydrosera*
	Fragilariophyceae	Fragilariophycidae	Fragilariales	Fragilariaceae	*Fragilaria*
					Diatoma
					Synedra
					Tabularia
	Bacillariophyceae	Eunotiophycidae	Eunotiales	Eunotiaceae	*Eunotia*
		Bacillariophycidae	Cymbellales	Cymbellaceae	*Placoneis*
					Cymbella
					Encyonema
					Encyonopsis
				Gomphonemataceae	*Gomphonema*
					Gomphoneis
					Reimaria
Bacillariophyta			Achnanthales	Achnanthaceae	*Achnanthes*
				Cocconeidaceae	*Cocconeis*
				Achnanthidiaceae	*Achnanthidium*
			Naviculales	Diadesmidaceae	*Diadesmid*
					Luticola
				Amphipleuraceae	*Frustulia*
				Brachysiraceae	*Brachysira*
				Neidiaceae	*Neidium*

Table1 (Continued)

Division	Class	Subclass	Order	Family	Genus
Bacillariophyta	Bacillariophyceae	Bacillariophycidae	Naviculales	Sellaphoraceae	*Sellaphora*
					Fallacia
				Pinnulariaceae	*Pinnularia*
				Diploneidaceae	*Diploneis*
				Naviculaceae	*Navicula*
				Pleurosigmataceae	*Pleurosigma*
					Gyrosigma
				Stauroneidaceae	*Stauroneis*
					Craticula
			Thalassiophysales	Catenulaceae	*Amphora*
			Bacillariales	Bacillariaceae	*Bacillaria*
					Hantzschia
					Tryblionella
					Nitzschia
			Rhopalodiales	Rhopalodiaceae	*Epithemia*
					Rhopalodia
			Surirellales	Surirellaceae	*Surirella*

2.3 Biological assessment of water quality

The biological assessment of water quality is seen as an essential part in the assessment of the ecological quality of running waters besides data obtained from other environmental information such as hydrology, ecomorphology, physical and chemical water analysis and ecotoxicological analysis (Chovanec *et al*., 2000). Traditionally, the application of macroinvertebrate based methods is standing in the centre of interest whereas periphyton methods are often overlooked. Nevertheless, benthic algae especially diatoms have been used successfully to assess specific water quality (Rott *et al*., 2003). In running ecosystem, some organisms that live in the sediments or attach to substrates could be used as biomonitors to assess the water quality. One of the most effective organisms were benthic diatoms that attached to the rock, stone, cobble, gravel, mud or other substrates (Whitton *et al*., 1991 ; Wetzel, 2001). Benthic algae especially diatoms frequently cover almost all surfaces within the wetted perimeter of river bed (Rott *et al*., 2003).

Diatoms are successfully used for monitoring aquatic environments around the world and for many years with centres of activities in Europe and USA (Rott *et al*., 2003).

Diatoms are cosmopolitan as many taxa have been identified from a range of sites throughout the world. They are sensitive to, and appear to have a consistent tolerance to a wide range of environmental parameters such as light, moisture, current velocity, pH, salinity, oxygen and inorganic and organic nutrients (van Dam *et al*., 1994). Diatoms often occur in large numbers and show considerable species richness: even a simply collected, small surface mud sample or a scraping from a rock can yield more than 106 valves, representing of between 20 and 50, and even up to 100, taxa (Battarbee, 1988). These characteristics provide diatomists considerable advantage over ecologists reliant on macroinvertebrates or other bioindicators.

Diatoms are successfully used for monitoring aquatic environments around the world and for many years with centres of activities in Europe, USA and Japan (Rott *et al*., 2003). The process for the application of diatoms to river quality monitoring is still in progress (Prygiel *et al*., 1999)

Peerapornpisal in 2007, proposed the method for assess the water quality, which based on the 35 water resources in Thailand. This water quality assessment is called "AARL- PP Score". It is composed of 2 parts scoring system. The first part is the standard score of water quality base on trophic status. The water quality was categorized into 6 status using 1-10 scores. Each status was divided by former research experience, i.e. clean (oligotrophic status), clean to moderate (oligotrophic-mesotrophic status), moderate (mesotrophic status), moderate to polluted (mesotrophic-eutrophic status), polluted (eutrophic status), and very polluted (hypereutrophic status). In the second part, the dominant genera of phytoplankton include planktonic diatoms from different water quality resources were given 1-10 scores. The lower scores indicated clean water whereas the higher scores indicated polluted water. The physical and chemical properties of water were used in combination to evaluate the water quality (Lorraine and Vollenweider, 1981; Peerapornpisal *et al*., 2007).

2.4 Diatoms in Asia region

The studies of diatoms in Asia were lead by Japanese scientist for long time. There are many documents published by Japanese researchers. However, there are now widely study in many Asian countries.

Ohno *et al.* in 1971 published the diatom flora of the Mekong water system in Cambodia. The material collections made by the member of the Society for Investigation of the Mekong Water System of Kochi University from December 1969 to January 1970. From the materials, 77 taxa of diatoms were found in Tonle Sap Lake and the Tonle Sap River which 36 taxa found in Mekong River. Fourteen taxa of diatoms were found in both regions. Main species of the Mekong Water System, Cambodia, were *Melosira granulata, Melosira granulata* f. *curvata*, *Synedra ulna* var. *oxyrhynchus* and *Gyrosigma kutzingii* (Ohno *et al*., 1971).

Foged in 1976, was investigated freshwater diatoms in Sri Lanka (Ceylon). In 22 freshwater samples collected in 1971 and 1973. A total of 310 taxa from 34 genera were recorded. A single new species namely *Caloneis gjeddeana* was described.

The diatom communities collected from artificial substrates along Pinang River Basin, Malaysia were analyzed by Maznah and Mansor in 2002. The diatom community structure and the specific sensitivity of certain diatom species can be related to the degree of water quality in Pinang River Basin. The abundance of certain diatom species could be used as biological indicators to measure impacts of river pollution (Maznah and Mansor, 2002).

In Nepal and India, diatom samples were collected from 30 and 53 streams in the Kathmandu Valley and Niddle Hill respectively. A total of 168 taxa were recorded. Sixty two taxa occurred only in the Kathmandu Valley with 56 found only in the Middle Hills. The remaining 50 were common to both areas. Most taxa found only in the Kathmandu Valley belonged to the genus *Navicula* while most taxa confined to the Middle Hill were *Achnanthes, Fragilaria* and *Gomphonema* (Jüttner *et al*., 2003).

Doung *et al.* in 2006 published impact of urban pollution from the Hanoi area on benthic diatom communities collected from the Red, Nhue and Tolich Rivers in Vietnam. A total of 291 diatom taxa were found from three rivers. These were mainly cosmopolitan taxa, with some tropical, subtropical and endemic species. The most abundant taxa at Red River were *Aulacoseira granulate*, *Achnanthidium minutissimum, Encyonema minutum* and *Navicula recens.* Diatom assemblages at the Tolich River consisted mainly of *Nitzschia umbonata*, *Nitzschia palea* and *Eolimna minima* (Doung *et al.*, 2006).

Peerapornpisal, *et al.* reported the diversity and distribution of diatoms in lower Mekong Basin. This project was a part of "Ecological Health Monitoring Program for the Lower Mekong River and Selected Tributaries" that supported by Mekong River Commission (MRC). The diatom samples were collected from 57 sampling sites in Mekong River and its Tributaries in Lao PDR., Cambodia, Thailand and Vietnam in the year 2004 to 2006. From this project, the Average Tolerance Score per Taxon (ATSPT) were reported. ATSPT is the average tolerance of all taxa of diatoms recorded in a sample, calculated regardless of their abundances. Most diatom taxa indicated meso-eutrophic status of water quality (MRC, 2006).

2.5 A study of diatoms in Thailand

Diatom flora was studies by foreign scientists for many years. The study of diatoms in Thailand was started in 1902, Östrup reported 81 different diatoms from the island of Koh Chang in the Gulf of Thailand. The samples were collected by a Danish Expedition to Thailand during 1899-1900 (Östrup, 1902). Then, in 1936, Patrick reported a total of 185 diatom species in the study of the intestinal contents of tadpoles from Thailand and the Federal Malay Satates (Patrick, 1936). Furthermore, the Joint Thai-Japanese Biological Expedition to Southeast Asia collected the diatoms samples in 1961-1962. The samples were identified by Hirano who published an account of 143 diatom species, 114 of them were found in the samples from Thailand in 1976. Most of these samples were collected in Chiang Mai areas and the others from localities in the central and southern parts of Thailand (Hirano, 1967). Foged in 1971 reported the freshwater diatoms in Thailand. The material collected in central and northern parts of Thailand in 1966. Three hundred and seventy eight taxa of diatoms were published. In this work, 8 new species, 5 new varieties and 2 forms were additionally reported (Foged, 1971).

In 1995, Ladda Wongrat published "The check list of the Algae in Thailand" (Lewmanomont *et al.*, 1995). This checklist has been prepared through a compilation of various publications including survey reports, scientific papers and results from the Environment Impact Assessment supported by the Office of Environmental Policy and Planning (OEPP) and Danish Cooperation on Environment and the Development (DANCED). The checklist was complied from 53 publications of both marine and freshwater algae. One hundred and sixty one genera, 1001 species, 287 variaties and 63 forms were reported. The diatom flora was recorded in Division Chromophyta, Class Bacillariophyta. A total of 46 genera, 385 species, 144 varieties and 43 forms of diatoms have been recorded.

In 1996, Peerapornpisal studied about phytoplankton in the reservoirs of Huai Hong Khrai Royal Development Study Centre in Chiang Mai Province. The species of diatoms were reported such as *Aulacoseira granulate, Cyclotella meneghiniana, Fragilaria capucina* var. *gracillis, Fragilaria fasciculate, Nitzschia intermedia* and *Nitzschia gracillis* (Peerapornpisal, 1996). In 1998, Wongrat published "The Status of Phytoplankton Diversity in Thailand" in which some species of the diatoms were reported. The samples were collected from all parts of Thailand, mostly from the northern and northeastern parts of the country (Wongrat, 1998). Then in 1999, Peerapornpisal *et al*. continued the work concerning water quality and phytoplankton distribution in the reservoir of Mae Kuang Udomtara Dam, Chiang Mai Province. The phytoplankton including diatoms were investigated during August 1996 to 1998. (Peerapornpisal *et al*., 1999).

Therefore, most works on diatoms have been done on plankton in standing water from many parts of Thailand and carried out by Thai scientists in various universities and institutions.

Although the arrangement of an exquisitely sculptured part of the silica cell wall of diatoms is the main features in identification. However, other features such a chloroplast shape, number position and arrangement within the living cell were also used to identify then. Cox (1996) provided a key to freshwater diatoms based on chloroplasts and other cytoplasmic features visible under light microscope. (Cox, 1996). In Thailand, Suphan *et al.* in 2002 published the use of chloroplast and other features of the living cell in the taxonomy of freshwater diatoms. The samples were collected from Huay Kha Yang watershad, Kanjanaburi Province. The living cells of *Synedra ulna, S. laceolata, Cymbella turgidula, Achnanthes crenulata* and *Gomphonema parvulum* were shown (Suphan *et al.*, 2002).

Electron microscopes were used for clearly identification of the diatoms. It was very useful for diatom identification at species level and help to distinguish related species for establishing a new genera or taxa (Lange-Bertalot, 1993).

In Thailand, some works used scanning electron microscopes to study the structure of the diatoms. Scanning electron micrographs of freshwater diatoms in diatomite from Lampang Province, Thailand. The materials were collected from Mae Ta and Koh Ka Districts. Both sampling sites seem to have only one single dominant species, *Aulacoseira granulata* throughout most of the deposit (Pekthong and Peerapornpisal, 2002).

In 2009, Suphan and Peerapornpisal published scanning electron micrographs of benthic diatoms in Mekong River and its tributaries, Thailand. The fine structure and other details of *Pleurosigma salinarum*, *Cymbella* sp., *Cocconeis pediculus, Nitzschia obtuse, Achnanthidium minutissimum*, *Bacillaria paxillifer*, *Encyonema* *silesiacum* and *Cymbella sumatrensis* were shown (Suphan and Peerapornpisal, 2009). Futhermore, benthic diatom diversity and water quality in Mekong River in the vicinity of Ubon Ratchathani Province were reported. Scanning electron micrographs of *Synedra ulna, Nitzschia palea, Cocconeis placentula, Cymbella turgidula* and *C. tumida* were also shown (Moonsyn *et al.*, 2009)

Description and scanning electron micrographs of some benthic diatoms in Ping River, Northern Thailand were reported by Leelahakriengkrai and Peerapornpisal. In this investigation, *Planothidium rostratum, Diadesmid contenta, Sellophora popula, Gomphonema pumilum, Brachysira* cf. *neoexillis* and *Surirella roba* were identied and described (Leelahakriengkrai and Peerapornpisal, 2009).

In addition, there are some works in Thailand on the use of diatoms in aquaculture for feeding mollusks and crustaceans. The publishing of "Utilisation of Algae : A Research and Development Potential in Thailand" was recorded. The use of *Chaetoceros muelleri, Chaetoceros calcitrans, Skeletonema costatum, Thalassiosira pseudonana*, *Nitzschia* spp. and *Navicula* spp. were used as feed for mollusks and crustaceans (Powtongsook, 2001)

There have been few studies of diatoms in Thailand in the past twenty years. Basic knowledge from the studies on diversity, distribution and ecology of diatoms are very important. It is very essential for algae applications such as agriculturally, nutritionally, medicinally, cultivation and environmental.

2.6 Use of benthic diatoms for monitoring rivers in Thailand

The use of benthic diatoms for monitoring the quality of surface running waters in Thailand has been carried out since 1998. Pekthong reported the phytoplankton and benthic algae from Mae Sa Stream, Chiang Mai Province. The samples were collected from April 1997 to February 1998. The majority of the phytoplanktons and benthic algae were diatoms in the Order Pennales. There were 172 species of benthic diatoms. The most abundant species were *Navicula lanceolata, Nitzschia dissipata, Cocconeis placentula, Achnanthes lanceolata,* and *Cymbella tumida*. Benthic diatoms that are characterized as tolerant towards eutrophication, organic pollution and high turbidity are *Gomphonema parvulum* and *Nitzschia palea*. The sensitive groups are *Cocconeis placentula*, *Gyrosigma nodiferum, Nitzschia* *dissipata* and *Gomphonema angur*. The species that is characteristic for unpolluted waters with low concentration of nitrogen is *Achnanthes minutissima* (Pekthong, 1998). In the same year, Waiyaka continued the studies and reported 102 species of phytoplankton and 106 species of benthic algae in the same stream. Diatoms were found to be the majority of phytoplankton and benthic algae in both studies. The majority of benthic algae were *Cocconeis placentula, Navicula viridula, Fragilaria ulna* and *Melosira varian* (Waiyaka, 1998). Kunpradid (2000) reported the phytoplanktons in Mae Sa Stream that carried out from April 1998 to September 1999. The majority of the phytoplankton was diatoms and the most abundant species were *Fragilaria ulna*, *Fragilaria capucina, Nitzschia dissipata*, *Navicula cryptotenella, Navicula viridula, Cymbella tumida* and *Melosira varians.* The diversity of phytoplankton and benthic algae in a Mae Sa Stream, Doi Suthep-Pui National Park were investigated during April 1997 to February 1998 by Peerapornpisal, *et al*. (2000). From the results, it was implied that the majority of phytoplankton and benthic algae were diatoms (Peerapornpisal *et al*., 2000).

In 2000, Wanathong reported the diatoms from the tributaries of Mae Jam Stream. *Nitzschia sigmoidal* and *Navicula cryptocephala* were found in oligotrophic status, low nutrient and low conductivity (Wanathong *et al.*, 2000).

A study on the relationship between diatoms and water runoff was investigated by Hempattarasuwon in 2001. The diversity of the diatoms in Pasak River was studied during February to September 2000. Two hundred and eight species of diatoms were found. The majority of the diatoms were in the Order Naviculales in which 119 taxa were identified. Dominant species were *Achnanthes minutissima*, *Aulacoseira granulate* var. *angustissima, Cocconeis placentula, Cymbella hustedtii* and *Gyrosigma scalproides*.

Pekthong and Peerapornpisal in 2002 continued their work on benthic diatoms and their applications as indicator species to monitor water quality in Mae Sa Stream. The samples were collected from April 1998 to September 1999. Thirty four genera complied with 278 species of diatoms were found. Twenty five species of diatoms were scored and listed in Mae Sa Diatom Index and could be properly used to indicate the physico-chemical property of water quality (Pekthong, 2002a). From their work, fifty one species of freshwater diatoms were newly records of Thailand (Pekthong and Peerapornpisal, 2001).

Suphan (2004) studied the diversity of benthic algae in Thong Pha Phoom Watershed, Kanjanaburi Province, Western Thailand. *Achnanthes minutissima* and *Brachysira neoexilis* could be used for monitoring oligotrophic – mesotrophic status of water quality.

The study of macroalgae, benthic diatom and their relationship with nutrients in the Ping and Nan Rivers were carried out from November 2001- February 2004. One hundred and thirty species of benthic diatoms were found. The dominant species of diatom in the Ping River were *Gomphonema parvulum*, *Nitzschia palea*, *Achnanthes lanceolata* and *Bacillaria paradoxa*, the Nan River were *Cocconeis placentula*, *Achnanthes crenulata*, *Achnanthes lanceolata* and *Rhopalodia gibba*. From this work, the Ping and Nan River Diatom Index were established (Kunpradid, 2005).

In the same year, Inthasotti reported the distribution of benthic algae in Mae Tang Stream, Chiang Mai Province during March 2005 – January 2006. A total of 118 diatoms species were found. Most of them were in Order Pennales (Inthasotti, 2006a). In 2006(b), Inthasotti continued her work in Num Kham Watershed, Chiang Rai Province, Northern Thailand from 2003-2004. One hundred and sixty seven species of benthic diatoms were found.

Leelahakriengkrai (2007a) studied diversity of macroalgae and benthic diatoms in Kok River and its tributaries in Northern Thailand between February to July 2006. Seventy-eight species, 26 genera of benthic diatoms were recorded. In addition, *Achnanthes pusila*, *Achnanthes minutissima*, *Achnanthes exigua* var. *constricta*, *Nitzschia dissipata*, *Cymbella turgidula* and *Diadesmis contenta* were used as bioindicator for moderate water quality (mesotrophic status). In the following year, Leelahakriengkrai reported diversity and monitoring water quality in Ping River from 2004-2005. Diatoms namely *Achnanthes minutissima* and *Cymbella tumida* were found in water low in nutrient (Leelahakriengkrai, 2007b).

From 1998 to date, the works on the use of benthic diatoms for monitoring river in Thailand were continued. Many works modified and developed the diatoms index in each location. Therefore, the Diatom Index of Thailand will be developed in the near future.

2.7 Diatom Index

Nowaday, diatom index are widely applied during routine water quality surveys from different parts of the world. Indices have been developed to monitor eutrophication, organic pollution and human disturbance (Jüttner *et al*. 2003). The details of some indices of diatoms are as follow;

2.7.1 The Diatom Assemblage Index of Pollution (DAIpo) (Watanabe *et al*.,1988)

Watanabe *et al*. in 1988 published the Diatom Assemblage Index of Pollution (DAIpo). This index was based on 548 diatom taxa identified from 1,343 samples taken from Japanese rivers. Diatom Assemblage Index of Pollution (DAIpo) was produced for each taxon and site based on the relative abundance of taxa in each of three components relating to saprobity. The index score represents the species' optimum tolerance to BOD (Biochemical Oxygen Demand: a proxy indicator for organic content) on a scale of 0 - 100. Taxa with scores of less than 30 were considered to be saprophilous, or pollution intolerant, while those with scores greater than 70 are saprophobic, that is, antagonistic to pollution. A simple weighted averaging approach can provide DAIpo values for sites and samples (Reid *et al*., 1995).

2.7.2 The Trophic Index of Schiefele and Kohmann (Schiefele and Kohmann, 1993)

This works were developed from the study on the distribution of diatoms from streams and rivers in Germany. The index classified the trophic status using the indicator weight evaluation method.

The good indicators are given more influence on sample index. The index was calculated by the following formula;

$$\text{Sample index} = \frac{\Sigma \text{ Relative Abundant} \times \text{Index} \times \text{Indicator weight}}{\Sigma \text{ Relative Abundant} \times \text{Indicator weight}}$$

A comparison of the saprobic water quality class or the trophic status was shown in Table 2.

Table 2 Trophic state lists of Schiefele and Kohmann Trophic Index (Schiefele and Kohmann, 1993)

Sample index	Trophic state	Level of inpact
1.0 – 1.4	oligotrophic	no impact
1.5 – 1.8	oligo- to mesotrophic	little impact
1.9 – 2.2	mesotrophic	district impact
2.3 – 2.7	meso-eutrotrophic	critical impact
2.8 – 3.1	eutrotrophic	significant impact
3.2 – 3.5	eu- to hypereutrophic	strong impact
3.6 – 4.0	hypereutrophic	very strong impact

2.7.3 The Trophic Index of van Dam (van Dam *et al*., 1994)

This index were developed from the distribution of diatoms from rivers and streams in the Netherlands. There was a diatom distribution with an index in 7 categories (Oligotraphentic to hypereutraphentic). The sample index was calculated using the following formula;

$$\text{Sample index} = \frac{\Sigma \text{ Rel. Abund. X Index}}{\Sigma \text{ Rel. Abund.}}$$

The value is compared to the trophic level in the Table 3.

Table 3 Trophic index (van Dam *et al*., 1994)

Categories	Species	Sample index	Trophic state
1	oligotraphentic	1.0 – 1.5	oligotrophic
2	oligo-mesotraphentic	1.5 – 2.5	oligo-mesotrophic
3	mesotraphentic	2.5 – 3.5	mesotrophic
4	meso-eutraphentic	3.5 – 4.5	meso-eutrophic
5	eutraphentic	4.5 – 5.5	eutrophic
6	hypereutraphentic	5.5 – 6.0	hypereutrophic
7	indifferent		

2.7.4 The Saprobic Index of Rott *et al*. (Rott *et al*., 1997)

This index was developed from a study of diatoms in streams and rivers of Austria. This method was used to indicate the saprobic water quality. The sample index was calculated using the same formula of van Dam (1994),as shown in Table 4.

Table 4 Trophic state list of Rott *et al*. Saprobic Index (Rott *et al*., 1997)

score	Categories	Water quality class	Saprobic level
<1.3	I or better	-no or very little impact	oligosaprobic
1.4-1.7	I-II	- little impact	oligo to betamesosaprobic
1.8-2.1	II	-moderate impact	betamesosaprobic
2.2-2.5	II-III	-moderate to strong impact	beta to alphamesosaprobic
2.6-3.0	III	- strong impact	alphamesosaprobic
3.1-3.4	III-IV	- strong to very strong impact	alphameso to polysaprobic
>3.5	IV	-very strong impact	polysaprobic

2.7.5 Mae Sa Index (Pekthong, 2002), and Ping and Nan Index (Kunpradid, 2005)

These method was adapted from Kelly (2000). Mae Sa Index and Ping and Nan Index are a measure of the effect of nutrients (predominately phosphorus) on stream communities, whilst the latter is a more general measure of water quality (Table 5-6). Factors such as biochemical oxygen demand, ammonia, salinity and nutrients were taken into account. Trophic Diatom Index is computed in the same way, using a "weight average" equation (Zelinka and Marvan, 1991 cited by Kelly, 2000). The formula for this equation is:

$$WMS = \frac{\Sigma avs}{\Sigma av}$$

Where a = relative abundance (proportion) of species in the sample

v = the indicator value (1-3)

s = pollution sensitivity (1-5) of the species

The Mekong River and its tributaries Diatom Index in this research was also developed based on this formula, as shown in Table 5 and 6.

Table 5 The six classes of alkalinity, conductivity, nitrate nitrogen and SRP and the scores for calculation the Mae Sa Index (modified from Kelly, 2000).

Scores	1	2	3	4	5	6
Alkalinity (mg.l^{-1})	<50	50-100	100-150	150-200	200-500	>500
Conductivity (µS.cm^{-1})	<50	50-100	100-250	250-500	500-1000	>1000
Nitrate nitrogen (µg.l^{-1})	<10	10-100	100-1000	1000-5000	5000-10000	>10000
SRP (µg.l^{-1})	<10	10-35	35-100	100-350	350-1000	>1000
Trophic Status	Oligotrophic	Oligo-mesotrophic	Mesotrophic	Meso-eutrophic	Eutrophic	Hyper-eutrophic

Table 6 The seven classes include ammonium nitrogen, nitrate nitrogen and SRP and the scores for calculating the Ping and Nan Index (modified from Kelly (2000), Lorraine and Vollenweider (1983), Wetzel (1983) and AARL water quality standard

Scores	1	2	3	4	5	6	7
BOD (mg.l^{-1})	<0.5	0.5-1.0	1.0-2.0	2.0-5.0	5.0-10.0	10.0-20.0	>20
Nitrate -N (mg.l^{-1})	<0.01	0.01-0.1	0.1-1.0	1.0-5.0	5.0-10.0	10.0-20.0	>20.0
Ammonium-N (mg.l^{-1})	<0.01	0.01-0.05	0.05-0.2	0.2-0.5	0.5-1.0	1.0-5.0	>5.0
SRP (mg.l^{-1})	<0.01	0.01-0.03	0.03-0.1	0.1-0.35	0.35-1.0	1.0-3.0	>3.0
Trophic Status	oligo saprobic	oligo-betameso saprobic	beta-meso saprobic	beta-alfa mesosaprobic	alfa-meso saprobic	alfa-poly saprobic	poly saprobic