Assessing phytoplankton distribution and water quality in constructed wetlands during dry and wet periods: A Case Study in USM Engineering Campus

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Abstract. Constructed wetlands (CW) are built to improve water quality while serving as an alternative sustainable habitat for aquatic life. In Malaysia, CWs are designed according to the guideline for Urban Stormwater Management Manual for Malaysia (MSMA 2nd Edition) 2012. This study shall evaluate the performance of constructed wetland design as stipulated by MSMA through assessment of water quality and phytoplankton growth, which acts as a biodiversity indicator. A 5-month sampling period with phytoplankton abundance in a constructed wetland was correlated with the selected water quality index (WQI) parameters comprising dissolved oxygen (DO), ammoniacal oxygen (AN), chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solid (TSS), and pH. The constructed wetland consists of three main zones, namely the forebay, macrophytes and micropool zones. The highest WQI value was obtained from the micropool zone with a range of 78.98-85.45 (82.71±3.35) while the lowest WQI value was collected from the macrophytes zone with a range of 68.13-77.96 (73.25±3.42). A total of 15 phytoplankton species from 5 different algal phyla were identified in the constructed wetland. Phytoplankton distribution was high in the macrophytes zone (521 cell m⁻³) during dry season due to high nutrient concentration as opposed to the other zones. The phytoplankton distribution was found to be closely associated with DO, AN, TSS and total phosphorus. At the same time, the macrophyte zone design also affects the distribution of phytoplankton.

1. Introduction
Phytoplankton not only serves as a primary producer in the food chain but also plays a significant part in the primary productivity system. In addition, it acts as a bio-indicator for numerous environmental factors aside from being a biological filter by accumulating and removing nutrients in the aquatic ecosystem. The diversity of algal or phytoplankton community is used in assessing the level of anthropogenic stress to the ecosystem [1]. Thus, it is necessary to measure environmental impacts of phytoplankton community due to land use changes such as the introduction of stormwater constructed wetlands. Constructed wetlands are engineered systems that have been designed and built to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating pollution and wastewater [2]. The free water surface (FWS) constructed wetland (CW) is a popular type of constructed wetland to treat wastewater, as indicated by [3]. Among the types of
wastewater treated by FWS CW are residential stormwater runoff [4] and highway stormwater runoff [5]. A typical FWS CW with emergent macrophytes constitutes a shallow sealed basin or sequence of basins, containing 20-30 cm of rooting soil, with the water depth of 20-40 cm. FWS CWs are effective in removing organics through microbial degradation and settling of colloidal particles [6]. Treatment studies of FWS CWs have also been well documented in tropical climate regions, especially in Malaysia [7-11].

The Urban Stormwater Management Manual for Malaysia (MSMA) is a standard design guideline of the Department of Irrigation and Drainage (DID) Malaysia [12]. Most evaluation and assessments pertaining to water quality studies are based on the first edition of MSMA, implemented since the year 2000. In 2012, the second edition of MSMA was introduced with various improvements in regard to design, monitoring, etc. Being new, only a few assessments and studies have been conducted to evaluate freshwater phytoplankton growth in reference to MSMA Second Edition. Thus, this study hopes to evaluate the distribution of freshwater phytoplankton in a constructed wetland and correlate such distribution with water quality as an environmental factor. Here, water quality shall be evaluated in accordance with the water quality index (WQI) as proposed by the Department of Environment (DOE) Malaysia.

2. Materials and methods

2.1. Site description

The constructed wetland in this study is located in Universiti Sains Malaysia (USM) Engineering Campus (Figure 1). Its exact coordinates are between latitudes 100°29.5’ South and 100°30.3 North and between longitudes 5° 9.4’ East and 5° 8.5’ West in Mukim 9 of Seberang Perai Selatan District, Pulau Pinang. The locality is known as Sri Ampangan, Nibong Tebal, Pulau Pinang which is about 2 km south-east of Nibong Tebal town centre, 1.5 km north-east of Parit Buntar (Perak) and nearly 1.5 km north-west of Bandar Baharu (across Kerian River in Kedah). The campus area is about 320 acres in size and made up of mainly flat land used for oil palm plantation. This constructed wetland receives storm water runoff from 17.6 acres catchment area of faculty buildings and car park areas.

Figure 1. Location of study area of stormwater constructed wetland in USM Engineering Campus.
2.2. Sampling and analysis

The constructed wetland comprises three zones, namely the forebay, macrophyte and micropool zones. The forebay is the initial point to receive discharge from drainage/swale; while the macrophyte zone is composed of high-marsh, low-marsh and deep-marsh zones. This zone is teeming with macrophytes or wetland plant species especially from the emergent types as explained in detail in Table 1. The last zone before water exits the constructed wetland is called the micropool. Table 1 also shows the water depth measurement and types of emergent plants present. Sampling was conducted in a once-a-month basis throughout the period beginning November 2014 until March 2015. Four (4) sampling points were selected in the forebay (IF, FA, FB and FC), seven (7) points were chosen in the macrophytes zone (IAM, IBM, MA, MB, MC, OAM and OBM) and three (3) sampling point were selected in the micropool zone (MCA, MCB, OM). In addition, the study employed a 3-hour sampling duration from 9 am until 12 pm to collect grab samples, with a standard 0.5m below surface water. The phytoplankton were sampled using plankton net, filtered 40L samples were preserved using formalin 5% solution and Lugol concentration. Identification was performed using the taxonomic keys [13] and [14]. Enumeration of phytoplankton samples was carried out in line with the method recommended [15]. The in-situ parameters were determined at the sampling point during the sampling process using YSI Professional Plus handheld multi-parameter meter while laboratory analysis was conducted in accordance with HACH, Water Analysis Handbook 5th Edition. Further, precipitation data were collected using rain gauge placed in the vicinity of the constructed wetland, while Pearson correlation and regression was utilized to quantify the relationship between phytoplankton abundance (cell/ml) and WQI.

**Figure 2.** The sampling point and zonation of constructed wetland; Forebay, Macrophytes zone (High marsh, Low marsh and Deep marsh) and Micropool zone.
### Table 1. Zone characteristics (water depth and plants/macrophytes) in the constructed wetland.

| Zonation          | Water depth (m) (min-max) | Types of plants                        | Plant Density | Picture |
|-------------------|---------------------------|----------------------------------------|---------------|---------|
| Forebay           | 0.6-1.0                   | -                                      | -             | -       |
| Macrophyte Zone   |                           | - Donax grandis, Eleocharis variegata  | 1.63 ind/m²   |         |
| High Marsh        | 0-0.3                     | Phragmites karka                      | 58 ind/m²     |         |
| Low Marsh         | 0.3-0.5                   | Typha angustifolia                    | 29 ind/m²     |         |
| Deep Marsh        | 0.6-1.0                   | -                                      | -             |         |
| Micropool         | 0.7-1.4                   | -                                      | -             | -       |

In this study, the water quality index (WQI) from the Department of Environment (DOE) Malaysia has been adopted to measure water quality based on the given formula;

\[
WQI = (0.22 \times SIDO) + (0.19 \times SIBOD) + (0.16 \times SICOD) + (0.15 \times SIAN) + (0.16 \times SISS) + (0.12 \times SIpH)
\]

Where;

- SIDO = Sub-index DO (% saturation); SIBOD = Sub-index BOD; SICOD = Sub-index COD
- SIAN = Sub-index NH3-N; SISS = Sub-index SS/TSS; SIpH = Sub-index pH

Whereas the sub-index was calculated based on the following formula;

- Sub-index for DO (In % saturation)
  - SIDO = 0 for \( x \leq 8 \)
  - SIDO = 100 for \( x \leq 92 \)
  - SIDO = \(-0.395 + 0.030x^2 - 0.00020x^3\) for \( 8 < x < 92 \)

- Sub-index for BOD
  - SIDOD = 100.4 - 4.23x for \( x \leq 5 \)
  - SIDOD = 108 \times \exp(-0.055x) - 0.1x for \( x > 5 \)
Sub-index for COD

\[ SICOD = -1.33x + 99.1 \quad \text{for } x \leq 20 \]
\[ SICOD = 103* \exp(-0.0157x) - 0.04x \quad \text{for } x > 20 \]

Sub-index for NH3-N

\[ SIAN = 100.5 - 105x \quad \text{for } x \leq 0.3 \]
\[ SIAN = 94* \exp(-0.573x) - 5* I x - 2 I \quad \text{for } 0.3 < x < 4 \]
\[ SIAN = 0 \quad \text{for } x \geq 4 \]

Sub-index for SS/TSS

\[ SISS = 97.5* \exp(-0.00676x) + 0.05x \quad \text{for } x \leq 100 \]
\[ SISS = 71* \exp(-0.0061x) + 0.015x \quad \text{for } 100 < x < 1000 \]
\[ SISS = 0 \quad \text{for } x \geq 1000 \]

Sub-index for pH

\[ SIpH = 17.02 - 17.2x + 5.02x^2 \quad \text{for } x < 5.5 \]
\[ SIpH = -242 + 95.5x - 6.67x^2 \quad \text{for } 5.5 \leq x < 7 \]
\[ SIpH = -181 + 82.4x - 6.05x^2 \quad \text{for } 7 \leq x < 8.75 \]
\[ SIpH = 536 - 77.0x + 2.76x^2 \quad \text{for } x \geq 8.75 \]

Note:

* Means multiply with

3. Results and discussion

3.1. Monthly precipitation and water temperature

Based on rainfall measurement as shown in Figure 3, the months of November and December 2014 were classified as wet season, with the highest rainfall amount measuring 17.5 mm in November and 27.7 mm in December. The graph also shows November to experience higher rainfall frequency compared to December. Dry season, on the other hand, occurred from January 2015 until the end of the sampling period in March 2015. Rainfall had influenced water temperature in the constructed wetland as thermometer reading was below 30°C, while the lowest reading at 28.09°C was obtained in December 2014. Water temperature rose during dry season, as thermometer reading peaked at 30.99°C in February. This is probably due to water in the constructed wetland being exposed to the sun for a longer period, which subsequently caused gradual temperature to rise throughout the sampling period. These three main factors, namely rainfall amount, water temperature and duration of sun exposure, will impact the concentration/value of water quality parameters as well as phytoplankton distribution and abundance.
3.2 Spatial and temporal water quality concentration

Figure 4 shows the monthly variation in water quality as well as the different sampling point results throughout the sampling period. These six parameters make up the main parameter for evaluation as they represent WQI value, which shall sum up the water quality between November 2014 and March 2015. Based on the results, ammoniacal nitrogen (AN) showed significant variation in terms of monthly comparison, whereby low AN value was obtained during the wet season as opposed to dry season. The abundance of water from runoff and precipitation had diluted AN concentration in the months of November and December 2014 in the constructed wetland. From the box plot, AN concentration decreased from the inlet Forebay to the outlet Micropool. The micropool area produced Class II AN in which the lowest concentration of 0.11 mg/l was obtained at OM. For the other parameters, there were variations between the forebay, macrophytes and micropool zones. For BOD5, a high concentration range of 2.12-2.37 mg/l was obtained at the Forebay, while low concentration range between 1.40-2.07 mg/l was obtained at the macrophytes zone. Biodegradable organic matter from microorganism and nutrient concentration affected BOD concentration. In other cases, oxygen production from the phytoplankton may have contributed to the amount of oxygen in the water, which affected degradation process from microorganism as well BOD reading. As for the range of COD concentration, most sampling points fell into Class III, with 24 mg/l and 21 mg/l except for MC and OM. Low COD concentration of 7 mg/l was obtained at MC whereas OM had 9 mg/l. This also shows that organic matter was reduced, starting from the inlet at the forebay before it exits the micropool. The level of organic matter can be ascertained by referring to the TSS concentration. TSS concentration experienced a reduction starting from the inlet at the forebay before it leaves the OM, micropool. In addition, organic particles from decomposing materials might also contribute to TSS concentration. As algae, plants and animals decay, the decomposition process allows small organic particles to break away and enter the water column as suspended solids. However, most sampling points indicate Class I TSS concentration, with reference to the box plot graph. The pH value indicates acceptable ranges between 6.5-8.5, with December 2014 showing low pH value while February 2015 showing high pH value. The pH value indicates low pollutant level in the constructed wetland and this argument can be supported by looking.
at all the graph plots. High DO concentration at the Forebay compared the other zones may be due to sufficient nutrients for phytoplankton to undergo photosynthesis with less amount of microorganism for degradation process. Compared to the macrophyte zone, although the nutrient amount was high, the amount of microorganism is predicted to be high as well due to the high number of plant density. The root plants offer a place for bacteria and microorganism to grow, which use oxygen for the degradation process [16]. Figure 5 shows the different nutrient concentration mean of total nitrogen (TN) and total phosphorus (TP) at each sampling point, with the highest mean obtained at the macrophytes zone, at 0.19-0.34 mg/l for TP and 2.35-16.47 mg/l for TN. The high nutrient concentration obtained in the macrophytes zone was due to the nutrient uptake capability of plants. Jinadasa and team [17] had found that for satisfactory plant establishment (which in this case in the newly planted plant in the newly constructed macrophytes zone) certain period of time required for their establishment such as *Typha* sp took 7-8 month for their establishment for nutrient uptake as well as growth. Plant decay also contributed to the high nutrient in the zone. However, such a problem can be solved through monitoring of the abundant plants and maintaining each marsh area via annual harvesting. This factor had also affected phytoplankton distribution as the density was high at the macrophytes zone compared to the other zones due to nutrient availability.

3.3. Monthly and sampling point variation of phytoplankton abundance

Figure 6 presents the phylum of phytoplankton distribution throughout the sampling period. Four main phylum were observed with Chlorophyta being the most abundant phylum at 585-770 cell/ml, followed by Bacillariophyta (239-298 cell/ml), Cyanophyta (59-103 cell/ml), Chrysophyta (21-46 cell/ml) and Pyrrophyta (9-24 cell/ml). Chlorophyta and Bacillariophyta were the most commonly sampled phylum and are major inhabitants of the freshwater as well as other aquatic ecosystems [18,19]. In addition, Figure 7 shows some of the species observed and obtained in the constructed wetland. The species of phytoplankton in the wetland is an important food source for herbivorous species [20]. Olurin and Awolesi [21] documented that the *Tilapia mariae* and *Chromidotilapia guntheri* fish species, mainly feed on phytoplankton consisting of the desmid genera *Closterium* and *Cosmarium*. Thus, *Closterium* sp suits this constructed wetland well due to the presence of the *Tilapia* sp. fish.
Figure 4. Monthly variation (left side) and sampling point variation (right) of mean concentration of six parameters; (from top to bottom) BOD, Ammoniacal nitrogen (AN), COD, DO, pH, and TSS from November 2014 to March 2015.
Figure 5. Mean total phosphorus (TP) and total nitrogen (TN) concentration from November 2014 until December 2015 in the sampling point constructed wetland.

Figure 6. Monthly distribution of phytoplankton density by phylum in the constructed wetland from November 2014 until March 2015.
3.4. Relationship between phytoplankton, WQI and water quality parameters

Based on Pearson correlation information in Table 2, DO concentration showed a significant high negative relationship with up to 0.01 significance level for phytoplankton phylum, except for Phyrrophyta. The pH value indicated a positive relationship only for Chrysophyta ($p<0.05$). For the other four parameters in WQI, no significance result was found for phytoplankton density. Phytoplankton density affected DO concentration in the water. Phytoplankton decomposed by bacteria was in the constructed wetland. Bauerfeind [22] reported that bacteria play an important role in using oxygen to breakdown the phytoplankton carbon during the process of sedimentation. Thus, the energy released as organic matter is returned to the main food chain through a microbial loop of bacteria and protozoa [23]. As for the nutrient, only TN showed a significant positive relationship ($p<0.05$) with Cyanophyta and Chrysophyta. TN typically stimulates phytoplankton growth in the freshwater ecosystem in lakes and wetlands [5]. However, TP registered no significant result for all phytoplankton and this outcome could be due to source of nutrient.

Table 2. Correlation between phytoplankton and water quality parameter.

| Parameters | Bacillariophyta | Chlorophyta | Pyrrhophyta | Cyanophyta | Chrysophyta |
|------------|-----------------|-------------|-------------|------------|-------------|
| BOD        | -0.309          | -0.253      | -0.089      | -0.29      | -0.263      |
| AN         | 0.336           | 0.339       | 0.196       | 0.091      | 0.053       |
| COD        | -0.185          | -0.201      | -0.052      | -0.406     | -0.414      |
| DO         | **-0.723**      | **-0.836**  | **-0.526**  | **-0.734** | **-0.836**  |
| pH         | 0.430           | 0.477       | 0.115       | 0.380      | 580*        |
| TSS        | 0.236           | 0.144       | 0.336       | -0.006     | -0.005      |
| TN         | 0.502           | 0.428       | 0.435       | **0.629**  | **0.623**   |
| TP         | -0.131          | -0.097      | 0.133       | -0.188     | -0.117      |
| Temp       | **-0.794**      | **-0.847**  | **-0.729**  | **-0.699** | **-0.721**  |
| WQI        | **-0.720**      | **-0.852**  | **-0.593**  | **-0.650** | **-0.736**  |

** Statistically significant at the 0.01 level ; * Statistically significant at the 0.05 level

Based on the information in Figure 8, WQI value developed from the six main parameters showed all a significant negative correlation ($p<0.05$) with all phytoplankton groups. As WQI value decreased in the macrophytes zone to 71.6-77.9, phytoplankton density increased which may be due to the amount of nutrients in this zone as compared to the rest. There are several possibilities which may contribute to this zone having low WQI value compared to the others. First, it may be due to the high amount of nutrient in this area, as indicated by the graph of AN in Figure 4 as well as TN and TP in Figure 5. The source of nutrient may originate from natural processes involving decomposition of plants and animals as well as surface water runoff entering this zone from the forebay. The WQI value also showed suitability of the macrophytes zone for the phytoplankton to inhabit as well as characteristics of the area.
itself, which had low water depth and was likely to have received direct sunlight for photosynthesis to occur. The outlet WQI value of this constructed wetland was high, with the range value of 83.7-85.5 which showed that the constructed wetland had improved the water quality received from the catchment area, treated and polished it, before it was discharged. Johari [24] showed the reduction of nutrient from inlet to outlet of constructed wetland had improve the WQI value to be high, thus it will affect the density of phytoplankton as nutrients play a vital role in the distribution and growth of phytoplankton [25].

In Table 2, it can be seen that DO and water temperature were the most important parameters affected by the abundance of phytoplankton. Figure 9 showed how such relationships were formed in the simple regression analysis. The relationship in DO is complex as it involves a lot of factors including the respiration and decomposition rate of bacteria, which affects the oxygen concentration level in the water. Bacteria is attached to the plants parts. The more plants, the more likely places for the bacteria to inhabit and it is presumed to have high decomposition rate, as the roots and rhizomes in the soil provide surface for bacteria and other microorganisms attachment [7]. Thus, the graph showed that as the density of phytoplankton is reduced, the concentration of dissolved oxygen increases. Smith and Piedrahita [26] found that high algal biomass has only an indirect linkage to low dissolved oxygen. Algal density affects turbidity, inorganic nutrient levels, and algal respiration. These, in turn, affect oxygen levels by influencing net primary production and total plankton respiration. The positive physiological effects of temperature increases might eventually turn into negative effects as soon as the optimal temperature range is exceeded [27]. This can be caused by cellular stress, due to high membrane fluidity and enhanced degradation rates of proteins and enzymes, and if these effects become significant they can negatively affect primary production [28].

**Figure 8.** WQI and phytoplankton density pattern for sampling station in the constructed wetlands
4. Conclusion
As a conclusion, this study proves that WQI has negative correlation with the density of phytoplankton. From the six parameters which constituted the WQI value, only DO and pH showed significant correlation with the distribution of phytoplankton density. Only TN showed slight relationship with some phylum of phytoplankton while water temperature showed significant impacts in relation to all phylum of phytoplankton. There was a slight difference in the monthly distribution of phytoplankton, as it slightly increased in density from the wet season to the dry season. However, by comparing the results from the sampling points, the distribution of phytoplankton was found to be high in the macrophytes zone compared to the other zones in the constructed wetland. As for the WQI parameters, only AN indicated an obvious difference between wet and dry seasons, while the rest only showed differences between the sampling points. For the future study, in this constructed wetland must take into consideration as they may affect the whole calculation, including WQI parameters and phytoplankton density.

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