

Phosphorous Concentration Upon a Rural-Urban Gradient in Lake Mendota

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Abstract

In order to determine if the concentration of phosphorus varies across different locations of Lake Mendota in Madison, Wisconsin, an analytical analysis of lake water samples collected at three different locations was conducted. Ascorbic acid method was used to determine the total phosphorous (TP) content and total reactive phosphorus (TRP) content of water samples collected at Downtown, Middleton Springs, and Picnic Point locations respectively.

Keywords: TP, TRP, Ascorbic acid method, Malachite green method, analytical methods

1. Introduction

Lake Mendota is a medium sized body of water that borders many different locations in Madison, from downtown/urban regions to more rural areas like Maple Bluff. Lake Mendota is a frequent victim to algal boom due to increased phosphorus and nitrogen concentration from runoff or pollution. Phosphorus is often the limiting factor to these algal blooms and as such, by controlling the concentration of Phosphorus, aquatic scientists can reduce the lake eutrophication. Do the different regions across Lake Mendota, specifically regions that can be differentiated as urban, suburban, and rural, have different concentrations of Phosphorus? To test this, 3 samples from across the greater Madison Area that bordered Lake Mendota were collected and analyzed for TRP (direct spectrophotometric analysis) and TP (digestion of samples followed by spectrophotometric analysis). Collection time happened around 9:00 am on Monday, November 11th, 2019. The 3 samples included one from Downtown Madison (urban), another from Middleton Springs (suburban), and the third sample from Picnic Point (rural).

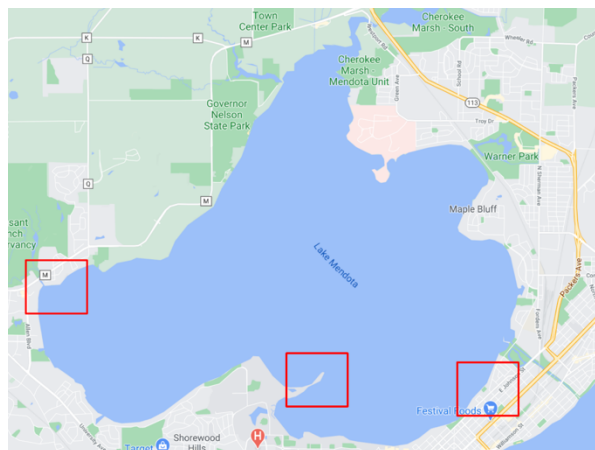


Figure 1: 3 sample locations: Middleton Springs (Left); Picnic Point (Middle); Downtown (Right)

Shuiwang Duan et al., analyzed lakewater in Baltimore, Maryland upon a rural-urban gradient to learn more about anthropogenic sources of watershed leading to an increased Phosphorus concentration and discovered greater pollution from urban sites led to an increased phosphorus concentration when compared to the rural sites. While Madison and Baltimore have different features along their lakes, Lake Mendota should still follow this pattern of having higher concentrations of Phosphorus along urban sites. So, this experiment's results should mirror this conclusion by having the highest PO₄-P concentrations at the Downtown collection site and the lowest at the Picnic Point site while Middleton Springs gives an intermediate concentration value.

2. Method

The lake water samples were collected on the shore of the lake, on November 11th, at Downtown, Picnic Point, and Middleton locations (Refer to Fig 1 for specific site locations). All samples are collected in properly cleaned plastic water bottles, and stored in a 4 degree celsius fridge. TP and TRP are determined using 5 experimental steps described below (TP Data Collected Mon Nov 11th, 2019, TRP Data collected Fri Nov 15th, 2019):

2.1 Ascorbic Acid vs Malachite Green (Method Comparison)

Generation of absorption spectra:

In the preparation of the standard solutions for both ascorbic acid method and the malachite green method, the highest concentration of the standard solutions are used to determine the wavelength of maximum absorption.

Generation of calibration curve:

- 1) Malachite green method: Method in Veldhoven, et. al, is used for Malachite Green Analysis using the concentration standard solution set below:

0M	0.2M	0.16M	0.14M	0.12M	0.10M	0.08M	0.06M	0.04M	0.02M
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- 2) Ascorbic Acid: Method in Ascorbic Acid Paper provided on Canvas (citation not found on paper)

0.1013g/L	0.0507g/L	0.02533g/L	0.0127g/L	0.0063g/L	0.00317g/L	0.00158g/L	0.000158g/L
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2.2 Generation of calibration curve for Ascorbic acid method

- 1) Purpose: In order to measure the concentration of phosphorus in the lake water samples, a standard curve was made by spectrophotometrically analyzing a series of known concentrations of phosphorus, so that accurate measurements of the concentration of P in the unknown lake water could be made later on.
- 2) Brief description: KH₂PO₄ was used to make a stock solution of 0.0005M PO₄⁻, and it was serially diluted to 0.00025M, 0.000125M, 0.0000625M, 0.00003125M, 0.0000156M, and 0.0000078125M. After the combined reagents and indicator were added appropriately (specifically 4mL of reagent A and 4mL of reagent C for a total volume of 20mL solution), a spectrophotometry analysis was

conducted to generate the calibration curve. And data points within the 0.1-2.0 mg P/L are kept.

2.3 Determination of MDL

- 1) Purpose: The purpose for finding MDL is to experimentally determine the lowest concentration of a solution in which the analyte can be reliably detected with an acceptable confidence interval (in Chem 329 lab, 99%). It is an important quality assurance technique because it ensures that the solutions prepared have a concentration that can be accurately and reliably measured by the lab equipment and our concentration determination method.
- 2) Brief description: The MDL of the calibration curve was determined by using the lowest concentration solution (3.91uM) and preparing 7 decreasing concentration aliquots: 3.75uM, 3.41uM, 3.12uM, 2.77uM, 2.45uM, 2.28uM. The MDL is further discussed in the results section.

2.4 Digestion of the lake water samples

- 1) Purpose: The phosphorus may occur in combination with organic matter which might interfere with the TP measurement. In order to reliably and accurately measure the TP concentration, effective oxidation of the organic matter is needed so that the phosphorus can be released as orthophosphate. Digestion will not be conducted for determination of TRP. Therefore, the purpose for conducting a digestion of lake water sample is to help us to accurately determine the TP concentration. And persulfate oxidation method was used due to its simplicity. (TRP concentrations were found without digestion).
- 2) Brief description: Indicator, H₂SO₄, and Ammonium Persulfate (NH₄)₂S₂O₈, was added to the lake water sample sequentially and the lake water was boiled and for a duration of 30-40 mins and let cool. And NaOH was added dropwise to the room temperature lake water sample until the solution reaches a pink-colored end point.

2.5 ICP analysis

- 1) Purpose: The purpose for conducting the ICP analysis is that it will allow us to determine the cation concentration in the solution. And we will be able to account for the interference caused by these cations. Matrix effects introduced by the presence of Ca²⁺, Al³⁺, and Fe³⁺ are of particular interest (refer to

Results and Discussion Section for further explanation).

- 2) Brief description: The standard stock solutions were made for generating calibration curve of each cation so we can use the calibration curve for the determination of the unknown concentration of the cations in the lake water sample. The cation standard solutions were made by Ammonium Iron Sulfate Hexahydrate (Fe^{3+}), Alum Potassium Sulfate (Al^{3+}), and Calcium Chloride dihydrate concentrations of 326 $\mu\text{g/L}$, 331 $\mu\text{g/L}$, and 330 $\mu\text{g/L}$ respectively.

2.6 Spike recovery

- 1) Purpose: Spike recovery was conducted to test for the matrix effect.
- 2) Brief Description: 1m of the spike (0.0000635M stock solution) is added to 20 mL of each properly digested lake water sample, and spectrophotometry analysis was conducted to measure the response of the lake water sample to the added spike. And the recovery will be calculated by $C(\text{spike}) - C(\text{unspike}) / C(\text{added}) * 100\%$.

3. Results

3.1 Malachite Green vs Ascorbic Acid Phosphorus Concentration Determination Method Comparison:

Absorption Spectra:

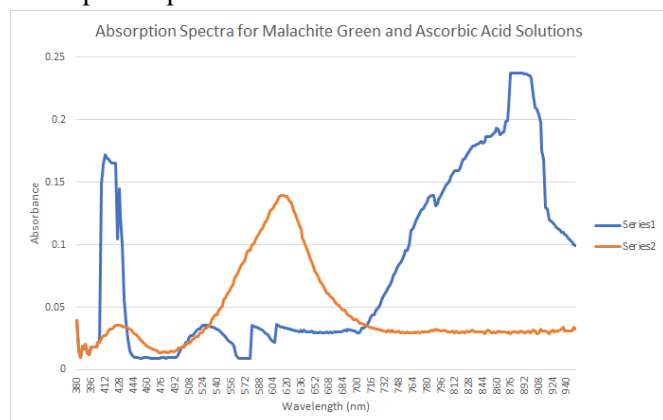


Figure 2: Absorption Spectra. Orange Curve: Malachite Green Abs Spectrum (max Wavelength: 616.0 nm). Blue Curve: Ascorbic Acid Abs Spectrum (max Wavelength: 878.0 nm)

Calibration Curves:

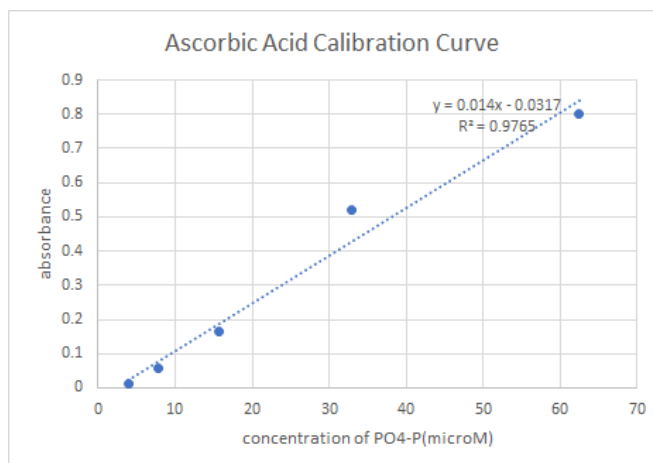


Figure 3: Ascorbic acid phosphorus determination method calibration curve

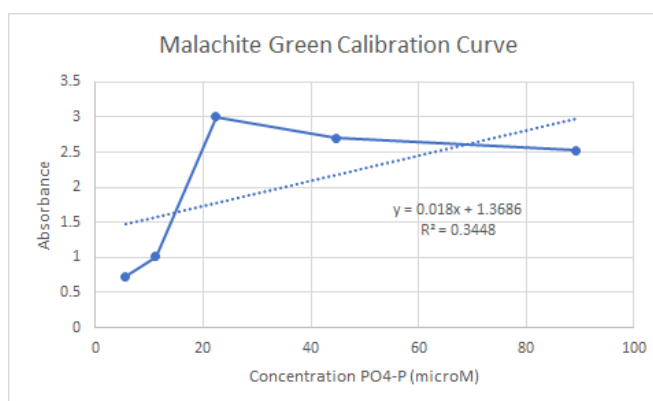


Figure 4: Malachite green phosphorus determination method calibration curve

From a quantitative standpoint, Ascorbic Acid's curve and method are clearly better for analysis of digested and undigested lake samples. First, the AA curve fits better to the linear model (indicated by the R^2 values), but more importantly, the sensitivity of the AA curve is higher. While the slope of the MG curve is higher, the large fluctuations of the data around the linear model decrease the sensitivity of the MG method considerably. Therefore, Ascorbic Acid is the far better method of the two when comparing linear fit and sensitivity.

Additionally, the AA method is a much more simple procedure where the preparation and use of solutions is more reliable and easier to do. The MG method only works in a certain analyte concentration range. Outside of that range there is a black precipitate that does not redissolve after gentle heating and stirring. Therefore, the AA leaves less room for systematic error and is more reliable. Also, the MDL for the ascorbic acid was a lower concentration value, indicating that the linear range of the Ascorbic Acid calibration curve was

larger than the Malachite Green curve (at least in the lower concentration values). So, the Ascorbic Acid curve will be more accurate at low concentration values, which are what we expect to obtain from our lake samples (another reason to use AA Method over MG Method). MDL data for Ascorbic Acid is given below.

3.2 Method detection limit (MDL): Ascorbic Acid

Method:

Table 1: Figure 4: MDL Data for Ascorbic Acid Phosphorus Concentration Determination. Standard deviation: 0.0086008 $t(6, 99\%) = 3.143$. MDL: Absorbance: 0.0270322 Concentration: 4.20 (4.195) μM

concentration(μM)	Abs
3.91	0.0201
3.75	0.0208
3.41	0.01604
3.12	0.01198
2.77	0.00708
2.45	0.0026
2.28	0.00022

The MDL Value (4.20 μM) is larger than some of our TP/TRP concentrations. So, certain concentrations must be taken with extra uncertainty as the accuracy of our equipment and method is not verified.

3.3 TP/TRP Figure/Data:

Tables below show % Transmittance data collected using the Ascorbic Acid method described in the Experimental section of this paper. These data were transformed into absorbance measurements ($\text{Abs} = -\log(T)$) and then applying our calibration curve equation (Fig. 3), were made into concentration values summarized in Figures 6 and 7.

Table 2: Tables showing raw % transmittance data collected by LabQuest for TRP data. These data are transformed into concentration values summarized in Figure 6.

TRP Data:			
Solution	Downtown	Middeton	Picnic Point
Trial #			
1	99.3	86	83.4
2	96.5	86.8	86.8
3	95.5	78.5	79.8
4	98.2	79.5	79.7
5	98.4	81.6	81.7
6	97.5	84.2	82.8
7	99.2	82.5	81.7

Table 3: Tables showing raw % transmittance data collected by LabQuest for TP data. These data are transformed into concentration values summarized in Figure 6.

TP Data:			
Solution			
Trial #	Downtown	Middeton	Picnic Point
1	90.2	92.8	95.2
2	88.9	91.4	92.4
3	91.7	91.6	91
4	91.9	94.7	94.6
5	91.9	94.5	97.1
6	91.4	94.7	95.1

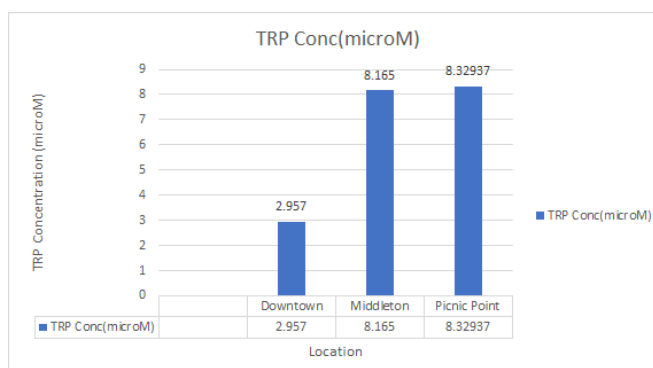


Figure 5: Bar Graph and Table show the relative TRP concentrations across the 3 sample locations

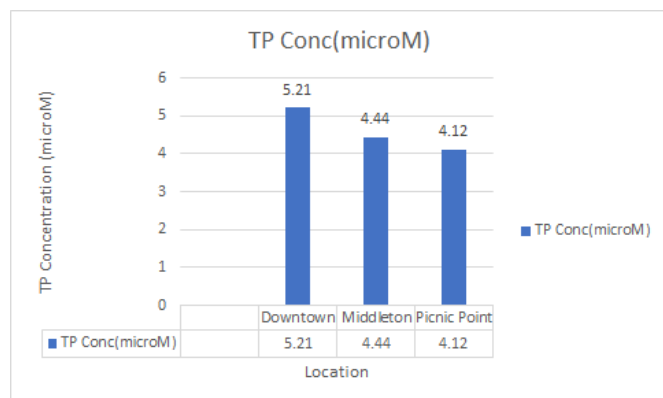


Figure 6: Bar Graph and Table showing Total Phosphorus Concentrations across the 3 sample locations.

Figures 5,6 and 7 summarize the TP and TRP concentrations across the 3 sites. The expectations for the data, based off the Shuiwang Duan et al. results, should be that Downtown has the highest concentration of both, Middleton should give an intermediate value, and Picnic Point should be the lowest. The TP Data follows this trend exactly as expected. Downtown (5.21 microM) has the highest concentration of analyte, while Picnic Point (4.12 microM) has the lowest concentration (a one way ANOVA Test was run to determine if the 3 means were statistically different). On the other hand, the TRP Data follows an odd trend and has values that do not necessarily make sense. First, the TRP values for Picnic Point and Middleton are higher than their TP values. This should not be the case as TP includes TRP concentration in its value. Second, Middleton and Picnic Point TRP values dwarf the Downtown TP concentration which should not be the case given the TP data. The reasoning for these odd TRP Values is given in the Discussion Section of this paper. We believe this has to do with the presence of algae in our downtown sample and the presence of phosphate precipitating ions in solution (explored further in Discussion).

3.4 ANOVA results for TP data:

Table 4: ANOVA test result for TP data, with $\alpha = 0.05$ (95% confidence interval)

ANOVA					
Response: Site Set					
Parameters	DF	SUM	MEAN	F-Value	Pr(>F)
sampleSites	2	3.7727	1.88637	5.8716	0.01308
Residuals	15	4.819	0.32127		

Since our $\text{Pr}(>F)$ is .013, which is smaller than 0.05 value for our Confidence Level, this indicates that the TP concentrations are significantly different at the 3 sample locations around our lake. Therefore, these results follow both the trend described in the Shuiwang Duan et al. paper and the results of that paper. In other words, the 3 locations around Lake Mendota had statistically different mean TP concentrations.

3.5 ICP-MS data:

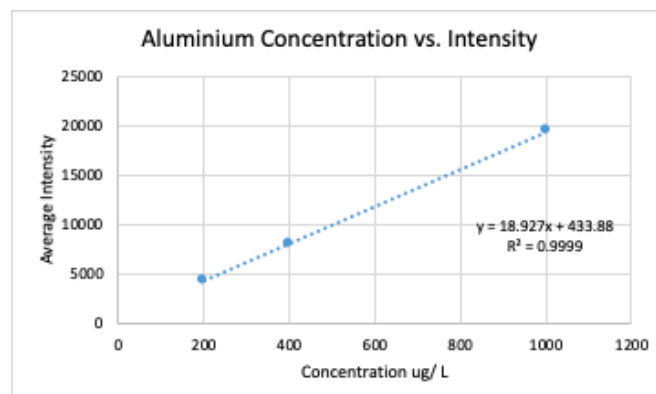


Figure 7: ICP-MS Curves relating Intensity measurements to concentrations of the respective ion. These were used to determine Al, Fe, and Ca ion concentrations in each of our 3 solutions.

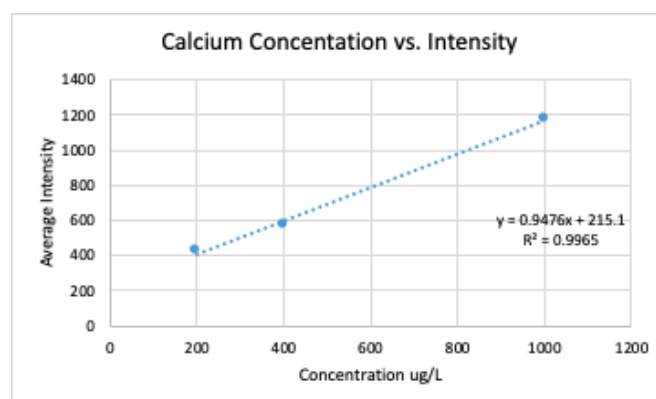


Figure 8: ICP-MS Curves relating Intensity measurements to concentrations of the respective ion. These were used to determine Al, Fe, and Ca ion concentrations in each of our 3 solutions.

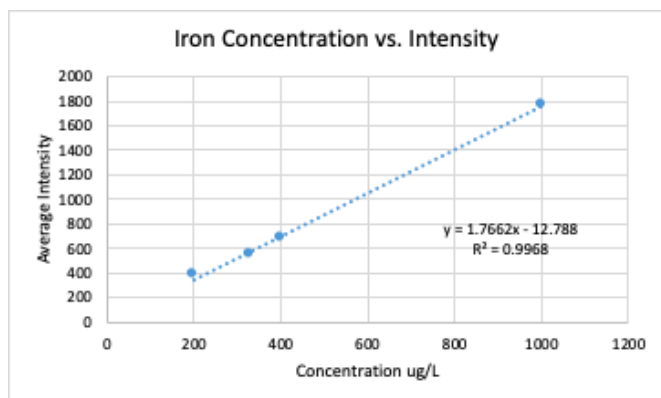


Figure 9: ICP-MS Curves relating Intensity measurements to concentrations of the respective ion. These were used to determine Al, Fe, and Ca ion concentrations in each of our 3 solutions.

Table 5: A summary of ion concentration present in the three lake water sample collected

Cation Conc(ug/L)/Location	Downtown	Picnic point	Middleton
[Al3+]	23.38	150.43	161.16
[Ca2+]	27191.02	33337.22	31784.18
[Fe3+]	31.85	89.11	119.28

According to Berkowitz et.al(2005), all the above cations contribute to the decrease of orthophosphate concentration (total phosphorus). Addition of aluminum to the lake water would cause the formation of $\text{Al}(\text{OH})_3$ floc which is insoluble in solution, which means that its presence will decrease the orthophosphate concentration. Berkowitz et.al, also argue that dissolved Ca^{2+} in the lake water may lead to precipitation of Ca-PO_4 while the loss of phosphorus from the lake water might be associated with the presence of Ca^{2+} and precipitation of the Ca-PO_4 , and that the molar ratio to be 1.25 ± 0.62 . Furthermore, Iron (Fe^{3+}) might have a similar effect to aluminum, and cause the loss of P-PO_4 concentration.

3.6 Spike recovery:

Table 6: A summary of Spike recovery of the three lake water sample

Location	Downtown	Middleton	Picnic Point
% Recovery	162.84%	104.3%	110.12%

The ideal range for % Recovery is 85% to 120%. The experimental values fall in and around this range, indicating that matrix effects were accounted for. In other words, analyte detection was not affected by the difference between the matrix of the standard solutions used to generate the calibration curves and matrix of the lake water samples.

Table 7: Capstone Figure: Summary of all results

Sample Locations Parameters	Downtown(Urban)	Picnic point(Rural)	Middleton (Suburban)
Mean Abs (TP)	0.04107009417	0.02589313433	0.03024643117
concentration(TP)(μM)	5.210	4.120	4.440
concentration(TRP) (microM)	2.957	8.329	8.165
ICP (microg/L)	Al: 23.38 Fe: 27191.02 Ca: 31.85	Al:150.43 Fe: 33337.22 Ca: 89.11	Al: 161.16 Fe: 31784.18 Ca: 119.28
Spike recovery	162.84	110.12	104.30

4. Discussion

The TP concentrations for the lakewater samples are proportional to what was expected based off of literary sources from Berkowitz's research and Duan's. The urban sample contains the most phosphorus which is possibly due to an increased land use and watershed while our rural sample contains the least phosphorus. The site samples were analyzed the same day of collection for TP concentration meaning that the possible confounding effects of the algae, ion concentrations, and sediment should not affect the TP concentrations the way they affected the TRP concentrations (discussed below). Based off the ANOVA Statistical Test (at the 95% Confidence Level), the 3 sites had different mean TP concentrations, indicating that the hypothesized trend of Downtown TP > Middleton TP > PP TP, is observed in and supported by the data.

This experiment's TRP data prompts further research into the effects of algae and various ion concentrations on phosphorus concentrations. The urban sample had relatively little reactive phosphorus in it by the time the analysis was run. Initially, after the sample was collected, algae was observed. The sample sat for three days and by the time it was analyzed, there was no observable algae. Smith E. and Kalff J. remark that algae need phosphorus to live. The observed algae possibly consumed some of the phosphorus and then decomposed when its nutrient sources depleted. So it is likely that by the time the samples were analyzed for TRP

concentration, the phosphorus had been partially depleted by the algae.

Additionally, depletion of Phosphorus in Downtown samples occurred because of the presence of certain cations in the lake water sample. According to Berkowitz et.al (2005), the presence of Ca^{2+} , Al^{3+} , and Fe^{3+} all contribute to the depletion of orthophosphate concentration in the lake water sample. Specifically, the presence of Al^{3+} will cause the formation of $\text{Al}(\text{OH})_3$, also known as the alum floc, which is insoluble in water and will precipitate (Fe^{3+} is believed to have similar effect as Al^{3+} according to Berkowitz et.al(2005)). Furthermore, Berkowitz et.al(2005) suggest that the presence of Ca^{2+} will lead to formation of Ca-PO_4 complex which will ultimately precipitate. In this experiment, the lake water samples contain a significant amount of the cations mentioned above. To determine which cation imposes the largest effect on the depletion of phosphorus concentration, further analysis and research is needed.

On the other hand, the Middleton and Picnic Point samples had an increase in TRP concentration relative to the TP concentration after 3 days. The observations made about the samples includes the presence of an amount of sediment (ie. soil) that was not there after the 3 day period. Boström et al. (1988) argues that the presence of sediment in a sample will dissolve after time, increasing the concentration of Fe, Al, P, among other ions. This helps explain why the TRP concentrations increased so dramatically after the 3 day period between TP and TRP measurements. However, further research is still warranted to discover the exact effect of sediment in lake water samples on phosphorus concentrations over time (ie. an experiment that would relate the rate of sediment dissolving and the change in the phosphorus concentration in that sample).

To further research this topic, we propose an analysis of the algae population density from each site. Further investigation of the algae density could lead to a greater understanding of the Phosphorus trends. We believe that by not accounting for the population of algae in this experiment our results may have been affected. Additionally, we propose a study to find the effect of sediment as well as ion concentration on the phosphorus concentration in lake water samples.

5. Conclusion

The Ascorbic acid method was used for phosphorus concentration analysis of three different locations upon an urban-rural gradient on Lake Mendota. The urban location had the highest TP concentration, while the rural location had the lowest. Contrary to our hypothesis, the urban location had the lowest TRP concentration and the rural location had the

highest. This could be due to unaccounted for algae and/or ion effects which prompts further research. However, statistical evidence in the unaffected TP measurements helps support the hypothesis that urban regions along Lake Mendota have the highest phosphorus concentrations, while rural areas have the lowest concentrations.

Acknowledgements

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