

Spatial Distribution of Dissolved Inorganic Nutrients and Phytoplankton around Kota Kinabalu Wetland, Sabah, Malaysia

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Abstract

Kota Kinabalu Wetland is importantly habited of mangrove, diverse aquatic flora and fauna as well as feeding stop for migratory birds. This wetland is inundated with the tidal flow, as connected with a small river and nearby coastal areas, thus. A study was carried out to determine the spatial distribution of dissolved inorganic nutrients and phytoplankton diversity at Kota Kinabalu (KK) Wetlands. Five stations, in which river mouth of Likas Bay, river channel (two stations) and inundated area (two stations) in KK Wetland were selected for this study. In-situ parameters of water, water for nutrients and phytoplankton samples were collected from May 2019 until October 2019. The highest concentration of nitrate (0.115 mg/L) was recorded at inundated area of wetland (S5) while the lowest nitrate concentration (0.0047 mg/L) was found at river (S3) flowing towards wetland. The concentrations of ammonia (0.2004 to 2.311 mg/L) were recorded relatively higher at every station compared to other dissolved inorganic nutrients (DIN). The concentration of phosphate was determined in the ranges of 0.0089 - 0.0513 mg/L. Nitrate, ammonia and phosphate showed no significant difference (P = 0.737) in terms of DIN concentration at all five sampling stations during the study period. Twenty-four genera of phytoplankton were identified, dominated by diatoms (55.29%), followed by dinoflagellates (24.95%), Chrysophyta (11.15%), Spirotrichea (5.28%) and Cyanophyta (3.33%). Dominating species throughout the study period include Chaetoceros sp., Pseduo-nitzschia sp., and Cylindrotheca closterium, Peridinium quinquecorne and Alexandrium sp. Phytoplankton species compositions were observed the highest in river mouth area in July with the highest density of 12.115×10^4 cells/mL. The study showed that nutrient concentration was insignificant (P = 0.614) in altering the phytoplankton density, as influences with the tidal water.

Keywords

Dissolved Inorganic Nutrients, Special Distribution Phytoplankton Species, Diversity and Richness

1. Introduction

In wetlands nutrients mainly originated from anthropogenic factors through domestic waste and housing area development, with the exception inundated with the river flashing from the estuaries. Estuaries are known for its strong environmental gradients and complex physico-chemical process is the main factor for large spatial and temporal variations in the structure and dynamics of any biological communities in that area, including phytoplankton [1]. Phytoplankton is susceptible to fluctuating the productivity therefore leading to variations in distributions and succession. Succession of phytoplankton was affected mostly by combination of biological, such as grazing and competition between species for physical and chemical quality aquatic ecosystem. Physical parameters like, hydrodynamics, rainfall, light and temperature, as well chemical factors, such as availability of dissolved inorganic nutrients, pH and dissolved oxygen in aquatic ecosystem key components in phytoplankton bloom [2]. Phytoplankton community fluctuates due to uptake of high concentration of nutrients, especially nitrogen in the form of nitrate and phosphorus as phosphate are essential phytoplankton growth [3]. Nitrate and ammonia represent as two main sources of dissolved nitrogen as nitrate is derived from anthropogenic origin [4] while ammonia originates from the discharge of wastewater [5]. Inorganic nitrates and ammonia limit phytoplankton growth [6] and their ecological significance in rivers and streams has been well documented [7]. There are many factors that affect the relative amount of nitrogen in various forms, which are influences with the discharge of wastewater into water, fertilization and types of wetland, reservoirs or lakes in the path of flowing water [3]. Correlation between nitrogen and phosphorus and abundance further supported the former conclusion that phosphorus is the controlling factor in phytoplankton growth in the estuary where light is not limited [8] while nitrogen is commonly limited in marine ecosystems [9]. Surface runoff after heavy precipitation initially resulted in increased accumulation of nutrients (nitrate and phosphate) concentration in the estuary. Nitrate is a limited factor for phytoplankton cells density rather than phosphate in Kota Kinabalu coastal water [10]. Abundance and composition of phytoplankton in estuaries are the consequences of nutrients concentration (phosphate, nitrate, ammonia) and might also be the tides as high tides condition allows diatoms from seawater to enter the freshwater and mix with freshwater phytoplankton [11]. On the other hand, the inundated area at KK Wetland is characterized by the environment's specific properties such as low hydraulic gradient, poor mixing of water columns, with high probability of water retention. Due to the wetlands located in estuary areas, tidal fluctuation plays a vital role in changes in concentration of nutrients. Water flowing in the river channel at KK Wetland is also affected by inputs of high loads of nutrients. The inputs are mainly due to main activities, known as anthropogenic factors, such as domestic waste and development of housing area strongly modify biomass of phytoplankton abundance. Information on the distributed of dissolved inorganic nutrients and the community structure of phytoplankton in wet land is limited, especially characteristics like KK Wetland. This study was attempted to identify the distributed pattern of dissolved inorganic nutrients and community structure of phytoplankton alone the adjacent areas of KK Wetland. It will contribute the scientific data about the concentration of dissolved inorganic nutrients and provide checklist of phytoplankton diversity in Kota Kinabalu Wetland.

2. Materials and Methods

2.1. Description of Study Site

The study was conducted at Kota Kinabalu (KK) Wetland located in west coast of Sabah, Malaysia (**Figure 1**). Five sampling stations were selected adjacent to KK Wetland as shown in **Table 1**. Samples were taken for period of period of six months (May 2019 until October 2019).

2.2. Collection of Water Samples for Dissolved Inorganic Nutrients



The water samples from the selected stations were collected by using Van Dorm



Station	Attributions of Area	Latitude (N)	Longitude (E)
Station 1 (S1)	The mouth of Likas Bay where the river meets the sea	5°59'36.12"	116° 5'33.12"
Station 2 (S2)	River situated around construction sites and housing areas	5°59'20.52"	116° 5'20.64"
Station 3 (S3)	River channel passing through KK Wetlands	5°59'13.26"	116° 5'19.44"
Station 4 (S4)	Inundated area inside Kota Kinabalu Wetlands	5°59'6.42"	116° 5'6.48"
Station 5 (S5)	Inundated area inside Kota Kinabalu Wetlands	5°59'6.06"	116° 5'9.96"

Table 1. Sampling stations with attributions and coordinates.

water sampler within one metre depth in each station. Water sample was then transferred into polyethylene bottles and labelled according to stations. The analysis of dissolved inorganic nutrients was conducted in oceanography laboratory at Borneo Marine Research Institute (BMRI).

2.3. Collection of Phytoplankton for Identification of Species and Cell Density

Phytoplankton net of 20 μ m mesh size was towed for 1 minute at the water surface against movement of tide for every station. Phytoplankton was concentrated at the bottom of nets and transferred into polyethylene bottles. They were preserved immediately with Lugol's iodine. Identification of phytoplankton species was executed thoroughly at Borneo Marine Research Institute (BMRI) based on guidance from [12].

2.4. Analytical Parameters

2.4.1. Collection of In-Situ Parameters

Water parameters such as salinity (ppt), temperature (°C), level of DO (mg/L) and pH were measured using YSI multi-parameter probe (Model: Pro Plus, GeoHydrOcean Services) at depths around 1 - 1.5 m.

2.4.2. Laboratory Analysis for Ex-Situ Parameters

The water samples were filtered with 0.45 μ m pore-size membrane filter for the determination of nitrate, ammonia and phosphate (mg/L). Nutrients were analyzed with spectrophotometric methods proposed by the American Public Health Association [13].

2.4.3. Cell Density (Cells/mL)

Phytoplankton cell count was carried out using Sedgewick-Rafter counting chamber under light microscope at $40 \times$ magnification. The total number of phytoplankton was counted according to formula by Stirling [14]:

$$N = A \times 1000 \times CV \times F \times L$$

where:

- N= Number of plankton cells per litre of original water
- A = Total of counted phytoplankton
- *C* = Volume of final concentrate of samples (mL)

- V = Volume of field (m³)
- F = Number of counted fields
- L = Volume of original water (L)

2.4.4. Statistical Analysis

Statistical analyses like one-way ANOVA and Pearson correlation were conducted using the SPSS (Version 19.0). Significant level of p < 0.05 was tested for normality and homogeneity of variances. Diversity indices, such as, species diversity, equitability index and species richness of phytoplankton community were calculated by using PAST software.

3. Results and Discussion

3.1. Spatial In-Situ Parameters

The average salinity during the study period among the stations were recorded in the range of 28 - 32 ppt while the mean of 32 ± 2.10 ppt and the lowest of $28 \pm$ 6.02 ppt observed in the station S1 and S3 respectively. The temperatures were recorded in the range of 30.00° C - 31.20° C, fluctuates in less than 1°C which indicates all five stations have almost similar surface water temperature. The highest dissolved oxygen of 4.46 mg/L was found at the river mouth (S1) while the lowest of 2.68 mg/L was determined at station S4 in the inundated area located inside KK wetland. The value of pH at inundated area of estuary, stations S4 and S5 (7.37 and 7.35) were found lower compared to the pH value of 7.87 in the river mouth, station S1 (**Table 2**). Significant difference (P = 0.008) was observed in salinity, temperature, dissolved oxygen and values of pH in when compared between months around KK wetland. Temperature, salinity and pH shared similar fluctuating trend throughout the six months while dissolved oxygen fluctuated drastically from June to July (the lowest) and with the increase in the month of August to September (**Figure 2**).

3.2. Spatial Nutrients Concentration

Concentration (mg/L) of nitrate observed had the lowest values compared to values in concentration determined in ammonia and phosphate. The concentration

Table 2. Values (mean \pm SD) of *in-situ* water parameters (salinity, temperature, dissolved oxygen and pH) collected at the five sampling stations.

	Stations						
Parameters	S1 River mouth	S2 River	S3 River	S4 Inundated area	S5 Inundated area		
Salinity (ppt)	32 ± 2.10	31 ± 1.72	28 ± 6.02	28 ± 2.34	31 ± 2.07		
Temperatrue (°C)	31.20 ± 0.32	31.03 ± 083	30.88 ± 1.13	30.00 ± 1.24	30.38 ± 0.78		
Dissolved Oxygen (mg/L)	4.46 ± 0.82	3.64 ± 1.53	3.38 ± 1.66	2.68 ± 1.57	2.80 ± 0.78		
pH	7.87 ± 0.21	7.68 ± 0.56	7.65 ± 0.55	7.37 ± 0.40	7.35 ± 0.40		

of nitrate was observed from 0.005 - 0.012 mg/L (**Table 3**). The highest concentration of 0.0115 mg/L nitrate was recorded at station S5 while the lowest concentration of 0.0047 mg/L nitrate was found at station S3. However, the concentrations of ammonia were relatively higher at every stations compared to other DIN (**Figure 2**), especially concentrations of ammonia of 0.2004 mg/L and 2.311 mg/L recorded at the river (stations S2 and S3). The lowest ammonia concentration of 0.0159 mg/L was recorded at station S4 (**Table 3**).

The concentration of phosphate was found within the range of 0.0089 - 0.0513 mg/L. The lowest of 0.0089 mg/L and the highest of 0.0513 mg/L concentrations of phosphate were determined in station S1 and S4 respectively (**Figure 3**).

In general, nitrate, ammonia and phosphate showed no significant difference (P = 0.737) in terms among the sampling stations during the study period, except ammonia from station S3. Ammonia and phosphate levels were observed the highest during the month of July and September respectively. Nitrate concentration was observed consistently similar throughout the study period (**Figure 3**).

The *in-situ* water parameters such as salinity (ppt), temperature (°C), dissolved oxygen (mg/L) and pH can greatly affect the dynamics of phytoplankton growth and abundance in the water [15]. There were no significant differences in spatial distribution among the salinity (P = 0.1613), temperature (P = 0.1441),



Figure 2. Trends in Salinity, temperature, dissolved oxygen and pH values from May 2019 to October 2019 (mean \pm sd).

Stations						
Nutrients	S1 River mouth	S2 River	S3 River	S4 Inundated Area	S5 Inundated Area	
Nitrate (mg/L)	0.003 ± 0.00	0.004 ± 0.00	0.005 ± 0.00	0.008 ± 0.00	0.012 ± 0.00	
Ammonia (mg/L)	0.19 ± 0.01	0.20 ± 0.07	2.31 ± 0.08	0.016 ± 0.03	0.06 ± 0.03	
Phosphate (mg/L)	0.009 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	



Figure 3. Trends of DIN (Nitrate, Ammonia and Phosphate) concentrations from May 2019 to October 2019 at Kota Kinabalu Wetland area.

dissolved oxygen (P = 0.1128) and values of pH (P = 0.0782) which were recorded in five sampling stations around Kota Kinabalu Wetlands. This might be due to the limitation of study area that were covered in the study, as well as fewer sampling stations which led to less sample variability in analysis. This was the same condition observed for *in-situ* water parameters between months during the dry season period [10]. In this study, the highest salinity was observed during the pre-monsoon (May) while lowest was at July and August, during the dry period of southwest monsoon, although high salinity was expected in less rainfall period during the dry season [16] [17]. Dissolved oxygen also plummeted incredibly from 4.34 mg/L on May to 1.34 mg/L on July. This was probably due to the environmental condition as the lowest tide during low tides occurred on July, which promotes decrease in salinity, dissolved oxygen and even values in pH. However, the tidal data was recorded during the study period. Similar conditions was observed recently in tidal lake of Indonesia where the salinity was lower during the lowest tide because saltwater intrusion from the sea did not occur while for dissolved oxygen, diffusion process was restricted hence limiting oxygen supply in water [18]. The same study also suggested the sudden low value of pH level in dry season of low tide was due to the intense decaying process of organic matter as a result of high intensity and frequent exposure of water to sunlight. Salinity and dissolved oxygen concentrations were the lowest in July during the dry season in Marudu Bay [19]. The present study showed that higher salinity was observed at the river mouth near to the bay area where similar finding was found in Brunei Bay during the dry season due to the mixing of seawater [20]. The temperatures were observed similar between sampling stations and months throughout the period of study which was probably due time of sampling therefore dependent to presence of sunlight and heating effect.

Since the samplings were done during the dry season, the concentrations of DIN were not affected by La Nina period. The concentration of nitrate was relatively similar and low compared to ammonia and phosphate in every sampling station and month during the present study. Low concentrations of nitrate were due to longer residence time, thus increases the chances of decoupling between

tidal cycle and residual drainage [21]. Decomposition process is known to be higher during the dry season, including the period of residence time [21]. Concentrations of ammonia were the highest at the river channel (S2 and S3) with not much of differences in mean concentration around estuary inundated area (S4 and S5) (Table 3). Across the six months of study, ammonia concentration was the highest in July. Ammonia concentrations were known to be higher than nitrate in estuary and mid-estuary area as a result of regeneration of ammonia from mangrove sediments [22] [23] with low precipitation of dry season also contributed to the sudden peak of ammonia [24]. The spike increase of ammonia concentration in July was highly due to wastewater effluents from municipal area and use of fertilizer from agricultural activity nearby Kota Kinabalu Wetland area associated with lowest precipitation of the dry period [25]. Similar to trend in ammonia, phosphate mean concentration was observed constant in stations located at the river channel and inundated area of estuary, slightly higher than concentrations at river mouth. Overall phosphate concentration was highest in July. The present average phosphate concentration (0.01 - 0.03 mg/L) is almost similar to Douala estuary [6] where the concentrations were ranged from 0.03 to 0.04 mg/L in dry season, thus indicating phosphate concentrations were generally low in dry season compared to other monsoon seasons. Phosphate concentrations around Kota Kinabalu wetlands is high around estuary and river channel passing through the housing areas, indicating phosphate levels is induced by sewage and wastewater nearby housing areas [26]. The nutrients level in the present study were lower than the Malaysia NWQS [27] acceptable limits of 7 mg/L, 0.1 mg/L and 0.2 mg/L for nitrate, ammonia and phosphate respectively which represents the waters around Kota Kinabalu Wetland is suitable for aquatic wildlife.

3.3. Abundance and Diversity of Phytoplankton

A total of 24 genera form 19 families were found in Kota Kinabalu Wetland waters during the period of study. Among them seven were from dinoflagellates (24.95%), 14 from diatoms (55.29%) and three genera, each from Spirotrichea (5.28%), Cyanobacteria (3.33%) and Crysophyta (11.15%). Diatoms were mainly dominated by *Chaetoceros* sp., *Pseduo-nitzschia* sp., and *Cylindrotheca closterium* throughout the study period while *Nitzschia* sp., *Pleurosigma* sp., *Coscinodiscus* sp., *Thallassionema* sp. and *Skeletonema* sp. occurred abundantly in certain months from June to September. Dinoflagellates were dominated by only *Peridinium quinquecorne* and *Alexandrium* sp. from May until October while massive numbers of *Gonyaulax* sp. was only observed on August. Cyanobacteria and *Codonella* sp. of Spirotrichea occurred consistently in small density throughout the June to September.

The highest density of 11.4×10^4 cells/L was recorded at station S1 while the lowest density of 6.29×10^4 cells/L phytoplankton was found at station S3 (**Table 4**). No significant (*P* = 0.284) differences were determined with the abundance

of phytoplankton among the sampling stations during the study period. Diatoms were found to be dominating every station with the highest percentage of 64.33% at station S1 and of 65.58% at station S2. Abundance of dinoflagellates with highest percentage of 37.56% were observed at station S5 and of 34.48% at station S4. Phytoplankton abundance (cells/L) was the lowest during pre and post-monsoon period (May and October respectively) with a total of 2.685×10^4 cells/L and 3.660×10^4 cells/L respectively, while the highest density of 12.115×10^4 cells/L and 10.995×10^4 cells/L of phytoplankton were observed during the dry season, July and August respectively (Table 4).

The Simpson's Diversity Index (D) for phytoplankton was found to be the highest (0.912) at station S2 and the lowest (0.886) at station S5 (**Table 5**). However, species evenness is relatively low compared to the diversity as the ranges of the evenness index (J') is from 0.283 to 0.291, with the lowest inside Kota Kinabalu wetlands inundated area (S5) and highest at the river (S2). Margalef Richness Index (d) was found the highest at station S3 while the lowest was at station S1 with the values of 3.082 and 2.843 respectively (**Table 5**).

Phytoplankton communities are generally represents as an indicator in determining the changes of nutrients in water and as a vital component for evaluating eutrophication in marine ecosystems [28]. The abundance of phytoplankton was found to be the most abundant (11.4560 \times 10⁴ cells/L) at river mouth (S1), as located at the mouth of the Likas Bay. This might be due to the lower turbidity level and mixing of saline and freshwater. Phytoplankton abundance in

Months	Phytoplankton abundance (×10 ⁴ cells/L)	
May	2.685	
June	5.645	
July	12.115	
August	10.995	
September	6.940	
October	3.660	

 Table 4. Phytoplankton abundance (cells/L) in respective months (May 2019 to October 2019).

Table 5. Diversity indices of phytoplankton	in	Kota	Kinabalu	Wetland	at five	e sampling
sites.						

Stations	Simpson's Diversity Index (H)	Evenness Index (J)	Margalef Richness Index (d)
S1	0.896	0.286	2.843
S2	0.912	0.291	2.997
S3	0.902	0.288	3.082
S4	0.895	0.285	2.941
S4	0.886	0.283	2.996

Brahmani estuary [29] and Perak estuary was observed to be increased with elevating salinity level [30]. The lowest phytoplankton abundance (6.2900 \times 10⁴ cells/L) was located at the river channel inside estuary (station S3) of Kota Kinabalu Wetland. The limited abundance of phytoplankton in S3 was due to high turbidity which limits photosynthetic rate for primary productivity. Most estuaries have vast amount of mineral sediments as a result of input from runoffs and suspended by tidal and wave fluctuations [31]. Since the average depth of waters around the study area was within 1 - 1.5 m, process of turbidity driven by mixing of sediments assumed to be high which narrows the photic zone therefore limiting light penetrating into the water column and reducing phytoplankton biomass. However, the fluvial discharges of sediment were not accounted in this study.

Other factor leading to low abundance in S3 is the location was far away from source of nutrients inputs. Phytoplankton abundance was also the highest during July of dry season. There observed huge difference in abundance of phytoplankton between July (12.115×10^4 cells/L) and May (2.685×10^4 cells/L). The phytoplankton abundance was the highest during drier season and the lowest during the inter-monsoon month of May [32] [33]. The low abundance in May was due to slow current during the inter-monsoon hence limits mixing of water [34].

Phytoplankton compositions in Kota Kinabalu Wetlands were mainly comprised of diatoms (55.29%), dinoflagellates (24.95%) and cyanobacteria. The species of the Diatom was accounted for 77% of total phytoplankton in marine ecosystem. The diatoms and dinoflagellates were the major dominated group among the phytoplankton in estuaries and marine ecosystem [32] [35]. Diatoms were observed abundant during the dry season (June, August, and September), pre and post southwest monsoon (May and October). However, densities of diatoms dropped staggeringly in July as the sampling was done during the low tide. The species of Crysophytes were observed as the dominating phytoplankton community at that specific month in low tide estuaries [36]. Dominating dinoflagellates (Peridinium quinquecorne and Alexandrium sp.) which were found in high density in estuary in Kota Kinabalu Wetland during the low tide was due to the tolerance to low salinity and warmer environment [37]. Diatoms such as Chaetoceros sp., Pseduo-nitzschia sp., and Cylindrotheca closterium were most abundant throughout the period of study where these genera represent groups of marine diatoms and can be found in estuary during high tides as the seawater flows into the river and estuary. Nutrient concentrations are not the only factor affecting the phytoplankton blooms because changing in environmental parameter might favor some species to blooms [11]. The environmental parameters such as temperature, salinity, Secchi depth (visibility), pH, and dissolved oxygen are the factors that can affect the algal cells community succession or limit the growth of certain groups of algae [11]. In addition enrichment in nitrogen particularly nitrate lead to an increase in phytoplankton biomass in marine habitat [13]. Tit was documented that salinity is an important factor that influence HABs in Kota Belud's water other than nutrients concentration [10]. The intrusion of seawater in Kota Kinabalu Wetland might control the composition of phytoplankton community. *Pseduo-nitzschia* sp., which is also a potential harmful phytoplankton, is capable of producing domoic acid [38]. In Sabah, only four *Pseduo-nitzschia* sp. had been recorded which are *P. pungens*, *P. calliantha*, *P. pseudodelicatissima*, and *P. delicatissima* [39]. Genus from diatoms (*Chaetoceros, Cylindrotheca*, and *Pseudo-nitzschia*) and dinoflagellates (*Peridinium* and *Alexandrium*) persist throughout the entire southwest, pre and post monsoon where their persistence could be stemmed from vast distribution and tolerance to brackish estuarine environmental conditions [40] [41]. Overall, the total numbers of genera in Kota Kinabalu Wetland (24 genera) were observed relatively smaller compared to studies done by other researchers in tropical estuaries. A total of 37 genera in Philippine mangrove estuary [32] and 30 genera in Sungai Brunei estuary [35] indicated the lower diversity of phytoplankton at Kota Kinabalu Wetland.

4. Conclusion

The phytoplankton abundance in Kota Kinabalu wetlands mainly depends on the nutrient inputs in the estuary with other anthropogenic sources outside of Kota Kinabalu wetlands. The dry season in Kota Kinabalu Wetland resulted in lower concentrations of dissolved inorganic nutrients (nitrate, ammonia and phosphate) while environmental *in-situ* water parameters were almost similar and did not greatly affect the phytoplankton growth. In Kota Kinabalu Wetland, a total of 24 genera of identified phytoplankton were confirmed with the highest occurrence of dominating phytoplankton diatoms. Among the nutrients nitrate and phosphate are observed with increasing numbers of phytoplankton density. The findings obtained from this study can be used as a guideline and basis for future research, especially during the wet season. Continuous monitoring of environment parameters and succession phytoplankton in different seasons may fulfil the overall goal to achieve sustainable healthy ecosystem of Kota Kinabalu Wetland, which initially cannot be conclude with short period of study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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