

# Veterans Lagoon Water Quality Study

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## Introduction

Veterans Park Lagoon is a 14 - acre lake located on the Milwaukee waterfront (43°02'50.6"N 87°53'33.6"W) between Lake Michigan and the City of Milwaukee. It is part of a series of parks managed by the Milwaukee County Park System along the waterfront and provides a source of recreation for city residents and tourists alike. It is a prominent fixture at the gateway to the City of Milwaukee. The lagoon runs parallel to the main thoroughfare along the waterfront, Lincoln Memorial Drive, and is adjacent to the War Memorial Building, Mckinley Marina, Milwaukee Art Museum, Discovery World and Summerfest grounds (Fig. 1). Visitors to the city of Milwaukee are brought to these attractions including the lagoon through a major interstate 795 by way of I-43/I-94. These are some of the most popular attractions in the city. As such, the lagoon along with the swan shaped paddle boats for rent have become an iconic landmark in the City of Milwaukee.



Figure 1. Google Earth rendering of Veteran's Park Lagoon and the surrounding landscape. Inset shows the location of the lagoon in the City of Milwaukee, Wisconsin, USA.

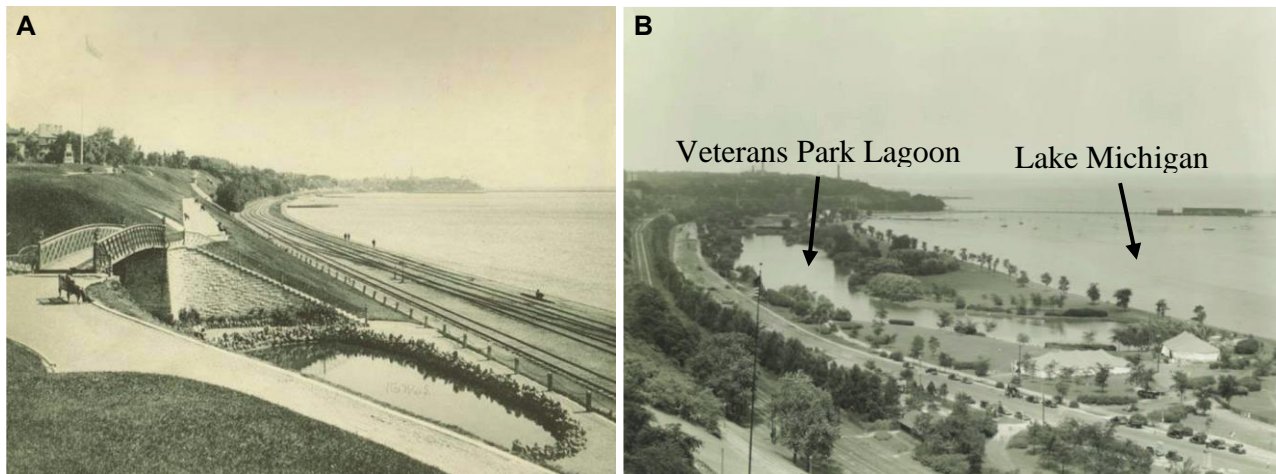


Figure 2. A) Juneau Park and the Lake Michigan waterfront in 1895 prior to construction of the lagoon. B) Veterans Park Lagoon in 1960, presumably with some narrow connection still to Lake Michigan.

The lagoon was formed in 1907 from the deposition of landfill extracted during various construction projects in the city (O'Reilly 1982). The lagoon became a popular swimming hole and technically was an embayment of Lake Michigan since a connection to the big lake remained until 1963. This opening to Lake Michigan narrowed as the landfill was expanded. A newspaper article from 1955 states the lagoon opening to Lake Michigan was only 3 feet across at that time (Dawson 1955). The eventual closing of the connection to Lake Michigan likely had profound effects on water retention time and water quality in the lagoon. Storm water connections have been made to the lagoon and/or existed prior to its creation which we hypothesize has also impacted water quality. The largest of these currently is a storm water pipe that drains runoff from Prospect Avenue along the bluff at significant elevation above the lagoon.

Today Veterans Park Lagoon experiences frequent and intense seasonal toxic cyanobacterial blooms (cyanoHABs) (Fig. 3A). Algal blooms of unknown species have been noted since at least the 1980's with spikes in chlorophyll-a to above 200  $\mu\text{g/L}$  (O'Reilly 1982), indicative of an aquatic environment containing a large amount of nitrogen (N) and phosphorus (P) that feeds algal growth. In 2016, we noted a large cyanoHAB in the lagoon at the north end

and for the first time took samples to measure the cyanobacterial liver toxin, microcystin (MC). In this bloom MC concentrations were greater than 300 times the United States Environmental Protection Agency's safe recreational limit for in-water activities (United States Environmental Protection Agency 2016). The Milwaukee Health Department was notified and signs (Fig 3B) were posted to warn recreationalists of the dangers that cyanobacterial toxins pose to the health of people and their pets. At that time, only two activities were taking place on the lagoon that would have been considered in-water activities which included an annual waterskiing event and paddle boarding, both of which have since been suspended. To our knowledge swimming in the lagoon has not occurred with any popularity since the 1950's. Paddle boats are the main recreational activity that occurs in the lagoon.

CyanoHABs are generally driven by warm water temperatures, nutrient pollution, and physical forcing events (wind and waves). Phytoplankton abundance, including cyanobacteria, is dependent on growth and loss processes where growth is dependent on water temperature, nutrients, and to a lesser extent light while losses primarily occur through zooplankton grazing (Kâ, Mendoza-Vera et al. 2012, Mohamed, Bakr et al. 2018, Ger, Naus-Wiezer et al. 2019), filter feeding organisms (e.g. mussels) (Hwang, Lee et al. 2021, Zhang, Yu et al. 2022), transport out of the system and sinking (Ibelings, Mur et al. 1991, Wu, Kong et al. 2010, Chen, Zhu et al. 2022). Physical forcings including wind and waves can alter light and temperature regimes or cause phytoplankton to accumulate along shorelines producing cyanoHABs (or other HABs) (Chen, Zhu et al. 2022), entrain phytoplankton under the euphotic zone and/or transport cells out of the system (Visser, Ibelings et al. 1996, Brookes, Regel et al. 2003). All of these variables in addition to the unique biological traits of cyanobacteria, discussed below, are important for understanding drivers of cyanoHABs.

Cyanobacteria have adapted mechanisms to allow them to dominate many water bodies, particularly eutrophic water bodies like Veterans Lagoon. Cyanobacteria compete well for inorganic carbon, N and P when standing stocks of these nutrients are low (Yamamoto and Nakahara 2005, Aubriot and Bonilla 2012, Aubriot and Bonilla 2018). In addition, they possess carbon, N and P storage mechanisms (Simon 1971, Jacobson and Halmann 1982). Some cyanobacterial genera are also able to fix atmospheric dinitrogen and/or use organic forms of N and P (Lee, Parker et al. 2015). Cyanobacteria buoyancy control allows them to maintain optimal depth for light and nutrient requirements (Bormans, Sherman et al. 1999, Wallace and Hamilton 2000, Brookes and Ganf 2001). Furthermore, cyanobacteria have relatively low light requirements (< 20 microeinsteins) as they are capable of growing in far red light using alternate forms of chlorophyll (Krüger and Eloff 1977, Gan, Shen et al. 2015). Many cyanobacteria that produce blooms are colonial, forming large macroscopic or filamentous colonies that at least partially inhibit feeding by zooplankton and filter feeding organisms (Wang, Qin et al. 2010, White and Sarnelle 2014). Cyanobacteria also produce a vast array of allelopathic compounds thought to deter predation by zooplankton or inhibit zooplankton digestive enzymes (Pawlik-Skowrońska and Bownik 2021).

CyanoHABs in Veterans Park lagoon contain species typically associated with the production of one or more toxic compounds, cyanotoxins, presenting acute and/or chronic toxicity to humans and other animals. Toxins produced by cyanoHABs in north temperate environments include a variety of peptides and alkaloids with varying potencies and mechanisms of toxicity. MC's are among the most prevalent toxins produced by cyanobacteria globally. These seven amino acid membered peptides vary considerably in structure such that over 200 congeners have been detected in nature, though many others (>300,000) are theoretically

possible (Jones, Pinto et al. 2021). MC's are liver toxins that can cause acute liver failure and in laboratory studies have also been shown to affect other tissues including reproductive organs and the central nervous system (as reviewed in (Miller, Beversdorf et al. 2017)). Their ability to inhibit protein phosphatases I and II in human cells makes them suspected carcinogens (Nishiwaki-Matsushima, Ohta et al. 1992, Campos 2010, Svirčev, Baltić et al. 2010). Thus, long term, low-level exposure to MC has been associated with liver and colorectal cancer in epidemiological studies (Zhou, Yu et al. 2000, Yu, Zhao et al. 2001, Fleming, Rivero et al. 2002, Zhou, Yu et al. 2002, Hernández, López-Rodas et al. 2009, SvirčEv, KrstiČ et al. 2009, Zheng, Zeng et al. 2017). Nodularin is a structurally related five amino acid membered peptide with similar human health effects (Ohta, Sueoka et al. 1994). It should be noted that cyanobacteria make thousands of other peptides including cyanopeptolins (Cpt's), anabaenopeptins (Apt's) and microginins (Mgn's), among others, but human health effects of these peptides are not as well known, although some have shown strong potency in animal studies (Gademann, Portmann et al. 2010, Faltermann, Zucchi et al. 2014, Lenz, Miller et al. 2019).

Besides the peptides, a variety of important small molecule alkaloid cyanotoxins have been described and/or detected in studies of north temperate lakes. These include the peripheral neurotoxins anatoxin-a (ATX) (Carmichael, Biggs et al. 1979) and anatoxin-a(s) renamed recently as guanitoxin (Matsunaga, Moore et al. 1989, Fiore, de Lima et al. 2020), the paralytic shellfish toxins (saxitoxins, STX and gonyautoxins) as well as the liver and kidney toxins cylindropermopsins (Cyl's). Considerably less is known about the ecology of these toxins in north temperate lakes compared to MC's.

In addition to cyanoHABs other water quality issues are a possible concern. The lagoon receives storm water input and local street runoff. An older unpublished report in the 1980's



Figure 3. CyanoHAB in Veterans Park Lagoon in 2016 containing  $>3,000 \mu\text{g/L}$  microcystin.

reported heavy metals and legacy contaminants including polychlorinated biphenyls and diphenyl ethers in sediments in the lagoon (O'Reilly 1982). Many storm water drains in urban settings including Milwaukee have been identified as possible sources of sewage contamination (McLellan and Sauer 2009, Olds, Corsi et al. 2018). As the lagoon receives water input from several storm drains, human sewage contamination is a possibility. In addition, the lagoon is commonly inundated with many geese and other water fowl. The feces of these animals are typically found on the land surrounding the lagoon. Thus there is a possibility that the lagoon contains unsafe levels

of harmful microorganisms.

### Objectives

This report describes results of our studies to characterize water quality and sediment characteristics in Veteran's Park Lagoon. The primary objectives of the study were to:

- 1) Identify the major cyanoHAB species, and their toxins.
- 2) Describe biogeochemistry and nutrient sources that may be feeding cyanoHABs.
- 3) Determine if the lagoon is receiving human sewage input.
- 4) Determine sediment thickness and sediment contaminants.

Below we provide data from multiple years of sampling since our monitoring work began in 2016. Not all results cover this time period. We provide results in the years as indicated where data is available.

## Results

### CyanoHABs in Veterans Park Lagoon. The

algal pigment, chlorophyll, is the major

indicator for algal abundance in any water body. In 2019 and 2020 the average concentration of chlorophyll in Veteran's Park Lagoon was  $25 \pm 45$  and  $72.7 \pm 127.8$   $\mu\text{g/L}$ , respectively making the lagoon a mesotrophic to eutrophic environment based on Carlson's Trophic State Index (Carlson 1977). While chlorophyll is an indicator for total algal production, phycocyanin is the blue pigment produced by cyanobacteria and is therefore used as a marker for the occurrence and intensity of cyanoHABs. A concentration of  $30 \mu\text{g/L}$  is equivalent to the World Health Organization's first alert level of 20,000 cyanobacterial cells/ml for recreational settings (McHau, Makule et al. 2019). This alert level suggests that waters pose moderate to high risk for in-water activities above this concentration. In 2020, the average concentration of phycocyanin in the lagoon was  $100 \pm 176 \mu\text{g/L}$  and in 2019 it was  $538 \pm 1,211 \mu\text{g/L}$  making in-water recreation a moderate to high risk activity due to potentially toxic cyanoHABs during the open water season. Qualitative microscopic observations consistently identified *Microcystis* as the most abundant cyanobacterial species accompanied occasionally with *Dolichospermum*, *Aphanizomenon* and/or *Cylindrospermopsis* (Fig. 4). All of these species have been associated



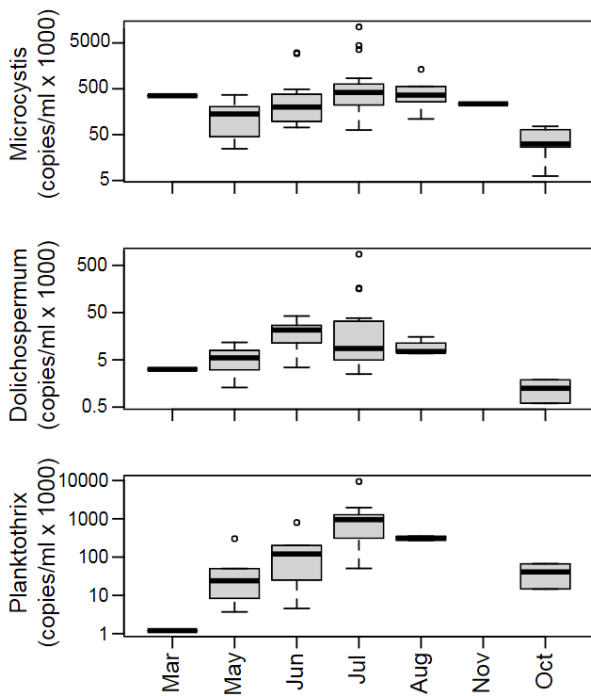
Figure 4. Micrograph of a water sample from Veteran's Park Lagoon

with the production of one or more toxins in other water bodies (Miller, Beversdorf et al. 2017). However, *Microcystis* is considered the most common toxic cyanoHAB species globally.

Given the high algal production in the lagoon and presence of *Microcystis* species we sought to quantify the abundance of all *Microcystis* spp. and the proportion of those *Microcystis* containing the microcystin synthesis genes. In addition, since we observed *Dolichospermum* and *Planktothrix* species in lagoon samples by microscopy and prior studies show that these genera can also produce microcystin we also quantified these species and the proportion of each that contain the microcystin synthesis genes. We analyzed 50 lagoon samples for total *Microcystis*, *Dolichospermum* and *Planktothrix* abundance and a subset for the microcystin-encoding gene *mcyA* from these three genera. The *mcyA* gene is a genetic marker indicating a particular strain has the capability to produce the toxin microcystin, as not all strains carry this ability. All analyses used a molecular approach (i.e. digital quantitative polymerase chain reaction) quantifying the abundance of each genera by targeting their 16S rRNA gene and genotoxic variants by targeting the *mcyA* gene specific to each genus. Sampling dates ranged from March 2019 to August 2021 to provide a look at seasonal patterns for cyanoHABs caused by these genera. We focused on near surface water samples for all analyses.

All samples were positive for all three genera, which were abundant throughout the sampling timeframe. Sample concentrations for *Microcystis* ranged from ~6000 to 11,000,000 gene copies per mL of lagoon water with the average concentration at ~770,000 copies per mL (Fig. 5). *Planktothrix* was as abundant or more abundant than *Microcystis* while

*Dolichospermum* was about ten- fold less abundant than *Microcystis* and *Planktothrix*. These cell abundances are high and similar to those observed in other lakes harboring large blooms, such as



**Figure 5.** Boxplot of *Microcystis*, *Dolichospermum* and *Planktothrix* abundance in Veterans Park lagoon averaged by month over three years (2019 – 2021) Boxes represent the median and 1<sup>st</sup> and 3<sup>rd</sup> quartiles. Whiskers indicate the 10<sup>th</sup> and 90<sup>th</sup> percentiles.

in the western basin of Lake Erie (Rinta-Kanto et al., 2005) or Lake Taihu in China (Li et al., 2017), two well-known water bodies supporting large cyanoHABs, mainly of *Microcystis* species. The high abundance of *Planktothrix* is expected in this shallow waterbody as these cyanobacteria compete well in low light turbid conditions.

While total *Microcystis* concentrations were always relatively high in our sample set, there was an increase in these concentrations in the summer months as compared to spring and fall months (Fig. 5). This result was expected as the warmer summer months tend to favor *Microcystis* growth. *Dolichospermum* and *Planktothrix* show similar trends

in the western basin of Lake Erie (Rinta-Kanto et al., 2005) or Lake Taihu in China (Li et al., 2017), two well-known water bodies supporting large cyanoHABs, mainly of *Microcystis* species. The high abundance of *Planktothrix* is expected in this shallow waterbody as these cyanobacteria compete well in low light turbid conditions.

While total *Microcystis* concentrations were always relatively high in our sample set, there was an

All samples tested also contained *Microcystis* carrying the *mcyA* gene. Toxin gene concentrations averaged ~44,100 gene copies per mL of lagoon water (Figure 6), which indicates the genetic capability for toxin production is always present in the ice-off season. The ratio of the *mcyA* gene to total *Microcystis* provides a rough estimate of the percent of cells carrying toxin-encoding genes. By this measure, on average 9.1% of cells had the capability to produce toxins. At maximum, this value was 37.6% of cells. Samples with higher percentages of toxin genes tended to occur when there were lower total cell numbers. Of note, these toxin gene ratios typically underestimate the true percentage, as *Microcystis* cells often contain more than one 16S rRNA gene, which we used to quantify the total number of cells.

By comparison, few *Dolichospermum* and *Planktothrix* cells contain the microcystin toxin synthesis genes in the lagoon. *Dolichospermum* and *Planktothrix* carrying the *mcyA* gene was detected in only 28% and 6% of samples, respectively, and at mean concentrations of 885 and 178 gene copies per ml, respectively, 100 fold lower than *Microcystis*. As such, *Microcystis* the dominant toxin producer in the lagoon. Since *Dolichospermum* and *Planktothrix* were highly abundant in the lagoon, but did not have toxin genes on most days, this suggests that they are non-toxic contributors to cyanoHABs in the lagoon.

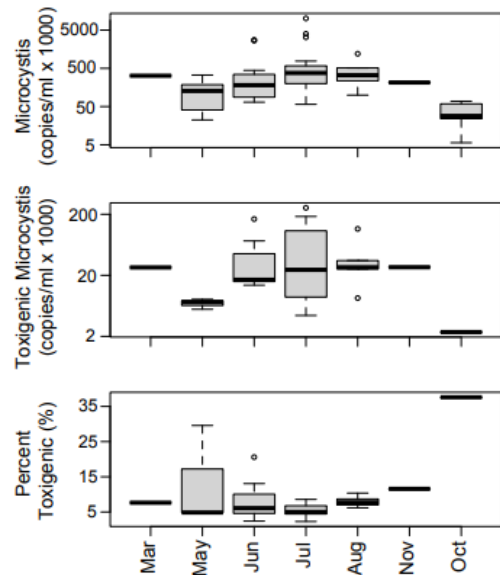


Figure 6. Abundance of *Microcystis* (top), *Microcystis* carrying genes for toxin synthesis (middle) and the percentage of *Microcystis* with the toxin genes (bottom)

Overall, our gene analysis results confirm the preliminary findings that *Microcystis* is a dominant bloom-forming cyanobacterium in the lagoon. This does not rule out the possibility for other bloom forming species. However, *Microcystis* is present throughout the ice-off season at very high levels and its blooms peak in mid- to late-summer. *Microcystis* strains carrying toxin genes are constantly present in the lagoon, but the levels vary throughout the year, which could explain at least some of the varying levels of toxin concentrations.

[Algal Toxins in Veterans Park Lagoon](#). *Microcystis* was shown to be the major toxic cyanoHAB species in the lagoon producing microcystin. Monitoring for the presence of microcystins in Veterans Park Lagoon has been conducted since 2016 using liquid chromatography tandem mass spectrometry (LC-MS/MS) using previously published methods (Beverdorf, Weirich et al. 2017). In 2016 regular monitoring did not start until August and lasted until November. Only samples from the center of the lagoon were taken. The concentration of microcystin peaked in October at 10.8 µg/L while the average concentration during the sampling period was 2.2 +/- 0.4 µg/L. A floating algal scum sample taken from the north shore in October contained well over 3,000 µg/L microcystin. The US EPA threshold for recreational exposure is 8 µg/L and if this is exceeded in 10% or more of samples taken over the open water season then the water body is considered impaired for recreation. Not enough samples were taken in 2016 to determine if microcystins exceeded the 10% threshold.

In 2017 monitoring for microcystins occurred between July and October. In this year floating scums were sampled separately from non-scum samples to determine if concentrations were significantly higher in scum samples. In addition, samples were taken along a north to south transect from four locations. The overall average concentration of microcystin was 6.6 +/- 18.6 µg/L in all samples, 69.8 +/- 4.0 µg/L in scum samples and 1.3 +/- 2.4 µg/L in non-scum

samples. The mean concentration of microcystin in scum samples was significantly higher than that of non – scum samples based on a Kruskal Wallis test ( $p < 0.001$ ). Microcystins exceeded the US EPA recreational guideline value in 15.4% of all samples. Thus, the data from this year indicated that the lagoon could be considered an impaired waterway for in-water activities and that if scums were observed then there would be a heightened risk to public health from in- water recreational activities. Prior to our monitoring, water skiing events had taken place in the lagoon, which is considered an in-water activity.

In 2018 an aeration experiment took place to determine if aeration could be used to control cyanoHABs and reduce toxicity. During this study we also measured microcystins by the Abraxis quick striptest method and compared results to the “gold standard” LC-MS/MS method to determine if this quick method could be used as a reliable indicator of water toxicity. There was no significant difference in microcystin concentration between aerated and non- aerated zones based on a Kruskal-wallis rank sum test ( $P = 0.45$ ) suggesting that aeration as applied was not successful in reducing cyanoHABs or at least their toxicity. The average concentration of microcystin in 2018 across all sites was  $12 \pm 25 \mu\text{g/L}$  and 28% of samples were above the US EPA recreational limits, suggesting again that the lagoon was impaired for in-water activities. In this year and since that time all in-water activities have been suspended. The striptest is based on visual examination of a red colored line on the strip. Therefore, we categorized the results in three levels as  $< 2.5$ ,  $2.5 - 10$  and  $>10 \mu\text{g/L}$  of microcystin. The striptest was deemed not accurate at the higher concentrations of microcystin where it should have indicated concentrations  $>10 \mu\text{g/L}$  but instead underestimated concentrations as  $2.5 - 10 \mu\text{g/L}$ . When the microcystin concentration was  $2.5 - 10$  or  $< 2.5 \mu\text{g/L}$  then the striptest was accurate, as compared to LC-MS/MS analysis. This suggests that the Abraxis striptest for microcystins is

unlikely to be reliable for detecting when microcystins exceed the recreational threshold set by the US EPA, at least within this lagoon environment.

In 2019 and 2020 we focused on paired measurements of algal pigments and microcystins. The average microcystin in 2019 and 2020 was  $20 \pm 49$  and  $X \pm Y$   $\mu\text{g/L}$  and microcystin exceeded the US EPA recreational guideline values in 34 and X% of samples, respectively. Microcystin was significantly correlated with chlorophyll in 2019 and 2020. This indicates that the dominant algal species are microcystin producing species or at least those with the potential to produce microcystins. This is likely due to the dominance of toxic cyanobacteria, most notably *Microcystis*, over other chlorophyll producing species in this water body. Thus, if the water is green in the lagoon there is a strong possibility for the presence of microcystin.

**Biogeochemistry.** Nutrients in the form of nitrogen (N) and phosphorus (P) are required for algal growth. While cyanoHABs can develop without growth, such as from wind induced concentration of cells piling up along shorelines, there first needs to be appreciable growth of algal cells in the lagoon for this to occur. As such, over the period between 2019-2021, we evaluated variations in nutrient concentrations in Veterans Park Lagoon. We also sampled water in the confluence of the Prospect storm drain on a few occasions as this storm drain is the largest point source of water to the lagoon. Total dissolved nitrogen (TDN) reported here includes the sum total of dissolved inorganic nitrogen (DIN), nitrate ( $\text{NO}_3$ ), nitrite ( $\text{NO}_2$ ), ammonium ( $\text{NH}_4$ ), and dissolved organic nitrogen (DON). On average, TDN was  $0.383 \pm 0.060$  ppm-N (mg-N/L) or  $27.32 \pm 4.75$   $\mu\text{M}$  in the lagoon (Table 1). Within the TDN pool, the average concentration of DIN was as low as  $0.0089 \pm 0.0031$  ppm (mg-N/L) or  $0.64 \pm 0.23$   $\mu\text{M}$  in the water column, leaving majority of the TDN in the form of DON, with an average of  $0.374 \pm 0.060$  ppm (mg-N/L) or  $26.7 \pm 4.3$   $\mu\text{M-N}$ . In terms of percentage or nitrogen phase distribution, DIN

comprised less than 3% of the TDN pool in the lagoon, while DON made up 97% of the TDN pool. Compared to Lake Michigan, the average DIN concentration and the DIN/TDN ratios observed in the lagoon are extremely low (For DIN: 25-45  $\mu\text{M}$  in Lake Michigan vs.  $0.64 \pm 0.23$   $\mu\text{M}$  in the Lagoon).

Interestingly, DIN concentrations were as high as 0.565 mg-N/L or 40.4  $\mu\text{M}$  in the Prospect storm drain collected on January 16, 2021 and 0.579 mg-N/L or 41.36  $\mu\text{M}$  in storm drain samples collected on April 9, 2021, which are orders of magnitude higher than the DIN concentrations in the lagoon (Table 1), indicating that the Prospect storm drain is likely a significant source of N to the lagoon.

Based on particulate N data, concentrations of total nitrogen (TN) were calculated from TDN and PN. As shown in Table 2, PN comprised over 57% of the TN pool, while TDN made up about 43% of the TN pool in the lagoon during 2019. Interestingly, DIN (nitrate, nitrite and ammonium) only comprised <1% of the TN pool in the water column during 2020 where DIN

Table 1. Comparisons in nutrient concentrations (mg/L) and molar nutrient ratios between Veterans Park Lagoon and other water bodies

Nutrient species	Veterans Lagoon	Lake Michigan (a&b)	Milwaukee River (c)	Storm Drain
TDN (mg/L)	0.383+/-0.060			
DIN (mg/L)	0.0089+/-0.0031	0.28-0.49	1.75+/-0.84	0.565
DON (mg/L)	0.374+/-0.060			
DIN/TDN (%)	2.3%			
DIP ( $\mu\text{g-P/L}$ )	6.82+/-5.27	0.341-1.17	30.1+/-24.5	373
DIN/DIP (molar)	3.9+/-1.7	>1,500	128	3.4
TN/TP (molar)	13.6+/-8.1			

a) Lin and Guo (2016); b) Cuhel and Aguilar (2013); c) Tember (2016)

data are available. Thus, high biomass in the lagoon was likely derived from cyanobacteria who

used both DON and PN in the water column. This needs to be further confirmed based on additional data and measurements.

Table 2. Partitioning of nitrogen between dissolved and particulate phases in VP lagoon (TN: Total nitrogen, TDN: total dissolved nitrogen; PN: particulate nitrogen).

N species	Sampling year	Concentration (mg-N/L)	SD (mg-N/L)	% Tot N (all data)	% Total N (Spring)	Remark
TDN	2020	0.383	±0.060	-	-	
DIN	2020	0.0089	±0.0031	<1%		
PN	2020	0.514	±0.081	-	-	PN estimated from 2019
TN	2020	0.897	±0.100			
TDN	2019	0.424	±0.110	42.7±16.6	55.7±17.3	
PN	2019	0.715	±0.470	57.3±16.6	44.3±17.3	
TN	2019	1.142	±0.489	-	-	

There was strong seasonal variability in the concentrations of all N species in the lagoon. For example, PN concentrations were generally higher between July and September than those in other sampling seasons. In addition, concentrations of PN could be significantly different between surface water and bottom water. Consistent sampling at the same locations is needed in future studies.

For dissolved inorganic phosphorus (DIP), the average concentration was 6.82 +/- 5.27 µg-P/L or 0.22 +/- 0.17 µM in the lagoon. This phosphate concentration in the lagoon is in general higher than those measured in Lake Michigan surface waters (0.341-1.147 µg-P/L or 0.011-0.037 µM, reported in Lin & Guo (2016, L&O)). However, it is much lower than those observed in the Milwaukee River (55.8±34.1 µg-P/L or 1.8±1.1 µM-P, reported in Lin and Guo, 2016). In addition, phosphate concentrations were as high as 373 µg-P/L or 12.03 µM in the Prospect storm drain (collected on January 16, 2021) and 514 µg-P/L or 16.58 µM collected on April 9, 2021. Similar to DIN, the storm drain could be a potential long term source of phosphate to the

lagoon (see also Table 1). Detailed variations in chemical speciation of phosphorus in the lagoon have been reported elsewhere (Wang, Yang et al. 2021).

Available data of different P species, including dissolved inorganic phosphorus (DIP or phosphate), dissolved organic phosphorus (DOP), particulate inorganic phosphorus (PIP), particulate organic phosphorus (POP), and total phosphorus (TP), are listed in Table 1 and Table 3. On average, TDP comprised  $43.82 \pm 32.80\%$  of the TP pool in the water column, while total particulate phosphorus (TPP) comprised  $56.18\%$  of the TP pool in the lagoon. Similar to TN pool, phosphate or DIP only comprised  $<10\%$  of the TP pool, but DOP comprised about  $35\%$  of the TP pool (Table G3). Within the particulate P pool, POP was highly variable, likely due to the heterogeneous nature of the lagoon with also input from a large goose population.

Table 3. Partitioning of phosphorus between dissolved and particulate phases in VP lagoon during 2019 sampling (March – September/October).

P species	Sampling year	Concentration ( $\mu\text{g-P/L}$ )	SD ( $\mu\text{g-P/L}$ )	% Tot P (all data)
DIP	2019	14.85	$\pm 24.83$	$9.21 \pm 7.63$
DOP	2019	47.84	$\pm 51.35$	$34.60 \pm 28.78$
PIP	2019	64.21	$\pm 64.08$	$34.55 \pm 45.90$
POP	2019	58.93	$\pm 56.07$	$31.71 \pm 42.13$
TP	2019	185.82	$\pm 133.07$	-

On the basis of DIN and DIP concentrations in the lagoon, molar ratios of DIN/DIP were also calculated and listed in Table 1. The average DIN/DIP ratio in the lagoon was as low as  $3.9 \pm 1.7$ , which is similar to those of the Prospect storm drain. Compared to the Redfield ratio (N/P=16) and the DIN/DIP ratios in the global oceans (also  $\sim 16$ ), low DIN/DIP ratios point to a nitrogen limited ecosystem in the lagoon. In contrast, both Lake Michigan (DIN/DIP ratios  $>1,500 - 3,000$ ) and Milwaukee River (average DIN/DIP ratio of  $\sim 128$ ) are both phosphorus limited ecosystems. Molar ratios of total nitrogen and total phosphorus are typically used to

gauge long term nutrient stoichiometry of aquatic ecosystems that takes into account both dissolved nutrients as well as those within biomass. Similar to the DIN/DIP ratio, the average TN/TP ratio in the lagoon of  $13.6 \pm 8.1$  suggests that algal growth is most likely limited by nitrogen and that there is an abundance of phosphorus in the water column available for algal growth.

In terms of major ions, the average concentrations in the lagoon were  $167.24 \pm 15.57$  ppm for chloride and  $11.81 \pm 2.11$  ppm for sulfate. Compared to Lake Michigan, higher chloride concentrations in the lagoon (167 ppm vs. 15 ppm in Lake Michigan) clearly indicate an accumulated influence of road salts application. On the other hand, slightly lower sulfate concentrations in the Lagoon (12 ppm vs 27 ppm in Lake Michigan) were somewhat surprising and is not consistent with influences from road salt application. It is likely that S is a non-conservative element and a significant portion of sulfate may have been reduced in bottom waters or the benthic layer/sediment-water interface (a highly reducing environment due to the accumulation of algal debris/detritus and particulate organic matter), causing either accumulation in sediment in the form of metal sulfide deposits or escape from the water column in the form of hydrogen sulfide gas. The bottom waters of the lagoon in some locations do get hypoxic (see below), which could favor sulfate reduction and formation of hydrogen sulfide.

The average concentration of dissolved inorganic carbon (DIC) was  $33.89 \pm 13.18$  ppm (or  $2,824 \mu\text{M-C}$ ) in Veterans Park Lagoon, compared to  $70 \pm 18$  ppm (or  $5833 \pm 1500 \mu\text{M}$ ) in the Milwaukee River and  $24.78 \pm 0.29$  ppm (or  $2065 \pm 24 \mu\text{M}$ ) in Lake Michigan. Compared to Lake Michigan, higher DIC in the lagoon likely results from the influence of road salt application and/or the addition of ground water, although both the lagoon and Lake Michigan have a much lower DIC concentration than the Milwaukee River.

The average water isotope (H and O) compositions ( $d^2H$  and  $d^{18}O$ ) were  $-5.70 \pm 0.54\text{‰}$  for  $d^2H$  and  $-45.56 \pm 3.34\text{‰}$  for  $d^{18}O$ . These values are somewhat similar to those of Lake Michigan ( $-5.9 \pm 0.2\text{‰}$  for  $d^2H$  and  $-44.8 \pm 0.9\text{‰}$  for  $d^{18}O$ ), indicating similar water sources overall between the lagoon and Lake Michigan, which is primarily precipitation plus storm drain input runoff (Hunter, Clites et al. 2015).

Concentrations of DOC ranged from 7.46 ppm (mg-C/L) to as high as 18 ppm, with an average of  $10.85 \pm 3.28$  ppm during 2020 sampling season. In addition to DOC, optical properties of the water, including chromophoric dissolved organic matter or DOM (CDOM, in UV-absorbance at 254 nm) and fluorescence DOM (FDOM) were also characterized using UV-visible spectrophotometry and 3D fluorescence spectrophotometry (DeVilbiss et al., 2016; Lin and Guo, 2020). Overall, CDOM followed the trends of water temperature and chlorophyll concentrations, but with a peak in early November 2019. In addition, DOC reached peaks slightly later than CDOM.

Excitation–emission matrix-parallel factor (EEM-PARAFAC) analysis divides fluorescent organic compounds into several independent components according to their unique properties and structures. Based on EEM-PARAFAC analysis, three humic-like fluorescent components (C1-C3) and one protein-like (C4) fluorescent component were identified in the lagoon which are indicative of the influence of terrestrial DOM in rainwater runoff, likely from the leaching of local soil. While C1, C2 and C3 followed major planktonic blooms, C4, the protein-like fluorescent component seemed to track other events, such as algal exudates, heterotrophic bacteria, and organic degradation processes during the late bloom stage.

Overall, the biogeochemistry results from surface water in the lagoon and the storm drain suggest that high algal production has resulted in low concentrations of dissolved inorganic N

and P as these organisms draw down these nutrients for growth. The high algal biomass has also led to high organic forms of N and P in the lagoon. Water in the Prospect storm drain contained dissolved inorganic N and P at orders of magnitude above what is already available for algal growth within the lagoon. As such, the storm drain should be considered a significant source of N and P to the lagoon. Given the high residence time of water in the lagoon it is likely that over time much of the P that enters the lagoon has been trapped in the sediments (discussed below) and or recycled into the water column through diagenesis and resuspension seasonally to feed cyanoHABs. It is possible that within sediments, some of the inorganic N in the lagoon may undergo denitrification to dinitrogen gas, but this has not been measured.

**Dissolved Oxygen and pH.** The concentration of dissolved oxygen and pH of a water body can have significant effects on water quality, chemistry and biology. Generally speaking day time high dissolved oxygen occurs with the productivity of algae due to high rates of photosynthesis. Dissolved oxygen in surface waters of the lagoon were supersaturated exceeding 100% saturation in 2019. However, the median bottom water dissolved oxygen concentration was 60% and occasionally hypoxic at 20% saturation. The draw down in carbonic acid from photosynthesis tends to increase pH and favor cyanobacterial growth. The average pH was above 8.5 in surface waters and almost circumneutral in bottom waters in 2019.

**Human Sewage Markers.** A total of 28 samples were analyzed for the presence of bacteria (*Bacteroides* species) known to be specifically associated with human fecal matter (Sauer, VandeWalle et al. 2011). A high abundance of such bacteria is indicative of contamination with human sewage, such as might occur if there are sanitary connections to the lagoon. Approximately 21% of samples tested positive for human *Bacteroides* by digital PCR, but it was not abundant at an average of only 3 gene copies per ml on those occasions when it

was detected. As such, there does not seem to be a significant source of human derived sewage contamination in the lagoon.

**Sediment Characteristics.** Sediment thickness and water depth was measured across the lagoon in 71 samples using a metered steel rod. The average sediment thickness was 2.13 feet with a maximum of approximately 5 feet. Sediment thickness was greatest at the southern end of the lagoon. The metered rod could not be inserted into many locations due to hard packed sediment (Fig. 8), except for the southern end where sediment was soft muck presumably high in organic content. Water depth data gathered with a metered rod shows that most of the lagoon is 6 to 8 feet with an average depth of 6 feet based on manual measurements with metered rod (Fig.

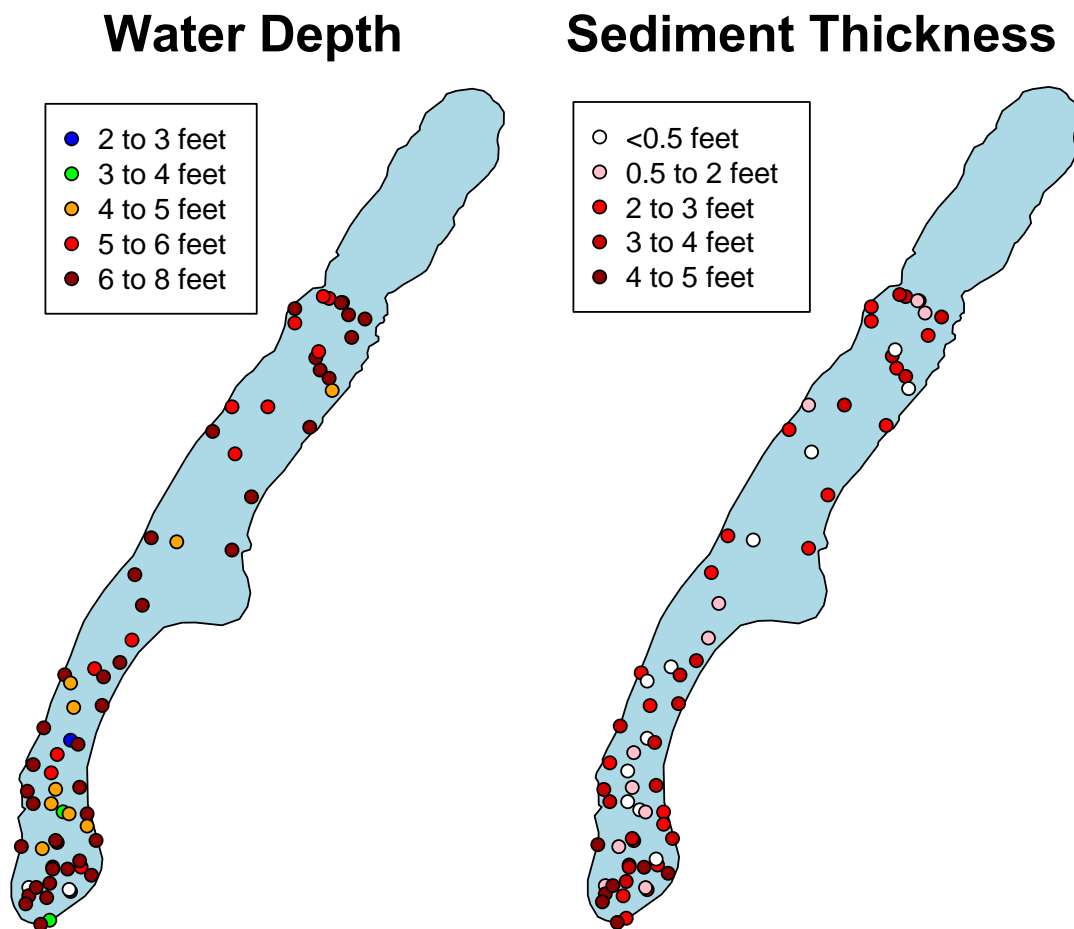


Figure 7. Water depth and sediment thickness in Veterans Park Lagoon.

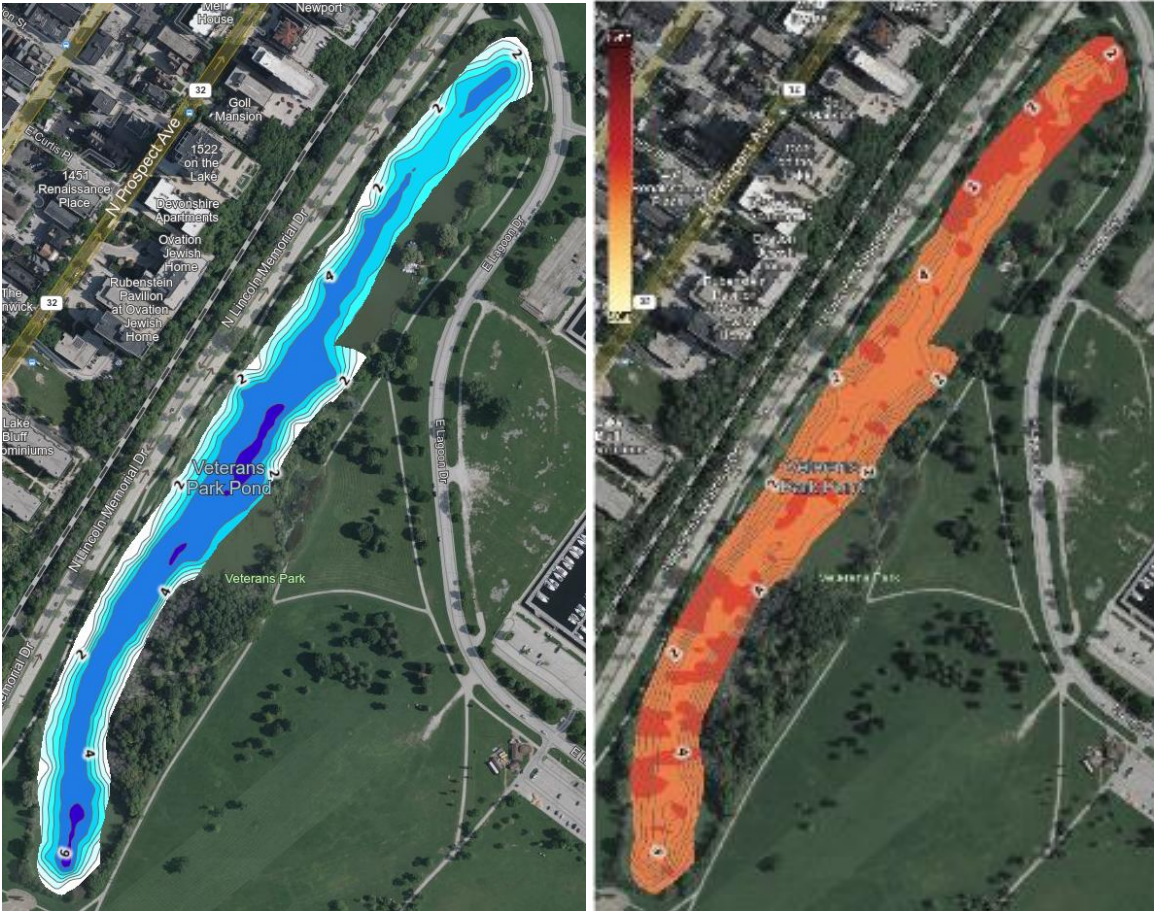


Figure 8. Water depth in feet based on sonar scan data (left) and locations of hard and soft sediment in Veterans Park Lagoon based on sonar data

7) or 4 to 6 feet based on sonar measurements (Fig. 8). There is a steep drop at the edges of the lagoon, especially on the western shore. The southern end of the lagoon is deepest where the maximum depth of 8 feet occurs based on manual measurements. The lagoon has a relatively flat even depth but becomes unusually shallow where it narrows approaching the southern end. Sonar scans of this area (data not shown) show a sharp decline in water depth and increasing hard packed sediment where sediment depth could not be determined or was negligible.

Two sediment cores of approximately 5 feet each were taken from the southern end of the lagoon. One of these cores is in the process of being dated. The top of the cores was characterized by soft muck transitioning gradually to grey reduced sediment and finally sand at

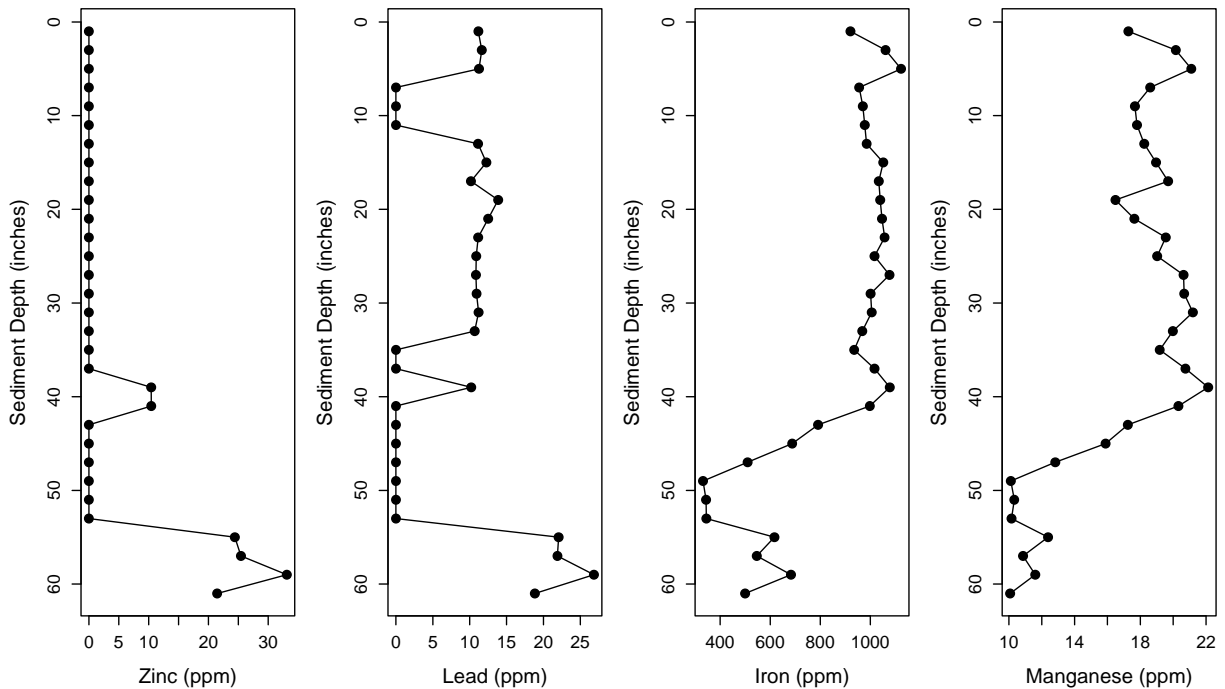


Figure 9. Concentrations of metals in a sediment core taken from Veterans Park Lagoon.

the bottom of the cores. This sand at the bottom of the cores is likely from the Lake Michigan lakebed (“beach sand”) prior to or during lagoon construction in the early 1900’s. Thus, these cores likely represent about 100 years of sediment deposition.

Heavy metals were measured in one of the cores. The following stable metals were measured including strontium-88 (Sr88), molybdenum-95 (Mo95), cadmium-111 (Cd111), barium-137 (Ba137), lead- 208 (Pb208), aluminum-27 (Al27), chromium-52 (Cr52), manganese-55 (Mn55), iron-56 (Fe56), cobalt-59 (Co59), nickel-60 (Ni60), copper-63 (Cu63), and zinc-66 (Zn66). Of these Ba-137, Pb-208, Al-27, Mn-55, Fe-56, and Zn-66 were detected. Assuming core depth increases with time, Fe-56 and Mn-55 increased early in the lagoon’s history to a stable maximum to modern day. This increase likely occurred as the lagoon was closed off to Lake Michigan in the 1950’s. Zn-66 and Pb-208 were highest at the bottom of the core. Zinc was likely highest at the bottom of the core due to the presence of sand since no zinc was detected in

other layers. Lead was likely high due to early contamination and runoff pollution. It was also detected at lower levels in more recent sediment layers up to modern day.

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