CHAPTER 2

LITERATURE REVIEW

2.1 Origin of benthic diatoms researching

The origin of the study of benthic diatoms began in 1703 by an English man who was looking at the roots of the *Lemna* sp. plant. He used a simple microscope and documented many of the intricate branches and exact squares. Presently, his descriptions and diagrams are believed to likely refer to *Tabellaria flocculosa*. The next known recording of benthic diatom data were put down by Baker in 1753, some of Baker's illustrations describe two plastids, the central bridge of cytoplasm identifying the nucleus and two granules that were likely *Craticula cuspidata*. Various descriptions of diatoms occurred in the second half of the 18th century and gave a Latin binomial reference. The work of Muller (1783) is worth a special mention because it was one of his species, *Vibrio paxillifer*, which served as an example of the first recorded diatom genus *Bacillaria* Gmelin to Muller, *V. paxillifer,* while the other *Vibrio* species were animals; he named them *animalcula infusoria*. Muller recorded *Vibrio paxillifer* into the same classification as ciliates, amoeba, volvox, dinoflagellates (Round *et al.*, 1990). The first real advances in the field came in the early 19th century when diatoms were in favor with microscopists utilizing the emerging improvements in microscope resolution, many monographs of diatom genera on a world-wide basis were done by Grunow and Cleve. In the early 20th century, fossil diatoms were first studied and most famously, Hustedt, produced the taxonomic and ecological study of diatoms which remains a key reference today. Probably the most complete treatment of diatoms is that of Round *et al*. (1990). Nowadays, various papers have been published on diatom physiology, diversity, biochemistry, ecology and the role of diatoms in the geochemical cycling of various elements, especially silicon, diatom genetics and genecology. Moreover, the study of diatoms in the present day has focused on the many topics of monitoring the environment and the establishment of a the diatom index in many countries around the world (Round *et al*., 1990)

2.2 Information and characteristics of diatoms

Diatoms have been identified as microscopic unicellular eukaryotes, photosynthetic algae, and may include colonial or pseudo-filamentous algae (Round *et al*., 1990; Lee, 2008). Diatoms are characterized by a silica-impregnated cell wall (frustule) and are known to be amongst the most common benthic primary producers, along with Cyanobacteria and Chlorophyta that are typically found in lakes and lake-type water bodies (Stevenson 1996). They are typically found in the soil as well as most aquatic environments. These include both fresh water and marine water ecosystems (Barber and Haworth, 1981). They have been classified as being non-motile and display only a limited degree of movement along a substrate, which is accomplished by secretion of mucilaginous material emerging from a slit-like groove or channel known as a raphe. Because they are considered autotrophic, they are limited to the photic zone (these include water depths down to about 200 m and their presence depends on the degree of water clarity). Benthic and planktonic forms are known to exist (Round *et al*., 1990). The size of the diatoms is usually between 5-500 µm in diameter or length; however, on occasion, they can reach up to 2 ml. in length (Barber and Haworth, 1981).

Formerly, diatoms have typically been classified into the Division Chrysophyta, Class Bacillariophyceae (Bold and Wynne, 1985). The Chrysophytes are an algae species that form endoplasmic cysts. This species of algae do not store starch but rather store oils. Additionally, at some stages of their life cycle, they possess a bipartite cell wall and secrete silica.

Diatom frustules are decorated in a significant manner and appear in a remarkable array of forms. The diatom frustule's shape is noticeably specific to the species. Originally, the evolutionary relationship of the diatoms with regard to their taxonomic identity was always based on the silica frustule. However, recently this has changed (although there are exceptions). Generally speaking, we can classify diatoms into their principle groups. The following descriptions identity those groups: 1) centric diatoms or Coscinodiscophyceae possess valves that display radial symmetry (Figure 1) pennate diatoms or Bacillariophyceae have valves displaying bilateral symmetry (Figure 2). The centric diatoms are incapable of movement, but it is possible for some pennate diatom.

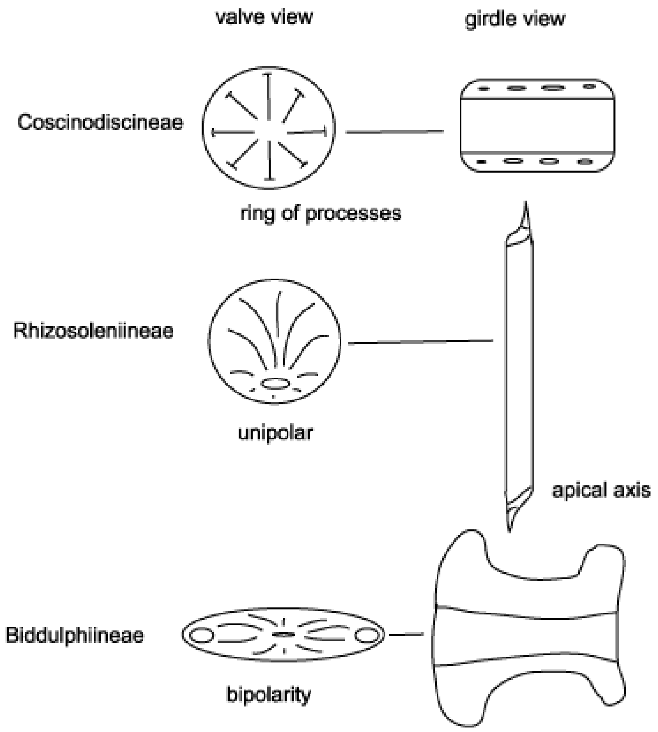

Figure 1 Schematic diagram of centric diatoms
Source: Hasle and Syvertsen (1997)

The diatom frustule structure has been investigated in significant detail through examinations using light and electron microscopy. Through laboratory cultures and in their natural habitats, diatoms have been deeply researched by diatomists. Consequently, there is a significant amount of information on both their biology and ecology.

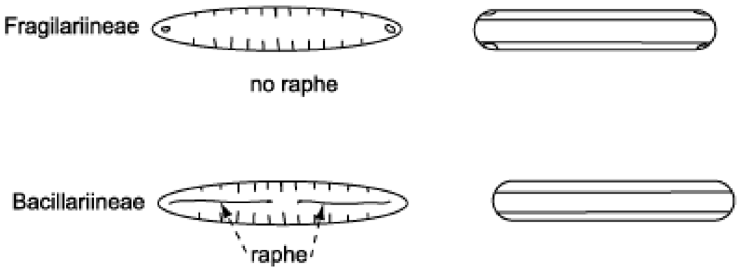

Figure 2 Schematic diagram of penate diatoms.
Source: Hasle and Syvertsen (1997)

The diatoms' protoplast is made up of a cytoplasmic layer lining the interior of the frustule and surrounding the large central vacuole. There is a diploid nucleus and two to several pigment-bearing plastids located within the cytoplasmic layer. The diatom frustule (Figure 3) is typically compared to an agar dish with an epitheca (a larger upper valve), and a hypotheca (smaller lower valve). The epicingulum refers to the vertical lip or edge of the epitheca, while the epicingulum fits over (slightly overlaps) the hypocingulum of the hypotheca. The girdle is made up of several connective bands joining the epicingulum and hypocingulum. Diatoms are typically heterovalvate, meaning that the two valves of the frustule are dissimilar. This is extrtemely apparent within the family Achnanthaceae because only one of the valves has a raphe and the other does not, and the Family Cymatosiraceae because one valve utilizes a tubular process while the other does not. Species that are chain-forming with cells linking together by siliceous structures usually possess separation valves. However, these valves are morphologically dissimilar to the valves that are located within (Round *et al.*, 1990 and Cox, 1996).

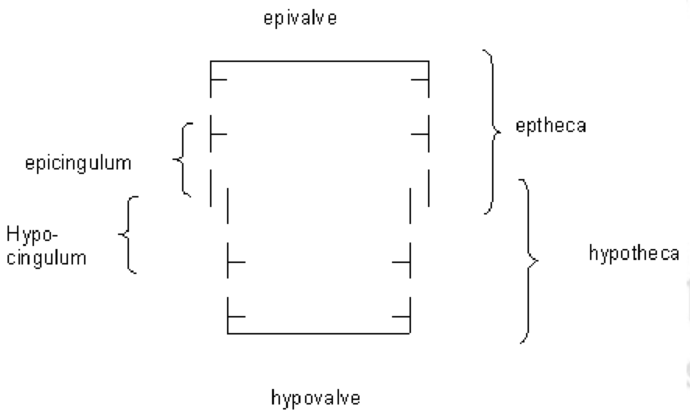

Figure 3 Schematic diagram of frustule terminology
Source: Round *et al.* (1990) and John *et al.* (2002)

2.3 Life cycle and cell reproductive properties of diatoms

Diatoms are classified as being capable of sexual reproduction by an amoeboid isogamy or auxospore and of performing asexual reproduction through anisogamy or oogamy (Chapman and Chapman, 1973). The reproduction of diatoms is considered primarily asexual via binary fission. After a cell is divided, each new cell grows from the epitheca valve of the parent frustule. After ten to twenty minutes, new growth establishes its own hypotheca. This process can often happen between one and eight times per day. The vegetative reproduction rate is limited by the availability of the amount of dissolved silica; however, this method has been known to significantly reduce the average size of the diatom frustule. There always exists a specific threshold where restoration of the size of the frustule becomes a necessity. Auxospores are primarily produced as cells that possess a unique cell wall structure that lacks a siliceous frustule but grows to the full frustule size (Figure 4). The auxospores of this species form an initial cell, which subsequently forms a new frustule of full size inside itself. Most neritic planktonic diatoms possess both a vegetative reproductive phase and a thicker walled resting cyst or what is known as a statospore stage. Typically, the siliceous resting spore develops after the active vegetative reproductive phase when nutrient levels are reduced. Upwelling that takes place annually is consequently an integral element of the life cycle of many diatoms. This is because it is a provider of nutrients as well as a mechanism of transformation, which produces statospores as well as related vegetative products being pushed up into the photic zone (Hasle and Syvertsen, 1997).

The morphology of the resting spore of some species is very much like that of the known corresponding vegetative cell. However, in alternate species, both the vegetative cells and the resting cells differ significantly. The two valves that are present on a resting spore are often similar but may be significantly different. Routinely, the first valve that becomes formed is closer to the valves of the vegetative cells than they are to the second valve. Within the same species, the diversity of the valve types requires care in identification work and the use of clean diatom material (John *et al*, 2002).

Generally diatoms attain their maximum dimension by the formation of auxospores. These are typically formed through sexual or asexual reproduction. Rather than possessing a rigid cell wall, an auxospore is a unique type of cell that possesses silica

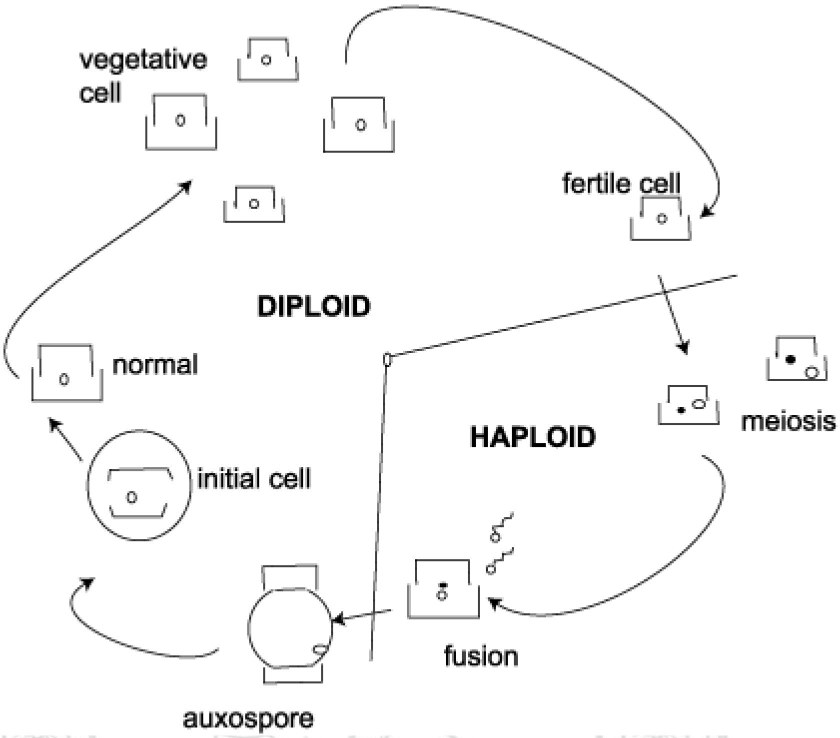

Figure 4 Diagram showing the simplified life cycle of a typical diatoms.
Source: Hasle and Syvertsen (1997) and John *et al*. (2002)

bands (perizonia). The cell is allowed to grow to its maximum size by the perizonium, and it then produces a frustule according to the normal cell morphology (Hasle and Syvertsen, 1997).

2.4 Habitat and adaptation of diatoms

Benthic diatoms grow while attached to surfaces. They are often attached to a rock or aquatic plant. The frustules of these species are typically shaped in a way that aids attachment. These frustules are typically arched or curved in order to fit smoothly into the stem of some aquatic moss. Some diatom species are known to form a stalk, which can be attached to a surface. Some of the species form short stalks, or mucilage pads. Other species form more long branching stalks. The stalks function so as to hold the cells in place, which makes them resistant to waves or high flow in rivers. Stalks can also allow the plant to obtain nutrients from the water (Smol and Stoermer, 2010).

In all aquatic habitats and in certain moist and dry habitats, diatoms have been found when the appropriate conditions of temperature, light, nutrients and chemicals are present for their growth. In both marine environments and fresh water, diatoms are abundant in terms of phytobenthos and phytoplankton. Benthos are more diverse than plankton, both in terms of the life forms that they are present in and the species number. Benthic diatoms are typically characterized by a brown color and are found to be growing on various types of substrates, such as gravel, cobblestones and boulders. These are known as epilithic diatoms. Additionally, diatoms can grow on sand grains, plants and animals and these are called episammic, epiphytic and epizoic diatoms, respectively. Benthic diatoms possessing various modes of attachment to different substrates can be divided into two categories. In the first category, diatoms are closely pressed to the substratum, as in the genus of *Amphora*, *Cocconeis* and *Epithemia*. In the second category, the substrata are attached to the valves by stalks, and this is common in the genus of *Gomphonema*, *Cymbella* and *Achnanthes* (Round *et al*., 1990; Stevenson *et al*., 1996; Lee, 1999). The environmental physical and chemical factors are considered the significant factors for the growth and survival of benthic diatoms. Because light is necessary for photosynthesis, benthic diatoms grow in a photosynthetic zone. Another one of the main physical factors that results in the occurrence of different species growing difference latitudes is temperature. In terms of the chemical factors, the nutrient requirements of benthic diatoms are not unlike those of most other plants. Phosphates, ammonium and nitrate nitrogen are very useful elements for the growth of benthic diatoms, while magnesium, potassium, sulphates, calcium, iron and silicon have also been found to be important elements in their growth (Patrick and Reimer, 1966).

2.5 River (running water ecosystems)

The natural watercourses that are known as rivers or other running water bodies that are typically comprised of freshwater and usually flow toward the ocean, or into a lake, sea or another river. The river ecosystem is considered a system that operates in its natural environment, and facilitates the biotic (living) interactions that exist between plants, animals and micro-organisms living in that water body. They also support the abiotic (nonliving) physical and chemical interactions that exist between these organisms (Frissel *et al.*, 1986). River ecosystems are a very important example of lotic ecosystems (Allan, 1995). Lotic ecosystems and related lotic systems, such as streams and springs, are classified as river-ecosystems. Lentic ecosystems involve relatively still terrestrial waters such as lakes and reservoirs or dams and can be contrasted with lotic ecosystems. These two fields form a broader study area of freshwater ecology. The characteristics that make the ecology of running water bodies unique from other aquatic habitats include water flow, water temperature, and light (Gordon *et al*., 1992; Giller and Malmqvist, 1998).

The most significant factor in lotic ecosystems is water flow or water velocity and this influences the ecology of these water bodies (Gordon *et al*., 1992). The strength of the water flow can vary between different systems. The systems may range from torrential rapids to slow backwaters that seem closer to lentic systems. The mean flow rate vector is based on the variability of the amount of friction that is measured against the bottom or the sides of the channel, and also involves sinuosity, obstructions, and the specific incline gradient of that water body (Allan, 1995). In addition, flow rate is affected by the amount of water that is routinely input into the system from either direct precipitation and/or groundwater. The shape of the streambed can be altered by flowing waters through erosion and deposition, and this creates a variety of habitats, which include riffles and pools (Cushing and Allan. 2001).

Water temperature varies in terms of seasons and from day to day in accordance with air temperature, but temperatures generally increase as we travel downstream because the cooler, higher elevation climate is displaced by the climate that pervades at lower elevations. Water is heated or cooled in a naturally occurring cycle and all living organisms rely on this system. Shallow streams are usually well mixed and the water temperature usually remains stable within them. However, in deeper, slower moving water systems, a significant difference may develop between the bottom and the surface temperatures. Strong diurnal fluctuations and seasonal variations are seen in many systems and some of the most extreme are found in arctic, desert and temperate systems (Allan, 1995). The temperature of lotic ecosystems can also be influenced by the amount of shading, climate and elevation (Giller and Malmqvist, 1998).

Light has a significant effect on running water systems because it provides the necessary energy to facilitate primary production through photosynthesis and it can also provide shelter for species that are prey for predators in shaded areas. Surrounding forests will shade, for example, areas that surround a small stream. The external variables in large river systems can be minimized because they are fairly wide and the sun can reach the surface. Particles in the water increasingly affect light in the deeper waters of rivers, so they are often more turbulent (Cushing and Allan. 2001). Seasonal and diurnal factors might also play a role in light. The availability of light is also affected by seasonal and other diurnal factors because the angle at which the light hits the water results in light being lost through reflection. Cloud cover, altitude, and geographic position also can affect light availability (Brown, 1987).

The most important chemical constituent of lotic systems is probably oxygen because all aerobic organisms require it for survival. Oxygen enters and mixes with the water at the point of contact. Streams and other fast moving waterways present more surface area to the air so they usually have lower temperatures and are more oxygenated than slower moving water bodies (Giller and Malmqvist, 1998). Because oxygen is a byproduct of photosynthesis, systems that reveal a high abundance of plants and aquatic algae also will reveal a high concentration of oxygen in the daytime (Wetzel 2001). During the night, these levels can significantly decrease when primary producers of oxygen switch to respiration. When circulation is poor, oxygen can be limited because usually the activity of lotic animals is very high at this time, or because of a large amount of organic decay (Cushing and Allan. 2001).

The geologic material composes most of the inorganic substrates in lotic ecosystems and is present in the catchment areas where it is eroded, transported and deposited by the current. As a matter of routine, inorganic substrates are classified by size on the Wentworth scale. The substrate scale ranges from boulders, to pebbles, to gravel, to sand, and to silt (Giller and Malmqvist, 1998). Particle size usually decreases downstream amidst larger boulders and stones in area that are mountainous and along the sandy bottoms of lowland rivers. This is because mountain streams have higher gradient slopes, which facilitate a faster flow of water and move smaller substrate materials downstream where they deposit (Cushing and Allan. 2001).

2.6 Reservoir (standing water ecosystems)

Around the world, reservoirs are considered important water sources in many countries. Reservoirs are artificially formed, usually by dam constructions across a river or by diverting a part of the river for the purposes of storing the water in a catchment area known as a reservoir. Upon completion of a dam, the river collects behind it and the artificially created basin fills (UNEP 2000). Both seasonally occurring water runoff and precipitation feed the reservoir. Reservoirs can be big artificial lakes or small pond-like water bodies. Reservoirs or standing water ecosystems are principle examples of lentic ecosystem as they range from ponds to lakes to wetlands. Lentic ecosystems and lotic ecosystems can naturally be compared. Light, temperature, oxygen and phosphorus along with other physical and chemical characteristics are of primary interest for most reservoirs (Dortch, 1997). In lentic ecosystems, light makes up the most significant energy source through photosynthesis, (Brönmark and Hansson 2005). Small reservoirs often have areas that are shaded by surrounding trees and cloud cover often affects the availability of light in all systems, no matter how big or small they are. Once the surface has been penetrated by light, the light may then be further scattered by particles that are suspended in the water. This can decrease the total amount of light in deeper areas (Kalff 2002). Reservoirs are typically divided into photic and aphotic regions. Photic regions receive sunlight and aphotic regions, which are below the area that is penetrated by light, are void of any photosynthetic activity (Brönmark and Hansson, 2005). With regard to the zoning of lakes, the pelagic and benthic zones are within the photic region, while the profundal zones are conclusively in the aphotic region (Brown 1987).

In lentic ecosystems, temperature is a critical physical factor because most of the biota are poikilothermic. Internal body temperatures are determined by the systems they are surrounded by. Light intensity typically determines water temperature. Stronger degrees of light intensity increase both the temperature and the photosynthesis process in the ecosystem (Ratanapanee, 1990). Water is naturally heated or cooled through the water cycle (Giller and Malmqvist 1998). Warmer waters often exist at the surface, while cooler waters are typically found at the bottom. Additionally, temperature fluctuations can vary greatly in these systems, both diurnally and seasonally (Brown 1987).

Oxygen is necessary for respiration in all human and organisms. The amount of oxygen that is present in standing waters is dependent on: 1) the air that is exposed to the transparent water, 2) the amount of water that circulates within the system and 3) the magnitude of oxygen that is generated and used by any organisms present in the water body (Brown 1987). Plants are typically plentiful in shallow water bodies because there are usually high fluctuations in the levels of oxygen. Extremely high concentrations of oxygen are often present during the day as a result of photosynthesis and values that are very low often occur at night because of the respiration from primary producers. In larger reservoirs, the amount of oxygen present can also be affected by the thermal stratification that occurs in different zones (Smith, 1990). Epilimnion is known to be oxygen rich because it can circulate quickly, gaining oxygen through contact with the air. Fewer green plants exist in the hypolimnion, and as a result, there is less oxygen released via the process of photosynthesis (Brown 1987). The profundal zone is characterized by low oxygen levels due to an accumulation of decaying vegetation and animal matter that falls down from the pelagic and benthic zones. There is also an inability to support the primary producers because of a lower oxygen level (Brown 1987).

2.7 The factors that influence the distribution of benthic diatoms

The survival of benthic diatoms is directly related to the physical and chemical factors in the environment. Temperature is one of the most significant physical factors, as it causes different species to occur at difference latitudes and in a variety of currents. Because light is necessary for photosynthesis, benthic diatoms are required to remain in a photosynthetic zone. With regard to chemical factors, the nutrient requirements of benthic diatoms are quite like those of most other plants. Phosphates and nitrogen (usually in the form of ammonium and nitrate) are known to be essential elements for the growth of benthic diatoms, while sulphates, calcium, magnesium, potassium, iron and silicon are also known to be elements of significance (Patrick and Reimer, 1966).

Each ecosystem and its ecological dynamic display an impact on the relationship that exists between 3 principal factors, such as the physical, chemical and biological factors. In running water, most algae live in the benthic form (Hynes, 1970). The algae typically grow on any surface of the substratum. All varieties of substratum rock, sand, silt and mud are affected by the behavior of benthic algae (Panha, 1998). The current velocity and the volume of water are related to the substrate type (Peerapornpisal *et al*, 2002); therefore, the substrate characteristics will directly impact upon the distribution of the benthic diatoms (Chapmand and Chapman, 1973). In the rocky and cobblestone substrates, the distribution of the benthic algae is usually in high abundance. Shade not only decreases the light intensity, but it also decreases turbidity, which is another very important factor. Turbidity values are dependent on the suspended particles that are present in the water-bodies and that also obstruct the amount of light that permeates throughout the water-body. High turbidity levels result in low light being emitted throughout the water and limits some groups of algae in terms of growth (Kochasaney, 1993). Additionally, running water typically displays a higher velocity of water than the standing water body. Fast flowing and sturdy currents result in more particles being dissolved in different forms than occur in the standing water of reservoirs and dams (Hynes, 1970). Typically, the morphology of the filamentous algae is primarily affected by the measurement of the velocity of the water. In some instances, certain groups of algae are found in fast flowing water (Patrick, 1948)

Chemical factors such as pH value and alkalinity are other significant factors that induce diatom distribution. These factors are limiting factors for some diatom distribution. Certain species of benthic diatoms, such as *Mastigloia smittii, Cymbella pusilla* and *Rhopalodia gibba* can be found in limited amounts in areas with high pH values, whereas *Eunotia exigua, Brachysira seriams, Frustulia megaliesmontria, Navicula affeari* and *N. frustiva* are often found in areas having low pH values (Kwandrons 1993). While the species *Navicula aumbriensis* can often be found in water bodies with low alkalinity levels (Hattunem and Turkia, 1990).

The dissolved components outside the environment in running water ecosystems were increased as a result of the dynamics of the water body (Wetzel, 2001). The Soluble components found in the water bodies can be measured in a variety of ways, such as by conductivity. The measurement of conductivity is revealed by the concentration of soluble substances that are recorded in the water body (O'neil, 1993). Conductivity was used to gauge the discharge of outer substances into the ecosystem (Sirisingh, 1982). The level of dissolved oxygen (DO) is another factor that is affected by the movement of the water. The amount of DO that is found to be present in standing water is dependent on the process of photosynthesis by aquatic plants and phytoplankton, but the DO concentration recorded in running water also depends on the amount of diffused oxygen and the mixing that occurs when the water body is flowing. Consequently the amount of DO in running water is usually higher than that found in standing water (Klapper, 1991). Another useful application of the measurement of DO concentration is the level of Biological Oxygen Demand (BOD). BOD is indicative of the measurement of the concentration of organic substances found to be present in the water. Clean water will reveal a low level of BOD, but waste water will typically be present in a high level of BOD (Klapper, 1991).

Most of the dissolved substances found in the water are the essential elements needed for algal growth. Each species of algae normally requires different amounts of macronutrient levels and micronutrient levels. Most of the nutrients in running water occur from run off (Meybeck, 2003). Most of the elements that are necessary for the growth of algae, are considered fundamental nutrients, which can include phosphorus and nitrogen compounds. The distribution of algae is dependent on the concentration of nutrients. In this type of aquatic ecosystem, nitrogen components are known to be the element in the highest concentration. Nitrogen is the principle element in the soil, which is easily discharged into reservoirs (Wetzel, 1983). Whereas, in tropical ecosystems, ERA (1997) reported that nitrogen and phosphorus compounds were the nutrients present in the highest concentration in the Mae Sa Watershed which is a Ping River tributary. The high concentration of nitrogen compounds is not only caused by soil erosion and run off, but is also a result of the presence of fertilizers and human waste.

Nitrogen is a significant source as it is a primary producer in aquatic environments (Goldman and Horn, 1983). Additionally, the amount of algae present is directly related to any nitrogen compounds present, such as those in the ammonium forms, which are more commonly used by algae than any other compound (Darley, 1982). Algae uses ammonium as a primary nutrient, but nitrate nitrogen has also been shown to be the most common nitrogen compound found in aquatic ecosystems because nitrates are diluted and discharged through both run off and erosion (Moss, 1998). Nitrogen compound concentrations are very important for algae. This is true with *Melosira varians, Synedra ulna* and *Navicula viridula*, all of which are often found to possess high levels of nitrate concentration at 2.0-3.0 mg/1 (Barnett, 2000). It was revealed by Underwood and Provot (2000) in their work that 4 isolated diatoms; *Navicula phyllepla, N. perminula, N. salinarum* and *Cylindrotheca clostium*, which were identified as being the dominant species in environments that are known to be high in ammonium and nitrates, while *N. phyllepta* was the dominant species in the environment with low nitrate concentrations.

Similarly, phosphorus is the primary nutrient compound that is present in rivers. Most of the phosphorus compound that is found in the water originates from household waste, detergent and fertilizers (Sirisingh, 1982). Phosphates that are found in water occur in 3 forms which are: orthophosphate or soluble reactive phosphorus (SRP), polyphosphate and organic phosphates (Kochasaney, 1993). The amount and distribution of algae is directly affected by SRP because this derivative phosphorus is a key element in the metabolism process. The energy conversion process in algae is dependent on the phosphorus level, especially in terms of the specific energy form as well as nucleic production (Wongrat, 1998). The forms of phosphates that are found in ecosystems are identified as orthophosphate and organic phosphates. Likewise, the non-reactive phosphorus that is present in the sediment can be re-mineralized into its reactive form (Brönmark and Hansson 2005). Sediments are known to be richer in phosphorus than mere lake water; however, this would indicate that the phosphorus would likely have had a long period of residency before it is re-mineralized and gets re-introduced back into the system (Kalff, 2002). Regardless, a significant volume of phosphorus discharges is likely another cause of eutrophication and algal blooms (Shapiro and Wright, 1984).

2.8 Study of diatoms in Thailand

Previously, diatoms were studied exclusively by western scientists. However, the study of diatoms in Thailand began in 1902 by Östrup who was a Danish botanist and who reported 81 different diatoms from collection sites on Koh Chang Island, in the Gulf of Thailand (Östrup, 1902). In 1936, Patrick reported a total of 185 diatom species in a study of the intestinal contents of tadpoles found in Thailand and the Federal Malay States (Patrick, 1936). Moreover, the Joint Thai-Japanese Biological Expedition to Southeast Asia collected samples of diatoms between 1961 and 1962. Hirano identified the samples and then published an account of 143 diatom species, and 114 of them were recorded in the samples collected from Thailand. The majority of these samples were collected from sites in Chiang Mai and the others were collected from localities in the central and southern parts of Thailand (Hirano, 1967). In 1971, Foged reported on the freshwater diatoms found in Thailand that had been collected in the central and northern parts of Thailand in 1966 and a total of 378 taxa were published. In this work, Foged reported an additional 8 new species, 5 new varieties and 2 forms (Foged, 1971).

Subsequently, the study of diatoms by Thai biologists began in 1995. Wongrat published a document entitled “The Check List of the algae in Thailand” (Lewmanomont *et al*., 1995). This checklist was prepared through a merging of various publications that included survey reports, scientific papers and relevant results that were published by 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 arranged from information found in 53 publications of both freshwater and marine algae. The diatom flora was classified in Division Chromophyta, Class Bacillariophyta. A total of 46 genera, 385 species, 144 varieties and 43 forms of diatoms were recorded in this publication.

The phytoplankton that was present in the reservoirs of the Huai Hong Khrai Royal Development Study Centre in Chiang Mai Province was studied by Peerapornpisal in 1996. *Aulacoseira granulata* and *Cyclotella meneghiniana* were reported as the species of diatoms that were found in this reservoir.

In 2003, Hunpongkittikul studied the distribution of phytoplankton in the Pasak Jolasid Reservoir, Lop Buri Province. *Aulacoseira granulata* was found in this reservoir and was presented as a dominant species during the rainy season (August).

Pekthong studied the biodiversity of benthic diatoms and their application in monitoring water quality of the Mae Sa Stream, Doi Suthep-Pui National Park, Chiang Mai in 2002, the 34 genera complied with 278 species of benthic diatoms were found and 51 species were added to the new records of Thailand. Moreover, *Gomphonema pumilum* var. *rigidum* E.Reichardt & Lange-Bertalot, *Eunotia minor* (Kützing) Grunow and *Gomphonema clevie* Fricke were found at upstream sites as a species indicator of clean water. While, *Nitzschia palea* (Kützing) W. smith, *Achnanthes lanceolata* (Brébisson) Grunow, *Gomphonema parvulum* (Kützing) Grunow, *Melosira varians* Agardh, *Gyrosigma scalproides* (Rabenhorst) Cleve and *Bacillaria paradoxa* Gmelin were found to be a species for monitoring the polluted water quality (Pekthong, 2002).

The diversity of benthic diatoms in the area of the Golden Jubilee Thong Pha Phum Project, Thong Pha Phum District, Kanchnaburi Province were studied by Suphan in 2004. A total of 162 diatoms species were recorded and 45 species were reported as new records for Thailand. Additionally, it was found that *Achnanthes minutissima* Kützing and *Brachysira neoexilis* Lange-Bertalot could be used as indicators for clean to moderate water quality. Conversely, *Achnanthes biasolettiana* Grunow and *Gomphonema lagenula* Kützing could be used as indicators for moderate to polluted water quality (Suphan, 2004).

The diversity of benthic diatoms and their relationships with nutrient compounds in the Ping and Nan Rivers were studied by Kunpradid in 2005. A total of 103 diatoms species were recorded in the Ping River and 102 species were recorded in the Nan River. The dominant species in the Ping River were *Gomphonema parvulum* (Kützing) Kützing, *Nitzschia palea* (Kützing) Smith, *Achnanthes lanceolatum* (Brébisson) Grunow and *Bacillaria paradoxa* Gmelin, whereas the dominant species in the Nan River were *Cocconeis placentula* Ehrenberg, *Achnanthes crenulata* Lange-Bertalot, *Achnanthes lanceolatum* (Brébisson) Grunow and *Rhopalodia gibba* (Ehrenberg) Müller (Kunpradid, 2005).

The diversity of benthic diatoms in the Kham Watershed, Chiang Rai Province was studied by Inthasotti in 2006. A total of 167 diatoms species were recorded and 32 species were reported as new records for Thailand. *Decussata placenta* (Ehrenberg) Lange-Bertalot, *Diadesmis paracontenta* Lange-Bertalot & Werum, *Nitzschia linearis* (Agardh) W.Smith and *Surirella elegans* Ehrenberg revealed a positive correlation with BOD5 and *Hippodonta hungarica* (Grunow), whereas Lange-Bertalot revealed a positive correlation with DO. Additionally, *Gomphonema lagenula*, *Navicula symmetrica* Patrick and *Nitzschia palea* were used to indicate the mesotrophic to eutrophic status (Inthasotti, 2006).

The diversity of the benthic diatoms found in the Ping River of northern Thailand were studied by Leelahakriengkrai in 2007. This study reported a total of two orders, forty genera and one hundred and twenty eight species of benthic diatoms. A positive correlation of the water quality was observed with benthic diatoms and DO with *Encyonopsis leei* var. *sinensis* Metzeltin & Krammer, BOD with *Cymbella* sp. 1, nitrate nitrogen Generally diatoms attain their maximum dimension by the formation of auxospores. These are typically formed through sexual or asexual reproduction. Rather than possessing a rigid cell wall, an auxospore is a unique type of cell that possesses silica *Achnanthes biasolettiana* Grunow, *Rhopalodia gibberula* Ehrenberg O. Müller and *Rhopalodia gibba* Ehrenberg O. Müller and TDS with *Synedra ulna* var. *aequalis* (Kützing) Hustedt. Additionally, *Nitzschia palea* (Kützing) W. smith, *Nitzschia* sp. 2 and *Synedra ulna* var. *aequalis* (Kützing) Hustedt were acknowledged as indicator species for moderate water quality, while *Cymbella turgidula* Grunow, *Gomphonema lagenula*

Kützing and *Navicula symmetrica* Patrick were recognized as the indicator species of clean to moderate water quality (Leelahakriengkrai, 2007).

The diversity of the benthic diatoms found in the Mekong River (in specific parts of Thailand), and their applications for water quality monitoring, were studied by Pruetiworanan in 2008. Consequently, a total of 42 genera and 168 species of benthic diatoms were found. These included *Achnanthes minutissima* Kützing, *Cymbella turgidula* Grunow, *Nitzschia dissipata* (Kützing) Grunow, *Cyclotella stelligera* Cleve, *Gomphonema lagenula* and *Aulacoseira granulata* Ehrenberg, all of which were found to be capable of monitoring clean to moderate water quality (Pruetiworanan, 2008).

In 2009, Leelahakriengkrai studied the diversity of benthic diatoms in the Mekong River passing through Thailand and their application for water quality monitoring, about 42 genera and 168 species of benthic diatoms were found and *Achnanthes minutissima* Kützing, *Cymbella turgidula* Grunow, *Nitzschia dissipata* (Kützing) Grunow, *Cyclotella stelligera* Cleve , *Gomphonema lagenula* and *Aulacoseira granulata* Ehrenberg, all of which could be used to monitor the clean to moderate water quality. (Leelahakriengkrai *et al.*, 2009)

Benthic diatoms were studied by Suphan and Peerapornpisal in terms of their potential for applications in the monitoring of the water quality of the Mekong River in 2010 in specific parts of Thailand. A total of 252 species were found to belong to 53 genera and 53 species were revealed as new records for the country of Thailand. It was found that *Luticola goeppertiana* (Bleisch) D.G. Mann, *Eolimna minina* (Grunow) Lange-Bertalot and *Mayamaea atomus* (Kützing) Lange- Bertalot could all be used to indicate certain parameters of water quality, including high conductivity, high concentrations of SRP and BOD, respectively (Suphan and Peerapornpisal, 2010).

Leelahakriengkrai studied diatoms in 2011 from six of the main rivers of Thailand. Consequently, a total of 214 species of benthic diatoms were reported belonging to 3 classes, 6 subclasses, 13 orders, 28 families and 50 genera. In Thailand, the most abundant species of the six main rivers were found to be *Nitzschia palea* (Kützing) W. Smith, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cyclotella pseudostelligera* Hustedt, *Gomphonema lagenula* Kützing *Navicula cryptocephala* Kützing and *Navicula symmetrica* R.M. Patrick (Leelahakriengkrai, 2011).

As recently as 2014, the seasonal variations of the benthic diatoms of the Yom River were studied by Yana. In this study, a total of 164 species of benthic diatoms were recorded belonging to 31 genera. *Achnanthidium caravalense, Achnanthidium latepcephalum, Cymbella parva, Encyonema malaysianum, Encyonema yuwadii, Navicula jacobii* and *Navicula suprinii* were all acknowledged as the most abundant species (Yana, 2014).

2.9 Water quality assessment through the use of diatoms index

There has been a long history of the assessment of the conditions of water quality in both running and standing water using benthic diatoms. Diatoms were first used as bioindicators in studies by Kolkawaitz and Marsson (1908); however, the findings of this study are now of minimal value and only a small number of diatoms have been associated with the stone's surface. In 1979, diatoms were used as bioindicators for water quality assessment fairly accurately by Lange-Bertalot (1979). In this study, diatoms were classified into 3 categories based on how sensitive they were to pollution: 1= the higher tolerant, 2= the tolerant and 3= the lower tolerant. Additionally, a study of diatom distribution in relation to organic pollution was attempted, particularly, with BOD. The above mentioned research study was conducted by Watanabe *et al* (1988). The use of diatoms as bioindicators has been successfully applied in various countries in Europe (Kelly *et al*., 1998; Lobo *et al*., 1998), North America (Stevenson and Pan, 1999), Asia (Rothfritz *et al*., 1997), South America Australia (Chessman *et al*., 1999) and Africa (Gasse *et al*., 1995). Diatoms in these countries have been applied to determine the hydro-chemical characteristics in lakes, while others were developed to monitor the current conditions in various bodies of water and have been even further developed into indices which can be used for the monitoring of routine water quality (Kelly, 2000).

Presently, the governments of many countries are applying bio-assessment programs which consider the analysis of diatom distribution as a version of pollution tolerance index (PTI). The use of diatom communities as a way to measure the health of the environment is done through the use of an index rating diatom taxa that takes into account their sensitivities. Barbour *et al.* (1999) outlined the level of tolerance of diatoms to pollution in a pollution tolerance index. This was done as one of a number of recommended metrics. The U.S. states of Kentucky (Metzmeier, 1991), Oklahoma (OCC, 1993) and Montana (Bahls, 1993) all have used some form of the pollution tolerance index (PTI) within their programs of diatom bioassesment. The majority of these indices have been based upon Lange-Bertalot (1979), who separated the taxa into three distinct categories based on: (1) tolerance to or (3) sensitivity to pollution. The second category (2) is used with taxa that do not have strong associations. The relative abundance of each taxa (ni/N) was then multiplied by its tolerance value (ti), and all data were then summed up (A). The result for each sample is presented as a composite value that represents the level of pollution tolerance of the community that is being sampled. Lange-Bertalot's designations were used to assign tolerance values, along with other autecological surveys, and other relevant water quality data, the equation is as follows:

$$\mathrm{PTI} = \frac{\sum(\mathrm{ni\ ti}\,)}{N} \qquad \text{(A)}$$

Where PTI is the total PTI value for the sample,

n_i is the number of organisms of that taxonomic breakdown (species in this instance).
t_i is the tolerance value for that taxon.
N represents the total number of organisms found at the site.

2.9.1 Trophic Index of Schiefele and Kohmann (Schiefele and Kohmann, 1993)

Trophic diatom index (TDI) of Schiefele and Kohmann identifies and characterizes the trophic status of running water using the indicator weight evaluation method and classifies the water into seven divisions of status (oligotrophic-hypereutrophic) (Table 1). Their work was developed mainly form the study of the calcareous streams and rivers in Germany. The sample index was calculated using the following formula (B);

$$\text{Sample index} = \frac{\sum \text{Relative Abundant X Index X Indicator weight}}{\sum \text{Relative Abundant X Indicator weight}} \qquad \text{(B)}$$

Table 1 Trophic status of Schiefele and Kohmann Trophic Index

Sample index	Trophic state	Level of impact
1.0 – 1.4	oligotrophic	no impact little
1.5 – 1.8	oligo - mesotrophic	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	eutro - hypereutrophic	strong impact
3.6 – 4.0	hypereutrophic	very strong impact

Source: Schiefele and Kohmann (1993)

2.9.2 The Trophic Index of Van Dam (Van Dam *et al*., 1994)

The trophic index of van Dam characterizes and identifies the trophic status of low land freshwater sites in the Netherlands by diatoms and also uses the indicator weight evaluation method and classifies the water into seven divisions of status (Table 2). The formula below was used to calculate the sample (C):

$$\text{Sample index} = \frac{\sum \text{Relative Abundant X Index}}{\sum \text{Relative Abundant}} \qquad \text{(C)}$$

Table 2 Trophic Index of Van Dam

Categories	**Species**	**Sample index**	**Trophic status**
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		

Source: Van Dam *et al*. (1994)

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

In 1977, Rot *et al* established the trophic index of running water in Austria by characterizing the benthic diatoms and by also using the indicator weight evaluation method and classified the water into seven divisions of status (Table 3). The sample index was calculated using the same formula of Van Dam applied in 1994 (D):

$$\text{Sample index} = \frac{\sum \text{Relative Abundant X Index X indicator weight}}{\sum \text{Relative Abundant X indicator weight}} \qquad \text{(D)}$$

Table 3 Trophic status of Rott *et al*. (Saprobic Index)

Score	Categories	Water quality class	Saprobic status
<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	alphameso to polysaprobic
>3.5	IV	very strong impact	polysaprobic

Source: Rott *et al.* (1997)

In Thailand, water quality assessment applications using benthic diatoms were studied in the early 20th century. The majority of the diatom indexes of Thailand were established by researchers at Chiang Mai University.

2.9.4 Mae Sa Diatom Index (Pekthong, 2002)

Mae Sa index was the first benthic diatom index of Thailand and was produced by Pekthong. This method was modified from the method of Kelly (2000), and the index was established on the Mae Sa Stream in the northern part of Thailand. A total of 25 of the most abundant species of benthic diatoms are listed in the Mae Sa index. The selected species are related to some physical and chemical factors, which included alkalinity, conductivity, nitrate nitrogen and SRP levels (Table 4). The Mae Sa index was calculated from the formula below (E):

$$\text{WMS} = \frac{\sum avs}{\sum av} \qquad \text{(E)}$$

Where a = relative abundance (proportion) of species in the
v = the indicator value (1-3)
s = pollution sensitivity (1-5) of the species

Table 4 The six classes and the scores for calculation of the Mae Sa Diatom Index

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	10-10	100-1000	1000-5000	5000-10000	>10000
SRP (µg.l^{-1})	<10	10-35	35-100	100-350	350-1000	>1000
Trophic Status	oligo trophic	oligo-mesotrophic	meso trophic	meso-eutrophic	eutrophic	hyper-eutrophic

Source: modified from Kelly (2000)

2.9.5 Ping and Nan Diatom Index (Kunpradid, 2005)

The Ping and Nan index was the first benthic diatom index applied in the rivers of northern Thailand. The 25 most abundant species of benthic diatoms are listed in the Ping and Nan Index. The selected species are related to some chemical factors, which include; BOD, ammonium nitrogen, nitrate nitrogen and SRP levels (Table 5). The sample index was calculated using the same formula as was used in the Mae Sa Diatom Index (E). This method was modified from Kelly (2000), Lorraine and Vollenweider (1983), Wetzel (1983) and Peerapornpisal *et al.* (2004).

2.9.6 Mekong Diatom Index (Suphan, 2009)

Improvements to the diatom indexes of Thailand have been made continuously by

Suphan (2009). The Mekong Diatom Index applied the methods used from previous studies. A total of 29 of the most abundant species of benthic diatoms are listed in the Mekong Diatom Index. The selected species are related with some chemical factors, which are; BOD, ammonium nitrogen, nitrate nitrogen and SRP levels (Table 6). The sample index was calculated using the same formula as was used in the Mae Sa index (E).

Table 5 The seven classes and the scores used for calculating the Ping and Nan Diatom index

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.	>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.	>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 meso saprobic	alfa-meso saprobic	alfa-poly saprobic	poly saprobic

Source: modified from Kelly (2000), Lorraine and Vollenweider (1981), Wetzel (1983) and Peerapornpisal *et al.* (2004)

Table 6 The seven classes and the scores used for calculating the Mekong index

Scores	1	2	3	4	5	6	7
BOD (mg.l^{-1})	<0.5	0.5-1.0	1.0-2.0	2.0-4.0	4.0-10.0	10.0-20.0	>20
Nitrate –N (mg.l^{-1})	<0.01	0.01-0.05	0.05-0.2	0.2-2.0	2.0-5.0	5.0-10.0	>10.0
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	0.1-0.30	0.30-1.0	1.0-3.0	>3.0
Trophic Status	hyper-oligo trophic	oligo trophic	oligo-meso trophic	meso trophic	meso-eutrophic	eutrophic	hyper-eutrophic

Source: Suphan (2009)

2.9.7 Thailand Diatom Index (Leelahakriengkrai, 2011)

The Thailand Diatom Index was established by Leelahakriengkrai (2011). It was carried out from the six main rivers of Thailand. The water properties and benthic diatoms, which revealed a high relative abundance (>1%) at each site, were selected to calculate the weight averages. A total of 104 species of benthic diatoms are listed in the Thailand Diatom Index. The selected species are related to some chemical factors, which are; BOD, ammonium nitrogen, nitrate nitrogen and SRP levels (Table 7). The sample index was modified from Kelly (2000), Lorraine and Vollenweider (1983), Wetzel (1983) and Peerapornpisal *et al.* (2004) and the Pollution Control Department (2010) (F).

$$WA_{jk} = \frac{\sum(Xij\ x\ Yjk)}{\sum Xij} \qquad (F)$$

Where WA_{jk} = the weight average of taxon j for water quality factor k
X_{ij} = the present relative of taxon j at site i
Y_{ik} = the k^{th} water quality factor at site i

Table 7 The seven classes and the scores used for calculating the Thailand Diatom Index

Scores	**1**	**2**	**3**	**4**	**5**	**6**	**7**
BOD (mg.l^{-1})	0.5	0.5-1.0	1.0-2.0	2.0-4.0	4.0-10.0	10.0-20.0	>20
Nitrate -N (mg.l^{-1})	<0.01	0.01-0.19	0.20-0.39	0.40-0.79	0.80-1.90	2.0-10.0	>10.0
Ammonium-N (mg.l^{-1})	<0.01	0.01-0.19	0.20-0.39	0.40-0.59	0.60-0.99	1.0-5.0	>5.0
SRP (mg.l-1)	<0.01	0.02-0.04	0.05-0.06	0.07-0.19	0.20-0.99	1.0-3.0	>3.0
Trophic Status	hyper-oligo trophic	oligo trophic	oligo-meso trophic	meso trophic	meso-eutrophic	eutrophic	hyper-eutrophic

Source: (Leelahakriengkrai, 2011)

2.9.7 Yom Diatom Index (Yana, 2014)

In 2014, the Yom Diatom Index was established by Yana (2014) as the most current diatom index at that time. This index was modified from Kelly (2000), Lorraine and Vollenweider (1983), Wetzel (1983) and Peerapornpisal *et al.* (2004). Benthic diatoms, which display a high relative abundance (>1%) at each site and revealed water properties for the establishment of this index were investigated from the Yom River in the north of Thailand. A total of 104 diatoms species are listed in the Yom Diatom Index. The selected species are related to some chemical factors, which are; BOD, ammoniam nitrogen, nitrate nitrogen and SRP levels (Table 8) The Yom Diatom Index was calculated from the formula below (G):

$$\mathrm{WA(Sp)} = \frac{\sum(\mathrm{En\ X\ Abund\ X\ IV})}{\sum(\mathrm{Abund\ X\ IV}} \qquad \text{(G)}$$

En_i = the value of the environment variable

Abund = abundance of species

IV = indicator of species

Table 8 The seven classes and the scores used for calculating the Yom Diatom Index

Scores	**1**	**2**	**3**	**4**	**5**	**6**	**7**
BOD (mg.l^{-1})	0.5	0.5-1.0	1.0-2.0	2.0-4.0	4.0-10.0	10.0-20.0	>20
Nitrate -N (mg.l^{-1})	<0.01	0.01-0.19	0.20-0.39	0.40-0.79	0.80-1.90	2.0-10.0	>10.0
Ammonium-N (mg.l^{-1})	<0.01	0.01-0.19	0.20-0.39	0.40-0.59	0.60-0.99	1.0-5.0	>5.0
SRP (mg.l-1)	<0.01	0.02-0.04	0.05-0.06	0.07-0.19	0.20-0.99	1.0-3.0	>3.0
Trophic Status	hyper-oligo trophic	oligo trophic	oligo-meso trophic	meso trophic	meso-eutrophic	eutrophic	hyper-eutrophic

Source: (Yana, 2014)