EFFECT OF NITROGEN AND PHOSPHORUS CONCENTRATION IN THE WATER OF STORMWATER CONSTRUCTED WETLAND TO FRESHWATER PHYTOPLANKTON DISTRIBUTION (10 YEARS ESTABLISHMENT): CASE STUDY IN UNIVERSITI SAINS MALAYSIA

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ABSTRACT:

The understanding the implication of biotic component, especially freshwater phytoplankton in the man-made stormwater constructed wetland under tropical climate condition in Malaysia still rare and less documented. Thus, the aim of the this study is to investigate the effect of nitrogen and phosphorus removal (as an important element for freshwater phytoplankton) in the stormwater constructed wetland in Universiti Sains Malaysia (USM), which designed based on Urban Stormwater Management Manual for Malaysia (MSMA) first edition, 2001, to the freshwater phytoplankton communities, as a primary food chain in aquatic life. This paper also will elaborate the detail design of stormwater constructed wetland during the first construction design was being made. Four (4) main nutrients was measured; nitrite, nitrate, ammoniacal nitrogen and orthophosphate, together with other supporting data such as water temperature, total suspended solid, biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, DO and conductivity. The phytoplankton sampling was conducted at the same time of water quality sampling. The results showed that the concentration of nutrient (especially nitrogen and phosphorus) continuously reduce when enter to throughout the constructed wetland for nitrite (80%), nitrate (61.56%), orthophosphate (60.98%) and NH4-N (51.49%) before it discharge to the outlet. Based on this nutrient concentration reduction pattern, species diversity (H') measured for phytoplankton showed decreasing in number as well as number of individual species and types of species. The dominance species represented by the dominance group Chlorophyta are *Coelastrum microporum*, *Mougeotia sp* and *Westella botryoides.* The freshwater phytoplankton distribution is strongly correlated with the reduction of nutrient concentration, however, for the other biotic component such as freshwater fish, inverse relationship found with the freshwater phytoplankton due to other factors such as the water depth design and water temperature in this constructed wetland.

Keywords: Constructed wetland, Water quality, Biodiversity, Stormwater runoff

1. INTRODUCTION

Freshwater phytoplankton is a primary producer in the static and low flow environment such as freshwater lake and ponds. Generally, the role of phytoplankton is a primary producers and significant portion of system primary productivity (Vollenweider, 1974), bioindicator for numerous environmental factors such as ph, nitrogen (nitrate, nitrite and ammoniacal nitrogen), phosphorus, salinity and oxygen and also act as biological filter, accumulating and removing nutrient flowing through the system (Cronk and Mitsch, 1994). The diverse algal or phytoplankton community, whose composition potentially maybe used as a way of assessing the level of anthropogenic stress to the system (Lowe and Pan, 1996). Thus, we cannot ignore biological assessment, by using organisms such as freshwater phytoplankton, to measure environmental impact, especially land use changes, for example the introduction of stormwater constructed wetland. Stormwater constructed wetland or free water surface (FWS) is an artificial wetland, purposely construct to solve the water quality problems from the stormwater runoff. The main function is to utilize the natural processes involve wetland vegetation, soils and the associated microbial assemblages, which are the active agents in the treatment process (Stottmeister *et al.,* 2003).In Malaysia, *Urban Stormwater Management Manual for Malaysia* (MSMA) from Department of Irrigation (DID) is one of the guidelines to be used to design the stormwater constructed wetland (DID 2001 & 2012). A lot of research had been conducted to identify the capability of stormwater constructed wetland for the improvement of water quality (Mohd Sidek *et al.,* 2001, 2004; Mohd. Noor *et al.,* 2004; Ismail *et al.,* 2008; Sim *et al.,* 2008; Shaharuddin *et al.,* 2013, 2014). The

constructed wetland design under MSMA guideline effectively improves water quality in Malaysia. However, lack of biological assessment was apply especially on phytoplankton community for constructed wetland in Malaysia under this guideline; limit us to explain the factors changes of water quality in more wide and holistic area, correlates with biotic communities. Minimal attention has been given to the relationships between abiotic environment factors and overall biotic communities in constructed wetland (Thullen *et al.* 2008). There are some association between water quality parameters and planktonic communities, including phytoplankton such as correlated to TP, NH4-N, DO, and pH levels (Chen *et al.* 2011).Thus, in this study, the main objective to understand the effect of nutrient removal (nitrate, nitrite, ammoniacal nitrogen and orthophosphate) to the phytoplankton distribution from the MSMA first edition design of stormwater constructed wetlands in a campus area and assessed.

2 METHODOLOGY

2.1 Study Site Description

The stormwater constructed wetland of this study area is located in the Universiti Sains Malaysia (USM) Engineering Campus, Malaysia. The location is located at Peninsular Malaysia at Seberang Perai Selatan District, Pulau Pinang (Figure 1-Left). It lies between latitudes 100°29.5' South and 100°30.3 North and between longitudes 5° 9.4' East and 5° 8.5' West. The area of the campus is about 320 acre and made up of mainly oil palm plantation land and is fairly flat. Seventeen sampling points had been chosen as the study area. The sampling consist of 4 main area or zone; (i) Inlet Wetland (Inlet Wet) which located at the inlet wetland before entering to Macrophyte zone, (ii) Macrophyte zone; which consist of 6 sampling point (W1, W2, W3, W4, W5, W6). The W1, W3 and W5 located at the right bank while for W2, W4 and W6 located at the left bank of the constructed wetland. The reason to divide 6 sampling point in the macrophyte zone was due to avoid potential bias associated with consistent timing or order of sampling. Symmetrical area divided and approximately same composition and density of macrophyte or wetland plants covered for each sampling point were the control variables which will not be factor affecting the value obtain for physico-chemical and phytoplankton distribution. (iii) Micropool zone or open water zone (DID, 2001); divided into three rows (MA, MB, and MC) and each row consists of 3 equally distant point of station (15 meter). The reason to divide three rows in the Micropool zone were (a) to identified the water quality concentration obtained after the affect of macrophyte zone (MC), (b) to identified the effect of decomposition and grazing of organic matter (DID, 2001) at the MB to the water quality concentration and (c) to identified the concentration of water quality before it discharges (MC) and (iv) outlet wetland (OutletWet) zone as the last zone measured for this studied (Figure 1-Right).
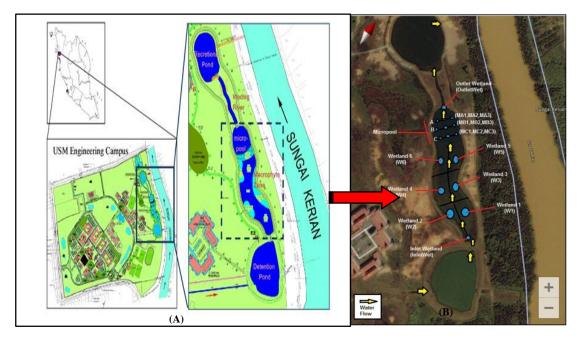

Figure 1. **(A)** Study location **(B)** Sampling point in the constructed wetland

2.2 Initial stormwater constructed wetland design
The stormwater constructed wetland was first being design based on MSMA 1st edition. Figure 2 showed that the actual condition for each sampling points in the wetland which obtained through on-site observation and Figure 3 showed the cross section as well as range of depths for each sampling station for this study by using River surveyor. Figure 4 showed that the detail design of this constructed wetland, base on guideline MSMA 1st edition and

previous study reported by Nor Azazi Zakaria et al. (2003), L.M. Sidek et al. (2004), A. Ab. Ghani et al. (2004) which consists of inlet zone, high marsh zone (0 – 0.6m) and low marsh zone (0.6 – 1.0m) area and open water zone or micropool zone. Table 1 showed detail design criteria of constructed wetland, with the catchment area covered most of the campus area, which include faculties and management buildings, lectures hall, assemble hall, hostels, library and cafeterias. The campus is surrounded by the ecological swale (grass swale) alternative to replace the conventional drain, and the water runoff enters into the stormwater constructed wetland system after passing through the detention pond. At the inlet wetland, the orifice pipe was plug in to reduce the inflow rate and to maximize the dispersion of water into the whole area of the wetland system. The inflow rate, which is design base on 3 month ARI (DID, 2001) was the main and important criteria of this stormwater constructed wetland system for the survival of wetland plant species or macrophyte as well as for the phytoremediation process to take place from the pollutant uptake of plants. Table 2 showed type of plants introduced into the systems, with the specific density and percentage area covered. 48% area covered with the wetland plant species in the area of macrophyte zone a while 12% for overall area of stormwater constructed wetland.
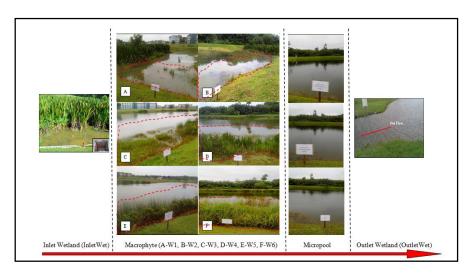

Figure 2. Actual condition for each sampling point in the constructed wetland for inlet wetland, macrophyte zone, micropool zone and outlet wetland
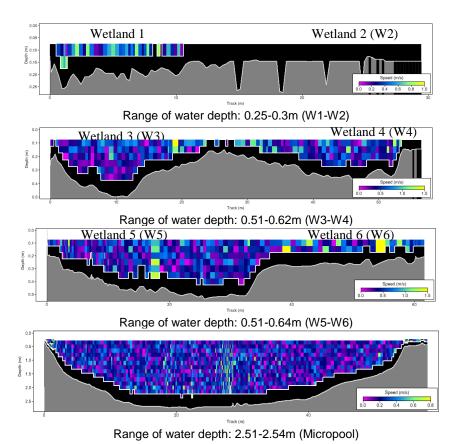
Figure 3.Cross section and range of depth of station in USM Stormwater Wetland (Shaharuddin et al. 2014)

Table 1: Design criteria for the constructed wetland, USM (Ab. Ghani et al. 2004)

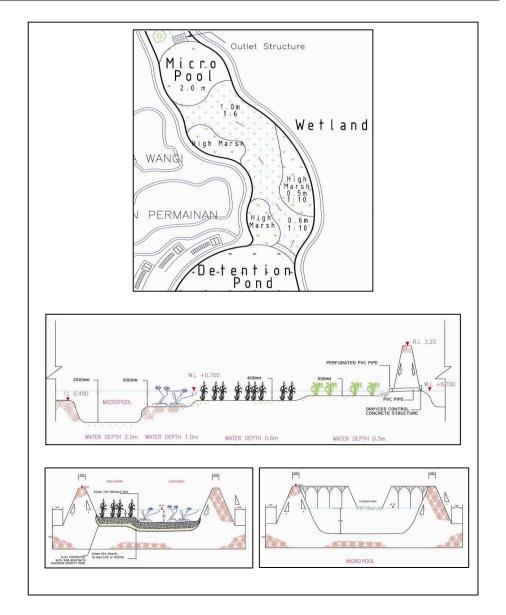| Design Parameter	Criteria
Catchment area	1.214 km^2
Design storm (3 month ARI)	22.5 mm/hr
Length	155 m
Width	60 m
Wetland surface area	$9,100 \text{ m}^2$
% Catchment area	0.7
Design inflow rate	$0.25 \text{ m}^3/\text{s}$
Mean residence time	3 days
Slope of wetland bed	1%
Bed depth	0.6 m
Mediae	Pea gravel and soil mixture
Hydraulic conductivity of gravel	10^{-3} m/s to 10^{-2} m/s

Figure 4. The detail design of constructed wetland from longitudinal section and section view

Table 2: Characteristics of wetland plant species (macrophytes) in the stormwater constructed wetland after 10 years establishment

Scientific name (Common name)	Natural/Planted	Type	Density (ind/m^2)	Percentage area covered in macrophyte area* (%)
Eleocharis variegata (Spike rush)	Planted and natural	Type 1 (less than 0.3m depth)	8.91 (1247 individual)	5 (140m^2)
Eriocaulun longifolium (Asiatic pipewort)	Natural	Type 1 (less than 0.3m depth)	2.52 (323 individual)	3 (92m^2)
Hanguana malayana (Common Hanguana)	Planted	Type 2 (0.3-0.6m depth)	1.96 (1023 individual)	19 (523m^2)
Lepironia articulata (Tube sedge)	Planted	Type 2 (0.3-0.6m depth)	10.39 (3356 individual)	12 (323m^2)
Typha angustifolia (cattail)	Planted	Type 3 (0.6m -1.0m depth)	5.97 (1523 individual)	9 (255m^2)

*area of macrophyte zone = 2736m^2

2.2 Water Quality Sampling

Water was samples in all seventeen sampling point in stormwater constructed wetland starting from December 2010 until December 2011 (13 month sampling periods), which included nitrogen (nitrite (NO_2-), nitrate (NO_3-) and ammoniacal nitrogen(NH_4-N)), orthophosphate (PO_4)and other parameter such as water temperature, pH, dissolved oxygen (DO) and conductivity, total suspended solid (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The water samples were collected using grab sample. Water sample were collected monthly and laboratory analysis were analyzed according to APHA (2005) methods. The physicochemical parameters were taken on-site by using multi-parameter Sonde meter. The summary of water quality data for each month will be presented by mean and standard deviation throughout the sampling period.

2.3 Phytoplankton community sampling

Phytoplankton communities were sampled monthly together with water quality sampling, starting from December 2010 until December 2011. The sampling point was exactly the same with sampling point of water quality sampling. The same sampling point was chosen to avoid variation and differences of topography, landscape and others influent by the environmental factors. Three replicates of phytoplankton samples were collected at the sub-surface water by filtering 40L of water, using plankton net with 35μm mesh size. The samples then preserved using Lugol's solution and buffered formalin 5% solution

2.4 Data analysis

The phytoplankton samples were identified and enumerate each species present. Phytoplankton were identify to the lowest taxon possible. The identification of phytoplankton was based on the taxonomic key from reference book (Bellinger and Sigee, 2010). Species diversity (H') index is measured by using Shannon-Weaver Diversity;

$$H' = -\sum (Ni / N) \log_2 (Ni /N) \qquad (1)$$

Where,   Ni/N =the probability to obtain species 'i' in a sample
     Ni = num. of individual for species 'i'

Evenness Index (J') is the ratio of Shannon-Weaver Diversity Index (H') to the maximum H' value;

$$(J') = \quad H' / (H' \max) \qquad (2)$$

Where, H' = Shannon-Weaver Diversity Index
    H' max = $\log_2$ S
    S = number of species

Margaleff's Index (d) can be used to calculated species richness.

$$\text{Margalef Index (d)} = \quad S\text{-}1/ (\ln (N)) \qquad (3)$$

Where, S= number of species in a community
N= total number of individual

3 Results and Discussion

3.1 Assessment of phytoplankton communities

A total of 15 different species of phytoplankton were recorded across all sampling stations, with the largest number of species recorded in the Chlorophyta (8 species), Bacillariophyta (3 species), Cyanophyta (2 species), Pyrrhophyta (1 species) and Chrysophyta (1 species) (Table 3). The dominant species found at most sampling station *Coelastrum microporum, Westella botryoides* and *Mougeotia sp.* Figure 5 showed the example of the species obtained which is observed under compound microscope. The less dominant and rare are *Hemidinium nasutum* from Pyrrhophyta and *Tribonema affine* from Chrysophyta. Table 4 showed the three biological indices measured for phytoplankton during the sampling period. Based on the resulted obtained, in the macrophyte area (W1 until W6), the higher diversity index (Shannon Weaver Index) can be seen at W2, which is 2.42. W2 also has high evenness (0.62) as compare to other site in the macrophyte area. The lowest diversity can be seen at W4, 2.11 and also possess lower evenness, 0.54. W5 showed higher richness index, 2.12 while W6 showed lower richness index value, 1.76. In Micropool area, the higher diversity and evenness can be seen at MA2, 2.22 and 0.58 while the lower diversity and evenness value can be seen at MB3, 1.96 and 0.51. The higher richness index can be seen MC2, 2.22 while the lower richness index can be seen at MA3, 1.83. Overall, the highest diversity and evenness can be seen at W2 while the lowest diversity and evenness can be seen at MB3. The higher richness index can be seen at Inlet Wet, 2.33 while the lowest richness index experience in W6. The biological indices showed fluctuation trend, which appears almost all sampling station for both in macrophyte zone and micropool zone

Table 3: Summary of species of phytoplankton at all sampling station in stormwater constructed wetland

Group	Species
Chlorophyta	*Ankistrodesmus falcatus,Coelastrum microporum**, Mougeotia sp.**,Pediastrum simplex, Scenedesmus quadricauda, Sphaerocystis schroeteri, Tetradesmus smithii, Westella botryoides**
Bacillariophyta	*Cyclotella comta, Navicula sp., Stephanodiscus astraea*
Cyanophyta	*Aphanocapsa elachista, Oscillatoria prolifica*
Pyrrhophyta	*Hemidinium nasutum**
Chrysophyta	*Tribonema affine**

** Dominant   * Rare   (Based on enumeration number of individual species)

Table 4: Summary of diversity index measured for phytoplankton at stormwater constructed wetland sites between December 2010 and December 2011

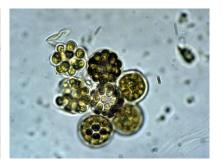| Sampling Station	H'	J'	d
Inlet Wet	2.07	0.56	2.33
W1	2.37	0.61	1.85
W2	2.42	0.62	1.85
W3	2.14	0.55	2.02
W4	2.11	0.54	2.07
W5	2.36	0.60	2.12
W6	2.14	0.58	1.76
MC1	2.10	0.54	2.17
MC2	2.13	0.54	2.22
MC3	2.17	0.56	2.16
MB1	2.03	0.52	2.18
MB2	2.08	0.53	2.21
MB3	1.96	0.51	2.01
MA1	2.19	0.56	2.21
MA2	2.22	0.58	2.12
MA3	2.01	0.56	1.83
Outlet Wet	2.00	0.53	2.12

Figure 5: Examples of freshwater species present in the stormwater constructed wetland (From left: *Westella botryoides*, *Navicula sp.*, *Coelastrum microporum*)

3.2 Assessment of water quality

To understand why this freshwater phytoplankton occurs in such trend in stormwater constructed wetland, the nitrite, nitrate, ammoniacal nitrogen and orthophosphate was to be determined. Based on Mohammadpour et al. (2014), the wetland was divided into three Cluster (Figure 6) (Cluster 1, Cluster 2 and Cluster 3) by using hierarchical cluster analysis (HACA). Thus, Table 5 below summarized the water quality concentration based on the classification made. Based on the table obtained, the important parameter for the purposed of this study showed high percentage removal of nitrogen (Nitrite-80%, Nitrate-61.56% and Ammoniacal nitrogen-51.49%) and phosphorus (orthophosphate- 60.98%). Removal was measured by using this formula; C_{in} (concentration inlet) and C_{out} (concentration outlet)

$$RR = \frac{C_{in} - C_{out}}{C_{in}} \times 100\%$$

Figure 6: Clustering of wetland. (a) Dendrogram; (b) location of three clusters. (Mohammadpour et al. (2014)

Table 5: Summary mean of water quality concentration for 17 sampling stations for 13 month period (December 2010- December 2011)

	Station	Tem °C	Con µS/cm	DO mg/L	pH	Nitrite mg/L	Nitrate mg/L	PO4-P mg/L	NH4-N mg/L	BOD mg/L	COD mg/L	TSS mg/L
C1	Inlet Wet	31.6	138	8.04	7.65	0.035	2.94	0.41	0.303	2.78	28	28
	W1	32.1	141	8.26	7.71	0.032	3.43	0.40	0.324	3.30	30	29
C2	W2	31.9	141	8.05	7.72	0.030	3.32	0.40	0.326	3.35	29	27
	W3	31.9	138	8.67	7.84	0.027	2.84	0.33	0.288	2.89	24	24
	W4	31.6	139	8.57	7.93	0.023	2.88	0.30	0.285	2.71	24	23
	W5	31.6	140	8.31	7.78	0.016	2.48	0.29	0.267	2.78	25	22
	W6	31.4	138	8.74	7.75	0.017	2.48	0.26	0.257	2.68	24	22
C3	MC 1	30.9	136	8.13	7.88	0.013	2.00	0.22	0.218	2.44	23	17
	MC 2	30.9	137	8.18	7.82	0.009	1.90	0.21	0.209	2.39	21	16
	MC3	31.0	137	8.20	7.80	0.009	1.92	0.22	0.191	2.36	21	15
	MB 1	30.4	135	8.09	7.78	0.008	1.80	0.20	0.180	2.24	20	13
	MB 2	30.7	136	8.01	7.77	0.009	1.67	0.19	0.174	2.40	19	14
	MB 3	30.8	136	8.11	7.80	0.010	1.66	0.18	0.171	2.33	17	13
	MA 1	30.5	132	8.04	7.26	0.009	1.43	0.17	0.165	2.36	19	11
	MA 2	30.5	134	8.03	7.70	0.009	1.38	0.16	0.160	2.19	18	11
	MA 3	30.6	134	7.95	7.72	0.009	1.36	0.16	0.151	2.08	16	10
	Outlet Wet	30.0	133	7.78	7.60	0.007	1.13	0.16	0.147	2.01	15	9
	Removal (%)	-	-	-	-	80	61.56	60.98	51.49	27.7	46.43	67.86

Based on the Figure 7, the nitrogen concentration and orthophosphate concentration trend were slightly affected the diversity distribution of freshwater phytoplankton. As the nutrient decrease it concentration, the diversity slowly decrease throughout the area. The source of nutrient was high at cluster 1 and 2 due to first nutrient concentration entering the wetland systems. Thus this high nutrient favorable to the distribution of freshwater phytoplankton in this wetland. Factor contributes to this high numbers distribution was due to high amount of nutrient availability concentration (Oyanedel et al. 2008). The high concentration such as phosphate and nitrogen compound helps the phytoplankton to grow and increase the numbers of phytoplankton. The densities of phytoplankton and phosphorus were positively and linearly correlated to each other (Cao et al. 2007). Nitrogen and phosphorus are the main elements for the matter and energy metabolism of the algae. As the nutrient concentration start to decrease gradually until it reaches micropool area, the phytoplankton distribution also start to decrease it numbers of individual gradually until it reaches micropool area. Westella botryoides is one of the common green algae found the freshwater area (Table 4.4), especially in the lake from temperate region country (Morabito et al. 2001) and most of the research in the topical country region.
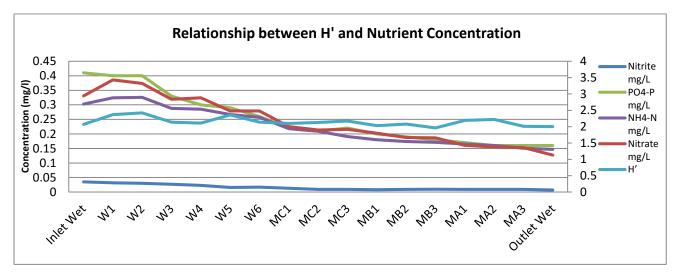

Figure 7: The relationship trend between the Shannon-Weaver Diversity (H') of freshwater phytoplankton and nitrogen and phosphorus concentration

4 CONCLUSION

As a conclusion, the baseline understanding of this distribution of freshwater phytoplankton in the constructed wetland can help for more holistic design of stormwater constructed wetland. Holistic design on which the design is takes into consideration of the impact of biotic component such freshwater phytoplankton, fish, birds and other mammals so the constructed wetland can sustain and produce ecosystem balance in future.

Acknowledgement

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