PHYTOPLANKTON AS BIOINDICATOR OF WATER QUALITY

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(16912)

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(Aquatic Resource Science and Management)
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Acknowledgement

"In the name of God, the most gracious, the most compassionate”

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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>B</td>
<td>Bottom</td>
</tr>
<tr>
<td>CANOCO</td>
<td>Canonical Community Ordination</td>
</tr>
<tr>
<td>Chl a</td>
<td>Chlorophyll a</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>DCA</td>
<td>Detrended Corresponded Analysis</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolve oxygen</td>
</tr>
<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>L</td>
<td>Liter</td>
</tr>
<tr>
<td>m</td>
<td>Meter</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>NH₃⁺</td>
<td>Ammonia</td>
</tr>
<tr>
<td>No.</td>
<td>Number</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>Phosphate</td>
</tr>
<tr>
<td>RDA</td>
<td>Redundancy Analysis</td>
</tr>
<tr>
<td>S</td>
<td>Surface</td>
</tr>
<tr>
<td>sp.</td>
<td>Species</td>
</tr>
<tr>
<td>Temp.</td>
<td>Temperature</td>
</tr>
<tr>
<td>Trans.</td>
<td>Transparency</td>
</tr>
<tr>
<td>UNIMAS</td>
<td>Universiti Malaysia Sarawak</td>
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</table>
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Phytoplankton as Bioindicator of Water Quality

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ABSTRACT

A study on phytoplankton and its relationship with the water quality was conducted at UNIMAS Lake, Malaysia. Specifically, the aim of this study is to determine the potential phytoplankton taxa for bioindicator of water quality. Sampling was conducted from September to December 2008. Quantitative sampling was done using the Van Dorn bottle sampler at four selected sampling stations. A total of 25 phytoplankton taxa consist of Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae, and Euglenophyceae classes has been identified. Switching in dominant taxa was observed during the study. Chlorophyceae was dominant in September and October while Bacillariophyceae dominated in November and December. Bacillariophyceae dominated the area with low light condition, Chlorophyceae identified as bioindicator for medium water temperature range from 30°C to 35°C, Trachelomonas sp. as indicator for high level of phosphorus, Ankistrodesmus sp. as bioindicator for high nitrate concentration, while Spirulina sp. can be considered as bioindicator for low dissolved oxygen concentration in water.

Key words: Phytoplankton, bioindicator, water quality, UNIMAS Lake

ABSTRAK


Kata kunci: Fitoplankton, petunjuk biologi, kualiti air, Tasik UNIMAS
1. Introduction

Phytoplankton have been used as biological classification tool for lakes and slow flowing water since the late 19th century (Heinonen et al., 2000). Phytoplankton are excellent indicators of ecological changes because they are relatively easy to detect, but quite hard to identify, and quantify, they conduct a large share of primary production and they are sensitive to diverse environmental stress (Paerl et al., 2007). As a sensitive bioindicator of aquatic ecosystems, they play significant role in the environmental quality control (Balode, 1999).

Krebs (1994) stated that phytoplankton reacts in two ways: bottom – up and/or top – down. Bottom – up control species growth such as light intensity, temperature, salinity, availability of nutrients, nitrogen and phosphorus ratio and chemical form, while top – down control its biomass like predation and competition. Thus, phytoplankton communities have been used as bioindicator through their response to the change of biological, chemical and physical parameters (Bianchi et al., 2003).

According to King (2009), populations of the phytoplankton will grow or diminish rapidly in response to changes in its environment. Changes in the trends for a given phytoplankton population such as its density, area distribution, and rate of population growth or diminishment will give information about the environmental conditions that are changing there. Then, by comparing these phytoplankton trends to other measurements such as temperature will tell more about how phytoplankton may be contributing to and affected by climatic and environmental change.

Alterations in community structure include a change from a more diverse to a less diverse community with fewer species or taxa and may also be composed mostly of species
that are tolerant of or adapted to polluted conditions and pollution-sensitive species (Connel & Miller, 1984). According to Askjar et al. (1989), phytoplankton are sensitive to some pollutants at levels which may not visibly affect other organisms in the short term or may affect other communities at higher concentrations.

The advantages for using phytoplankton as bioindicators are: (1) phytoplankton have very short life cycles and rapid reproduction, (2) phytoplankton tend to be most directly affected by physical and chemical environmental factors, (3) sampling for phytoplankton is easy, inexpensive, only requires few people to do the sampling and minimally impacts other organisms, (4) the most important thing, there is standard method exist for measuring biodiversity of phytoplankton.

1.1 Justification

UNIMAS Lake was purposely built as natural landscape for the university environment and also for recreational activities like kayaking. The policy made for the lake is to maintain the lake condition as clean as possible by controlling the direct discharge of sewage into the lake. Besides, boats with engine power were not allowed to use in the lake to avoid pollution from oil. Even if all the precaution steps had been applied still there is pollution occur in term of non-point sources like drainage, decaying plants, fertilizer seepage, and etc. Threats of nutrient increase in the water as a result of nutrient runoff from land could cause irreparable damage to the aquatic ecosystem. The measurement of nutrient concentration (orthophosphates, ammonical nitrogen and nitrate) alone may not effectively evaluate the quality of water in the habitat thereby a potentially more informative way of monitoring for an increased nutrient is through the use of bioindicator.
1.2 Objective

Chemical and physical parameters may not be sufficient to furnish a complete picture of the environment. Therefore, examination of biological components such as phytoplankton may supply further elements determining the quality of the water. Specifically, the aim of this study is to determine the potential phytoplankton taxa for bioindicator of water quality.
2. Literature Review

2.1 Phytoplankton

Phytoplankton are free-floating organisms that are capable of photosynthesis (Nyabakken & Bertness, 2005). Maximum production generally occurs at the top layer of the water, but shifts to the subsurface layers on hot days due to photo-inhibition at the surface (Yusoff et al., 1984). Plamer (1962) classified freshwater phytoplankton into seven classes: (1) Cyanophyceae, (2) Chlorophyceae, (3) Euglenophyceae, (4) Bacillariophyceae, (5) Chrysophyceae, (6) Cryptophyceae, and (7) Pyrophyceae.

2.2 The reaction of planktonic algae to environmental changes

Phytoplankton are the most important producer of organic matter in natural water bodies (Balode, 1999). Thus, they are among the first to react to water quality changes and by this they initiate a chain reaction which is successively reflected within other groups of organism (Willen, 2000). According to Heinonen et al. (2000), the following changes in the planktonic algal community are expected in a trophic gradient of increased nutrient availability:

1. A prolonged water blooming season.
2. Increasing biomass variations within the growth season of a year.
3. A change in size structures of the community, in which the proportion of large species increase – many of these are considered as stress tolerant.
4. A decreasing evenness, in which a few species become dominants.
5. A decrease in species richness, especially in hypertrophic environment.
6. A change structure among the algal classes.

The following changes are expected in a gradient of increasing acidity:

1. Decreasing biomasses, species richness and evenness.
2. Increasing trivialization of the plankton flora, in which only a few algal classes occur.
3. The number of life forms decreases and in very acidic water flagellated species prevail.

2.3 Phytoplankton as bioindicator

Important parameters for identification of indicator species were pH, transparency, color, conductivity, alkalinity, total nitrogen and total phosphorus (Rosen, 1981). Other than that, the use of individual species or a community structure as bioindicator involves the identification, classification and quantification of biota in the affected area (Connel & Miller, 1984). The single most widely approach used as a bioindicator index is the listing of species collected from different sampling stations and the presence or absence of that particular species has been used as a bioindicator (Wilhm, 1975).

Researchers from Egypt, Issa & Ismail (1994) have conducted an experiment regarding the sensitivity of phytoplankton species with samples of water from River Nile treated with four different detergents. The names of detergent that they used in the experiment were Biocleana, Lang, Omo and Tide. The water samples treated with detergents were incubated for
14 days after which the algae were counted and identified. Based on the finding, ten algal species that could tolerate all detergent doses were found; one from Chlorophyta (Chlorella sp.); seven from Bacillariophyta (Amphora australis, Caloneis amphipsera, Cyclotella austriaca, Cymbella vertricosa, Nitzschia amphibian, Nitzschia palea and Synedra vina); and two from Cyanophyta (Oscillatoria formosa and Oscillatoria limosa) (Issa & Ismail, 1994). According to Issa & Ismail (1994), these species could be considered as pollution – detergent tolerant species and could be regarded as biological indicators of water pollution.

Algae have been used as indicators for evaluating the degree of water quality. Several authors have come out with different environmental – biological relationship. Plamer (1962) reported that various species of Pediasirum tend to be tolerant to various industrial and organic wastes. The example of Pediasirum species according to Plamer (1962) are: (1) P. boryanum indicator for polysaprobic degree in water, (2) P. duplex indicator for water contaminated by paper mill waste which is toxic to most algae, and (3) P. simplex (salt tolerant species) indicator for brine contaminated water.

Euglenophyceae mainly from Trachelomonas spp. predominated the Amazonian floodplain lake in the dry season (Ibanez, 1998). High standing crops of Euglena sp. in the lake were recorded during low water when the total phosphorus was found highest together with filamentous Cyanophyceae. During the low water, the lake becomes totally isolated from the river. Diatom blooms mainly from Nitzschia palea also occurred in the dry season of lowest precipitation.

A study on species diversity of phytoplankton and its relationship to the tropical lake water quality was conducted at Lake Chini, Pahang, Malaysia by Kutty et al. (2001). Based on
the research, Chlorophyta was quantitatively and qualitatively the most dominant division, which was dominated by *Staurastrum* sp., *Cosmarium* sp., and *Ankistrodesmus falcatus*. The water quality of the lake was within the natural concentration except for nitrate that was detected slightly higher than the natural range. Furthermore *Ankistrodesmus falcatus* was found highly abundant in the sampling site. Thus, *Ankistrodesmus falcatus* is used as indicator for the high level of nitrate – nitrogen.

Rakocevic – Nedovic & Hollert (2003) used phytoplankton community and chlorophyll a data to predict the trophic state of Lake Skandar. Quantitatively, based on this study the most important taxonomic groups of phytoplankton are Bacillariophyceae, Chlorophyceae and Cyanophyceae. They also found that two species of diatoms *Cyclotella glomerata* Bach. and *C. ocelata* Pant. were typical perennial species of Skandar Lake. Based on the phytoplankton data, Lake Skandar was classified as being mesotrophic (Rakocevic – Nedovic & Hollert, 2003). This is due to *Cyclotella* species especially *Cyclotella glomerata* that have small cells with rapid nutrient uptake and turnover rate and are well adapted to moderately nutrient – poor systems.

Phytoplankton study along Tagus estuary by Brogueira et al. (2007) reveal that salinity, temperature, silicate and phosphorus as variables that explain the phytoplankton spatial pattern structure in the estuary. The dominant of algal group Bacillariophyceae in the estuary was correlated by the inclusion of silicates, phosphorus and low light penetration.

Sabater (2000) reported that diatom community characterized by nutrient rich and high mineral content waters was replaced by another diatom community characterized by pollution tolerant taxa. This is due to an accident of mine tailings dam occurred on 1998 in the
Guadianar River S – W Spain that caused the outflow of mud and water rich in heavy metals into the river. The comparison between the reference and affected sites showed a shift from a diatom community dominated by *Flagilaria construens*, *Achnanthes minutissima* and *Amphora pediculus* to another dominated by *Nitzschia palea* and *Gomphonema parvulum*. Correlation analysis between the diatom descriptors and the environmental variables confirmed that heavy metals in the water and sediment had an effect on the diatom communities of the Guadianar (Sabater, 2000).

According to Gao & Song (2005), phosphorus concentration in the Changjiang Estuary, China decreased quickly with the increase of phytoplankton abundance while DO and pH increased with the rise of phytoplankton abundance. Average suspended matter concentration was low on the surface of water, thus phytoplankton in the estuary are not limited by light. Based on this characteristic of rapid *in situ* growth of phytoplankton with high nutrients and sufficient light, a bloom in abundance of certain phytoplankton species especially *Prorocentrum dentatum* and *Skeletonema costatum* was occurred (Gao & Song, 2005).
3. Materials and Methods

3.1 Description of study site

Lake UNIMAS (Figure 1) is located in the central area of west campus of Universiti Malaysia Sarawak, Malaysia. The lake occupies around 45ha, with the shallowest depth is 3.5m and the deepest depth is around 7m deep. This lake has an automatic outlet channel which control the amount of water retained in the lake. When the water in the lake exceed the level, the gate in the outlet will open automatically and released the water to the adjacent area. Fish in this lake include lampam jawa, haruan, jelawat, and tilapia.

Figure 1: Map of Lake UNIMAS with marked sampling location. "S" indicated as station.
3.2 Data collection

Monthly water samples were collected at four stations, at the surface and at 2m depth intervals, with a Van Dorn bottle from September 2008 to December 2008. The locations of each sampling station are shown in Figure 1.

3.21 Water quality

The following parameters were measured in situ: transparency, with a Secchi disk; temperature and dissolved oxygen, by using Hanna HI 9143 microprocessor auto cell; and pH, with Hanna instrument HI 991300. Water samples with 2L were collected at the same depth and preserve in cold, dark conditions for laboratory analysis of phosphorus, ammonia, nitrates, phytoplankton identification and chlorophyll a. Phosphorus analysis was done according to the Method 8048 Powder Pillow Method, ammonia with the Method 8038 Nessler Method and nitrates with the Method 819 Cadmium Reduction Method (Hach, 2000).

3.22 Phytoplankton and chlorophyll a

Phytoplankton was analyzed in terms of biomass and abundance. Phytoplankton biomass was determined by performing chlorophyll a analysis. Water samples were filtered through Whatman GF/F filter papers and pigment extraction was performed with 90% acetone. Pigment concentration was measured by spectrophotometry (Parsons et al., 1984) and calculation was done according to Lorenzen (1967). Pigment diversity was determined based on absorbance at 664, 647 and 630nm.
Counting and identification of phytoplankton cell was made at 400x magnification with an inverted microscope equipped with phase contrast and image analyzer, following Sedgwick Rafter counting chamber (Plamer, 1962). Using the concentrated, 1ml sample was placed on a Sedgwick Rafter counting chamber. 50 field counts and one strip count were made for each sample with a quantitative record of each genus being kept. From this information the number of organisms per liter was determined by multiplying the total number of organisms in each genus according to the equation obtains from Plamer (1962). Identification of phytoplankton to the genus level was made according to Plamer (1962), Wang & I – Cheng Tseng (1984) and images from online materials.
The concentration of chlorophyll $a$ was calculated according to Lorenzen (1967). Concentration are in unit mg m$^{-3}$.

\[
\text{Chlorophyll } a = (11.85^*E_{664}) - 1.54^*(E_{647}) - 0.08^*(E_{630})
\]

Calculation of Chlorophyll $a = (C^*v)/(V^*L)$

Where: $C$ = Chlorophyll $a$

$v$ = Volume of 90% aqueous acetone (ml) used

$V$ = Volume of water filtered for the extraction of the chlorophyll (L)

$L$ = Path length (cm) of the cuvettes used in the spectrophotometer

The number of cell per millimeter of phytoplankton sample was calculated according to Plamer (1962).

\[
\text{No./ml} = (C^*1000\text{mm}^3)/(L^*D^*W^*S)
\]

Where: $C$ = Number of organism counted

$L$ = Length of each strip in mm (length of Sedgwick Rafter)

$D$ = Depth of strip in mm (Depth of Sedgwick Rafter)

$W$ = Width of strip in mm (Width of Sedgwick Rafter)

$S$ = Number of strip counted

Figure 2: Mathematical equation for chlorophyll $a$ concentration and number of cell per millimeter of phytoplankton sample.
3.3 Data analysis

Phytoplankton community in each sampling station was analyzed in terms of taxonomic composition and abundance (Coelho et al., 2007). The differences in species abundance and water quality parameters between month, station and depth during the study period were tested with two-way ANOVA ($p < 0.05$) (Alvarez-Gongora & Herrera-Silveira, 2006). Once the differences exist among the means, Tukey HSD test was performed (Haris & Omar, 2008). Both the statistical analysis was conducted using SPSS 15 for Windows. Correlation analysis (Pearson coefficient) was carried out between environmental variables of water (Sabater, 2000). The environmental variables used were temperature, dissolve oxygen, pH, transparency, phosphate, ammonia, nitrate and chlorophyll $a$.

To investigate the relationships between phytoplankton data and environmental variables, redundancy analysis (RDA) was performed using the software program CANOCO for Windows 4.5 (Lindstrom, 2000; Bianchi et al., 2003; Alvarez-Gongora & Herrera-Silveira, 2006; Brogueira et al., 2007; Coelho et al., 2007). Data were first tested with detrended correspondence analysis (DCA) to determine the appropriate analysis. DCA analysis showed that the length of gradient for phytoplankton data is less than 3. Thus, RDA analysis was chosen. Environmental variables significantly related to phytoplankton data were determined by forward selection and Monte Carlo permutation. All data were transformed using $\log_{10}(x+1)$ to obtain normally distributed. Only significant ($p < 0.05$) environmental variable was shown in the diagram. Ordination diagrams of relationship between phytoplankton data and the significantly environmental variables were constructed by using CanoDraw for Windows 4.14 (ter Braak & Smilauer, 2002).
4. Result

4.1 Physical and chemical water parameters

Result for water temperature, dissolve oxygen, pH, transparency, phosphate, ammonia, and nitrate discuss in this section was referred to Table 1. As the result tested with two-way ANOVA shown that there is no significant different between each station and depth, the data was compile according to month (Table 1 & Figure 3, 4, 5). It was assumed that the reading in each station and depth was same in that month but different between each sampling month.

Water temperature in Lake UNIMAS remained higher in September and October (30.63°C and 30.49°C) and slightly lower in November and December (28.74°C and 28.58°C). No significant different were found between month, station and depth for water temperature in Lake UNIMAS.

Dissolve oxygen concentrations varied significantly with month (p < 0.05) but not with station and depth. Tukey HSD test showed that samples collected in September and November were significantly different from the samples collected in October and December. Dissolve oxygen concentration reading is higher in September and November (3.90 0.71 and 6.89 1.35mg/l) compare with samples collected in October and December (2.84 0.27 and 2.22 0.18mg/l).

pH in the lake varied with month (p < 0.05) but not with station and depth. Tukey HSD test showed that pH reading in October and November were differed significantly with pH reading in September and December. Minimum pH 6.00 0.28 was found in November and maximum pH 7.37 0.28 was found in September.
Water transparency varied with month (p < 0.05) but not with station. Tukey HSD test showed that water transparency in September is significantly different with water transparency taken in October, November and December. The highest water transparency recorded was in September (0.75 m).

Mean phosphorus concentration varied significantly with month (p < 0.05) but not with station and depth. Tukey HSD test showed that the concentration of phosphate was differing between sample taken in September and December with the sample taken in October and November. This is due to the higher phosphate recorded was in September and December (0.88, 0.93 and 0.38, 0.28mg/l). While the lower phosphate recorded was in October and November (0.01, 0.01 and 0.14, 0.14).

Mean ammonia concentrations of samples ranged from 0.01mg/l in September to 0.29 mg/l in December. No significant different were observed for ammonia between month, station and depth.

Mean nitrogen concentration varied significantly with month (p < 0.05) but not with station and depth. Tukey HSD test showed that nitrate concentration in November was differ with nitrate concentration in sample taken in September, October and December. The actual reading for nitrate concentration in September was 0.0225mg/l, October (0.0300mg/l), November (0.0150mg/l), and December (0.0325mg/l). Thus, the slightly differences in this reading create the significant differences between the month.
Table 1: Summary of environmental variables (mean ± SD) at four months observation. Variables that are significantly different at p < 0.05 are indicated as bold.

<table>
<thead>
<tr>
<th>Variables</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>30.63 ± 0.53</td>
<td>30.49 ± 0.60</td>
<td>28.74 ± 3.11</td>
<td>28.58 ± 2.09</td>
<td>0.076</td>
</tr>
<tr>
<td>Dissolve oxygen (mg/l)</td>
<td>3.9 ± 0.71</td>
<td>2.8 ± 0.27</td>
<td>6.9 ± 1.35</td>
<td>2.2 ± 0.18</td>
<td>0.000</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.28</td>
<td>6.35 ± 0.05</td>
<td>6.06 ± 0.26</td>
<td>7.21 ± 0.12</td>
<td>0.000</td>
</tr>
<tr>
<td>Transparency (m)</td>
<td>0.76 ± 0.05</td>
<td>0.49 ± 0.05</td>
<td>0.60 ± 0.12</td>
<td>0.55 ± 0.06</td>
<td>0.001</td>
</tr>
<tr>
<td>Phosphate (mg/l)</td>
<td>0.88 ± 0.93</td>
<td>0.01 ± 0.01</td>
<td>0.14 ± 0.14</td>
<td>0.38 ± 0.28</td>
<td>0.008</td>
</tr>
<tr>
<td>Ammonia (mg/l)</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.29 ± 0.67</td>
<td>0.367</td>
</tr>
<tr>
<td>Nitrate (mg/l)</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Figure 3: Temperature, DO, and pH of Lake UNIMAS. Bar and cap represent the means and standard deviation values of eight samples in every month.