



*Research article*

## **Correlation of cyanobacterial harmful bloom monitoring parameters: A case study on western Lake Erie**

**Hesam Zamankhan Malayeri<sup>1</sup>, Mike Twardowski<sup>2</sup>, James Sullivan<sup>2</sup>, Timothy Moore<sup>3</sup> and Hyeok Choi<sup>1\*</sup>**

<sup>1</sup> Department of Civil Engineering, The University of Texas at Arlington, Arlington, TX 76019-0308, USA

<sup>2</sup> Harbor Branch Oceanographic Institute, Florida Atlantic University, Fort Pierce, FL 34946, USA

<sup>3</sup> Institute for the Study of Earth, Oceans, and Space, University of New Hampshire, Durham, NH, 03824-3525, USA

\* **Correspondence:** Email: [hchoi@uta.edu](mailto:hchoi@uta.edu); Tel: +18172725116; Fax: +18172722630.

**Abstract:** Occurrence of cyanobacterial harmful blooms (CHBs) in water has caused serious concern to environmental and health authorities because of their potential to produce and release lethal biological toxins. Among many toxins, microcystins (MCs) are of particular interest. There have been significant efforts to observe the harmful algal bloom events and cyanotoxin levels, including: (i) manual field sampling followed by lab analysis to directly measure MCs, (ii) remote sensing based on satellite image analysis to estimate cyanobacterial index (CI), and (iii) in-situ sensing of proxy parameters to cyanobacterial blooms such as phycocyanin. This study compared the observation systems in western Lake Erie to find any potential correlations among these CHB monitoring parameters based on the Pearson Product-Moment equation. We found the relationships among the parameters to be site-specific and so we compared geographical, ecological, meteorological, and analytical factors specific to the locations to explain the observed correlations and variations. The CHB observing parameters (MCs, CI, and phycocyanin) were generally well correlated because they inherently represented the same phenomenon. In particular, we found the measured biological toxin concentration (MCs) to be strongly correlated with the cyanobacterial bloom activity (CI) estimated by satellite image analysis. The phycocyanin concentration also had a strong correlation with CI, implying that measuring an easy-to-detect proxy parameter in-situ and in real-time is effective for monitoring CHBs. The results support the notion that key environmental management parameters such as CHB toxicity can be inferred from remotely-sensed ocean color through proxy variables such as CI.

**Keywords:** cyanobacterial harmful blooms; harmful algal blooms; Lake Erie; microcystins; cyanobacterial index; phycocyanin; monitoring and observation

## 1. Introduction

Several environmental factors, such as nutrients, light, wind, temperature, pH, and hydrology, stimulate frequent massive and prolonged blooms of cyanobacteria and algae, forming cyanobacterial harmful blooms (CHBs), more commonly known as harmful algal blooms. Direct economic impacts of coastal harmful blooms events in U.S. have increased significantly each year [1]. The majority of the impacts are associated with public health and commercial fishery sectors. There have been increasing research activities for developing algal bloom mitigation strategies categorized into mechanical, physical/chemical, and biological control [2,3]. For the effective implementation of such strategies with given operating budgets and efforts, location and degree of CHBs should be identified on time or preferentially in advance [3].

Cyanobacteria (blue-green algae) are of particular concern in freshwater bodies because more than 50 species of cyanobacteria are known to produce cyanotoxins such as microcystins (MCs), anatoxin-a, and cylindrospermopsin [4]. In particular, MCs are among the most powerful natural poisons and up to 50% of the recorded blooms can be expected to contain MCs [5]. The CHB and MC outbreak in 2014 in drinking water resources in the city of Toledo, OH triggered close attention of general public all around the nation [6]. Cyanobacteria and their toxins are currently in the U.S. Environmental Protection Agency's Drinking Water Contaminant Candidate List [7].

As a result, monitoring and publicizing CHB activity can provide a major mechanism for reducing or preventing exposures to toxins during CHBs and for deploying bloom mitigation strategies in advance [8,9]. Cyanobacteria and toxins can be directly identified while measuring easy-to-detect surrogate (i.e., proxy) parameters to them can be an alternative to the direct measurement in order to simplify the monitoring process [10]. On-site, in-situ, and remote observing approaches can be selected for the monitoring [8]. There have been huge efforts to monitor CHB activities and toxin releases in U.S. particularly by National Oceanic and Atmospheric Administration (NOAA) [11]. Both direct (microscale) and indirect (macroscale) observational systems for monitoring CHBs and their characteristics have been adopted (Table 1).

**Table 1.** Current observation systems for monitoring cyanobacterial harmful blooms.

Observing approach	Measuring target	Directness	Scale <sup>a</sup>	Final information
On-site sampling followed by lab analysis	Biological toxins (MCs) and cyanobacterial species	Direct	Microscale	Exact cyanobacterial community and toxin release
Remote sensing based on satellite image analysis	Cyanobacterial index (color image)	Indirect	Macroscale	General cyanobacterial blooms
In-situ sensing for monitoring a proxy parameter	Phycocyanin, an accessory pigment to chlorophyll	Indirect	Macroscale	General cyanobacterial blooms

<sup>a</sup> Microscale data: detailed information for a confined area and macroscale data: general information for a vast area.

First, on-site manual sampling followed by lab analysis is commonly used to identify cyanobacterial species and to assay biological toxins [12]. In spite of its high accuracy and reliability, this approach is neither sustainable nor practical to meet the vast spatial and temporal measuring need. Second, remote monitoring relies on spectral images taken from satellites and aircrafts and provides the large spatial scale and high frequency of observations required to assess bloom locations and movements. The remote sensing approach is useful for monitoring general cyanobacterial bloom activities by allowing the construction of a cyanobacterial index (CI) from analysis of pixels in remote sensing images [13]. Third, in-situ sensing is a recent monitoring approach [14]. For example, an in-situ autonomous observing approach employing a fluorescence probe mounted on buoys optically senses phycocyanin as an accessory pigment to chlorophyll often associated with CHBs (i.e., phycocyanin is a surrogate chemical or proxy to CHBs) [15]. The remote and in-situ (real-time) monitoring approaches improve opportunities for immediate decision-making and timely response. However, these color-based products are not specific to CHBs because high level of chlorophyll may or may not be associated with toxic blooms and thus not all cyanobacterial blooms are associated with release of biological toxins [16,17].

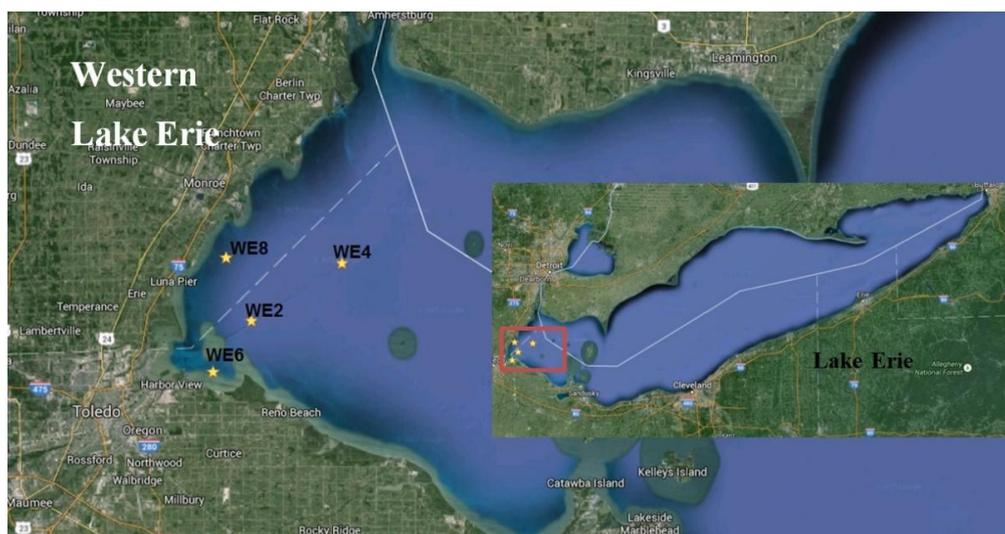
In spite of their utility, no one has attempted to collect and compare these CHB observing parameters to quantify correlations among the parameters to better assess, interpret, and even forecast CHBs and the presence of cyanobacterial toxins. The objective of this present study, therefore, is to compare and correlate the current observation systems and thus to evaluate the effectiveness of each system to monitor and assess CHB events and associated toxins. The observation systems monitor different CHB parameters such as biological toxins (e.g., MCs), general cyanobacterial blooms (e.g., CI), and proxy targets (e.g., phycocyanin). We hypothesize that they are correlated because they are inherently designed to reflect the phenomenon associated with cyanobacterial blooms. Since most of the CHB data and information are open to public, this study is not intended to present already-publicized CHB parameters but to compare the CHB parameters in order to find any correlation between the parameters.

We collected CHB abundance, meteorological conditions, and geographical information in western Lake Erie in 2013 only for which all of the three monitoring parameters were available. We interpreted correlations among the CHB data to test the hypothesis above and to better understand relationships between CHB outbreak and toxin release. This is the first study to compare and correlate the different observation parameters that exclusively target at monitoring and interpreting the same phenomenon, CHBs.

## **2. Materials and methods**

### *2.1. Selection of a CHB study site*

Western Lake Erie was selected as a CHB study site due to the frequent observation of cyanobacterial blooms and toxin releases there [18]. The Maumee River flows through northern Ohio and Toledo and then into Maumee Bay in the western basin of the lake [6]. We selected four study sites (WE2, WE4, WE6, and WE8) in western Lake Erie (Figure 1) because significant monitoring activities around these locations have provided useful CHB-associated information [18].



**Figure 1.** Study locations in western Lake Erie: WE2 ( $N41^{\circ}45.825$ ;  $W83^{\circ}19.701$ ), WE4 ( $N41^{\circ}49.663$ ;  $W83^{\circ}11.649$ ), WE6 ( $N41^{\circ}42.454$ ;  $W83^{\circ}23.000$ ), and WE8 ( $N41^{\circ}49.998$ ;  $W83^{\circ}21.895$ ). Inset shows the whole Lake Erie and the rectangle in the inset shows the western Lake Erie (adapted and modified from Google map at <https://www.google.com/maps>).

## 2.2. On-site monitoring of MC concentration

The NOAA Great Lakes Environmental Research Laboratory in collaboration with the Cooperative Institute for Limnology and Great Lakes Research at the University of Michigan has operated a sampling program for Lake Erie and publicized the distribution of MCs in many locations around western Lake Erie [19]. The NOAA laboratory collected samples from the four different locations denoted as WE2, WE4, WE6, and WE8 during typically May-October when cyanobacterial blooms were abundant [19]. The samples were taken at the surface (the upper 0.5 m of the water) to be most representative of the portion of water column that recreational users contact. The surface portion also corresponds to the focus of satellite images. As one of the most powerful biological assays, neurochemical and enzyme-linked immunosorbent assay (ELISA) was introduced to quantify the intracellular concentration of MCs in the water samples [12]. Basically, the MC information published by NOAA was used in this study.

## 2.3. Satellite-based remote sensing for CI

The NOAA National Centers for Coastal Ocean Science and Great Lakes Environmental Research Laboratory have analyzed satellite images around Great Lakes and published the Lake Erie Harmful Algal Bloom Bulletins [20]. The NOAA uses CI to quantify blooms because CI indirectly corresponds to the amount of algal biomass. The estimated threshold for cyanobacteria detection is at 35,000 cells/mL. We analyzed the satellite images published in the Lake Erie Harmful Algal Bloom Bulletins to extract values of CI. A number between 1 and 250 was assigned to each pixel in a satellite image. We calculated CI, based on Eq 1, where  $DN$  is pixel number based on color from 1 (coolest color) to 250 (warmest color) and CI ranges from 0.0001 to 0.031 [13,18].

$$CI = 10^{\frac{DN}{100}-4} \quad (1)$$

#### 2.4. *In-situ sensing of phycocyanin*

The Erie Land and Ocean Biochemical Observatory (LOBO) has monitored and publicized phycocyanin concentration in western Lake Erie as a surrogate parameter to CHBs [21]. The Erie LOBO location (N41°49.533; W83°11.617) is very close to WE4. It is an autonomous observing buoy for monitoring and collecting water quality and environmental data such as temperature, dissolved oxygen, nutrient level, etc., which researchers can use in their statistical ecological niche models to develop predictive capabilities for CHBs. The LOBO buoy is equipped with a phycocyanin fluorescence probe that has been calibrated based on regular field sampling. The phycocyanin information published by the LOBO was used in this study.

#### 2.5. *Correlation of CHB parameters*

The observing systems monitored three CHB parameters without any functional dependence, and thus we applied correlation analysis rather than simple linear regression to investigate potential linear relationship between parameters. Furthermore, because the monitoring parameters were measured at different time schedules, we used the Pearson Product-Moment (PPM) correlation equation (Eq 2), where X and Y are all independent variables and  $r$  is the PPM correlation coefficient ranging  $-1 \leq r \leq +1$  [22]. The equation is widely used as a measure of the degree of linear dependence between two independent variables. We quantified the relationships between two parameters with the correlation coefficient. If  $r$  is greater than zero, the two parameters show positive relationship. Very strong, strong, moderate, weak, and negligible (or no) relationships are indicated by  $r$  values at 1.0–0.7, 0.7–0.4, 0.4–0.3, 0.3–0.2, and 0.2–0.01, respectively. Correlation refers to quantitative relationship between two variables that are measured on either ordinal or continuous scales. Correlation implies an association between two variables rather than causation [23].

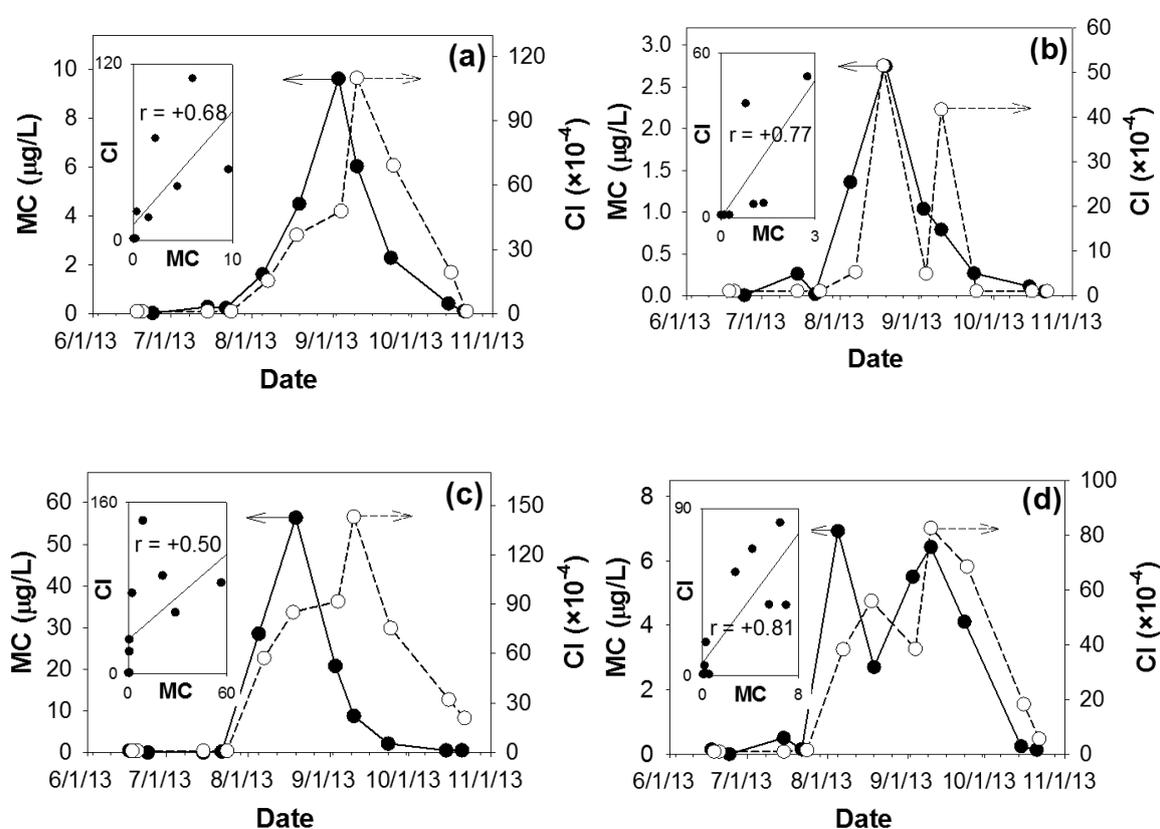
$$r = \frac{n(\sum xy)(\sum x)(\sum y)}{\sqrt{[n(\sum x^2) - (\sum x)^2][n(\sum y^2) - (\sum y)^2]}} \quad (2)$$

### 3. Results and discussion

The NOAA has measured concentrations of MCs through weekly water sampling at WE2, WE4, WE6, and WE8 locations during May-October since 2009 [19]. The NOAA has also developed a time series of CI-embedded satellite images for western Lake Erie weekly since 2009 and we extracted CI values from the images for WE2, WE4, WE6, and WE8 locations [20]. The LOBO has estimated phycocyanin concentration every hour at the Toledo Harbor Light since 2013, which is close to WE4 location (less than 0.2 mile) [21]. Considering availability of the spatial (measuring locations should be close enough) and temporal (measuring times should be close enough) monitoring data, comparison between MCs and CI was valid for WE2, WE4, WE6, and WE8 in 2013 while comparison between phycocyanin and MCs and comparison between phycocyanin and CI were valid only for WE4 in 2013.

### 3.1. Correlation of MCs with CI

We compared MCs (biological toxins) with CI (cyanobacterial blooms) for WE2, WE4, WE6, and WE8 based on data collected in 2013 (Figure 2) to find any correlations between two CHB parameters, and thus to ultimately predict MCs from CI information in the areas once established later. It should be noted that the two parameters were not measured simultaneously because the two observing systems were operated independently. Observing dates showing high cyanobacterial bloom tendency labeled with CI also showed high MC concentrations in water. For some dates and locations, MC concentrations were very low or negligible in spite of high CI (e.g., WE4 and WE6 in middle September). Based on the observation of MCs and CI for the locations, in general, the production of biological toxins was found to be highly associated with CHB activity. WE8 has the highest correlation of MCs and CI.



**Figure 2.** Comparison of microcystin concentration (MCs; biological toxin; solid dots) and cyanobacterial index (CI; cyanobacterial bloom; empty dots) in 2013 for (a) WE2, (b) WE4, (c) WE6, and (d) WE8 in western Lake Erie (data source: [19] and [20]). It should be noted that different axis scales (i.e., scales for double Y axes in main figures and scales for X and Y axes in insets) were used for each location to align the maximum values of the two parameters so that correlation of the two parameters can be easily visualized in each location, while thus varying the MC/CI ratios for the sites. Insets show correlation between MC and CI paired and measured within 48 hours time difference and thus actually indicate the varying MC/CI ratios specific to the sites. The correlation coefficient ( $r$ ) of the Pearson Product-Moment equation is shown.

In order to investigate the degree of correlation for a set of two parameters, we paired and plotted variables measured within 48 hours difference, and then applied the PPM equation to calculate a PPM correlation coefficient,  $r$ , as shown in the insets in Figure 2. Although there were some outliers, in general MCs were linearly correlated with CI (i.e.,  $r$  is greater than 0). WE2, WE4, and WE8 locations showed very strong correlation at  $r$  of +0.68, +0.77, and +0.81, respectively, while WE6 showed strong correlation at +0.50. We did a t-test to evaluate the significance of the correlation coefficients. The  $p$ -values (at 95% confidence level) were 0.026, 0.034, and 0.007 for WE2, WE4 and WE8 locations, respectively. All  $p$ -values were less than 0.05, which confirms that the correlation coefficients at the locations are statistically significant. Meanwhile the  $p$ -value at WE6 was 0.079 which is at the margin of statistical significance. We also found relationship between MCs and CI to be site-specific. For example, MC level for WE4 changed within a very narrow range of only 0–2.8  $\mu\text{g/L}$  while its CI changed greatly from 0 to  $50 \times 10^{-4}$ . Meanwhile, MC level for WE6 changed within a wide range of 0–57  $\mu\text{g/L}$  while its CI changed from 0 to  $142 \times 10^{-4}$ . This means the MC concentration at WE4 was very low compared to the MC concentration at WE6, with given CIs expressing cyanobacterial bloom tendency.

Many geographical, ecological, meteorological, and analytical factors specific to the locations might have been involved in the observed variations. In cloudy weather, satellites cannot properly capture high resolution images for the areas of concern, which impacts calculation of CI. The accuracy of MCs measurement also significantly decreases at low concentrations due to the nature of the ELISA method [24]. The average water temperature increased up to 3 °C between mid-August and early September. Since high temperature is favorable for the growth of cyanobacteria, both CI and MCs were high at that period. As cyanobacteria concentrate near the water surface, blue green scums are generated and can be clearly identified on satellite images.

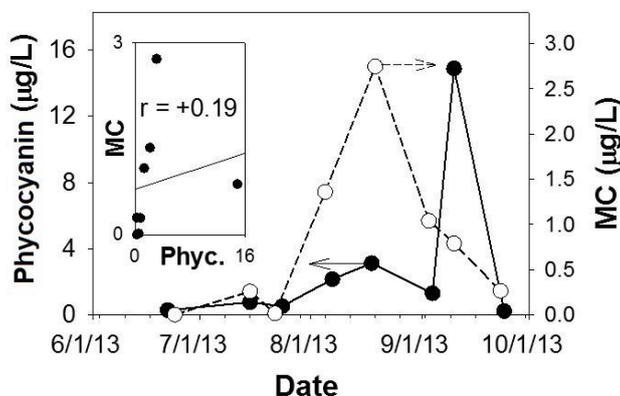
Looking at MCs and CI carefully for all the locations (particularly WE2 and WE6), CI peaks slightly followed MC peaks in 1–2 weeks. This might be particularly true to the end of cyanobacterial bloom season. During new and peak bloom periods, the intercellular MCs measured by the ELISA are very close to total MCs in cells and water because most of MCs are retained in the cells until cell death (i.e., negligible MCs in water) [25]. However, ageing cells during a dying bloom release MCs into the water, which are not counted by the ELISA method, while satellite images still keep capturing cyanobacterial blooms and proposing high CI. The early stages of a CHB in the Lake Erie tend to be more toxic per biomass than its later stages. These all might partly explain low MCs but still high CI in each observing time during September.

In fact, the situation is more complicated when vertical movement of cyanobacteria over time is considered. Some cyanobacteria, such as *Anabaena flos-aquae*, have gas-filled cavities that allow them to float and rise from near the bottom level to the water surface. Other cyanobacteria, such as *Planktothrix agardhi*, can be found in bottom sediment and may float to the water surface when mobilized by severe storm events and other sediment disturbance [26]. Such a cyanobacterial movement also depends on light conditions, nutrient levels, water temperature, and wind speed, and the movement typically takes several days. For example, unusually significant decreases in air temperature (from 24.5 to 23.7 °C) and rapid increase in wind speed (from 2.6 to 10.3 m/sec which is above 7.7 m/sec, a threshold wind speed strong enough to mix blooms through water column) were reported for WE4 location in September 4, 2013 [19].

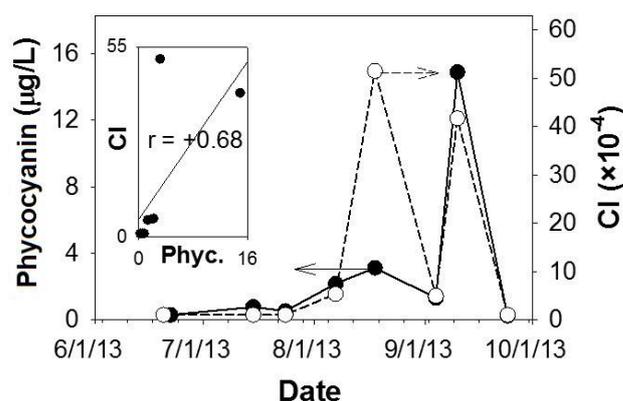
### 3.2. Correlation of phycocyanin with MCs and CI

Phycocyanin (as a proxy to cyanobacterial blooms) was compared with MCs (biological toxins) and finally CI (cyanobacterial blooms) monitored around WE4 location, as shown in Figures 3 and 4, respectively, to find any correlations between the CHB parameters, and thus to ultimately predict MCs from information on phycocyanin and CI in the areas once established later. Peak points for phycocyanin in accordance with MCs and CI occurred in the mid-August and early September, most probably due to the rapid increases in water temperature (from 22.5 to 25.5 °C) which is favorable for cyanobacterial blooms. Dates showing high MCs and CI generally exhibited high phycocyanin concentration, implying the production of phycocyanin is highly associated with CHB activity.

Unlike paired data of MCs and CI measured within 48 hours difference, a pair of phycocyanin and MCs (or CI) was measured almost simultaneously. The correlation coefficient of phycocyanin with MCs was at only +0.19, indicative of weak relationship (but still positive relation). As a result, the  $p$ -value (at 95% confidence level) was 0.616, indicating no statistical significance for the correlation coefficient. It is known that not all of cyanobacterial blooms produce MCs [17]. Up to 50% of recorded blooms are expected to contain such toxins [5]. Meanwhile, the correlation coefficient of phycocyanin with CI was at  $r = +0.68$ , indicative of strong relationship. The  $p$ -value was 0.061 which is close to 0.05 cutoff for statistical significance. In fact, phycocyanin is an accessory pigment to chlorophyll generally associated with cyanobacterial blooms.



**Figure 3.** Comparison of phycocyanin concentration (a proxy to cyanobacterial bloom; solid dots) with microcystins concentration (MCs, biological toxins; empty dots) in western Lake Erie in 2013 (data source: [19] and [21]). Phycocyanin has been monitored since 2013 only for the location (N41°49.533 and W83°11.617) very close to WE4. Inset shows correlation between two comparing parameters paired and measured almost at the same time. The correlation coefficient ( $r$ ) of the Pearson Product-Moment equation is shown.



**Figure 4.** Comparison of phycocyanin concentration (a proxy to cyanobacterial bloom; solid dots) with cyanobacterial index (CI; empty dots) in western Lake Erie in 2013 (data source: [20] and [21]). Phycocyanin has been monitored since 2013 only for the location (N41°49.533 and W83°11.617) very close to WE4. Inset shows correlation between two comparing parameters paired and measured almost at the same time. The correlation coefficient ( $r$ ) of the Pearson Product-Moment equation is shown.

#### 4. Conclusions

The CHB observing parameters (MCs, CI, and phycocyanin) were generally well correlated because they inherently represent the same phenomenon, CHBs. In particular, measured biological toxin concentration (MCs) was strongly aligned with cyanobacterial bloom activity (CI) estimated by satellite image analysis. The relationships between MCs and CI seemed to be site-specific. Phycocyanin had strong correlation with CI, implying that measuring an easy-to-detect proxy parameter in-situ and in real-time is effective for monitoring cyanobacterial blooms. Although it was hard to make solid conclusions due to the limited amount of the CHB data available in this study, we would say combining data by integrating the current CHB monitoring systems and observing programs is helpful to reliably assess CHB activities with high accuracy. This study comparing only three major CHB parameters can be extended to include many other observing targets associated with CHBs, including chlorophyll and phycoerythrin. More observing locations and longer monitoring periods, once established in the future, would enable us to propose more comprehensive correlations of the current monitoring systems and to understand the behavior and functioning of CHBs. When such a site-specific correlation is found through this kind of study, we will be able to better forecast biological toxin release from other CHBs-associated data such as CI and to better understand relationship between CHB activity and toxin release (ultimate goal). Publicizing the CHB activity and correlation can provide a major mechanism for reducing exposures to toxins and for deploying bloom mitigation strategies in advance. As a result, this study can significantly contribute to the areas of water supply, water quality, and algal bloom monitoring.

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## Conflict of interest

The authors declare there is no conflict of interest.

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