Indexes for Evaluating and Predicting Toxic Algal Blooms, application to Mattatall Lake (Nova Scotia, Canada)

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Abstract - **The bloom patterns Mattatall Lake (ML) have many particular aspects: non-toxic species domination in the summer and toxic species domination in the fall-winter; nutrients increasing monthly basis; and blooms co-exist with icy conditions. In this paper, we suggest a systematic investigation for ML via a *'standard process'* of sampling and analysis of governing parameters of the bloom patterns.**

Keywords - **Mattatall Lake, Harmful Algal Blooms (HAB), Redfield ratio, Cyanobacteria, Cyanophyta.**

I. Introduction

Many watercourses in Nova Scotia (Canada) have recently had algal blooms booming and surprisingly increasing in frequency and diversity without any good understanding or explanation about causes and effects. Mattatall Lake (Nova Scotia) started to experience severe algae blooms in 2013. During the fall of 2014, massive algal blooms appeared in ML, and persisted until late December 2014. This is an odd phenomenon because generally cyanobacteria are favored by warmer waters [1]. The duration of this phenomenon was extremely unusual. Toxic 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* 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 [2].

Although reasons for the algal bloom appearing are known by literature, there are certain combinations of multiple factors to push HAB triggering specific to each waterbody. However, their coupling effects are not yet well understood. No research nor data related to ML have been done. Moreover, in Nova Scotia, even cyanobacterial bloom patterns recently boomed in many watercourses at the scale of the entire province, no systematic investigation has been sketched, especially for coupled physical-biochemical effects. In this paper, we try to use different indexes to estimate the thresholds for bloom occurrence and to predict the bloom patterns in function of environmental factors. This is a preparation for the next step to evaluate different governing parameters and their coupling effects on the bloom phenomenon occurring as well as for a predictive model in the future.

II. Experimental processes

Different equipment for the field sampling and Lab analyzing processes were used, such as YSI probe (Professional Plus, Hoskin scientific LTD, USA) for pH, Dissolved Oxygen (DO), conductivity, and temperature in the water; weather station (E-348-H21-002, Onset, Hoskin scientific LTD, USA) for rainfall, wind speed/gust speed, wind direction, temperature/RH/dew point, and PAR (Photosynthetically Active Radiation); Fluorometer for Chlorophyll-a; and Photometer for evaluating chemical components. Samples were taken tri-weekly or every month depending on the weather conditions starting from June 2015 systematically, at the surface and bottom levels. It is also noticed that the lake is quite swallow with the maximum depth around 8m. Predetermined sampling locations are represented in Fig. 1 and their coordinates are given in Table I. If there some other places that are not included in the predetermined points with appearing algal bloom, samples are taken at these points of bloom concentration. HOBO weather station was installed in one fixed location of the lake. Samples for taxonomy were preserved with Lugol and sent to taxonomy Lab at University of Quebec at Montreal (Canada) after the field trip. Immediately after sampling, analysis for micronutrients at the lab including Phosphates, Total Phosphorus (TP), Iron, Nitrates, Nitrites, and Ammoniacal Nitrogen occurred to avoid the sample degrading. Samples for Chlorophyll-a were filtered. If measurements were not in the same day, filters were wrapped into the foil and frozen at -80˚C. The next step was the extraction with acetone, after two steps of centrifugation in 3 500*g* room temperature condition during 10 minutes and 13000*g* at 4˚C during 1.5 hour to purify the extraction.

III. Taxonomic Results: Microalgae species and cyanobacterial proportion

Our observation from last 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** were identified.

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The maximum species number in a single sample was found at 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).

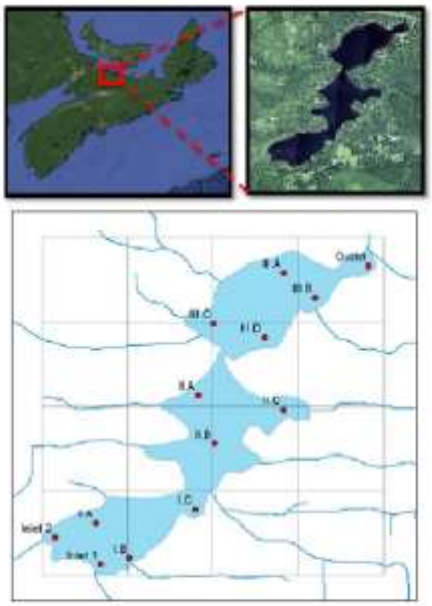


Fig.1. *Above :* Google Earth view of Nova Scotia highlighting ML area (left) and Government of Nova Scotia aerial photograph of ML (right)
Below: Sample stations (locations) predetermined and monitored. Coordinates of ML are 45° 42' 17" N, 63° 19' 39" W.

TABLE I: GSP COORDINATES FOR EACH REGULAR SAMPLING LOCATIONS IN ML

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

It is noticed 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 comparing 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 appearing in July-August period almost decreased even disappeared in certain locations in September-October, and the water turned in light green color with many features representing for cyanobacterial presence (Fig. 3, below). The taxonomy has confirmed the domination of *Anabeana planctonica* in both biomass and cell abundance.

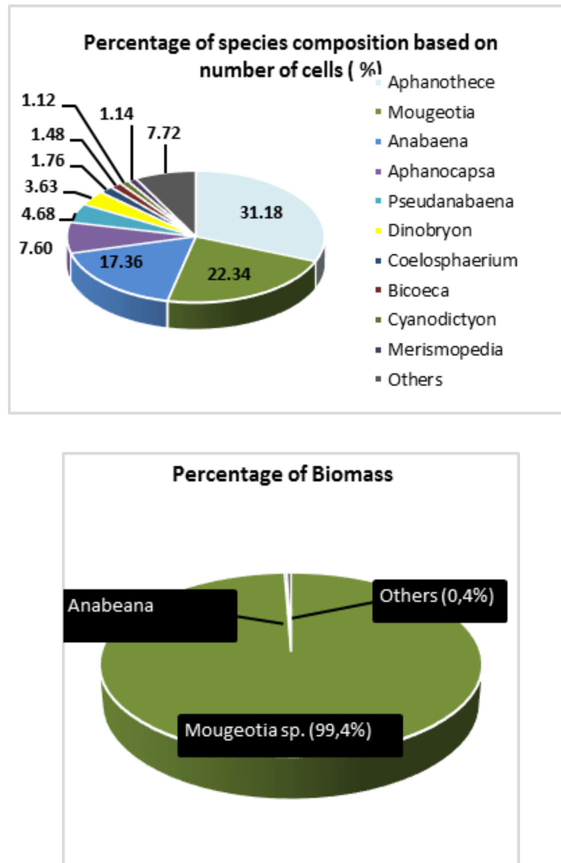


Fig.2. Approximation for microalgae species composition and cyanobacterial proportion in the whole ML via taxonomy tests from December 2014 to October 2015: *based on cell number (above) and based on biomass (below)*

IV. Indexes for the Estimation and Prediction of Algal Bloom Occurrence

The attempt to determine the eutrophic level of a waterbody and to well predict the algae bloom occurrence in this condition is the reason for the definition of many indexes for the estimation of eutrophication. Most of research on indexes are based on chemical components, i.e. micronutrient factors of the waterbody such as TP and its component Phosphates, and Dissolved Inorganic Nitrogen (DIN), which contribute significantly to algal growth. The simplest index based only on one factor such as the TP level, Chlorophyll-a or cell counts suggested by World Health Organization [3] and Canadian Council of Ministers of the Environment [4]. It is stated that if the number of cell is:

> 200 cells per ml, the *vigilance warning is placed on the water source*

> 2,000 cells per ml: *Alert Level One*

> 100,000 cells per ml: *Alert Level Two*

Fig. 3. *Above:* New algal scum appearing around the end of July, however observed on 30 July 2015 in Part I (South section). *Below:* Unique green color by algae on 4 October 2015 (Middle section)

Other similar indexes (based one or several coupled parameters) were also used by many researchers [5]-[6] to define a lake's trophic status. One common index for many cyanobacterial bloom research is the mass ratio of Total Nitrogen to Total Phosphorus [7].

A. *Modified Redfield ratio*

Redfield [8] 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 [9] defined two ranks of this ratio (N/P) with that the *Cyanophyta* species domination would be considered (from 5 to 10) or the green algae growth would be favored (from 20 to 50). In this study, the same idea of mass conversion was processed but the ratio was calculated on the mass conversion of <u>*Nitrates to Phosphates*</u> 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* (Fig. 4). 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*

Our analyses for all samples based on this modified ratio showed clearly two episodes of bloom developing in August *(10 cases of dominant green algae versus 5 cases of dominant cynobacteria)* and in October (*5 cases of dominant green algae versus 8 cases of dominant cynobacteria*). For *green algae* bloom possibilities, the number of cases were increasing from 8 to 10 (June to August) and decreasing till 5 favorable cases in October. For cyanobacteria *(blue-green algae)* blooms, the number of cases were decreasing from 6 to 1 (June to September) and increasing till 8 favorable cases in October. Field observations correspond very well with these calculations because dominant green algal blooms with *Mougeotia* species from July to August were obviously recorded and they were replaced by *Anabaena* blooms from September to October.

The modified Redfield ratios Nitrates/Phosphates have showed that the maximum potential cases leading to blooms were in September and less in the beginning of October (Table II). This could be explained by the fact that blooms were dispersed by strong circulations and the lake turned into a homogenous green color, as observed in 4 October field trip (Fig.3 below). However, most of the cases we calculated herein for potential growth in all of October were less than limit for *Cyanophyta* as above mentioned. It would be suggested that the rank for potential growth of cyanobacteria could be from <u>***0 to 6.5***</u>. We expect this suggested rank of cyanobacteria occurrence for the modified Redfield ratio would be validated and adjusted with more future data.

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

Fig.4. *Nitrates/Phosphates* ratio based on the mass conversion [9]

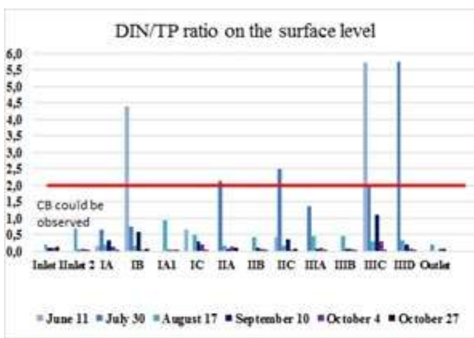
TABLE II: CASES OF POTENTIAL BLOOMS BASED ON THE MODIFIED RATIO *NITRATES/PHOSPHATES*

	11 June	30 July	17 August	10 Sept.	4 Oct.	27 Oct.
Cyanobacteria domination	6	4	5	1	6	8
Green algae domination	8	9	10	4	1	5
Potential cases	12	10	18	26	18	13

B. *DIN/TP ratio*

Another ratio is suggested to better evaluate the bloom occurrence: Dissolved Inorganic Nitrogen to Total Phosphorus DIN/TP. Cyanobacteria blooms (CB) can occur when the DIN/TP ratio was below than two [7].

Fig.5 evaluates the DIN/TP ratio at the surface and bottom level during the summer-autumn 2015. It is interesting to note that our data from all sampling locations at different levels of the lake have the DIN/TP ratio less than 2 and the Part I of ML seemed being most favorable for the CB development. According to this observation, the lake had the necessary condition for the occurrence of CB blooms in all summer and autumn (DIN/TP were less than 2 from July to October). Our taxonomic tests also showed that there were some cyanobacteria, however we did not have very prominent blooms from cyanobacteria during the summer (May to September), but *Mougeotia* sp. (green algae) blooms. This is also confirmed by the measured value of Phycocyanin (that we will mention in another article). From September we see the change in diversity of species and the domination of cyanobacteria (*A.planctonica* is 62% near WS in the end of October). The October trips have shown the very low DIN/TP values, less than 0.3. So, this ratio cannot reflect well the real condition of CB occurrence in ML.



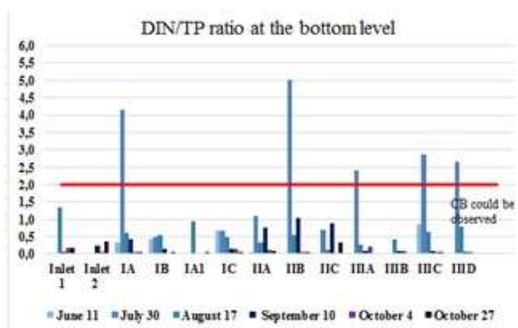


Fig.5. Estimation of algal bloom occurrence in using DIN/TP ratio from June to October 2015: on the surface *(above)* and at the bottom *(below)*

C. Trophic Index or TRIX number

In combining different parameters which could influence on the eutrophication, Vollenweider et al. [10] have suggested the TRIX number, that means the integral indicator for trophic level (TRIX standing for *Trophic Index*). This indicator was defined by a linear combination of the logarithms of four state variables: 1) Chlorophyll-a as the primary production; 2) Dissolved Oxygen as absolute percent of deviation from saturation (DO%) in the phase of photosynthesis and in the phase of respiration predominance; 3) Mineral Nitrogen (DIN) and TP as the presence of main nutrients for algal development. The TRIX number can be calculated according to the following formula.

$$TRIX = \log([Chl]\times[DO\%2]\times[TP]\times[DIN]\times k) / m$$

Where:
Chl – sum concentrations of Chlorophyll-a and Pheophytin-a, *µg/L*
DO%– deviation of oxygen saturation from 100%
TP – concentration of total phosphorus, *µg/L*
DIN – mineral nitrogen, *µg/L*

Two constants appearing in the formula (1) *k* and *m* are named *'scale coefficients'* with the value *k =1.5* and *m =1.2*. They are introduced to the formula in order to fix the lower limit value of the TRIX number and the extension of all related trophic scale, from 0 to 10 TRIX units [11].

Different trophic levels are defined based on TRIX number as follows.

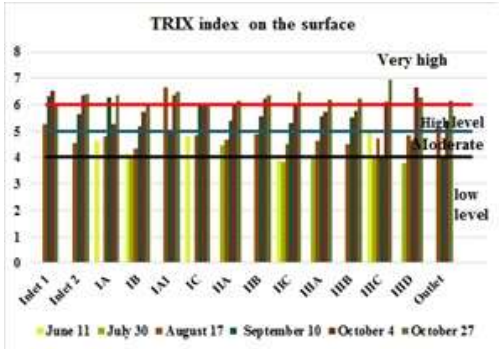
- *TRIX < 4:* Trophic level is low. Water quality is high.
- *TRIX= 4-5:* Trophic level is moderate. Water quality is good.
- *TRIX= 5-6:* Trophic level is high. Water quality is moderate.
- *TRIX > 6:* Trophic level is very high. Water quality is degraded. High water turbidity, large areas of colour anomalies of water, regular hypoxia over a large area and frequent anoxia of bottom waters, death of benthic organisms [12].

According to the above classification, the trophic level in ML based on the TRIX number was calculated for all field trips for the surface and bottom levels: from June to October 2015 (Fig.6). It is noticed that TP level is *0 mg/L* in many locations on 11 June and a few locations in 30 July. In August and September, based on TRIX number, Parts I and III of the lake seem being highly eutrophic with the degraded quality of water and in October all of the lake is in the same situation. The TRIX number showed ML had a degraded water quality and a very high level of eutrophication all over the surface of the lake in the end of October.

V. Discussions about these indexes

Despite every index previously mentioned can evaluate at certain level the occurrence of blooms, it is evident that just one parameter (such as TP index or Chlorophyll-a index) is not enough to estimate the possibility of blooms due to the complex nature of coupling effects, and hence these indexes showed very often contradictory results.

For example, the limit of TP, a major factor of eutrophication considered as the main nutrient of algae, is very low (0.02 mg/L = 20 µg/L). In the same time, the suggested limits of other parameters such as Chlorophyll-a and DO in the same guideline are not as much as strict as TP to determine eutrophic level and to estimate the bloom occurrence. Different field trips we made show a high concentration of TP everywhere in ML and some are much higher than the value of 0.02 mg/L. As a result, we have the incompatible conclusions when we use these limits to evaluate the water quality and eutrophic level, for example some points have very high trophic level by TP limit but good quality of water if using the Chlorophyll-a and DO limits. This conclusion was valid for almost all water samples collected.



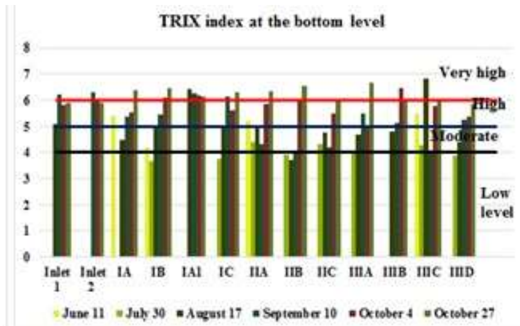


Fig.6. Evaluation of Trophic level in the lake based on TRIX index from June to October 2015: on the surface *(above)* and at the bottom *(below)*

The ratio of main nutrient components *(Nitrates/Phosphates)* seemed to be *a good prediction of algae development* in ML. Using this modified Redfield ratio to define locations considered as 'appropriate conditions for blue-green algae growth and for algae blooms', it was really observed the corresponding blooms in June (section I), in July (section II), in August (section III), in September (section II and III) and all over the lake in October. This Nitrates/Phosphates ratio therefore could be a good index for the location where the growth of algae (green or blue-green) might lead to the blooms with appropriate conditions. However, as above mentioned, the rank suggested here for modified Redfield ratio Nitrates/Phosphates needs to be validated more by future data. DIN/TP indicated that almost everywhere on the lake were favorable for bloom occurrence. In the meantime, TRIX number indicated the good water quality in all summer periods even when we had observed many algae scums in the water. Although TRIX number was used by literature just only for the salty water for most of the cases, it seems that its usage herein for fresh water is quite significant. Only in September and October we can notice that the eutrophic level was very high in the lake according to TRIX number and the water quality was bad.

In general, the south section (Part I) is the most vulnerable part due to the fact that there are two brooks flowing directly into the lake and bringing the high quantity of nutrients (W1 and W3, Fig. 7). Different tests during the summer at these two inlets showed always the worrisome results about nutrients and algal growths with high diversity of species.

However, the index based on single or duo factors cannot reflect all factors affecting and trigging the bloom pattern. The mathematical modeling seems to be the unique way being able to estimate the coupling effects of all factors.

VI. Conclusion

Mattatall Lake is a moderately eutrophic lake containing algal toxic species. Although the eutrophication is clearly determined in the lake waterbody and in its watershed, unfortunately the main sources of contamination cannot be identified and the level of nutrients fluctuated very much during the summer time, hence massive bloom consequences in the lake. With no industrial zones nor agricultural activities nearby, the contamination of ML might be explained by human-based activities of residents and the beaver dams within the watershed

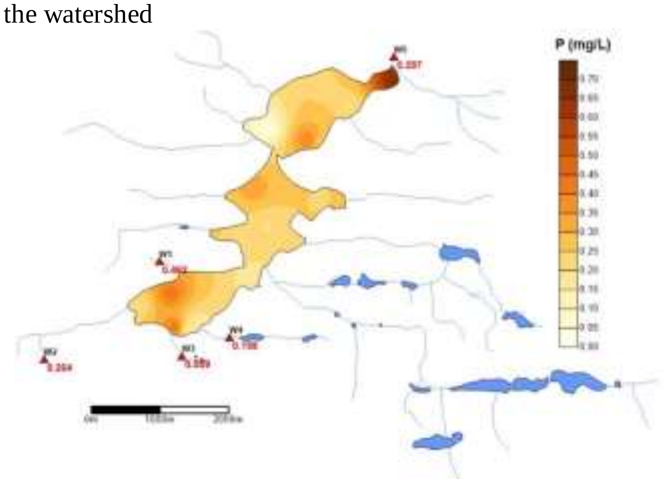


Fig. 7. Total Phosphorus distribution at the lake surface and brook surface on 17 August 2015

The lake including its own watershed with 3 different sections together interconnected, shows a complex system of ecology and biology. The whole lake is quite shallow (maximum depth is around 8m and minimum depth is around 1m) so the effect of stratification is certainly not significant. The flow speed is very slow (0.4 cm/s in average) with the residence time around 8 days, hence the lake may be considered as static and even stagnant.

Chemical data (nutrients) of the lake were detected and analyzed in summer and autumn months. There was a part of nutrients released from sediments due to the circulation in the lake by wind and warm weather. In the meantime, there was a strong consumption of these nutrient elements in all points of water body, especially at the water surface level due to phytoplankton development. As other lakes, the main supply of ML is from its own watershed including brooks and tributaries. Soils within watershed, which are contaminated by human or natural activities, certainly affect the water source flowing into the lake. For example, some cottages on the southwestern shore were built at the same level of the lake. Hence, there is a risk of leaching septic tanks into the lake. Other possible reasons making the nutrient level of the lake higher could be the decomposition and defrosting of various organic materials that had been frozen through winter and spring periods. Multiple brooks flowing into ML have the Phosphorus concentration areas by human activities (clear-cutting, spraying, etc.). Consequently, dissolved nutrients coming from those sources by rainfall-runoff and/or brooks wasting directly into the lake contribute to the growth of nutrient level in the waterbody. Micronutrients due to human activities rejected directly into the watershed contribute to the causes of bloom development. A profound study on the entire watershed is envisaged for a long term solution to this issue.

The formation of blooms can occur only when an optimal combination of favorable conditions including micronutrients, water temperature, PAR, etc. Two main categories of blooms were observed in the ML seasonally – one normal category of algal bloom from July to September, formed with non-toxic species *Mougeotia sp.* and another one with *Anabaena sp.*, especially *A.planctonica* starting from September until December, and this species generated blooms in the fall term lasting till December.

The research through this paper showed a very important insight: bloom patterns can be only well explained and predicted by coupling effects of all involving parameters. In order to combine all effects of all possible parameters, only mathematical model can help us to deal with this complex issue. Mathematical modeling is hence one of our avenues of future research.

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