Tropical Life Sciences Research, 21(2), 51–67, 2010

Perspectives on the Use of Algae as Biological Indicators for Monitoring and Protecting Aquatic Environments, with Special Reference to Malaysian Freshwater Ecosystems

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Abstrak: Komuniti alga mempunyai banyak sifat-sifat sebagai penunjuk biologi perubahan ruang dan masa alam sekitar. Parameter alga, terutamanya struktur komuniti dan variabel berfungsi yang dapat digunakan dalam program pemantauan biologi dibangkitkan dalam dokumen ini. Penunjuk biologi seperti alga hanya sejak kebelakangan ini dimasukkan ke dalam penilaian kualiti air di beberapa kawasan di Malaysia. Penggunaan parameter alga untuk mengenal pasti pelbagai bentuk pendegradan air adalah perlu dan pelengkap kepada penunjuk alam sekitar yang lain.

Kata kunci: Pencemaran Air, Alga, Diatom, Pemantauan Biologi, Penunjuk Biologi

Abstract: Algal communities possess many attributes as biological indicators of spatial and temporal environmental changes. Algal parameters, especially the community structural and functional variables that have been used in biological monitoring programs, are highlighted in this document. Biological indicators like algae have only recently been included in water quality assessments in some areas of Malaysia. The use of algal parameters in identifying various types of water degradation is essential and complementary to other environmental indicators.

Keywords: Water Pollution, Algae, Diatom, Biological Monitoring, Biological Indicators

INTRODUCTION

Governmental agencies and the general public have become increasingly concerned about maintaining the quality of aquatic resources. Physical and chemical measurements provide quantitative data on the presence and levels of aquatic pollution and degradation, but these parameters do not reflect the extent of environmental stress reaching the living organisms or the subsequent effects of this stress. Karr and Chu (1999) stated that our ability to protect biological resources depends on our ability to identify and predict the effects of human actions on biological systems; thus, the data provided by indicator organisms can be used to estimate the degree of environmental impact and its potential danger for other living organisms. Kovacs (1992) defined biological indicators as organisms (or populations) whose occurrence reflects the environmental conditions. Biological monitoring is the specific application of biological response for the evaluation of environmental change for the purpose of using this information in quality control program. In an effort to characterise more precisely the cumulative impact of human activities on

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ecosystems, it is important to shift environmental monitoring from sole reliance on chemical indicators towards the increased use of biological conditions (McCormick & Cairns 1994).

Algae are an ecologically important group in most aquatic ecosystems and have been an important component of biological monitoring programs. Algae are ideally suited for water quality assessment because they have rapid reproduction rates and very short life cycles, making them valuable indicators of short-term impacts. Algal assemblages are typically species rich, and algal species exhibit wider distributions among ecosystems and geographical regions. As primary producers, algae are most directly affected by physical and chemical factors. Algal assemblages are sensitive to some pollutants and they readily accumulate pollutants, and algal metabolism is also sensitive to the variation of environmental and natural disturbances. Algae are easily cultured in the laboratory and sampling is easy, inexpensive and creates minimal impact on resident biota; relatively standard methods exist for the evaluation of functional and non-taxonomic structural characteristics of algal communities (Stevenson & Lowe 1986; Rott 1991; Round 1991; Van Dam *et al.* 1994; McCormick & Cairns 1994; Stevenson & Pan 1999). Alterations and shifts in the species composition and productivity of algal assemblages in response to anthropogenic stresses should be considered in order to predict the effects on food web interactions and other ecosystem components (McCormick & Cairns 1994). Algae affect the taste and smell of water, and forecasting the movement and growth of algae in river systems is important for operational managers responsible for the distribution and supply of potable water (Whitehead & Hornberger 1984).

Periphyton are one of the most important algae associated with substrates in aquatic habitats. Periphyton have been widely used as a tool for biologically monitoring water quality (e.g., Leland & Carter 1985; Newman *et al.* 1985; Cosgrovea *et al.* 2004). These organisms exhibit high diversity and are a major component in energy flow and nutrient cycling in aquatic ecosystems. Many characteristics of periphyton community structure and function can be used to develop indicators of ecological conditions in the aquatic ecosystem (Hill *et al.* 1999). Periphyton are sensitive to many environmental conditions, which can be detected by changes in species composition, cell density, ash free dry mass (AFDM), chlorophyll, and enzyme activity (e.g., alkaline and acid phosphatase). Each of these characteristics may be used, singly or in aggregation, to assess conditions with respect to societal values, such as biological integrity and trophic condition. The advantages that periphyton communities have over other organisms for monitoring purposes include the following: fixed habitats, so they cannot avoid pollution; relatively quick recolonisation after perturbations in water quality or flow, the ability to enable a rapid resumption of monitoring; the ease of sample preparation for analysis; and widespread, common taxa, enabling their pollution tolerances to become well known (Biggs 1985).

Diatoms have been used extensively in water quality monitoring (Round 1991). They exist in a wide range of ecological conditions, colonising almost all suitable habitats; they can thus provide multiple indicators of environmental change (Stevenson & Bahls 1999). Indices of water quality using diatoms gave the most precise data compared to chemical and zoological assessment (Leclercq 1988).

Algae as Bioindicators of Aquatic Ecosystem Health

Aquatic populations are impacted by anthropogenic stress, resulting in a variety of alterations in the biological integrity of aquatic systems. Algae can serve as an indicator of the degree of deterioration of water quality, and many algal indicators have been used to assess environmental status. Kolkwitz and Marsson (1908) were the pioneers who classified algal species based on their tolerance to various kinds of pollution. They stated that the presence of certain species of algae could define various zones of degradation in a river. Palmer (1969) published a composite rating of algal species that could be used to indicate clean and polluted waters. Patrick (1949) developed community indices and provided information that demonstrated that a healthy community is made up of numerous species in several groups of organisms, including algae. Patrick (1971) proposed a numerical approach to study water quality using diatom flora attached to glass slides as artificial substrates. Dixit *et al.* (1992) discussed diatom flora as a powerful indicator of environmental change and its emergence as a preferred indicator in monitoring studies. Algae are also used in laboratory bioassays to study water quality, using media for culturing indicator species from the field or defined media to which varying degrees or concentrations of the pollutant are added (Ho 1980; Guckert *et al.* 1992; Grimshaw *et al.* 1993; Knauer *et al.* 1997). Table 1 summarises the algal attributes and indicators that were used in biological monitoring programs. To define the effects of various types of river degradation, it is important to use a variety of algal parameters (Patrick 1973).

Nutrient enrichment is one of the most common anthropogenic stresses in lakes, and limnologists have demonstrated the strong relationship between nutrient loading and phytoplankton biomass. Algae can grow in abundance to the extent that they change the colour of water, which can significantly impair the recreational uses of aquatic systems. Blue-green algal toxins are contained within the living cells and will be released by cell decay. Eutrophication is a fundamental concern in the management of all water bodies and has been one of the focal points of contemporary research in lakes, leading to the development of several statistical models to predict the effects of nutrient loading on phytoplankton biomass. Blue-green algal blooms are an environmental hazard that impairs the quality of water in lakes, reservoirs and rivers. Predictive models based on the microbial and ecological processes in freshwater bodies are useful for developing management responses aimed at reducing the negative consequences of algal blooms on the community.

Table 1: Main algal attributes and associated indicators commonly used in monitoring programs (modification from McCormick & Cairns 1994).

Attribute	Indicator	Reference
Community structure		
Biomass	Ash-free-dry-weight (AFDW)	Ho* (1976); Vymazal and Richardson (1995); Putz (1997); McCormick *et al.* (1997); Hill *et al.* (2000a)
	Chlorophyll *a*	Ho* (1976); Joy *et al.* (1990); Welch *et al.* (1992); Putz (1997); Hill *et al.* (2000a); Biggs (2000)
	Autotrophic index (AFDW: chlorophyll *a*)	Putz (1997); Bourassa and Cattaneo (1998); Wan Maznah *et al.** (2000); Hazzeman* (2008)
	Cell biovolume	Stevenson and Lowe (1986)
Diversity	Species diversity (diatom)	Stevenson (1984); Nather Khan* (1991); Ho and Peng* (1997); Stewart *et al.* (1999); Maznah and Mansor* (1999); Wan Maznah and Mansor* (2002); Hazzeman* (2008)
	Species richness	Anton* (1981); Nather Khan* (1990); Maznah and Mansor* (1999)
Composition	Multivariate analysis (diatom)	Sabater *et al.* (1988); Kelly *et al.* (1995); Stewart *et al.* (1999); Hill *et al.* (2000a); Winter and Duthie (2000); Wan Maznah and Mansor* (2000, 2002); Nor Ashidi *et al.** (2006); Makhlough* (2007)
	Similarity indices (diatom)	Heckman *et al.* (1990); Stevenson (1984); Maznah and Mansor* (1999)
Community metabolism		
Net production	Change in biomass	Ho* (1976); Keithan and Lowe (1985); Biggs (2000)
	Relative specific growth rate	Rosenfeld and Roff (1991); Rier and King (1996)
Productivity	Oxygen evolution	Tease *et al.* (1983); Blanck (1985)
	Radioisotopic tracer (^{14}C)	Keithan and Lowe (1985); Shamsudin* (1987); Napolitano *et al.* (1994); Vadeboncouer and Lodge (2000)
	Photosynthetic capacity	Napolitano *et al.* (1994); Rier and King (1996)
Bioaccumulation	Nutrients	Grimshaw *et al.* (1993)
	Metals	Knauer *et al.* (1997); Paweena* (2005)

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Table 1: *(continued)*

Attribute	Indicator	Reference
Metabolic state	Adenylate energy charge	Hino (1988)
Biomolecules	Ribonucleic acid	Guckert *et al.* (1992); Napolitano *et al.* (1994)
Enzyme activity	Alkaline phosphatase activity	Guckert *et al.* (1992)
Population analyses		
Indicator species	pH index	Cox (1990); Whitmore (1989)
	Pollution tolerance index	Palmer (1969); Descy (1979); Lange-Bertalot (1979); Kelly *et al.* (1995)
	Saprobien index	Pantle and Buck (1955); Lange-Bertalot (1979); Friedrich *et al.* (1992); Ho and Peng* (1997); Wan Maznah and Mansor* (2002); Makhlough* (2007); Hazzeman* (2008)
	Diatom index	Prygiel and Coste (1993); Kelly *et al.* (1995)
	Microalgae spectral analysis	Vanlandingham (1976)
	Trophic index	Whitmore (1989); Makhlough* (2007)
Growth	Algal growth potential	Ho* (1980); Pringle (1987); Lukavsky (1992); Fujimoto and Sudo (1997); Wan Maznah *et al.** (2007)

Note: * indicates research conducted in Malaysia

Running waters dominate the Malaysian inland aquatic environments and support a rich diversity of flora and fauna (Khoo *et al.* 2003), whereas man-made lakes dominate among the lentic ecosystems. In Malaysia, development has inevitably resulted in adverse changes in the hydrology and ecology of wetland ecosystems. The developments are associated with more land use, increases in population urbanisation and industrialisation, and the expansion of irrigated agriculture, all of which have affected the quantity and quality of the water supply (Ho 1995; Sánchez *et al.* 2007). It was expected that Malaysia will face a water shortage by 2010, when its existing water production capacities will be reduced due to demands such as human population and economic growth (Chan & Nitivattananon 2006). The Department of Environment (DOE) has classified 40 rivers as polluted and about half of that number is on the "most polluted" list. In Malaysia, biological aspects have only recently been included in the integrated water quality monitoring program study, and few algal studies have been conducted in relation to water pollution. Most of the information from water quality studies remains unpublished, and some of it is available through regional seminars or internal university press.

One of the earliest algal studies conducted in relation to water pollution was carried out by Ho (1976), who studied periphyton production in the disturbed Renggam Stream, Selangor. Nather Khan (1985, 1990, 1991) conducted studies on the pollution status of the Linggi River Basin, Seremban, Negeri Sembilan using diatoms and reported that there was a marked variation in species between the unpolluted and polluted stations. Anton (1981) recorded a decrease in periphytic algal species in the downstream stations due to heavy siltation in the Langat River, Selangor. Phytoplankton composition changed in response to the addition of both NO_3-N and PO_4-P in the Ulu Langat Reservoir, Selangor (Anton & Abdullah 1982), and Cyanophyta was dominant when nitrogen was the limiting factor. Mansor and Lidun (1992) reported the presence of several species of filamentous algae and a high nutrient concentration in the Pulau Pinang rivers, which strongly indicated that some of them are polluted. Maznah and Mansor (1999) studied diatom diversity and its relation to river pollution and concluded that diversity values could be related to changes in water quality. In a related study, Wan Maznah and Mansor (2000) reported the occurrence of clean, polluted and brackish diatom species collected from artificial substrates (glass slides) along the Pinang River Basin, Pulau Pinang and its tributaries (Table 2). The differences in the specific sensitivity of certain diatom species to pollution were a reliable and useful means of assessing the degree of pollution in the Pinang River system, but the diversity of diatoms could not be directly related to water quality (Wan Maznah & Mansor 2002).

Table 2: Clean, polluted and brackish water species of diatoms attached to artificial substrates (glass slides) at the Pinang River Basin (Wan Maznah & Mansor 2000).

Clean water species	Polluted water species	Brackish water species
Achnanthes minutissima	*Achnanthes exigua*	*Cocconeis* sp.
Achnanthes oblongela	*Achnanthes exigua* var. *heterovalva*	*Coscinodiscus argus*
Achnanthes woltereckii	*Hantzschia amphioxys*	*Coscinodiscus antiquus*
Cocconeis placentula	*Nitzschia amphibia*	*Coscinodiscus excentricus*
Cocconeis pediculus	*Nitzschia fonticola*	*Coscinodiscus decipiens*
Cocconeis thumensis	*Nitzschia palea*	*Coscinodiscus symmetricus*
Eunotia pectinalis var. *minor*	*Pinnularia biceps*	*Cyclotella comta*
Fragilaria capucina	*Pinnularia biceps f. petersenii*	*Cyclotella striata*
Gomphonema acuminatum	*Pinnularia microstauron*	*Diploneis ovalis*
Psammothidium bioretii		*Diploneis interrupta*
Surirella linearis		*Diploneis bombus*
Surirella tenuissima		*Nitzschia littoralis*
		Nitzschia obtuse
		Nitzschia obtuse var. *scalpelliformis*
		Nitzschia sigma
		Surirella ovalis

Algal studies were also included in the river monitoring program by the Department of Environment (DOE 1998, 1999) to indicate the trend and status of the water quality of Malaysian river systems. Ho and Peng (1997) classified the water quality of the Perlis River (Perlis), Perai River (Pulau Pinang) and Juru River (Kedah) based on the abundance and species composition of phytoplankton. Yeng (2006) reported that water pollution in the Ahning Reservoir, Kedah was associated with the appearance of certain species of phytoplankton, especially dinoflagellates. Yap (1997) used the Shannon index and the saprobic index of phytoplankton for water quality assessment of a river ecosystem and concluded that ecological knowledge can be used in the management of a water body. In Malaysia, the determination of trophic state has been conducted primarily by measurements of physico-chemical parameters, primary productivity and chlorophyll-*a* concentration. In a study conducted in the Muda and Pedu Reservoirs, Kedah (Zulkifli 1980), it was found that both reservoirs were slightly eutrophic based on the phytoplankton assemblages, with moderate levels of nitrogen, alkalinity and pH. In another study conducted in the Mengkuang Reservoir, Pulau Pinang (Makhlough 2007), Carlson modified the trophic state index (Carlson 1977) and showed that the reservoir was near to a mesotrophic state based on the chlorophyll-*a* and Secchi disk transparency data, but the Shannon and saprobic indices of phytoplankton indicated that the reservoir was slightly polluted (class III) and moderately polluted (class II), respectively. The study also recorded the presence of *Anabaena*, *Microcystis*, *Oscillatoria*, *Nostoc*, *Dinobryon*, *Chroococcus*, *Staurastrum paradoxum* and *Mallomonas*, which are indicators of toxicity and pollution in aquatic ecosystems, thereby showing that algological studies are important for water quality assessment and can provide an early warning sign of water degradation.

Approaches for Biomonitoring of Aquatic Ecosystems Based on Algae

The oldest approach for using algae to assess stream water quality is based on the indicator species concept (Saprobien system). The Saprobien system is widely used in municipal and wastewater monitoring (Hill *et al.* 2000a) and discriminates between polluted and clean streams (Ho & Peng 1997). In our monitoring study conducted at the Pinang River Basin, Pulau Pinang (one of the polluted river basins in Malaysia), the saprobic index (Pantle & Buck 1955), which was based on diatom species assemblages on glass slides, successfully divided the sampling stations into four zones of saprobic contamination: the Polysaprobic Zone, the Alpha-mesosaprobic Zone, the Beta-mesosaprobic Zone and the Oligosaprobic Zone. The zones of saprobic contamination were characterised by the occurrence of certain groups of diatom species, namely Saprobiontic species, Saprophilic species, Saproxenous species and Saprophobous species (Wan Maznah & Mansor 2002). The saprobic index was calculated based on periphytic algal species composition in the Petani River Basin, Kedah (Hazzeman 2008) and revealed that water quality evaluation using diatom indices was consistent with the physical and chemical determination (Lehmann & Lachavanne 1999; Almeida 2001). Chemical stresses in aquatic ecosystems modify the taxonomic composition of the algal population, causing a reduction of sensitive species and an increase in the number of tolerant species (Genter & Lehman 2000; Biosson & Perrodin 2006).

A hierarchical framework is being used in the development of the periphyton indices of aquatic ecosystems. The framework involves the calculation of composite indices for biotic integrity, ecological sustainability, and trophic condition. The composite indices are calculated from the measured or derived first-order and second-order indices. The first-order indices include species composition (richness, diversity) (e.g., Winter & Duthie 2000; Soininen & Niemelä 2002; Potapova *et al.* 2005; Yallop *et al.* 2009), cell density, AFDW, chlorophyll, and enzyme activity (e.g., Saylor *et al.* 1979), which individually are indicators of the ecological conditions in an aquatic ecosystem. Second-order indices are calculated from periphyton characteristics, such as the autotrophic index (Weber 1973) and community similarity, compared to reference sites. Irvine and Murphy (2009) used a 'weight of evidence' approach to assess the trophic status and phytoplankton community characteristics in the Buffalo River, USA Area of Concern (AOC). They found that the phytoplankton community exhibits some anthropogenic impact, but that these impacts do not indicate extreme stress.

The approach based on the algal indices of community structure (diversity, evenness, richness, similarity), with the assumption that a pristine and healthy environment is typified by a greater diversity of organisms than found in degraded environments, has been used for monitoring rivers. However, several studies have questioned its reliability (Archibald 1972; Descy 1979; Stevenson 1984; Nather Khan 1991; Ho & Peng 1997; Maznah & Mansor 1999), arguing that the relationship between diversity and environmental quality is more complex than was previously thought and that the diversity might be high in stressed environments. Although the study conducted at the Pinang River Basin demonstrated that the difference in diatom species diversity could be related to changes in water quality (Wan Maznah & Mansor 2002), comparing diversity as a tool to discriminate water quality conditions was restricted to sampling stations upstream from those near the estuary. To accurately estimate the water quality using species diversity, it is necessary to precisely define the species that compose the community and to have thorough knowledge of their autecology (Archibald 1972).

Multivariate analysis that is based on the correlation of organism assemblages (especially diatoms) with environmental data has been developed to assess water quality (Sabater *et al.* 1988; Kelly *et al.* 1995; Hill *et al.* 2000a; Winter & Duthie 2000). Researchers found that the methods that compare the distribution patterns of diatom communities in the rivers with physico-chemical parameters allow for the analysis of the relationship between biotic and abiotic variables. Discriminant analysis using the density of diatoms attached on glass slides from our observation at the Pinang River Basin successfully discriminated sampling stations into clean, polluted and brackish waters (Wan Maznah & Mansor 2002).

Non-taxonomic measures of algae (e.g., wet, dry and ash-free weights, caloric contents, chlorophyll *a* and other photosynthetic pigments, and biochemical components such as ATP and DNA) can also be useful for detecting effects not indicated by taxonomic analysis (Hill *et al.* 2000b; Yamada & Nakamura 2002; Cosgrovea *et al.* 2004). Periphytic algae fatty-acid biomarkers revealed differences in the taxonomic composition of periphyton between reference and polluted sites (Napolitano *et al.* 1994). Estimates of community biomass based on the AFDW and chlorophyll *a* have been an integral part of ecological studies of aufwuchs concerning production (as biomass accumulation overtime) (Ho 1976), both as a productivity indicator or index of the photosynthetic potential and as an indicator of nutrient stress or community conditions (Clark *et al.* 1979). Autotrophic index (AI), determined as the ratio of AFDW:chlorophyll *a* in the periphyton collected on an artificial substrate, indicates the degree of organically polluted waters. AI increases in proportion to the concentration of organic matter (or BOD) because heterotrophs occupy a greater portion of the biomass as organic pollutants increase (Welch & Lindell 1992). In the study conducted at the Pinang River Basin (Wan Maznah *et al.* 2000) and Petani River Basin (Hazzeman 2008), AI reflects the conditions of the sampling stations, which have different levels of pollution; the results were consistent with those reported by Weber (1973), Clark *et al.* (1979), Matthews *et al.* (1982), Bourassa and Cattaneo (1998) and Hill *et al.* (2000b).

The problems associated with blue-green and toxic algae have prompted long-term monitoring of the physical, chemical and biological parameters of freshwater ecosystems worldwide. Data from these monitoring activities contain a wealth of information about the behaviour of these ecosystems that is rarely fully explored.

Concluding Remarks

There is a great variety of methods by which algae may be used as indicators of river water quality. Although the biotic indices and non-taxonomic measurements of algae clearly reflect the conditions of water quality, it is important to note that such measurements should not be taken as an absolute measure of the river perturbations but may be considered as a helpful description of the algal community response to such disturbances that complements other environmental indicators. Because no one group of organisms is always best suited for detecting and assessing the environmental disturbance associated with human activities, indicators derived from several groups of organisms should be included in water quality monitoring programs to provide a comprehensive signal of ecosystem change.

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