PHYTOPLANTON COMPOSITION OF MUARA TEBAS AND ITS RELATED ENVIRONMENTAL CONDITION

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Phytoplankton composition of MuaraTebas and its related environmental condition

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DECLARATION

I hereby declare that the research project is based on my works except quotations and citations which have been properly acknowledged. I also declare that this project have not been previously or concurrently submitted for any other degree at UNIMAS or other institutions.

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LIST OF ABBREVIATIONS

DO Dissolve oxygen

pH Potential of Hydrogen

PO_4^{3-} Orthophosphate

PSP Paralytic Shellfish Poisoning

HAB Harmful Algae Bloom

NEM Northeast monsoon

NO_3^- Nitrate

SW Southwest monsoon

SiO_2 Silicate dioxide

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Phytoplankton composition of MuaraTebas and its related environmental condition

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ABSTRACT

Harmful Algae Bloom (HABs) is not negligible as it is threat to human health, ecosystem and environment. Occurrences of phytoplankton are highly dependent on the environmental condition. This study is initiated to understand the influence of physicochemical to the production and growth of phytoplankton species in tropical countries. Study was carried out at Muara Tebas estuary as anthropogenic activities and development site is commonly seen. Qualitative and quantitative samples were collected from October 2013 to February 2014. Chemical parameters such as nutrient concentration and chlorophyll a showed to have direct impact on cell density trend. Cell abundance had correlation with the presence of macronutrient especially orthophosphate and silicate. Conversely, physical parameters showed weak relation to cell density with exception to temperature.Cell density also influenced by northeast monsoon (NEM) as the precipitation and upwelling in west coast of Sarawak. A total of 18 taxa of phytoplankton were found and identified to genus level, with 15 diatom (8 penates and 7 centric), 2 dinoflagellates and 1 cyanobacteria.

Key words: MuaraTebas; estuary;phytoplankton;chemical;physical

ABSTRAK

Kejadian ledakan alga berbahaya (HAB) tidak boleh diabaikan kerana kejadian tersebut membawa ancaman kepada kesihatan manusia, ekosistem dan kesekitaran. Kewujudan fitoplankton amat bergantung kepada keadaan persekitaran. Kajian ini dijalankan untuk memahami taburan dan penumbuhan fitoplankton di kawasan tropika. Kajian ini dijalankan di MuaraTebas kerana kawasan tersebut mempunyai banyak antropogenik aktiviti dan terdapat banyak tapak pembangunan. Sampel kualitatif dan kuantitatif telah dikumpul dari Oktober 2013 hingga Februari 2014. Kimia parameter seperti kepekatan nutrient dan klorofil menunjukkan kesan terhadap trend kepekatan sel. Kehadiran makronutrien terutamanya orthophosphate dan silikat menunjukkan korelasi dengan kepekatan sel. Disebaliknya, fizikal parameter menunjukkan hubungan yang lemah dengan kepadatan sel, kecuali suhu. Kepadatan sel juga dipengaruhi oleh monsoon timur laut yang memberi kesan pada taburan hujan dan upwelling di kawasan pantai laut Sarawak. Terdapat 18 taxa fitoplankton telah ditemui dan dikenal pasti ke tahap genus. Terdapat 15 diatom (8 pennatedan 7 centric), 2 dinoflagellatedan 1 cynobakteria.

Kata kunci: MuaraTebas;muara;fitoplankton;kimia;fizikal

1.0 INTRODUCTION

Phytoplankton refers to microscopic organisms that found drift in water column, both salty and fresh. It is among the most important marine organisms that account for the world's primary production. It produces organic compound and carbon dioxide to dissolve in water environment. Such process is used to sustain the aquatic food web. However, Harmful Algae Bloom (HABs) related toxicity brought implication on human health and ecosystem, in both estuaries and coastal water environment.

Malaysia is characterized with two main monsoon seasons that greatly affected the weather, which are Southeast (SE) and Northeast (NE) monsoons. SE normally is from late May to September while NE is from November to March. During NE monsoon, the area of east coast state of Peninsular Malaysia and western Sarawak normally receives a large amount of rainfall (Yoshida *et. al*, 2006). The high rainfall increases the amount of input water into the estuary system, thus indirectly affect the water quality of a particular area.

In Malaysia, the abundance of phytoplankton community can be found in estuary and coastal water ecosystems, where the mixing process of freshwater and sea water occurs. Such process influences the physicochemical properties and biological processes which then indirectly characterized the phytoplankton composition. Research done found high fluctuation of water influence the water parameters in estuary. Salinity, temperature and light are among the factors contribute to the growth, nitrate uptake and toxic production of HABs (Lim & Ogata, 2005).

In this study, the sampling site selected was Muara Tebas. This location was selected on the fact that many of anthropogenic activities such as tourism and industrial. Besides, this site also acts as main route for ship and cruise. Such condition provides an opportunity for

some of the harmful algae bloom to occur and eventually susceptible for algae blooming. Hence it is important to monitor the environment and the occurrences of species of phytoplankton. There are several aims for carrying out this experiment:

- To determine the phytoplankton community in Muara Tebas estuary
- To determine selected environmental parameters related to phytoplankton distribution

2.0 LITERATURE REVIEW

2.1 Occurrences of toxic phytoplankton in Malaysia

Several cases related to toxic harmful algae bloom had been reported in Malaysia waters. In Malaysia, harmful algae bloom cases reported was mainly related to paralytic shellfish poisoning (PSP). It is due to contamination of shellfish by several toxic marine dinoflagellates. *Pyrodnium bahamense* had long been considered as the most important PSP toxic-producing species in Southeast Asia (Usup & Azanza, 1998). These species had caused many poisoning events including several fatalities (Usup *et. al*., 1989). In the 1970s, *P.bahamense* is found abundance in the west coast of Malaysia. The very first case of PSP reported in Malaysia was in Sabah during 1976 (Roy, 1977). Besides, in early 1991, PSP had occurred in Sebatu, Straits of Malacca. This incident had involved to three people from suffering food poisoning due to consumption of the toxic mussels from a mussel farm contaminated with toxic algae. *P.bahamense* was suspected to be the toxic producer in this event, but to date, no *P.bahamense* has been found in plankton samples collected from a few locations in that area. However, one harmful alga has been identified as *A.tamiyavanichii* in that area (Usup *et al*., 2002). Dinoflagellates from the genus *Alexandrium* has been well known as producers for the potent neurotoxins that cause PSP in many coastal countries throughout the world (Anderson *et al*., 1994; Halegraeff *et al*., 1995).This species is found in tropical and subtropical waters. (Fukuyo *et al*., 1989; Montojo *et al.*, 2003; Nagai *et al*., 2003). Other *Alexandrium* species such as *A. taylori* and *A. peruvianum* (Dinophyceae) are also PSP toxic producers in Malaysia waters (Lim *et al.*, 2005). Consequently, there were six people were poisoned, including one fatally, was reported due to the consumption of benthic clam,

Polymesoda sp. which was contaminated by toxic harmful algae bloom at Tumpat, Kelantan. Researches done had confirmed that this incident was caused by *A.minutum* (Lim *et al*., 2004).

2.2 Distribution of phytoplankton

Bloom of phytoplankton occurs in abundance throughout the coastal region of the world which cause by different environmental issue at various locations. There are many different ways to aid the dispersal of phytoplankton, ranging from natural mechanisms of species dispersal to a host of human-related phenomena such as pollution, climatic shifts, increased number of observers, and transport of algal species via ship ballast water.

Abundance of species of phytoplankton is influenced by the chemical and physical elements. In estuary and coastal water, salinity gradient is one of the major factors that influence the growth and diversity of phytoplankton community (Macedo *et al*., 2001; Khatoon *et al*., 2010). For marine phytoplankton, they had adapted to high salinity and usually unable to survive in freshwater environment. Similarity, freshwater phytoplankton is found to be less tolerant with high salinity water. However, some species of the plankton has the ability to withstand large salinity fluctuation and inhabit brackish water estuaries. *Alexandrium* sp. is found at different environment with different salinity range. *A.minutum* can tolerate large salinity change followed by *A.peruvianum*, *A.tamarense*, and *A. tamiyavanichii* (Lim & Ogata, 2005). In Perak estuary, most of the plankton is found abundant in marine water while estuary is dominated by green algae and diatom (Nursuhayati *et al*., 2013).

Other water parameters such as water temperature (Ogata *et al*. 1987, Anderson *et al*. 1990a, b, Hamasaki *et al*. 2001) and turbidity also contribute to the dispersal of phytoplankton community. These parameters determine a suitable environment for some of the phytoplankton to bloom. Meanwhile, nutrient availability can reflect the condition of the

environment. It plays significant role as it able to induce the rapid growth of certain phytoplankton communities.

3.0 MATERIALS AND METHODS

3.1 Scope of study

The sampling site was located at Kuching area, Muara Tebas estuary, positioned at N 01^{o} 38 'E 110^{o} 29'. It is an inlet of the sea reaching into the Sarawak River valley, the water quality is influenced by thermal and salinity fluctuations. The water sample were collected from the jetty at Muara Tebas. Anthropogenic activities such as tourism and industrial are the main sources that contribute to the water parameter of the estuary. Besides, Muara Tebas also is the main route for ships and cruises. Thus, ballast water discharge is another factor that influences the phytoplankton species community. A small scale aquaculture activity is carried out by the local peoples at this site.

Figure 3.1: Map of Kuching, Sarawak showing the sampling location of Muara Tebas Estuary

3.2 Sample collection

Water samples were collected twice per month from September 2013 to February 2014 at Muara Tebas estuary (Fig 3.1). Both quantitative and quantitative samples were taken for identification of phytoplankton community and nutrient analysis. One litre of water sample was collected by using Van-Dorn for chemical water parameters measurement. The physical parameters such as pH and temperature were measured by using pH meter (HANNA, HI 98127). Other parameters such as water transparency and salinity were measured with Secchi disc and Refractometer (HANNA, HI 96822) respectively.

3.3 Water Quality

3.3.1 Water Filtration and Chlorophyll-a Analysis

One litre of the seawater sample was filtered through 47mm GF/C glass microfiber filter with electric pump. The filtrates were kept in sample bottles and kept at -20 for nutrient analyses. Meanwhile, the filter paper was transferred into a 15 mL centrifuge tube with 10 mL of 90% acetone. The samples were sonify in ice bath for 20s followed with centrifugation process at 3000 rpm for 10 minutes. The supernatant was transferred into a 1cm path length quartz cuvette and the extraction was measured on the DR 2800 spectrophotometer (HACH, USA) at the wavelengths: 750, 664, 647, and 630 nm (max adsorption wavelength of chl *a*, *b*, and *c*).

The chlorophyll concentration was calculated according to equation below:

1. Apply the correction for small turbidity blanks:
   $E_{664}\_C = E_{664} - E_{750}$
   $E_{647}\_C = E_{647} - E_{750}$
   $E_{630}\_C = E_{630} - E_{750}$

   Where E_{750} = absorption at 750 wavelength
   E_{664} = absorption at 664 wavelength
   E_{647} = absorption at 647 wavelength
   E_{630} = absorption at 630 wavelength
   Ex_C = corrected values of absorption for turbidity blanks

1. Calculate individual Chlorophyll concentrations (µg /mL) in water samples:
   Chl *a* (*Ca*) = $11.85*E_{664}\_C - 1.54*E_{647}\_C - 0.08*E_{630}\_C$
   Chl *b* (*Cb*) = $21.03*E_{647}\_C - 5.43*E_{664}\_C - 2.66*E_{630}\_C$
   Chl *c* (*Cc*) = $24.52*E_{630}\_C - 1.67*E_{664}\_C - 7.60*E_{647}\_C$

   Where *Ca*, *Cb* and *Cc* are Chlorophyll concentrations in µg/mL if 1 cm light path cuvette is used

2. Conversion of Chlorophyll concentrations to mg/L in water samples:

   $\boldsymbol{Chl\ (\mu g/L) = C_x * v / V1}$

   Where $\boldsymbol{C_x}$ = *Ca*, *Cb* and *Cc* are Chlorophyll concentrations in µg/mL
   v = volume of acetone in mL
   V= volume of seawater in L

3.3.2 Nutrient Analysis

Nutrients that were analysed in this study include nitrate (NO_3^-), reactive phosphorus (PO_4^{3-}) and silicate (SiO_2). These chemical parameters were determined through calorimetric method (Hach, 2005).

Different method was carried out to conduct different types of nutrient analysis. NO_3^- was analysed using Cadmium Reduction Method at wavelength 507nm. Nitra ver 3 was added to cuvette that contains 25ml of water sample. PO_4^{3-} was determined at wavelength 880 nm by using PhosVer 3 (Ascorbic Acid) whereby PhosVer 3 was added to 10ml of water sample. SiO_2 was determined at wavelength 651nm by adding Molybdate 3 Reagent and Citric Acid Reagent powder pillow into 10 ml of the samples. Concentration of each nutrient was analysed by using DR 2800 spectrophotometer (HACH, USA).

3.4 Phytoplankton Analysis

3.4.1 Cell enumeration

Water samples were collected using Van Dorn water sampler for cell enumeration. The entire water sample collected was preserved with acidic Lugol's iodine solution and placed at room temperature. Sedgwick-Rafter counting chamber was used for cell density calculation. The process was carried out under light microscope (LEICA, CME) with three replicate for each sample.

3.5 Data analysis

Statistical analysis of phytoplankton composition was analysed by Principal Component Analysis (PCA) and diversity indices was calculated using PAST program. Graphpad® Prism 5 was used for Graphical representation.

4.0 RESULTS

4.1 Qualitative data: Relative abundance of phytoplankton composition

A total of 18 phytoplankton taxa at Muara Tebas were identified to the generic level (Table 4.1). There were 15 genera of diatoms including 8 pennate and 7 centric diatoms. Pennate diatoms comprised *Amphiprora, Bacillaria, Ballerocheo, Frustulia, Nitzchia, Pleurosigma, Syndera* and *Thalassionema.* Meanwhile, centric diatoms that were appeared at that site were *Chaetoceros, Cerataulina, Coscinodiscus, Ditylum, Odontella, Rhizosolenia* and *Skeletonema.* The remaining two taxa were dinoflagellates (*Alexandrium* and *Ceratium*) and one cynobacteria (*Trichodesmium*).

Overall, diatoms were the phytoplankton that distributed in abundance in the water environment at Muara Tebas. *Skeletonema,* the centric diatom that had the highest relative abundance throughout the 9 sampling dates (Figure 4.1), particularly during end of year 2013 and first month of 2014 where its abundance dominated more than one quarter of the total phytoplankton species. This species was dominant in 19 Nov, 29 Nov, 12 Dec 2013 and 9 Jan 2014 sample where the species abundance reached 40.15%, 33.33%, 37.70% and 29.89% respectively. The second most abundant taxa found was *Coscinodiscus* spp. especially where it relative abundance shown the highest in 2014, particularly in 20 Jan, 19 Feb and 25 Feb (Figure 4.1). Other most abundance phytoplankton that present in water column in Muara Tebas included *Pleurosigma* and *Thalassionema*.

Generally, there were 3 taxa that could be found throughout the sampling periods. There were pennate diatoms *Nitzchia* and *Thalassionema,* as well as centric diatom *Skeletonema,* although with rather inconsistent abundance.

There were four uncommon phytoplankton found in Muara Tebas that with very low frequencies of occurrence. These uncommon phytoplankton were present only once throughout the sampling dates. Three of the uncommon phytoplankton was pennate diatoms, *Bacillaria, Ballerocheo* and *Frustulia* .There were only one rare cyanobacterium found, which was Trichodesmium, where it was found on 31 Oct 2013 only. There were 8 potentially harmful phytoplankton taxa found, comprises of 2 pennate diatom, 4 centric diatom and 2 dinoflagellates.

Micrographs of some phytoplankton taken using X50 Olympus inverted microscope are in Figure 4.1.2.

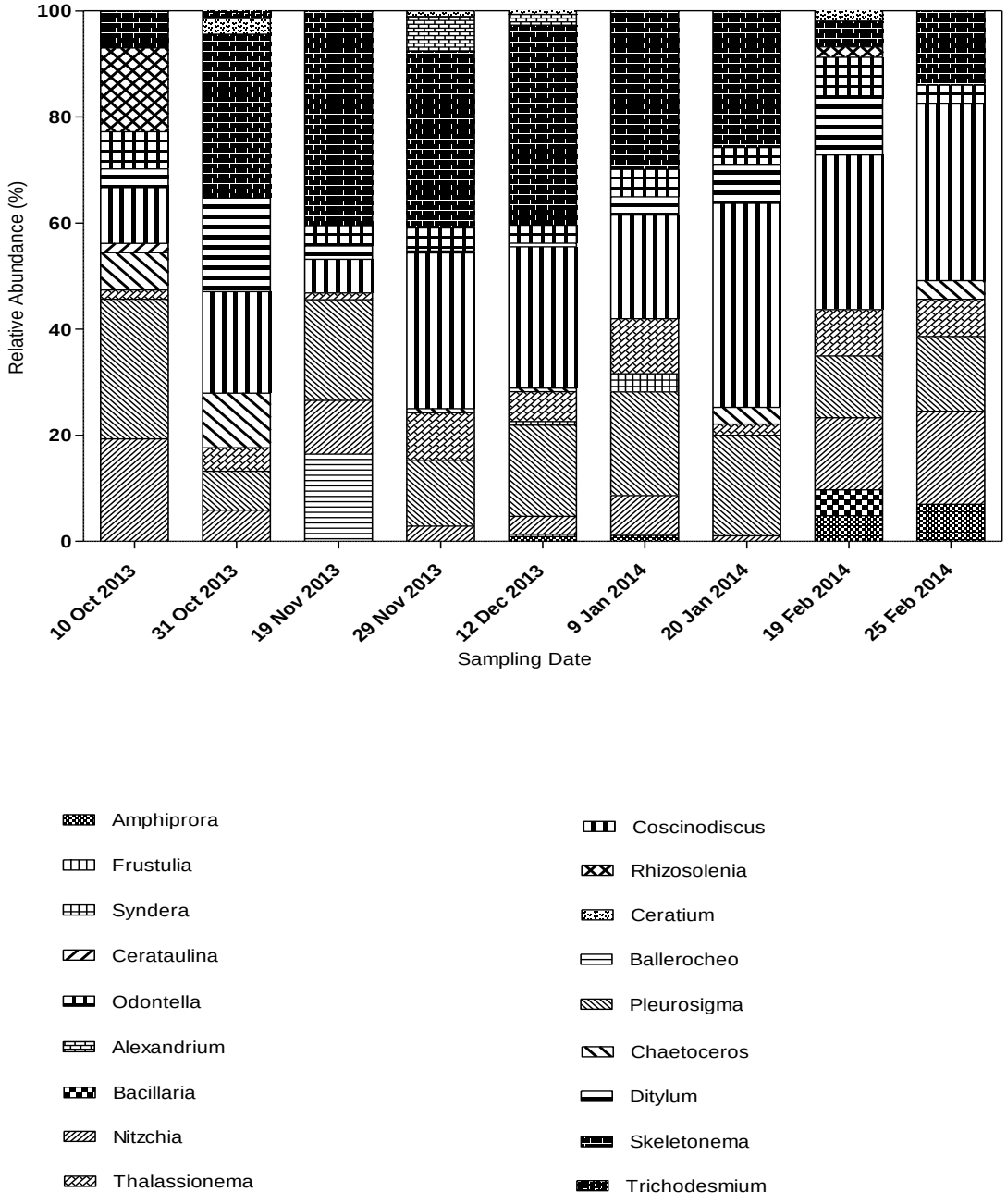

Figure 4.1.1: Relative abundance of various phytoplankton taxa for 9 sampling dates ranging from October 2013 to February 2014

Table 4.1: Occurrence of phytoplankton over 9 sampling dates

	Occurrence of Phytoplankton								
	2013					2014			
Taxa	10-Oct	31-Oct	19-Nov	29-Nov	12-Dec	9-Jan	20-Jan	19-Feb	25-Feb
DIATOMS (Pennate)									
Amphiprora					+	+		+	+
Bacillaria								+	
Ballerocheo			+						
Frustulia					+				
*Nitzchia**	+	+	+	+	+	+	+	+	+
Pleurosigma	+	+	+	+	+	+	+		+
Syndera					+	+		+	
*Thalassionema**	+	+	+	+	+	+	+	+	+
DIATOMS (Centric)									
*Chaetoceros**	+	+		+	+		+		+
Cerataulina	+								
Coscinodiscus	+	+	+	+	+	+	+	+	
Ditylum	+		+	+	+	+	+	+	+
*Odontella**	+		+	+	+	+	+	+	+
*Rhizosolenia**	+							+	
*Skeletonema**	+	+	+	+	+	+	+	+	+
DINOFLAGELLATES									
*Alexandrium**				+	+				
*Ceratium**		+		+	+			+	
CYNOBACTERIA									
Trichodesmium		+							
TOTAL	10	8	8	10	13	9	8	11	8

*=potentially harmful phytoplankton

Highlighted = phytoplankton that occurred during all sampling period

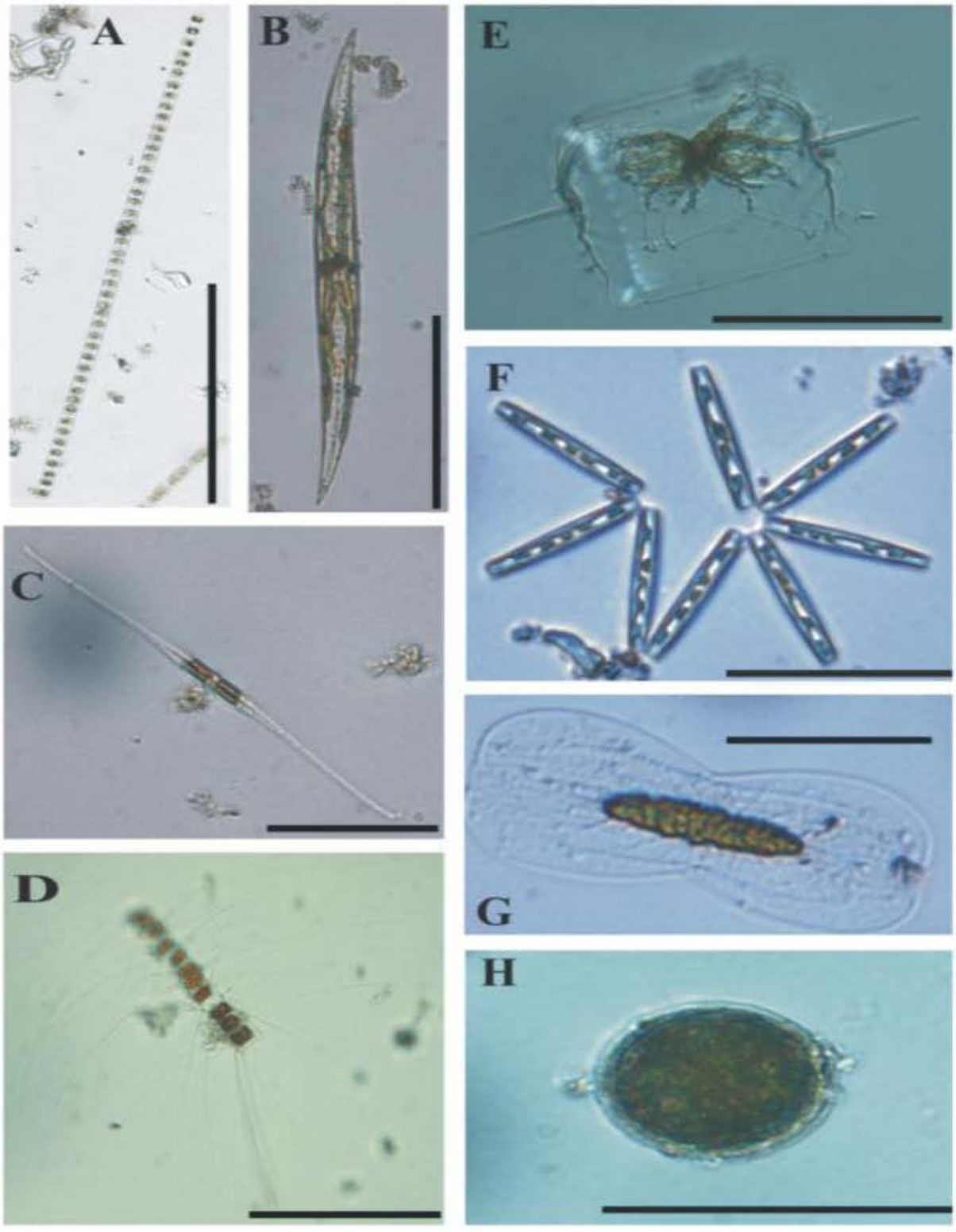

Figure 4.1.2 Micrograph taken for (A) *Skeletonema* sp., (B) *Pleurosigma* sp., (C) *Nitczhia* sp. , (D) *Chaetoceros* sp. , (E) *Ditylum sp* , (F), *Thalassionema* sp. , (G) *Amphiprora* sp. , (H) *Coscinodiscus* sp.

4.2 Qualitative data: Total cell density

The total cell density of phytoplankton range from 4833 cell L^{-1} to 36499 cell $L^{-}1$. The total cell density throughout the sampling period shown inconsistency, with obvious density resulted by amount of diatoms. The cell density showed a slightly decrease in October 2013 sampling followed by a drastically increase to the highest peak in 12 Dec 2013, then a huge decrease of cell density occur in 9 Jan 2014 and continuously decrease of cell density until the end of the sampling date.

The phytoplankton was classified into three different groups, the diatom group, dinoflagellates and cyanobacteria. It could be observed that the total cell density was dominated by diatom, followed by dinoflagellate and cyanobacteria where it could be observed in 19 Nov 2013 (Figure 4.2.2).