CHAPTER 5

GENERAL DISCUSSIONS AND CONCLUSIONS

5.1 General Discussions

In spite of the fact that much of the data already exists on the biology of diatoms in both lentic and lotic aquatic environments, little consideration has been given to diatoms that occur epiphytically in hot spring streams. These investigations were principally of a descriptive, taxonomic nature; however, relevant data on the seasonal succession of the species and their relative abundance were excluded. Hot springs are specific environments in which microbes can survive under severe conditions such as high temperature, pH, conductivity or alkalinity. This means that the type and number of diatom species present will be different from those of general ecosystems.

Benthic diatoms are important organisms as they assume a crucial role in the sustenance chain (Lamberti 1996). They react directly and rapidly to various ecological parameters, for example, topography (Stevenson 1997), existing velocity and available supplements (Potapova and Charles 2007). These reactions can shift as indicated by species physiology and the ability to display species–particular reponses to parameters while prompting an expansive board of assemblage compositions, as has been shown by the environmental states of the reservoir. In aquatic habitats including moist and dry environments, diatoms have been discovered when the suitable states of temperature, light, supplements and chemicals are available for their development. Diatoms are abundant with regard to phytobenthos and phytoplankton. Benthos can exist in greater variety than plankton, but both are life forms that are extant in terms of 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 develop while they are attached to surfaces. The frustules of these species are ordinarily molded in a way that guides connection. These frustules are regularly angled or bent with a specific end goal to fit easily into some aquatic plants. Some diatom species are known to shape a stalk, which can be attached to a specific surface. The different species structure had longer stretching stalks. The stalks hold the cells input, which makes them impervious to waves or high streams that are present in waterways. Stalks can likewise enable the plant to acquire supplements from the water (Smol and Stoermer 2010). Diatoms serving as bioindicators have been investigated for quite a while, but diatom examination has yet been accomplished for the purposes of recreation of the paleo–condition and water quality or considerations of ecological wellbeing.

Microbial community composition and diversity in terrestrial hot springs have been broadly studied, especially in Yellowstone National Park (YNP) (Blank *et al*. 2002; Meyer-Dombard *et al*. 2005; Spear *et al*. 2005; Hall *et al*. 2008; Miller *et al*. 2009; Osburn *et al.* 2011), Japan (Nakagawa and Fukui 2002-2003; Kubo *et al*. 2011; Otaki *et al*. 2012; Everroad *et al*. 2012), Great Basin (Costa *et al*. 2009; Dodsworth *et al*. 2011; Cole *et al.* 2012), Iceland (Hobel *et al*. 2005; Tobler *et al*. 2011), Thailand (Sompong *et al* 2005; Kanokratana *et al*. 2004; Purcell *et al*. 2007), and Russia (Bonch-Osmolovskaya *et al*. 1999; Belkova *et al*. 2007; Gumerov *et al*. 2011; Lebedeva *et al*. 2011). Most of these phylogenetic studies have focused on cyanobacteria while only a few of the studies focused specifically on diatoms. The results of these studies (Meyer-Dombard *et al*. 2005; Nakagawa and Fukui 2002; Everroad *et al.* 2012; Cole *et al.* 2012; Tobler *et al.* 2011; Purcell *et al*. 2007; Gumerov *et al*. 2011; Reysenbach *et al*. 2009; Jiang *et al*. 2010; Lau *et al*. 2006) have extended our insight into thermophilic microorganisms that regularly occupy thermal springs. To better understand diatom environments and capacities in geothermal niches, an expanding number of studies have tried to set up the linkage between diatom community composition/diversity and the relevant physicochemical conditions such as temperature, pH, and water chemistry (Cox *et al*. 2011; Skirnisdottir *et al*. 2000; Pearson *et al*. 2008). Among these ecological conditions, the impact of temperature on diatom community structures has received much attention. In another study, Miller *et al.* (2009) concentrated on the relationship between microbial diversity and temperature; typically, when the different microbial variety was conversely associated with temperature, or temperature was a key factor in controlling assorted microbial variety in hot springs.

From 1964 to 1967, the yearly varieties in terms of the relative abundance of diatoms in a thermal stream that can deplete a hot spring were inspected for changes in species diversity and redundancy. Despite large seasonal changes in abundance and shifts in the species composition, the values, in general, did not diverge significantly from the calculated mean diversity value. This relative constancy of species diversity was inferred as an indication of diatom community steadiness. The species revealed a characteristic pattern of seasonal abundance that is assumed be to moderately attributable to a light adaptation phenomenon (Stockner 1968).

Although microscopic diatoms are not significant contributors to biomass in springs, they are abundant (Poulícková *et al.*, 2004) and play a vital role in the functioning of these systems (Cantonati *et al.*, 2006). Diatoms have particular salinity, temperature, light, pH, and other natural requirements (Zalat and Vildary 2005). They are also influenced by the geochemistry of the substratum, conductivity, current velocity and shading (Cantonati *et al.*, 2006), which specifically influence their abundance and distribution. This examination broadened and enhanced information on the spring diatom groups of some of the hot springs situated in northern Thailand, and included a few further helpful perceptions. Seasonality was a principal factor in the first investigation in determining the variability of species composition along with the relevant physical and chemical factors, sampling sites and the distribution of selected hot spring diatom between the wet and dry seasons. A variation was found in the range of water chemistry at each sampling site. The non-significant differences of most of the physical and chemical characteristics and diatoms distribution in the springs were found between the wet and dry seasons ($p<0.01$). A total of forty–six species of hot spring diatoms were established with regard to the community diversity. These belonged to 2 classes, 14 orders, 18 families and 27 genera. Of these, four species; *Caloneis molaris* (Grunow) Krammer, *Craticula acidoclinata* Lange-Bertalot & Metzeltin, *Navicula subrhynchocephal*a Hustedt and *Pinnularia saprophila* Lange–Bertalot, Kobayashi and Krammer were determined to be new records in Thailand. The dominant genera according to high relative abundance (more than 1%) were *Diatomella* (41.7%) followed by

Achnanthidium (20.9%), *Anomoeoneis* (11.2%), *Rhopalodia* (6.4%), *Sellaphora* (5.7%), *Navicula* (2.9%), *Nitzschia* (2.4%) and *Craticula* (2.1%).

Hot springs are specific environments in which microbes can survive under severe conditions, such as high temperature, pH, conductivity or alkalinity that cause some types and numbers of diatom species to differ from those of the general ecosystem. A fluctuation of water temperature occurred at all sampling sites for 24 hours especially in SK sampling sites because a human disturbance. The benthic diatoms in this study could adapt to the changing temperatures over the period of a single day. Insignificant differences were found in terms of species richness or the diversity index values between epilithic and epiphytic assemblages, but temperature changes were the main environmental factors that influenced diatom assemblages. This means that a negative correlation with increasing water temperatures was found among the diatom assemblages. Even though absent in some samples, diatoms occur minimally in all parts of wetlands. This is true with regard to abundance, species composition and varying levels of diversity.

The NMDS ordination revealed the differences in species composition among the specimens collected from eight different hot springs and were in correlation with the existing environmental variables. Based on R^2 values, silicon dioxide (SiO_2), pH, conductivity, water temperature and total hardness, it was determined that there was a strong relationship between the environment and some species composition. Although the results indicated that physico-chemical factors could have an effect on some diatom speices, this was not the case except with *Diatomella balfouriana* Greville. This species seems to prefer lower temperatures and had a non-relationship with all of the water properties in this experiment. Sompong (2001) reported that *D. balfouriana* Greville was a dominant species at temperatures between 30 and 59°C, whereas only a small number of the publications in the world have identified this species as a dominant species in hot springs. The results of this study seem to indicate that it is difficult to establish a characteristic diatom flora for spring waters. In fact, none of the taxa were common to the eight hot springs. In any case, there are different species for which the physical and chemical characteristics of the individual hot spring conditions might be more conclusive in controlling their appropriation. Along these lines, different combinations of temperature and conductivity could clarify the distinctions in the synthesis of diatom arrays in the Galician springs (Leira *et al*. 2017). In any case, these components are needed to clarify the distinctions at the point when the collections are contrasted with samples collected from comparable situations somewhere else. The Cuntis spring can be viewed as having similarities to conditions in other hot spring frameworks such as those in Iceland, New Zealand or Kenya (Owen *et al*. 2008).

Hot springs offer the opportunity to test hypotheses regarding the biogeography of microbial organisms. Kociolek (2000) detailed some families, genera, and species that appear to be localized with regard to their distribution. However, the situation that different habitats can be located in very different geographic locales but still may have similar temperatures and water chemistries could be informative of hot spring ecosystems in general. This has led to the study of the factors that support similar or different species of diatoms (Vyverman *et al*. 2007). Likewise, Owen *et al*. (2008) compared hot springs in Iceland, New Zealand, and Kenya, and noted that the dominant taxa that were present in these systems were all quite notable regarding the significant groups represented. With regard to certain diatoms found in South Africa (*Synedra*, *Aulacoseira*, *Nitzschia*, *Cyclotella*, *Gyrosigma*, *Craticula*), these occurred exclusively at temperatures of <45°C and pH values of <8 (Jonker *et al*. 2013b).

Additionally, sediments can be seen as living spaces for benthic organisms, where they offer a place for resting and refugia. Organisms can be enlisted or re-suspended from sediments in the water body (Dobson and Frid 2008). Further, residue provides a source of chemical compounds that exist in the catchment area. All the hot spring sampling sites were approachable to the public, while some were particularly attractive as tourist destinations and were serviced as catering resources by local communities. Thus, human activities may have led to disturbance or disruption to the fluctuations of the physicochemical features and diatom distribution at some of these hot springs. The sampling intention did not recognize the scope to which human activities had changed the natural ecosystem and impacted upon the diversity of the diatom communities. Thus, to evaluate the effects of human disturbances, continuous monitoring should be done.

From the experiments, it was found that some diatoms found only in hot springs and some can be found in common water resources. In this study, *Achnanthidium exiguum* (Grunow) Czarnecki could grow within a wide range of water properties. They were found as the common species in most sampling sites during temperature ranges of between 37-55.3°C related with Owen (2008) that this species were the most common taxa in their study. As has been previously reported, *A. exiguum* was found to be present in all samples collected from the limnocrene springs of Bunica that were located in the south of Bosnia and Herzegovina, and as often as possible this species was found in alkaline hot springs (Dedić *et al*. 2015). Besides, Mannino (2007); Stavreva Veselinovska (2010); Nikulina and Kociolek (2011); Quintela *et al.* (2013); Covarrubias *et al.* (2016) reported that *A. exiguum*, *G. parvulum* and *A. ovalis* have been previously identified as thermophiles at temperatures in a range of 40–58°C. Consequently, this kind of diatom was prevalent and could prosper in conditions that had attempted to develop *A. exiguum* for lipid production. However, this diatom could only be grown at 30°C as isolation using Bold Basal's Medium (BBM) was not achieved at 40°C. Hence, it should be noted that specific factors affecting the physiology of the diatom suppress its thermostability. Guschina and Harwood (2006) revealed that environmental conditions such as nutrient availability, light and temperature have been found to influence algal growth and lipid accumulation. Conversely, a factor that has not yet been given much consideration is that of pH value. pH value can result in incremental to larger numbers of diatoms as decreases in structural lipid numbers can be considered influential with regard to the developing conditions and physiology of algal cells (Spilling *et al.*, 2013). All things considered, the impact of pH on the thermotolerance of diatom cultivation has not yet been examined. A particular pH range is one of the components of effectiveness for diatom growth and diatom biochemical synthesis. The effects of pH on the thermotolerance and lipid production of *A. exiguum* AARL D025-2 at various temperatures were emphasized in this research study. The use of the fatty acid methyl ester in this diatom was indicated in a scope of C16-C18 with a moderately high extent of palmitic acid (C16:0) and Cis-10-Heptadecenoic acid (C17:1n-1cis). Knothe (2008) claimed that these palmitic and stearic acids were considered preferable fatty acids for biodiesel production. Thus, the FAME of this diatom may be used for biodiesel production. Notwithstanding, certain elements of the distinctive fatty acid profile may affect the parameters of the physical properties of the oils (Martínez-Force *et al*. 2009).

Over the previous decade, diatoms have been perceived as a fundamental sort of algal biomass that has potential as lipids for the production of biodiesel feedstock (Popovich *et al*., 2012). *Cyclotella* and *Aulacoseira* have been distinguished as oleaginous species (Graham *et al*., 2011). Accordingly, comprehension of this thermotolerant property might be a favorable position for cultivation in high-temperature arable territories that exist without temperature control, for example, in deserts. Also, diatoms are heat tolerant species and their silica frustules can exist under extraordinary conditions, for example, hot springs. However, the cultivation of hot spring diatoms has not yet been explored. In this manner, it is obviously critical to obtain essential data on the variables associated with the development and lipid creation of each specific species.

5.2 Conclusions

Regardless, the investigation on diatoms is focused just around the water assets in typical conditions. Indeed, diatoms can develop in extraordinary natural surroundings, for example, hot springs with high temperatures and diverse physical-substance attributes in various environments. The investigation of diatoms in such natural surroundings may prompt the revelation of potential diatoms for different applications. Diatom samples from nine hot springs in northern Thailand were collected and a total of 30 species were recorded. Some of the physicochemical parameters of each station were not substantially inconstant with the changing seasons. *Diatomella balfouriana*, *Rhopalodia gibberula* and *Sellaphora lanceolata* were the most abundant and most common species in nine thermal samplings. This information enhanced our intent to understand how a number of diatoms were scattered in warm water living spaces with a concession to the centralization of ammonium nitrogen, alkalinity, light power and water temperature. This enabled the researcher to manage the appropriate autecological information for the reason that diatoms could be used all the more effectively in fulfilling evaluations of ecological varieties. All the hot springs that were examined were receptive to the general population, and some were determined to be attractive for vacationer goals and adjusted as being attractive for tourist destinations, while being seen as assets by local communities. Thus, human activities possibly will have led to disturbances or disruptions to fluctuations in both the relevant physicochemical features and diatom distribution. The testing goal did not perceive the degree to which human exercises have changed the characteristic biological system and have affected the varieties of diatom communities. The distribution of algae growth in the selected springs, therefore, does not only prescribe the effects of land seclusion on account of mineral substance. Regardless, in a like manner, there is a likely effect of human activity on species quality under these conditions.

Furthermore, the FAME of this diatom was additionally evaluated for its capability to determine the way the diatom possesses a capacity to accomplish thermostability. In addition, the FAME profile could be utilized to assess the capability of this diatom for use as a crude material for biodiesel creation. Moreover, the development and use of this diatom will provide imperative essential data to both the scholastic and mechanical segments. Diatoms can photosynthesize and deliver O_2; further, they can store lipids and are being considered as another promising source of biofuel. Our information bolsters the speculation that pH influences the growth and heat resilience of *A. exiguum* AARL D025-2. Cell development acquired at 40°C at a pH estimation of 9 uncovered the most substantial amounts of biomass and lipid generation. This investigation was basically directed to evaluate culture conditions and FAME profiles of hot spring diatoms. The acquired knowledge from this study will be worthwhile for the development of certain thermotolerant diatoms and could be valuable for the future advancement of diatom development with regard to creating elective vitality sources. It would not be difficult to think about applications in different fields, particularly in the modern field. At present, lipid generation is being directed in high-temperature life forms. Cell culture and lipid extraction should be possible at room temperature to diminishe the costs associated with generation since it does not require a temperature controlled refrigeration framework.

5.3 Recommendations

Except for the work of cyanobacteria, the previous accumulated knowledge on hot spring diatoms in Thailand has been considered inferior. This is stated in light of the fact that thermal spring diatom groups have for a long time been pulled into consideration by microbial biologists because of their extraordinary adjustments to harsh conditions (Sompong *et al*., 2005). The existing knowledge of hot spring diatoms should be reevaluated to create a greater understanding of the existing biodiversity records. It also appears that there is a need for taxonomic revisions of many of the established taxonomic groups.

We would have liked to retrieve more samples from nearby locations with cool or normal temperatures. This would have allowed us to compare species richness and diversity in non-hot spring locations to the species found to be living in hot springs. Likewise, a study on the diversity of algae that can grow in high temperatures will provide an initial basis for understanding the diversity and distribution of algae in Thailand.