Faculty of Resource Science and Technology

EPIPHYTIC MICROALGAE ASSEMBLAGE ON THE SELECTED MANGROVE PNEUMATOPHORES IN MELABAN RIVER AND ASAJAYA, KOTA SAMARAHAN, SARAWAK

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Bachelor of Science with Honours

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UNIVERSITI MALAYSIA SARAWAK

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EPIPHYTIC MICROALGAE ASSEMBLAGE ON SELECTED MANGROVE PNEUMATOPHORES OF MELABAN RIVER AND ASAJAYA, KOTA SAMARAHAN, SARAWAK

Siti Nabila Binti Mohd Sharif

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DECLARATION

I hereby declare that no portion of the work referred to this dissertation has been submitted in support for another degree of qualification or another university or institution of higher learning.

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ABSTRACT

This study aimed to document the epiphytic microalgae assemblages in mangrove areas of Melaban River and Asajaya. The sampling included the harvesting of selected pneumatophores in order to obtain the microalgal cells. The epiphytic microalgae from both sampling sites were identified until the genus level. There were 19 diatom genere were identified, and also an unidentified microscopic green algae. The most common diatoms occur at both sampling sites were *Fragilaria, Navicula* and *Pleurosigma* along with other dominant genera such as *Frustulia, Bacillaria, Skeletonema* and *Stauroneis*. There were no cyanobacteria and dinoflagellates identified in this study.

Keywords: epiphytic microalgae, diatoms, pneumatophores, mangroves, Sarawak

ABSTRAK

Kajian ini bertujuan mendokumen kumpulan mikroalga epifit di kawasan paya bakau Sungai Melaban dan Asajaya. Pensampelan melibatkan pengambilan pneumatofor untuk mendapatkan sel-sel alga mikro. Alga mikro epifit dari kedua-dua lokasi pensampelan telah dikenalpasti hingga ke peringkat genus. Terdapat 19 genera diatom yang telah dikenal pasti dan juga satu alga mikroskopik hijau yang tidak dapat ditentukan identitinya. Diatom yang paling kerap ditemui dikedua-dua kawasan pensampelan adalah Fragilaria, Navicula *dan* Pleurosigma *bersama-sama genera dominan yang lain seperti* Frustulia, Bacillaria, Skeletonema *dan* Stauroneis. *Tiada alga biru-hijau dan dinoflagilat yang dikenalpasti di dalam kajian ini.*

Kata kunci: mikroalga epifit, diatom, pneumatofor, paya bakau, Sarawak

1.0 INTRODUCTION

Mangrove forests are best developed in a tropical climate, where the temperature is above 20°C and are fairly constant throughout the year (<5°C of variation) (de Lacerda, 2002). This ecosystem is unique as they are distinguished on the accumulation of muddy sediments which are retrieved from the terrestrial system and receiving wave action from the marine system. Currently, the most developed mangrove forests are located along the coasts of Malaysia and Indonesia (Karleskint, Turner and Small, 2010). Such ecosystem is colonized by several groups of plants that possess adaptations to withstand the harsh conditions of mangrove forest (Tomlinson, 2008).

One of the pioneer plants is *Avicennia* species which can be found at the open area of river edges or mudflats (Rodtassana and Poungparn, 2012). According to Tomlinson (1986), the pneumatophores of *Avicennia* sp. are emerging in vast amount and become habitat for several biota including the microscopic algae. This community which encrust on the surface of other plant kingdom are called epiphytic algae (Sharma, 2007).

Epiphytic microalgae can also be assumed as benthic microalgae or microphytobenthos where their occurrences are remarkably documented at shoreline until the brackish waters up to the freshwater inlets (Durai and Pandiyan, 2011). As stated by Chung and Lee (2008), epiphytic algae are important as their existence can contribute up to 40% of the total community production. A study did by Newell, Marshall, Sasekumar and Chong (1995), showed that benthic microalgae at the mangrove acted as a dietary source of penaeid prawns such as the juveniles of *Penaeus merguiensis* when they graze on the substrates at mangroves.

According to Rajvanshi and Sharma (2012), benthic microscopic algae could be divided into three main groups; diatoms (Bacillariophyceae), the blue green algae (Cyanobacteria) and dinoflagellate (Dinophyceae).

Diatoms can be found at almost all aquatic body (Hedges and Kumar, 2009) hence it explains their dominance among the microscopic algae groups. Also according to Hedges and Kumar (2009), diatoms are one of the best characterized microalgae groups as they exhibit the unique features of their silicified cell wall. This group contributes to the primary production by covering at least 23% of the earth total production based on a carbon fixation estimate of 25.8 X 10^15g C.yr- (Snoeijes, Busse and Potava, 2002).

The existence of cyanobacteria is significant as they have achieved diversity since 2 billion years ago and acted as active organisms in accumulating earth’s early atmosphere (Vincent, 2009). The group is distinguished as photosynthetic prokaryotes and possess chlorophyll-a pigments (Whitton and Potts, 2012). Their occurrence had been documented in many water bodies as they are popular by causing toxicological problems when the blooming is uncontrollable.

Dinoflagellates are unicellular and biflagellate microorganism and typically autotrophic while there are few which are heterotrophic, saprophytic or even symbiotic (Thomas, Moore and Coffroth, 2007). They are known due to their association with the red tides, fish kills and shellfish poisoning which broaden the ecotoxicology field for many scientists as stated by Thomas et al. (2007). The success of this group is related to its tremendous diversity in form and nutrition and also by fossil documentation which dates a few hundred million years ago (Hackett, Anderson, Erdner and Bhattacharya, 2004).

2.0 OBJECTIVES

The objectives of this study are:

- To identify the epiphytic microalgae up to the lowest practical taxon
- To establish a baseline data of epiphytic microalgae on the plants' pneumatophores at the mangrove forests.

3.0 JUSTIFICATION

Studies related to microalgae classification and identification has been done in many countries especially the benthic organisms. In Malaysia researches regarding the benthic microalgae have been done in Sampadi Island, Sarawak (Tan, Lim, MujahidUsup and Leaw, 2013 and Harith (2006) where the focus are on the non-plant substrates such as sediments and corals. The information of epiphytic algae on the pneumatophores of mangrove plants such is very scarce.

It is important to carry out a research for epiphytic microalgae in Sarawak mangrove area because the data can be used as baseline information for more research in the future. The information about microalgae communities in the mangrove is essential for the references to the development in the next days.

4.0 LITERATURE REVIEW

4.1 Mangrove forest

Mangrove forest is a type of habitat that predominantly occurs in the tropical and sub-tropical coastal zone (de Lacerda, 2002). The word "mangrove" in the more limited sense may be defined as tropical trees restricted to intertidal and adjacent communities (Tomlinson, 2008). More than 80 species of plants including trees, shrubs, palms, ferns, epiphytes and algae are recognised in mangrove of which 50-60 species make a significant contribution of the constitution of the forest (Rahim, 2000). The mangrove forest of Sarawak covers 1.4% of total land and occupies 60% of 740 km coastline. Sarawak mangrove forests are divided into four classes as stated by Rahim (2000) and the pioneer species such as *Sonneratia* and *Avicennia* grow extensively seaward as they are replaced by *Rhizophora* and *Bruiguiera* species as the forests are heading to land.

The drier areas at higher intertidal zone are dominated by *Xylocarpus, Pandanus, Acrosthicum* and *Nypa* species as mentioned by de Lacerda (2002). Some species show adaption towards the harsh condition in mangrove areas by extending prop roots and pneumatophores into the intertidal and subtidal area as proposed by Nagelkerken et al. (2008). These aerial roots are responsible for the stabilisation of the sediment in which they provide substratum for the flora and fauna to inhabit. The space between roots of the trees provides habitat for the juveniles of fish, prawns and shrimps to graze and became shelter from the predation upon those juveniles (Nagelkerken et al., 2008). Apart from that, mangrove areas are also being utilized by humans for commercial purposes such as aquaculture and timbers harvesting as well as their existence as a natural defence against tsunami and wave action (Tomlinson, 2008).

4.2 Pneumatophores

Pneumatophore is a component that constitutes the root system of several groups of mangrove vegetations (de Lacerda, 2002). In general, pneumatophore is part of a root system along with three other components namely; cable, feeding and the anchor roots (Rodtassana and Poungparn, 2012). According to Purnobasuki and Suzuki (2005), the pneumatophore achieved its growth by extend vertically upward into the air and just above the soil surface by measurable small length (Figure 1). There is difference in terms of height and morphology between the species of mangrove plants. For example, in *Avicennia*, the roots are of limited height and usually less than 30cm while developing a second thickening internally (Tomlinson, 2008). On the other hand, the genus of *Sonneratia* is characterised by flaky barks that grow in young roots.

Typically, the soils of the mangrove forests have very low oxygen concentration. So, the underground organs of any plant must grow in anaerobic condition or find an alternative to be ventilated (Hovenden and Allaway, 1994). This is where the pneumatophores can be very functional. According to Rodtassana and Poungparn (2012), the main function of the pneumatophore is to allow gases exchange during low tide via lenticles on the surface of the roots. The lenticle is the circular and bulk outgrowth of the pneumatophores or at some species it appears as dark spot on the root surface (Purnobasuki and Suzuki, 2005). Hence, the presence of pneumatophores facilitates the diffusion of oxygen through the tissues in order to maintain the adequate level of gases concentration for cellular respiration.

On the other hand, the pneumatophores have been found to support a rich flora and fauna that are attached onto the root surfaces (Naidoo, Steinke, Mann, Bhatt and Gairola, 2008). The organisms found according to a study by Naidoo et al. (2008), on the pneumatophores of *Avicennia marina* at a particular mangrove forest in Africa were comprised of epiphytic micro and macroalgae.

4.3 Microalgae

Microalgae can be considered as a vital component in the ecosystem of many habitats particularly mangroves and estuaries. In the intertidal areas, microalgae are involved in the primary production and as food to the organisms of the higher trophic level (Dalsgaard, 2003). The benthic microalgae can occur in variety of medium as their habitat where the attached microalgae are called as epilithic, on mud or sand as the epipelic, or on other algae or plants as the epiphytic microalgae (Barsanti and Gualtieri, 2006). According to Rajvanshi and Sharma (2012), the benthic microalgae can be divided into three groups which are diatom, cyanobacteria and dinoflagellate.

4.3.1 Diatom

Diatoms (Bacillariophyta) are eukaryotes, unicellular and mostly photoautotroph microalgae where they are directly involved in the primary production at significant amount of 20% (Zimmermann, Abarca, Enk, Skibbe, Kusber and Jahn, 2014). According to Hedges and Kumar (2009), the occurrences of diatoms are very vast where they are recorded at almost every type of water bodies. Every diatom cell is enclosed within two siliceous shells or frustules and is connected by the presence of girdle bands (Hedges and Kumar, 2009).

This group of microalgae is ecologically important. It holds a crucial key in balancing the ecology in respect of its function in biogeochemical cycling of silica and global carbon fixation (Kesici, Tuney, Zeren, Guden and Sukatar, 2013). At the open sea, some of the diatoms would sinks into the bottom and become food for demersal organisms while in mangroves and estuaries areas they are responsible for the supply of oxygen via photosynthesis process (Snoeijas et al., 2002).

According to Lokman (1991), the Bacillariophyceae are divided into two orders; centric diatoms with radial and bipolar symmetry of frustules (order Centrales) and pennate diatoms (order Pennales) which differ in the presence and reduced or absence of raphe (Round, Crawford, and Mann, 2000). The diatoms can be classified as follows (Lokman, 1991) (Table 1).

Table 1: Classification of diatoms

Phylum	Class	Order	Suborder	Famili
Chrysophyta	Bacillariophyceae	Centrales	Discineae	Melocereceae
				Coscinodiscaceae
			Soleniineae	Rhizosoleniaceae
			Biddulphiineae	Chaetoceraceae
				Biddulphiaceae
		Pennales	Araphhidinaeae	Fragilariaceae
				Tabellariaceae
			Monoraphidineae	Achnanthaceae
			Biraphidineae	Naviculaceae
				Epthemiaceae
				Nitczchchiaceae
				Surirellaceae

As the diatoms are directly based on the concentration of macronutrients of the water, they could also being used as bioindicators for monitoring the water quality (Zimmermann et al., 2014). This is because they are sensitive towards pollution, nutrients availability, acidity and salinity.

4.3.2 Cyanobacteria

Cyanobacteria or the blue green algae are a type of photosynthetic prokaryote that uses chlorophyll pigment to capture sunlight (Vincent, 2009). Presently, the blue green algae are comprised of 2000 species in 150 genera and 5 orders (Whitton, 2012). According to Vincent (2009), the success of this group is contributed by several features such as tolerance to desiccation, favouring a higher temperature optimum compared to other algae, and the photosynthetic character.

Ecologically, cyanobacteria are often associated with the nitrogen fixation (Vincent, 2009). This is very significant, especially at the open sea where they would convert the nitrogen (N_2) to ammonium (NH_4^+) before being utilized as amino acids and other nitrogen-containing compounds. The blue green algae are considered as ancient oxygen producers as they were dominating the habitats during earth's early days (Barsanti and Gualtieri, 2006). Their long existence was proved by the presence of stromatolites; fossilised blue green algae.

The wide distribution of cyanobacteria had enabled them to be classified into five major groups (Vincent, 2009) as follows (Table 2):

Table 2: Classification of Cyanobacteria (Vincent, 2009).

Class	Order	Characteristics	Illustrative genera
Cyanophyceae	Chroococcales	Coccoid cells that reproduce by binary fission or budding	*Aphanocapsa, Aphanotheca, Gleocapsa, Merismopedia, Microcystis, Synechococcus, Synechocystis,*
	Pleurocapsales	Coccoid cells, aggregates or pseudo-filaments that reproduce by baeocytes.	*Chroococcidiopsis, Pleurocapsa*
	Oscillatoriales	Uniseriate filaments, without heterocysts or akinetes.	*Lyngbya, Leptolyngbya, Microcoleus, Oscillatoria, Phormidium, Planktothrix*
	Nostocales	Filamentous cyanobacteria that divide in only one plane, with heterocysts; false branching in genera such as *Scytonema*.	*Anabaena, Aphanizomenon, Calothrix, Cylindrospermopsis, Nostoc.*
	Stigonematales	Division in more than one plane; true branching and multiseriate forms; heterocysts.	*Mastigocladus (Fischerella), Stigonema*

4.3.2.1 Filamentous and non-filamentous cyanobacteria

In terms of form, the blue green algae are divided into two categories; the filamentous and non-filamentous (Cushing and Allan, 2001). The non-filamentous cyanobacteria are usually spherical and elongated in shape. The cells can exist into solitary form or aggregate themselves into colonies. These cyanobacteria are surrounded with mucilaginous material. According to Cushing and Allan (2001), the filamentous cyanobacteria can occur with or without the specialised cells which are called as heterocysts and are responsible for nitrogen fixation. Species that do not have specialised cells are *Oscillatoria* and *Lyngbya*.

4.3.3 Dinoflagellates

The group of dinoflagellates (division Pyrrophyta, class Dinophyceae) is the major phytoplankton in marine and freshwater bodies (Hackett et al., 2004). The abundance of dinoflagellates is supported by its behaviour pattern. According to Hackett et al. (2004), dinoflagellates could flourish in aquatic bodies that are unsuitable for the non-motile microalgae. This is due to the fact that they exist as the swimming cells and even exhibit a diel vertical migration pattern.

Sometimes, the dinoflagellates can be grouped according to the type of pigments they produce (Doak, Moore, Guldberg and Coffroth, 2005). The two major groups are the fucoxanthin and peridinin-containing dinoflagellates. The dinoflagellates are considered as unique as they are able to cause bioluminescent or the production of light in organelles which termed as scintillons (Valiadi and Iglesias-Rodriguez, 2013). In fact, the dinoflagellates are the light-emitting organisms that appear at the surface of ocean.

A significant number of dinoflagellates have been identified to produce potent neurotoxins which are often linked to the red tide phenomenon (Hackett et al., 2004). However, in the current field, the scientists are preferred the term of harmful algal blooms (HABs) after proper definitions about this phenomenon were published past the 1970s (Kim, 2010). The HABs toxins are capable to affect humans, other mammals, seabirds and fish (Hackett, et al., 2004) where in severe cases, there have been records on the human deaths.

Generally, the classification of dinoflagellates is based on morphology, overall shape and position of girdle. The botanists have regarded the dinoflagellates as plants due to the presence of chloroplasts (Austin, 1988). The following (Table 3) is the outline of dinoflagellates classification (Austin, 1988).

Table 3: Classification of dinoflagellates.

Order	Family	Representative genus
Procentrales (anteriorly inserted flagella; 2 main thecal plates)	Procentraceae	*Procentrum*
Dinophysiales (flagella inserted near anterior end; transverse groove present; thecal plates present)	Amphisoleniaceae Dinophysiaceae	*Amphisolenia* *Dinophysis*
Gymnodiniales (naked cells, i.e: no substantial thecal plates)	Gymnodiniaceae Lophodiniaceae Polykrikaceae Pronoctilucaceae Warnowiacea	*Gymnodinium* *Aureodinium* *Polykrikos* *Pronoctiluca* *Warnowia*
Noctilucales (large naked cells often with single tentacles; reproduction)	Noctilucaceae	*Noctiluca*

Pyrocrystales (non-motile coccoid cells; reproduction is by a motile male and female reproductive cells)	Pyrocystaceae	*Pyrocystis*
Peridiniales (distinct thecal plates resembling armour; transverse groove present)	Cladopyxidaceae Gonyaulacaceae Heterodiniaceae Oxytoxaceae Peridiniaceae Podolampaceae Pyrophacaceae Tridiniaceae	*Micracanthodinium* *Gonyaulax* *Heterodinium* *Oxytoxum* *Protoperidinium* *Podolampas* *Pyrophacus* *Triadinium*
Blastodiniales (parasitic; reproduction is by a motile gymnodinioid cell)	Dissodiniaceae	*Dissodinium*

4.4 The lack of epiphytic microalgae species data

Presently, there is still insufficient data on the documentation of the species number of epiphytic microalgae particularly in mangrove areas. The latest specific study about epiphytic algae on pneumatophores of mangrove vegetation was done by Naidoo et al. (2008) in Africa. However, the studies on benthic microalgae especially on diatoms are becoming a subject of interest in many academic institutions. For example studies are done by Harith (2006) which include the assemblage of microalgae in mangrove area while Rawi (2008) and Bayang (2007) were focusing on the benthic diatoms. There is optimism that the future research on epiphytic microalgae can be done more frequent.

5.0 METHODOLOGY

5.1 Sampling Sites

The mangrove forests at Melaban River and Asajaya were selected as study sites. The coordinates for both sites were N 01° 28. 479' E 110° 26.772' and N 01° 35.757' E 110° 36.206' respectively. GPS (Global Positioning System) 76 model Garmin was used to retrieve the coordinates of the sampling sites.

The mangrove forest in Melaban River was covered by various mangrove vegetations such as *Sonneratia alba, Avicennia* and *Nypa.* On the other hand, the mangrove area in Asajaya was much diverse with the presence of *Sonneratia, Avicennia, Nypa, Rhizophora* with variety of species for each genus. The samplings were conducted from February 2015 to March 2015.

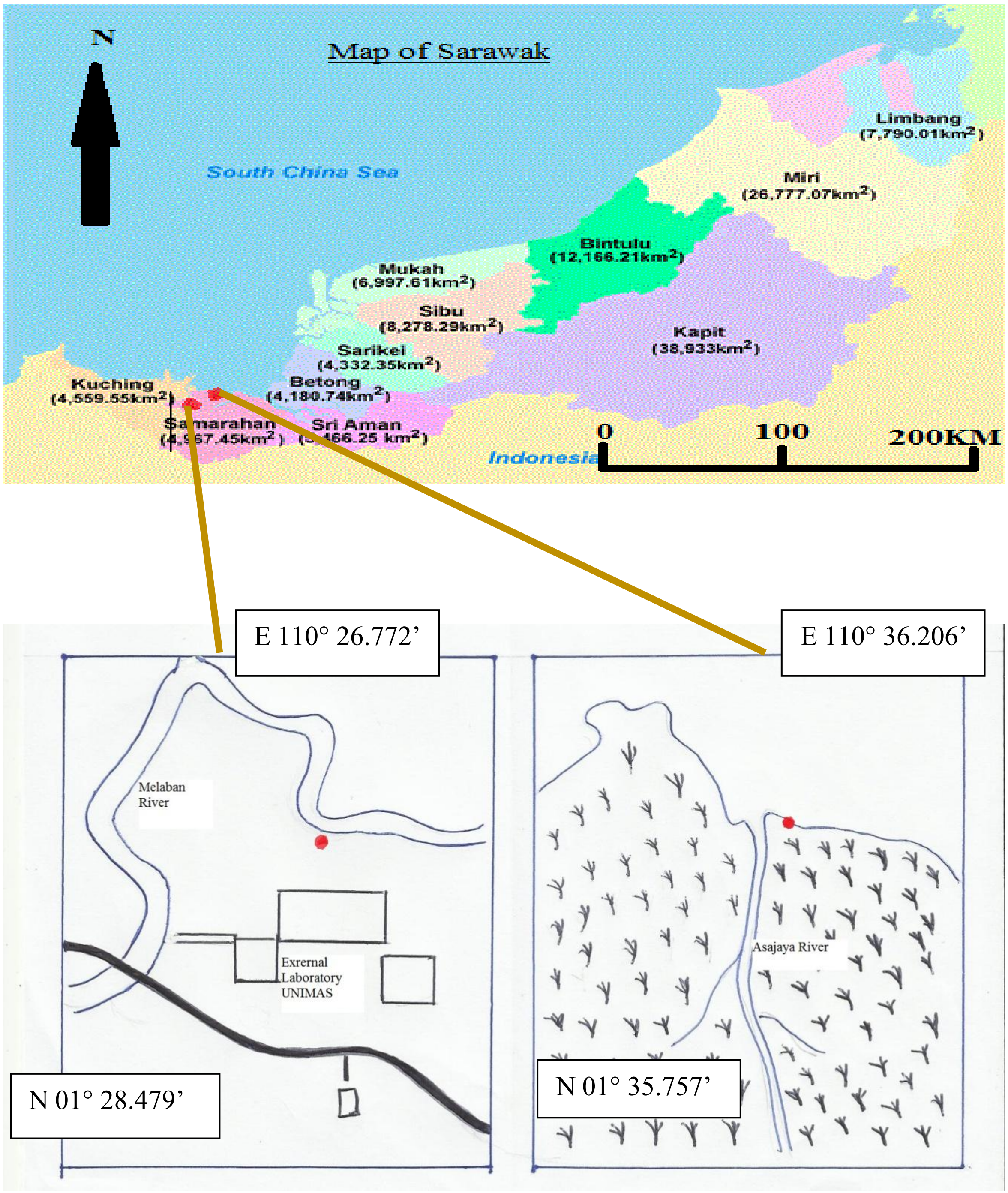

Figure 1: Map of Sarawak, Melaban River and Asajaya mangrove (From top to bottom)

Legend: ● = sampling site