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USING THE MODIFIED REDFIELD RATIO TO ESTIMATE HARMFUL ALGAL BLOOMS

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Abstract. Many waterbodies across Nova Scotia (Canada) have been experiencing algal blooms occurring in large numbers and diversity, without knowledge or understanding about their causes and effects. Algal blooms have appeared in Mattatall Lake (ML) and other lakes of the province in recent years. ML experienced severe algal blooms in 2013. During the fall of 2014, massive algal blooms appeared in ML, and persisted until late December 2014. The blooms have a pattern of being nontoxic in the summer and potentially toxic in the fall-winter season, with nutrients increasing on a monthly basis. This phenomenon is unusual as algal blooms have not been known to last until the winter season or coexist with icy conditions. The dominant species in this bloom was identified to be *Anabaena planctonica* (*Dolicospermum planctonicum*) with a cell count around 250 000 cells/mL, which may produce the neurotoxin *Anatoxin-a.* This cell count is approximately two and a half times higher than the alert level 2 guideline from World Health Organization's drinking water standards (WHO, 2003).

The goal of this paper is to use an index (Modified Redfield Ratio, hereafter called MRR) to estimate the HAB occurrence the Mattatall lake (Nova Scotia, Canada), served as a pilot site for our pioneering systematic study in order to evaluate the recent bloom phenomena in the entire province of Nova Scotia.

Key words: Cyanobacteria, Harmful Algal Blooms (HAB), Modified Redfield Ratio (MRR), Mattatall lake.

1. Introduction

The Nova Scotia province has been experiencing Harmful Algal Blooms (HAB) since 2008 (Brylinsky, 2012; Brylinsky and Sollows, 2014). Different works on HAB have mainly focused on two regions in the province: Yarmouth and Digby counties, where there is a large number of mink farms. In the visit to five lakes (four in the Carleton system and one in Meteghan system) organized in the middle of July 2016, we detected that there was the dominant presence of *Anabaena flos-aquae* (Nowlans, Fanning Lakes and Lake Vaughan) and *Microcystis aeruginosa* (Ogden Lake) with a high percentage of abundance (greater than 83 %).

Massive blooms have also been reported in Lake Torment (Kings County) since 2014. In August 2015, during a visit to the County, we detected blooms generated by a species from the *Anabaena* family in the south part of Lake Torment, equivalent to 285 000 cells/mL (33 %) in the sampled water containing a bloom. This is a very alarming percentage as it is higher than Alert level 2, according to World Health Organisation (WHO, 2003). Visible algal blooms had appeared in Lake Torment in the summer of 2016 and the taxonomy test showed the presence of the species *Anabaena sp.*

Mattatall Lake (ML) in Nova Scotia (Fig. 1) started to experience severe algal blooms in 2013. During the fall of 2014, massive algal blooms appeared in ML, and persisted until late December. This phenomenon was observed and recorded by our team in the fall-winter of 2014 (Nguyen-Quang, 2015). The duration of this phenomenon was extremely unusual as algal blooms have not been known to last until the winter season or coexist with icy conditions. The dominant species in this bloom was identified to be *Anabaena planctonica* (*Dolicospermum planctonicum*) with a cell count around 250 000 cells/mL, which may produce the neurotoxin *Anatoxin-a.* This cell count is approximately two and a half times higher than the alert level 2 guideline from World Health Organization's drinking water standards (Chorus and Bartram, 1999).

There are certain combinations of multiple factors that trigger HAB specific to each waterbody. However, their coupling effects are not yet understood. No research or data related to ML has been done. Moreover, in Nova Scotia, no systematic investigation has been sketched for cyanobacterial bloom patterns. Our main goal in this paper is to suggest the Modified Redfield Ratio (hereafter called MRR) to estimate the HAB occurrence for Mattatall lake, which is served as a pilot site for our pioneering systematic study in the province for the HAB issue.

2. Sampling process and analysis

Different equipment for field sampling and Lab analysis were used, such as a YSI probe (Professional Plus, Hoskin scientific LTD, USA) for pH, Dissolved Oxygen (DO), conductivity, and temperature in the water; a Fluorometer for Chlorophyll-a and Phycocyanin measurements; and a Photometer for evaluating nutrient components. Samples were taken tri-weekly or every month, depending on the weather conditions, starting in May through to November, at the surface and bottom levels. It is also noted that the lake is quite shallow with the maximum depth around 8 m. Predetermined sampling locations are represented in Fig.1 with corresponding coordinates in Table 1. If there were some other places that are not included in the predetermined points that had an algae bloom present, samples were then taken at these points as well. A HOBO weather station was installed in one fixed location at the lake. As soon as sampling was finished, they were taken to the lab for micronutrient analyses.

Chlorophyll-a (Chl-a) is usually used as a parameter to determine the quantity of primary photosynthetic pigment in cells of aquatic micro plants. Measurement and determination of this parameter are the basic analysis to evaluate the characteristics of the algal blooms used in many research works in the world. However, Chl-a represents just the whole quantity of photosynthesis pigment released from all algae and micro-plants present in water, hence cannot help to distinguish cyanobacteria existence among all living micro-plants and algae in the waterbody. To be able to define and confirm the presence of Cyanobacteria species in the composition of aquatic microalgae in the waterbody, another pigment form, Phycocyanin (PC), is used. This pigment can determine different cyanobacteria species from another planktonic species.

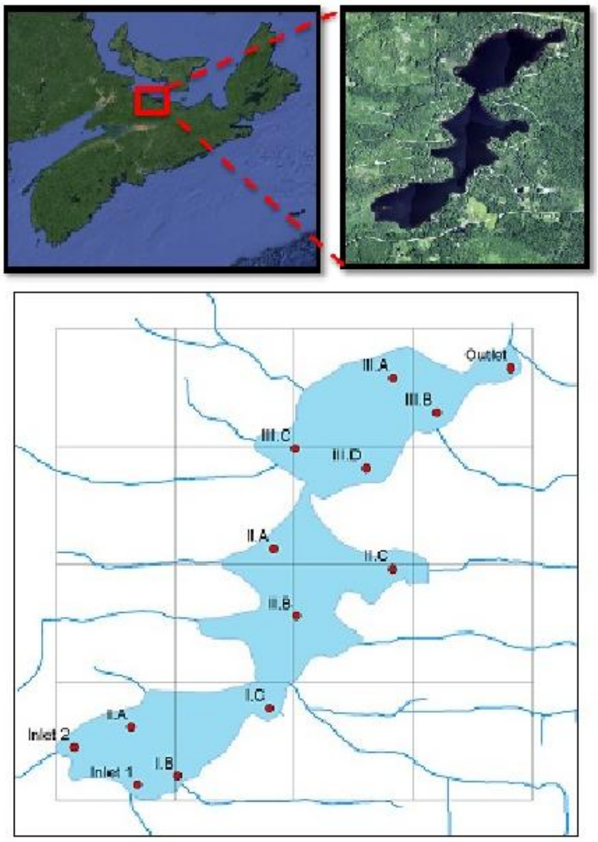


Fig. 1. Above: Google Earth view of Nova Scotia highlighting ML area (left) and Government of Nova Scotia aerial photograph of ML (right). Coordinaties of ML are 45° 42' 17" N, 63° 19' 39" W
Below: Sample stations (locations) predetermined and monitored

Table 1

Coordinates of sampling points in Mattatall Lake

Point	X (Longitude/Latitude)	Y (Longitude/Latitude)
IA	-63.483759 W	45.683914 N
IA1	-63.482013 W	45.681338 N
IB	-63.480922 W	45.681845 N
IC	-63.475384 W	45.684767 N
IIA	-63.475148 W	45.691568 N
IIB	-63.47378 W	45.688689 N
IIC	-63.467932 W	45.690711 N
IIIA	-63.467907 W	45.698875 N
IIIB	-63.46526 W	45.697408 N
IIIC	-63.473843 W	45.695859 N
IIID	-63.469584 W	45.695011 N
Inlet 1	-63.483369 W	45.681467 N
Inlet 2	-63.487229 W	45.683072 N
Outlet	-63.460799 W	45.699303 N

3. Taxonomy composition

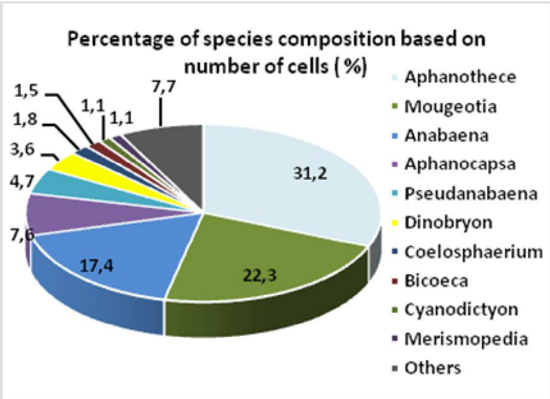
2015: Our observation from November 2014 till October 2015 with different taxonomic tests gave the result that a total of 19 classes, 72 genera and 113 microalgae species, among them there are 21 cyanobacterial species identified.

The maximum species number in a single sample was found at the Weather Station (WS) on 30 July 2015 – *47 species* and the minimal number of species at one location of observation– *8 species* in November 2014.

The proportional presence of individual microalgal genera (based on the cell count) from our survey was: *Aphanothece*, *31.2* %, *Mougeotia* 22.3 %, *Anabaena* 17.4 %, *Aphanocapsa 7.6* %, *Pseudoanabaena* 4.7 %, *Dinobryon 3.6 %* and the remainder 13.2 % (see Fig. 2).

It is found that if *based on the biomass* (Fig. 2) the non-toxic species *Mougeotia* was dominant during the whole period of survey while the presence of cyanobacteria *Anabaena* was very modest compared to the first one, due to the cell size of *Mougeotia* versus *Anabaena* one.

In September, the quantity of *Anabaena planctonica* cells appeared in the water as much as the quantity of *Mougeotia* cells. We noticed that various big scums of algae appeared in the July-August period, then decreased and even disappeared in certain locations in September-October. The water turned light green in color with many features representing for cyanobacterial presence. The taxonomy has confirmed the domination of *Anabaena planctonica* in both biomass and cell abundance.



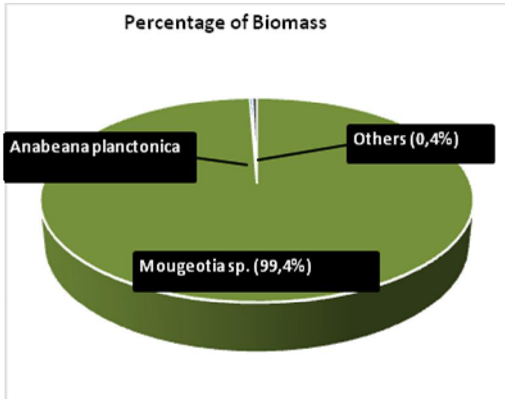
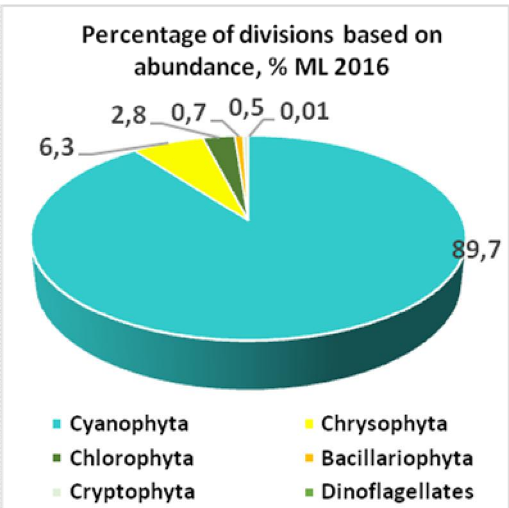


Fig. 2. Approximation for microalgae species composition and cyanobacterial proportion in the whole ML via taxonomy tests from April to October 2015: *based on biomass* (left) *and based on cell number* (right)

2016: Taxonomy from July to October 2016 showed that ML contained 7 divisions, 19 classes, 52 genera, and 75 taxa. Percentage distribution of divisions is presented in Fig. 3.

The maximum species in a single sample was found at section 1 on 12 July 2016: 41 species. The minimum number of species (15) at one location was registered at the outlet in the middle of October.



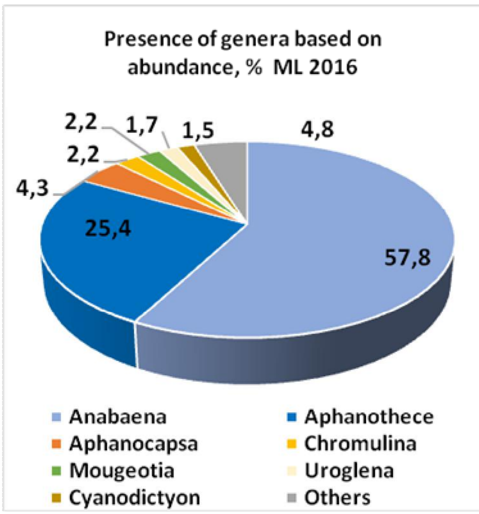
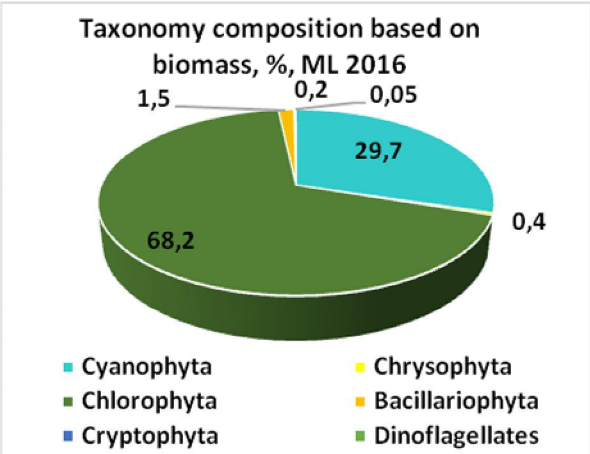


Fig. 3. Percentage of phytoplankton divisions and genera based on the abundance (%) in ML in 2016

In 2016 samples were taken during the summer to autumn period at the bloom points. Cyanophyta was the main division (89.7 %) dominating during the blooms based on the abundance. *Anabaena* and *Aphanothece* genera were dominant in abundance. *Mougeotia* represented green algae and *Chromulina mikroplancton* with *Uroglena Americana* represented golden algae in abundance.

As we can see from Fig. 4 below, Chlorophyta algae had the greatest biomass during the survey. *Mougeotia sp.* was dominant (68.1 %) based on biomass due to its property to create a big mats. Its cells are big, and even a small presence in the sample gave a large biomass. Cyanophyta gave 29.7 % of the total biomass. *Anabaena planctonica* was the dominant species. All other divisions gave very low biomass.



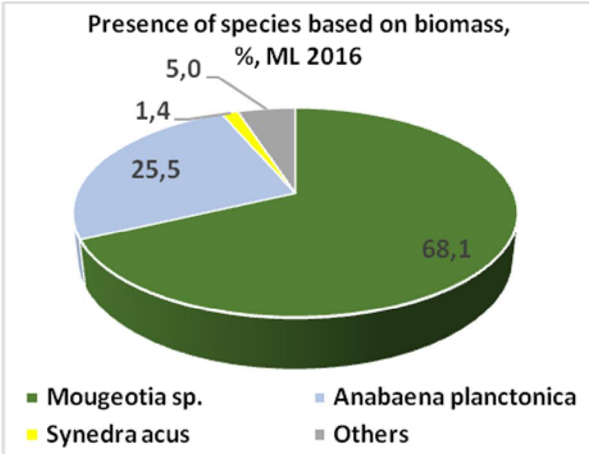


Fig. 4. Percentage ratio of phytoplanktonic divisions and species based on the biomass (%) in ML in 2016

In the middle of July *Mougeotia sp.* was 80 % of the biomass in the samples. *Uroglena americana* (golden algae) was 8 % of the biomass, *Anabaena planctonica* (blue-green algae) was present at 4 % of the biomass.

In the end of July 2016 there was a presence of *Mougeotia sp.* in the sample. The percentage of *Anabaena planctonica* was 1.8 % of the biomass in the first part. However, on the second and third part the *Anabaena planctonica* presence was 35 % and 52 % of total biomass respectively. It is interesting to note that *Anabaena planctonica* coexisted with *Cryptophyta* algae *Uroglena americana*. The biomass of *Anabaena planctonica* was 1.4 (second section)-1.7 (third section) times more than *Uroglena americana*. These algae species are mixotrophic. They prefer low nutrient environments that probably help it to survive with Cyanophyta algae.

In mid-September *Anabaena planctonica* was dominant in the entire lake. At the south and middle parts it was more than 90 %, but at the north part it was 75 % with the high presence of a degraded *Mougeotia sp.* (23.7 %). Samples from October (beginning and middle) show the dominance of *Anabaena planctonica* (98 %).

Anabaena planctonica started to develop one month earlier in 2016 than in 2015. In the end of July their presence in the samples was high and increased to the outlet part. The first section was almost free from this species due to the development of Mougeotia sp. From September onwards, *Anabaena planctonica* was completely dominant.

If we compare the two years of survey from 2015 and 2016, the results are represented in Fig. 5. From these pie-charts, the species of Chlorophyta (or green algae) were common in the phytoplankton community during the period of observation. Species from Bacillariophyta division (diatoms) took second place as they were 22–20 % respectively. Species of Cyanophyta (blue-green algae) had a higher rate in 2015 compared to 2016. The number of species from Chrysophyta (golden algae) division increased from 2015 to 2016 and have almost the same number of species as cyanobacteria in 2016.

The total number of species dropped down from 29 registered during 2015 to 13 in 2016. Such genera from the cyanobacteria division as *Aphanizomenon, Oscillatoria, Merismopedia, Planktolyngbya* were not found in the samples in 2016.

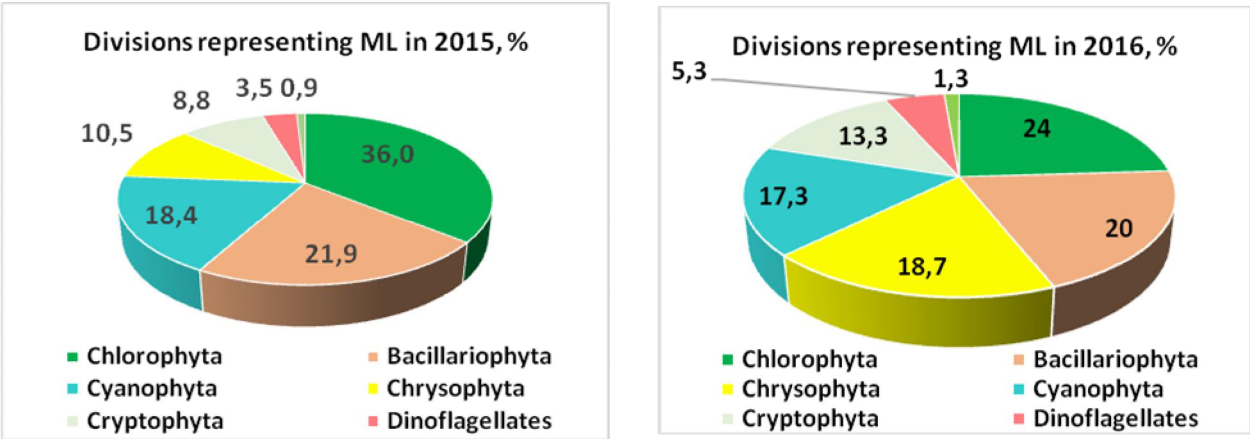


Fig. 5. Phytoplankton divisions found in ML in 2015–2016

4. Redfield ratio and Modified Redfield ratio (MRR)

Redfield (Redfield, 1958) suggested an optimal ratio between Nitrogen and Phosphorus which were contained in living organisms. This ratio, known under the name of *'Redfield ratio'*, can be used to estimate the algal status in a water body. With a mass conversion, Bulgakov and Levich (1999) defined ranges of this ratio (N/P): 1) *from 5 to 10: Cyanophyta* species domination would be considered; 2) from *20 to 50:* the green algae growth would be favored.

Inspired from this idea, our suggestion however did use Nitrates and Phosphates (not TP and TN) as we observe that mineral form of these nutrient components triggered the bloom in ML and could be the main cause of it. The mass conversion is based on the ratio of *Nitrates to Phosphates* to define the blooming possibilities as well as their limits for the development of two main potential groups leading to bloom patterns: *green algae* and *blue-green algae* (Table 2).

We detected that with the ratio Nitrates/Phosphates:

i) from 13 to 32.5: the green algae would be favored

ii) from 3.25 to 6.5: the blue-green algae or Cyanophyta would be favored

Table 2

Nitrates/Phosphates **ratio based on the mass conversion**

Phosphates (mg/L)	Nitrates (mg/L)									
	0.1	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**1.2**	**1.4**	**1.6**	**1.8**
0.01	10	20	40	60	80	100	120	140	160	180
0.02	5.0	10	20	30	40	50	60	70	80	90
0.03	3.3	6.7	13	20	27	33	40	47	53	60
0.04	2.5	5.0	10	15	20	25	30	35	40	45
0.05	2.0	4.0	8.0	12	16	20	24	28	32	36
0.07	1.4	2.9	5.7	8.6	11	14	17	20	23	26
0.09	1.1	2.2	4.4	6.7	8.9	11	13	16	18	20
0.2	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0
0.4	0.3	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
0.6	0.2	0.3	0.7	1.0	1.3	1.7	2.0	2.3	2.7	3.0
0.8	0.1	0.3	0.5	0.8	1.0	1.3	1.5	1.8	2.0	2.3
1.0	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8

Green algae bloom when ratio from 13 to 32.5

Blue green algae bloom when ratio from 3.25 to 6.5

5. Results and Discussions

The attempt to predict the HAB occurrence and proliferation under complex context of environmental conditions (nutrient, light, meteorological factors, etc.) led to many indexes for the estimation of HAB risks based on chemical components such as micronutrient factors of the waterbody that contribute significantly to algal growth. The simplest index is based only on one factor such as the TP level, Chlorophylla or cell counts.

In the context of this paper, we suggest to use our Modified Redfield ratio (MRR) mentioned above to estimate the HAB risk in a ‘simple’ way in combining Chlorophyll-a and PC indexes, shown in Table 3 below. For Chl-a and PC criteria, we refer to Novotny and Olem (1994), Bulgakov and Levich (1999), Brient et al. (2008) and Brylinsky (2009).

Table 3

Risk indexes, adapted from Novotny and Olem (1994), Bulgakov and Levich (1999), Brient et al. (2008), Brylinsky M. (2009), Hushchyna and Nguyen-Quang (2016)

Risk	Chl-a index	PC index	Our Modified Redfield Ratio (MRR)
Low risk of HAB	0-5 *µg/L*	≤ *30 ±2 µg/L*	≥ 6.6
Medium risk of HAB	5-10 *µg/L*	Between 30 and 90 *µg/L*	≤ 3.25
High risk of HAB	≥ 10 *µg/L*	≥ *90±2 µg/L*	3.26 - 6.5

Based on the field data obtained from 2015-2016 with our field observation (174 points), we established the relationship between PC and Chl-a measured and MRR, and is illustrated by the graph in Fig. 6.

When MRR value is 0-10, the graph contains the most Chl-a values, as well as the highest (120 points, 68.96 %).

From Fig.6 (with the log scale for MRR), we can see that when MRR is between 3.25 and 6.5, all Chl-a are greater than 20 µg/L and PC greater than 100 µg/L. In this range, we also have the highest values of Chl-a (>90 µg/L) and PC (>220 µg/L) at MRR=3.5. In combining Table 3, this shows a high risk of HAB. There were in total 27 points that fell into this category of having a ratio between 3.25 and 6.5.

With points having a MRR value less than 3.25, we can observe that Chl-a values vary between 5 and 30 µg/L, while PC values vary from 20 to 150 µg/L. There were 69 points (39.6 %) with a ratio MRR less than 3.25, and of those points, there was only one point that had the low Chl-a value: 9.91 µg/L. In referring to Table 3, it could be said that there was a medium to medium high risk of HAB.

When a MRR value of 10–30 occurred: This section of the graph has the second highest number of points in the total data distribution (45 points, 25.86 %). Among them, there are 29 points having Chl-a values between 0 and 5 µg/L, and the rest varied from 5 to 30 µg/L. Regarding PC, the distribution was not homogenous: some points had high values (over 70 µg/L) and some points had less than 30 µg/L. If we consider both PC and Chl-a criteria in the same time as indicated in Table 3, this MRR range should be potentially a low to medium risk.

When a MRR value > 30 was obtained: All points have a very low risk of HAB (9 points, 5.17 %). All of the points have Chlorophyll-a values below 5 µg/L. The PC values vary between 10 and 40 µg/L, except for some outliers with high values (over 80 µg/L). This was determined a low risk of HAB.

Actually, our field observations recorded during two summer seasons 2015–2016 showed different bloom scenarios fitting quite well with the above estimation, as illustrated in Fig. 7 and Table 4.

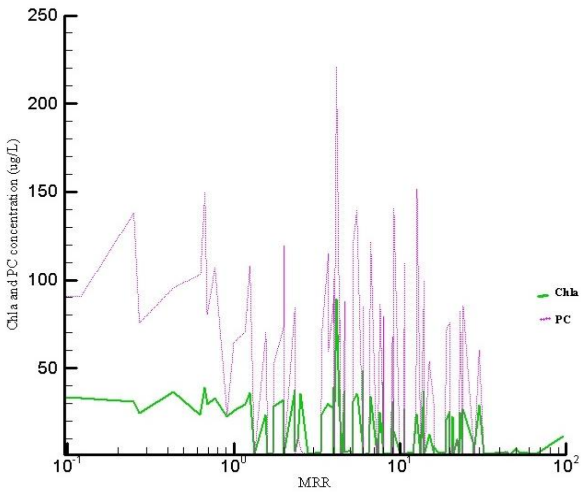


Fig. 6. Chlorophyll-a and Phycocyanin versus MRR (field data 2015 and 2016)

Fig. 7. Algal blooms in Mattatall Lake in August 2015

In Table 4, MRR values calculated from 2015 data showed two possibilities of blooms either caused by blue-green algae (cyanobacteria) or by green algae: one in August (*10 cases of dominant green algae versus 5 cases of dominant cyanobacteria*) and one in October (*5 cases of dominant green algae versus 8 cases of dominant cyanobacteria*). Bloom possibilities of *green algae* went from 8 to 10 and then to 5 (June to August to October). For *blue-green algae* the bloom possibilities went from 6 to 1 and then to 8 (June to September to October). These predictions are comparable to the field observations: *green algae* (such as *Mougeotia*) blooms were present from July to August, while *blue-green algae* (such as *Anabaena*) blooms were present from September to October.

Table 4

Potential cases for Blooms based on the Modified Redfield ratio (Nitrates/Phosphates)

	2015						2016				
	11-Jun	30-Jul	17-Aug	10-Sept	04-Oct	27-Oct	14-Apr	10-May	17-Jun	16-Aug	04-Oct
Cyanobacteria Domination	6	4	5	1	6	8	0	4	4	0	0
Green Algae Domination	8	9	10	4	1	5	3	0	3	0	0
Potential Cases	12	10	18	26	18	13	0	1	4	0	18

In April of 2016, Table 4 showed that there were three cases of green algae domination in the lake, no potential case of cyanobacterial presence and no bloom was observed as some favorable conditions such as temperature and nutrients were still low. However, in May, there were four favorable cases for cyanobacteria growth and one potential possibility of bloom. Generally, the absence of Phosphates and low temperature did not allow for the development of cyanobacteria. In June, concentrations of Nutrients in the water was high enough to develop both green algae and cyanobacteria. However, as the temperature was around 16 °C, green algae were favored to grow over cyanobacteria. The increase of the water temperature turned the dominance toward cyanobacteria (*Anabaena planctonica*). In August, we observed the complete depletion of Nitrogen and Phosphorus in the water hence no significant MRR values were found (all are 0). In October, the Nitrogen depletion was still occurring, but the presence of high Phosphates could be favorable for cyanobacterial growth and hence leading to many potential possibilities of blooms (18 cases total from Table 4). Via this Table, it is assumed that from April to October 2016, the probability of occurring blooms increased.

We can also assume that a range of Redfield ratio going from 0 to 6.5 could be more accurate for the potential cyanobacteria growth. This range can be also applicable to our proposed MRR. Nevertheless, more data will be needed to confirm this range.

6. Conclusions

With the data from two years (2015-2016), ML showed a moderately eutrophic level and contained potential toxic algal species. The formation of blooms can occur only when an optimal combination of favorable conditions including micronutrients, water temperature, etc. Two main categories of blooms were observed in ML seasonally – one common species of algal bloom from July to September, formed with non-toxic species *Mougeotia sp.* Another one potentially toxic *Anabaena sp.*, especially *Anabaena planctonica* (*Dolicospermum planctonicum*), which started in September and lasted until December, this species generated blooms in the fall term.

The MRR range going from 0 to 6.5 could be more accurate for the potential cyanobacteria growth with the MRR values between 3.25-3.5 can be considered as the threshold for the HAB pattern. The Modified Redfield ratio showed a reasonable reliability to be used for estimating quickly the HAB risks.

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