Release Mechanism of Available Phosphorus from Sediments in Grand Lake St. Marys

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ABSTRACT

Grand Lake St. Marys (GLSM) is Ohio's largest inland lake and the public drinking water supply for the City of Celina with over 10,000 in population. The 2007 National Lake Assessment (NLA) has discovered extreme levels of *Microcystin* and eutrophication in GLSM, jeopardizing public safety and aggravating difficulties in drinking water treatment. This project aims at estimating the internal releasing rate of available phosphorus – a main contributor to phytoplanktonic growth and blue-green algae bloom – from GLSM's sediments, complementary to external P sources such as agricultural runoffs. Water and sediment samples were collected in summer 2013 at six locations along the lake bank. Initial tests showed an average phosphorus concentration of 0.258mg/L in lake water and 41.16ppm in sediments. Later tests in six months displayed significant decrease of average phosphorus contents to 0.056mg/L in lake water samples and 13.92ppm in sediment samples. The release rate of phosphorus from sediments was estimated by lab simulation of stable lake condition. Hourly sampling and testing were performed for multiple trials. Resultant plots of phosphorus concentration versus time indicate that the model mirrors the general trend of first order decay reactions. This study aims to provide some perspectives on internal phosphorus release in GLSM and suggest possible improvements on similar studies in future.

Keywords: available phosphorus, concentration, colorimetric test, Olsen P Method, release mechanism, trend

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LIST OF ACRONYMS, ABBREVIATIONS & SYMBOLS

aka	Also Known As
CV	Coefficient of Variation
DI	Deionized
g	Gram
GLSM	Grand Lake St. Marys
Hr	Hour
$Hr_{adjusted}$	Adjusted Hour (+1 to Hr)
IC	Ion-Exchange Chromatography
ICP	Inductively Coupled Plasma
kg	Kilogram
mg	Milligram
P	Phosphorus
[P]	Concentration of Phosphorus
PO_4^{3-}	Phosphate Ion
R^2	Coefficient of Determination
StDev	Standard Deviation
Vol.	Volume
vs.	Versus

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I. INTRODUCTION

Grand Lake St. Marys (GLSM), covering an approximate area of 13,500 acres in Mercer and Auglaize counties, is Ohio's largest inland lake. Constructed in early 1840s, it primarily served as a feeder to the Miami-Erie Canal [4]. Nowadays it is the drinking water source for the City of Celina (over 10,000 in population) as well as a recreational site for local events and tourism.

Anthropogenic activities in recent years have adversely affected water quality. A lake assessment by Ohio Environmental Protection Agency (OEPA) in 2007 discovered high level of *microcystin* in GLSM. *Microcystin* is a toxic product of cyanobaterial cell-wall lysis that damages the liver and devastates health of humans and livestock; it is also relatively stable under natural aquatic conditions [2, 3]. Cyanobacteria (aka "blue-green algae") tend to proliferate in stable, warm and nutrient-rich water. Considering that over 80% of the Grand Lake/ Wabash watershed is cropland [2, 3], the algal bloom in GLSM is likely aggravated by agricultural runoff transporting major nutrients such as phosphorus to the lake. Phosphorus (in the form of P_2O_5 in fertilizers and PO_4^{3-} when dissolved in water and deprotonated) is adsorbable on sediment surfaces, with dependence on supernatant conditions, characteristics of the sediments, and biological characteristics of the water [4]. In the event when external phosphorus loading (that dissolved in incoming runoff) is reduced, the internal release of available phosphorus from sediments can still hinder the improvement of lake water quality over a certain period of time [8]. This brings attention to looking into the internal release mechanism of phosphorus, which would facilitate the estimation of phosphorus level in lake water at given time points.

This study focused on suggesting a possible model for the release of available phosphorus from sediments in GLSM. Lake water and sediments in shallow water were sampled near the Dog Tale Peninsula in the northeast of GLSM. Multiple bench-scale phosphorus release experiments simulating stable water conditions were conducted. A combination of Olsen P Method [5] and colorimetric tests [7] were implemented to estimate available phosphorus concentration in water and sediment samples over time. The model was obtained by fitting trend-lines to data series in Microsoft Excel.

II. SAMPLING

Sediment and water samples were collected at six different spots to the east and west banks of Dog Tale Peninsula located in the northeast part of GLSM, as illustrated in the following figures:

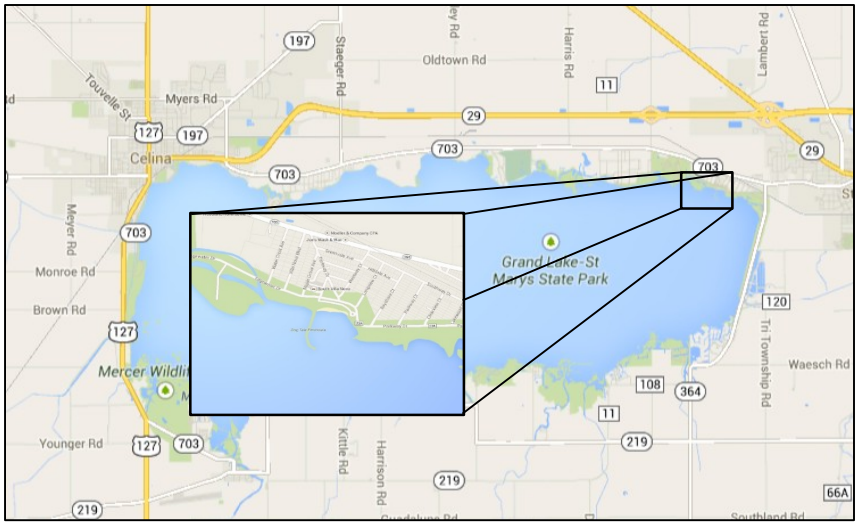

Figure 1: Sampling Site at GLSM (Google Map)

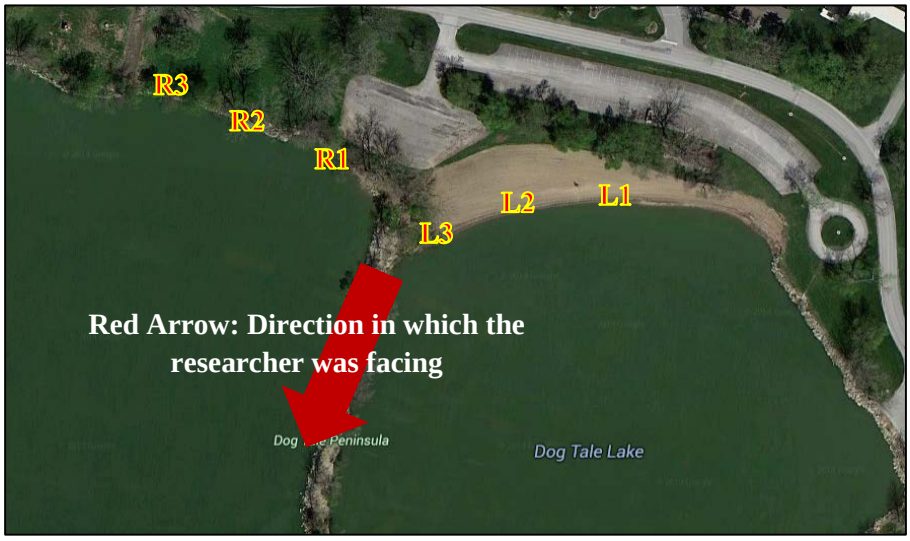

Figure 2: Sampling Locations at Dog Tale Peninsula (Google Earth)

Sediments in shallow water were sampled with a garden soil scoop and stored in six plastic zipper bags, labelled as ‘L1’, ‘L2’, ‘L3’, ‘R1’, ‘R2’, and ‘R3’. Lake water samples were collected at the same locations as sediments and stored in six acid-bathed plastic bottles with likewise labelling. Sediments were added to their corresponding bottles of lake water samples to preserve water samples overnight [9]. Four bottles of raw water samples (without sediments) were collected and labeled as ‘LR’ (left, raw), ‘LpH’ (left,

pH-adjusted), ‘RR’ (right, raw), and ‘RpH’ (right, pH-adjusted). 1 M hydrochloric acid was added drop-wise to the pH-adjusted samples, LpH and RpH, to bring their pH values (estimated with pH indicator paper) down to around 2 for preservation [9]. All samples were stored overnight in a cooler box with ice prior to initial testing. A conceptual illustration of the samples is provided below. Each large rectangle represents a plastic container; the light blue rectangles enclosed within represent water samples; the smaller, brown rectangles represent sediments.

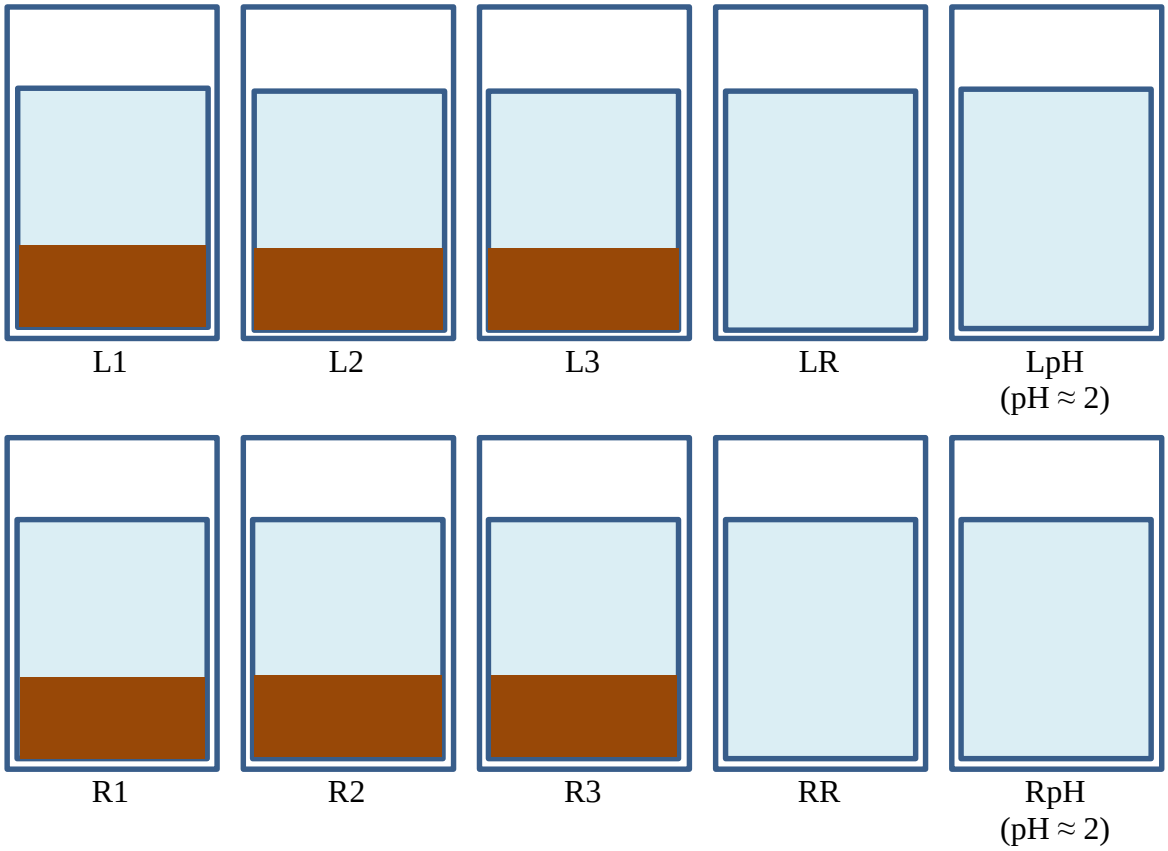

Figure 3: Conceptual Illustration of Water Samples

III. MATERIALS & METHODS

This section includes chemicals and equipment used for the research. Standard procedures for Olsen P Method and colorimetric test are included in APPENDIX A.

1. Chemicals and Reagants:

Sodium Bicarbonate Powder Certified (ACS 99.7 – 100.3) was ordered online from OSU Stores. KH_2PO_4 stock solution, molybdate reagent and stannous chloride reagent were prepared with available chemical stock in the teaching laboratory (Hitchcock 026). Hoffman 66005 Triple Super Phosphate Fertilizer (NPK: 0-46-0; 5 lbs) was purchased online from Amazon.

2. Equipment:

Whatman Grade No.42 filter paper (5.5 cm) was ordered online from Fisher Scientific. Vacuum filtration apparatus and six-paddle stirrers were borrowed from Dr. Cheng (cheng.160). Spectrophotometer was borrowed from the teaching laboratory with permission from Dr. Lenhart (lenhart.49). Millipore DI systems, glassware, 50-mL falcon tubes and tube racks were available in the laboratory for general usage.

3. Experimental Procedures:

(Conducted in Au2013)

Available phosphorus in the initial sediment samples was extracted with the Olsen P Method and determined with colorimetric test [5, 7]. Available phosphorus in the lake water samples was determined with colorimetric test after vacuum-filtration through Whatman Grade No.42 filter paper. The conductivity and pH of lake water samples were measured. Ion-exchange chromatography (IC) and inductively coupled plasma (ICP) tests were performed with help from graduate students to determine concentration of other chemicals present in the lake water. After the above-mentioned tests, samples were stored in the cold room in Hitchcock 026 at 38°F.

(Conducted in Sp2014)

After a storage period of 5 months, available phosphorus in sediment samples (both those in the zipper bags and those in the bottles of lake water) was extracted with the Olsen P Method [5]. Colorimetric test [7] was performed on the extraction solutions and lake water samples to determine concentrations of available phosphorus. Fertilizer solution was prepared by mixing 25 grams of the Triple Super Phosphate fertilizer with 500 mL of deionized (DI) water. Filtration was done to the fertilizer solution to remove suspended particles on the following day; concentration of phosphorus (in the form of PO_4^{3-}) in the filtrated fertilizer solution was thereafter determined via colorimetric test.

With regard to the release experiments, sediments stored in the bottles were transferred to six 1-liter beakers labeled as ‘L1’, ‘L2’, ‘L3’, ‘R1’, ‘R2’ and ‘R3’. A certain volume of fertilizer solution was added to each beaker of sediment using a 25-mL graduated cylinder and thoroughly mixed by stirring with a spatula. The sediments in beakers were then left in a hood for air drying.

After the sediments are sufficiently dry, the beakers were transferred to a laboratory bench and positioned under a six-paddle stirrer. 900 mL of water sample was measured with a 1-L graduated cylinder and transferred carefully to each beaker. DI water was used in the first two trials to obtain a general idea on the model; lake water was used in Trial 3 and 4. In an attempt to uniform phosphorus concentration in the supernatant while minimize disturbance to the sediments, the stirrer was elevated with pads so that its paddles rotated near the water surface in each beaker. Rotational speed of paddles was set to 10 rpm.

Sampling and testing procedures: A total of four trials were conducted. Timer started when the stirrer was switched on at the beginning of each trial. On an hourly basis, a small volume (~2mL) of supernatant was transferred from each beaker to its corresponding 50-mL falcon tube and diluted at with DI water. pH values and temperature of supernatants were recorded at each transferring. Colorimetric test was performed on the supernatant samples in the falcon tubes to determine phosphorus concentration. Necessary adjustments to the degree of dilution were made to achieve reasonable colorimetric readings (accuracy of measurement would decrease if the absorbance reading were too far beyond the phosphorus standard curve). Each trial lasted about 20 hours. The resultant phosphorus concentrations (mg/L) were plotted versus time (hr) for trend-line fitting.

IV. RECORDED DATA

Trial 1:

The concentration of phosphorus (in the form of phosphate, PO_4^{3-}) in supernatants over time is included in Table 3 under APPENDIX B. Plots are shown below:

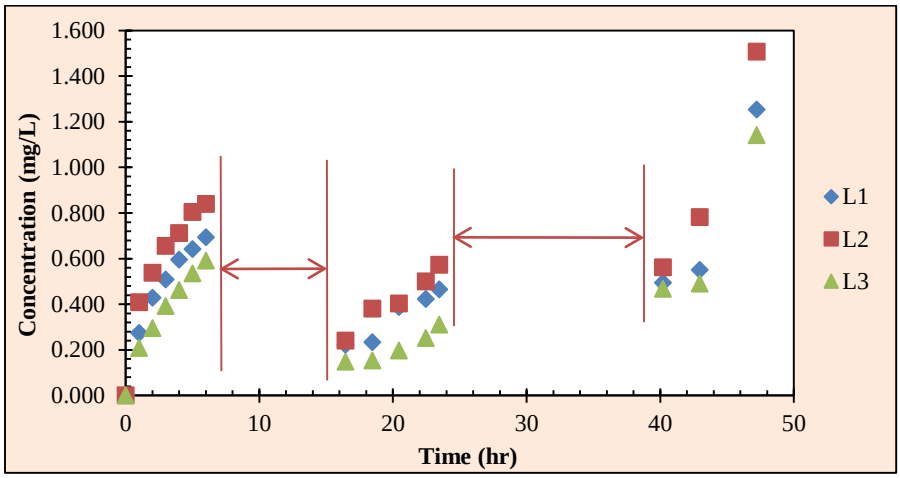

Figure 4: Trial 1, R – [P] vs. Time

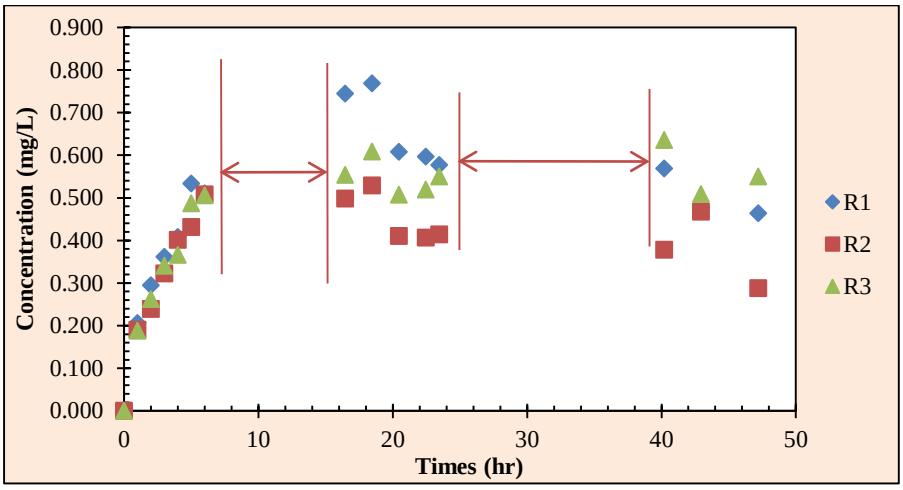

Figure 5: Trial 1, R – [P] vs. Time

Sampling for Trial 1 was not done on a consecutive hourly basis. As shown in both plots, although in the first 6 hours consistently increasing trends of PO_4^{3-} is observed in all samples, the trends become unexplainable in subsequent hours, particularly during the two big time gaps (marked by red double-headed arrows). No reasonable overall trend was derivable. After consulting the research advisor, the researcher made the adjustment that future trials be conducted through consecutive hours in order to acquire adequate data points for trend analysis.

Trial 2:

The second trial was conducted overnight for 16 consecutive hours. Phosphorus concentration in supernatants over time is included in Table 4 under APPENDIX B and plotted as follow:

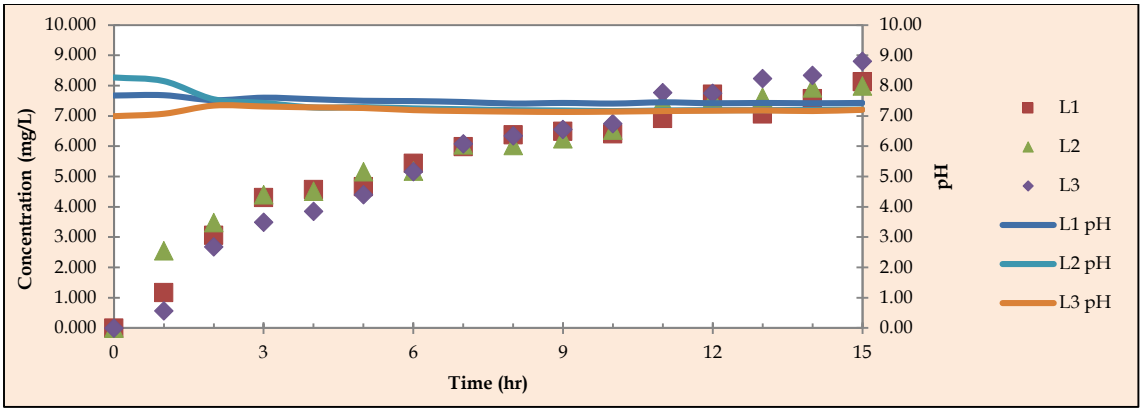

Figure 6: Trial 2, L – [P] vs. Time

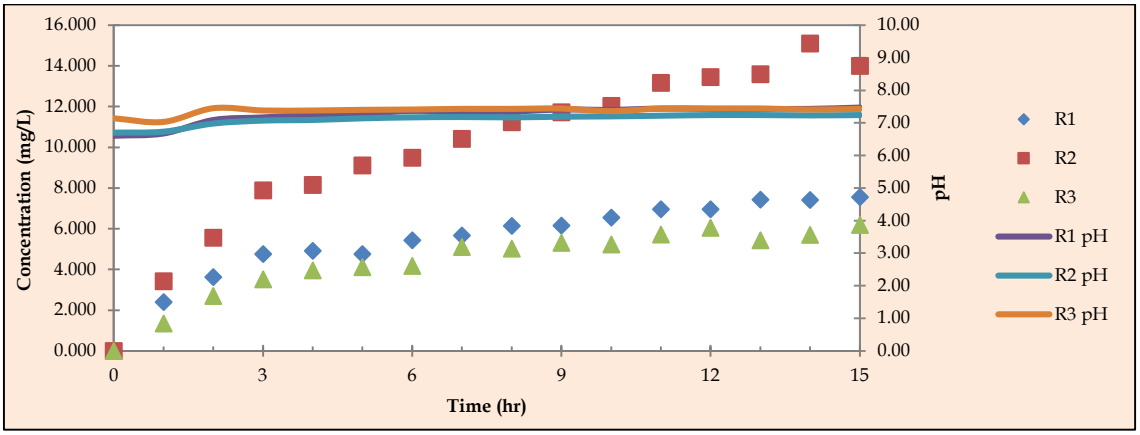

Figure 7: Trial 2, R – [P] vs. Time

All trends in Trial 2 have shown high consistency (unlike those in Trial 1). Every set of data points closely resemble the form of a shifted logarithmic function, or inversely the form of a shifted exponential function, i.e.:

Equation 1: General Form of Shifted Logarithmic Function

$$[\boldsymbol{P}] = \boldsymbol{C} \cdot \boldsymbol{log}_{\boldsymbol{a}}(\boldsymbol{t}+\boldsymbol{1}) \,,$$

Equation 2: General Form of Shifted Exponential Function

$$(\boldsymbol{t}+\boldsymbol{1}) = \boldsymbol{a}^{\boldsymbol{k}\cdot[\boldsymbol{P}]}$$

where '[P]' stands for the concentration of phosphate in supernatants in mg/L; 't' stands for time in hours; 'C', 'k' and 'a' are constants. Further analyses on the trends are included in Section V of this report.

In comparison of Figure 6 and 7, despite sharing similar forms, phosphorus concentrations in the R (R1, R2 and R3) supernatants increased at noticeably different rates, whereas data series of the L (L1, L2 and L3) supernatants were compact to one another. This was presumably caused by the initial difference in available phosphorus content in the sediment, difference in sediment/soil structures, and so forth. However, results obtained thus far were inadequate to derive a definitive correlation between the data and these factors.

Also found in Trial 2 was that the release of phosphorus is not correlated to change in pH as can be inferred from the minimal pH variation (continuous lines) in Figure 6 and 7. Nevertheless, pH was monitored in subsequent trials for control purpose. Temperature of supernatant recorded during the trial remained stable at about 24°C, which eliminated potential thermal variation of releasing rates.

Trial 3:

In light of the results from Trial 2, Trial 3 was likewise configured except that lake water was used as the supernatants in place of DI water. Before starting Trial 3, 5 mL of fertilizer solution was added to and thoroughly mixed with each sediment sample. Sediment samples in beakers were allowed to air dry in a hood. Afterwards, the Olsen P Method [5] and colorimetric test [7] were performed on about 1 gram of each sediment sample to estimate their initial available phosphorus content.

Due to time constrain, the releasing experiment was started before L2 was adequately dry. Hourly sampling from the supernatants was conducted for 20 consecutive hours for the other supernatants and for 10 consecutive hours for L2 (L2 was not ready for testing until 10 hours into the experiment). Measurements and recorded data are included in Table 6 and 7 under APPENDIX B. Plots of phosphorus concentration versus time are as follow:

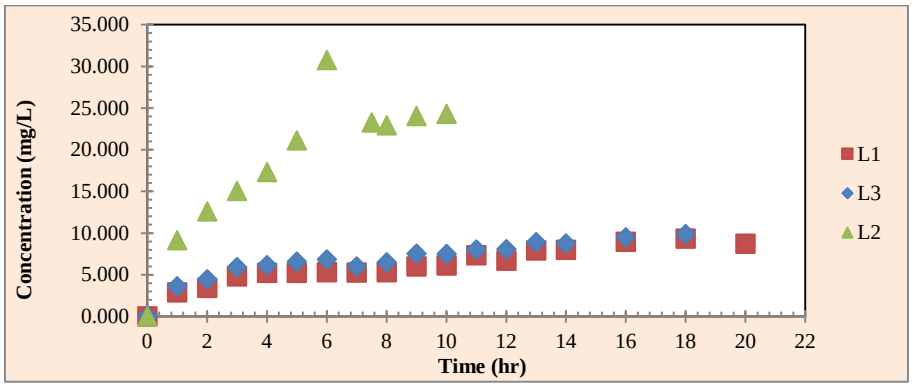

Figure 8: Trial 3, L – [P] vs. Time

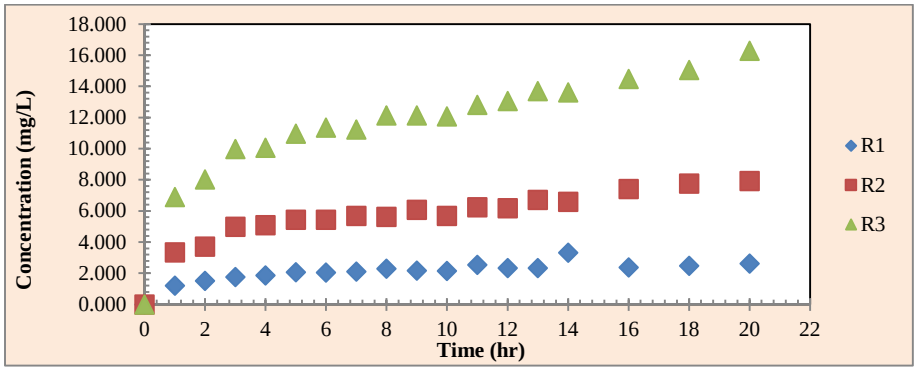

Figure 9: Trial 3, R – [P] vs. Time

Other than a few diverged data points, increasing trends for all series are similar to their counterparts in Trial 2. Furthermore, Figure 12 through 17 (see APPENDIX B) show that the trend mirrors that of **first order decay reactions**. Also discovered was that even though the same volume of fertilizer solution was added to all sediments before the trial, the samples differed in release rates and initial phosphorus availability.

Trial 4:

A final trial was conducted with no addition of fertilizer to any sediment as available phosphorus remained in all samples after Trial 3 was considered excessive [10]. After Trial 4, the Olsen P Method [5] and colorimetric test [7] were performed on about 1 gram of sediment from each beaker to determine the final available phosphorus contents. Measurements are included in Table 8 and 9 under APPENDIX B. Plots of phosphorus concentration in supernatant versus time are shown below; trends depicted are similar to those obtained in previous trials:

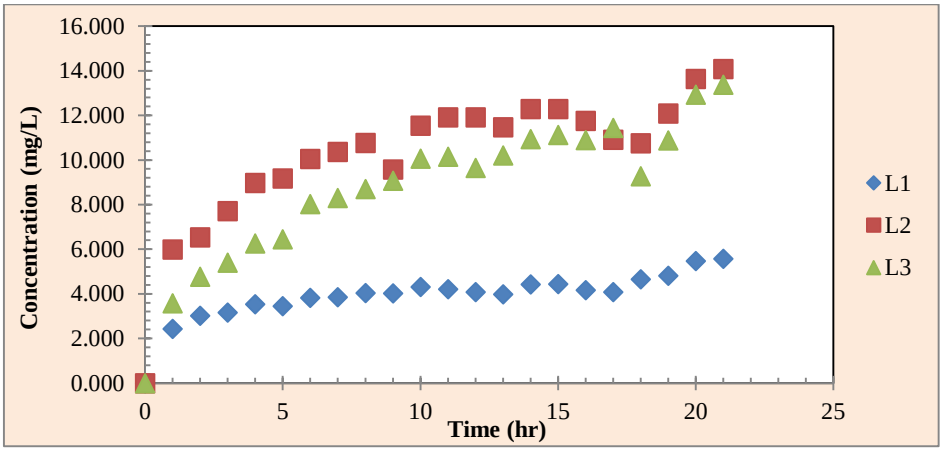

Figure 10: Trial 4, L – [P] vs. Time

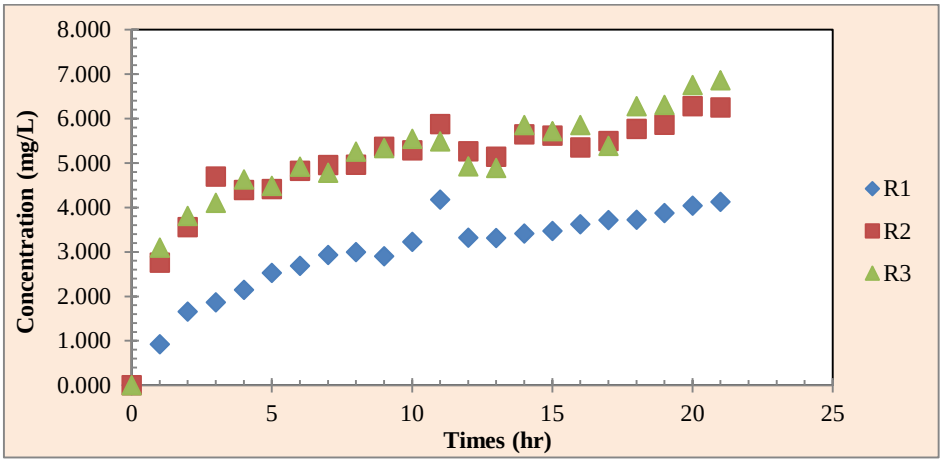

Figure 11: Trial 4, R – [P] vs. Time

V. ANALYSIS AND DISCUSSION

1. Section Overview:

Trend-line fitting was the approach in this study to develop a generic model for the release mechanism. This inevitably involved determining the relationship between the coefficients in the trend-line equations and the concentration of available phosphorus. However, due to unexplainable discrepancy in measurement, such a correlation was unable to be derived. Eventually, arithmetic means were calculated based on the measurement and plotted with standard deviation, which returned a data range for each sediment sample.

2. Trend-Line Fitting:

As mentioned in Section IV, the trends are in the form of a shifted logarithmic function (or inversely a shifted exponential function). The reason it is "shifted" is that basic logarithmic functions of the form of $\boldsymbol{y = log_a x}$ are asymptote to the negative y-axis and always pass through the point (1, 0). Phosphorus concentration measured at the beginning of every trial was zero. This would violate the default setting of Microsoft in trend-line fitting. Hence, 1 hour was added to all time points, which shifted the entire time axis toward the negative x-direction by 1 unit to make (1 hr, 0 mg/L) the new starting point of each data series. Such would enable Microsoft Excel to recognize the logarithmic trend and fit a logarithmic function to the data points. Afterwards, replace '***x***' in the trend-line equations obtained with '***t+1***' to account for the shift.

Below is a segment of Table 6, illustrating the above-mentioned shifts. An additional highlighted column named "Adjusted Hr" was inserted. The "Adjusted Hr" values were used as the horizontal axes for new plots, which are listed under APPENDIX C.

Hr	**Adjusted Hr**	Concentration of Phosphorus (mg/L) in Supernatant					
		L1	L2	L3	R1	R2	R3
0	**1**	0.000	0.000	0.000	0.000	0.000	0.000
1	**2**	2.430	5.989	3.582	0.923	2.762	3.100
2	**3**	3.022	6.532	4.765	1.659	3.558	3.812
3	**4**	3.164	7.710	5.404	1.867	4.695	4.107
…	**…**	…	…	…	…	…	…

3. Initial/Final Condition Approach:

It was speculated that a decline in available phosphorus in the sediments would occur after each trial. Initial concentration of available phosphorus in each sediment sample before Trial 3 and after Trial 4 was tested. The results are summarized in the following table:

Table 1: Initial and Final Conditions for Trial 3 and 4

	Concentration of Available Phosphorus in Sediments (mg/kg sediment)		
	Trial 3 Initial	Trial 3 Final/ Trial 4 Initial	Trial 4 Final
L1	**162.21**	**146.69**	**74.19**
L2	116.54	173.15	44.52
L3	**148.09**	**108.59**	**31.91**
R1	64.29	249.83	29.33
R2	95.77	241.40	35.69
R3	147.91	171.75	43.53

Note:

(1) No fertilizer solution was added to the sediment samples after Trial 3. The final concentrations for Trial 3 were regarded as the initial conditions for Trial 4.

(2) In **BOLD**: consistent in declination.

The overall decrease in concentration from before to after Trial 4 was as expected. However, for Trial 3, decline was only seen in L1 and L3; an increase was instead observed in all other samples, especially in R1 and R2 where the final concentrations were significantly higher than the initial. This was possibly a result of the thorough mixing before Trial 3; the vigorous stirring motion could have potentially altered or destroyed certain soil matrices within the sediments which helped in binding phosphorus [3]. Consequently, the unbound phosphorus contributed to an increase in concentration of available phosphorus. For illustrative purposes, plots of coefficients in the trend-line equations versus initial available phosphorus contents are included in APPENDIX D. No definitive trends or correlations can be determined based on the widely dispersed data points. The researcher speculated that factors such as concentration of other types of chemicals in the supernatant could have influenced the release of phosphorus. Also possible was that availability of phosphorus in sediments could have spontaneously changed over time. However, under current experimental settings, monitoring changes of phosphorus availability in sediments while testing for supernatants was extremely difficult.

4. Arithmetic Mean Approach:

The arithmetic means of measurements from Trial 3 and Trial 4 were calculated and summarized in Table 11 through 16 and plotted on Figure 26 through 31 (see APPENDIX E). Also included are standard deviations (StDevs) and coefficients of variation (CVs). The StDevs are calculated using built-in features of Excel. CV is a further indication of data discrepancy in addition to StDev: large CVs imply great deviation, and small CVs imply high precision. CV is calculated using the following formula:

Equation 3: Coefficient of Variation

$$CV = \frac{Standard\ Deviation}{Average} \times 100\%$$

Trend-lines are fitted to the arithmetic means with adjusted hours (likewise as Section V.1). The equations are as follow:

Table 2: Trend-Lines, Arithmetic Mean Approach

Trend-Line Equations for Arithmetic Mean Approach		
L1	$[P] = 2.021 \cdot \ln(t+1) + 0.684$	(R^2 = 0.953)
L2	$[P] = 7.480 \cdot \ln(t+1) + 1.334$	(R^2 = 0.914)
L3	$\mathbf{[P] = 3.321 \cdot \ln(t+1) + 0.693}$	(R^2 = 0.977)
R1	$\mathbf{[P] = 1.027 \cdot \ln(t+1) + 0.315}$	(R^2 = 0.947)
R2	$\mathbf{[P] = 1.887 \cdot \ln(t+1) + 1.273}$	(R^2 = 0.918)
R3	$[P] = 2.999 \cdot \ln(t+1) + 1.935$	(R^2 = 0.928)

Note:

(1) Although all R^2 are above 0.90, indicating high degrees of linear regression of data points to models, the trend-lines only account for the averaged data points. How precise these data series are should be inferred from the standard deviation bars on graphs or CV values listed in the tables (see APPENDIX E).

(2) In **BOLD**: high precision shown in data series.

With regard to Figure 26 through 31, decent levels of linear regression are observed in L3 (Fig. 28), R1 (Fig. 29) and R2 (Fig. 30), whereas the excessively long StDev bars in L1 (Fig. 26), L2 (Fig. 27) and R3 (Fig. 31) suggest low precision in models.

VI. CONCLUSION

1. Current Progress:

The researcher was able to develop a preliminary model applicable to all samples:

$$[P] = A \cdot ln(t + 1) + B$$

where [P] stands for phosphorus concentration in supernatant, t stands for time, A and B are coefficients. The original objective to acquire a definitive model was unsuccessful due to unexplainable relationships between the initial available phosphorus concentration in sediments and the coefficients. On the other hand, plume-shaped plots were created based on the arithmetic mean approach (see APPENDIX E) to account for the possible ranges of phosphorus concentration over time. The overall results explained short-term (~20 hrs) changes in phosphorus concentration. In the long term, an equilibrium between the supernatant and sediments is expected. Long-term studies are required to confirm the saturation point.

2. Future Perspectives:

Time constrains, sample insufficiencies and lack of sophisticated experimental skills were the main factors limiting the researcher from performing more thorough and well-rounded tests. Should tests of similar nature be conducted in future, it would be recommended that in complementary to hourly sampling and testing of supernatants test on the sediments be performed at a similar rate and comparison between the two be made; number of sampling sites be increased in light of variation in sediment and soil characteristics; contents of other chemicals in the supernatants and sediments be monitored to check for any potential influence on the release mechanism; bioactivity in the water be monitored as phosphorus may be consumed in the process; more iterations and longer periods be allowed for each trial to account for long term variations.

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APPENDIX A: TEST PROCEDURES

The following are test procedures exactly stated on their manuals [5, 7]. In an attempt to minimize usage of chemicals, scaling of the amount of chemicals used was made during experiments. More background information on the tests can be found in their respective reference materials (under Bibliography).

Olsen P Method [5]

Procedure:

1. Scoop or weigh 1 g of soil into a 50 mL Erlenmeyer flask, tapping the scoop on the funnel or flask to remove all of the soil from the scoop.

2. Add 20 mL of extracting solution to each flask and shake at 200 or more epm for 30 minutes at a room temperature at 24 to 27^0C.

3. If it is necessary to obtain a colorless filtrate, add 1 cm^3 (~200 mg) of charcoal to each flask.

4. Filter extracts through Whatman No. 42 filter paper or through a similar grade of paper. Refilter if extracts are not clear.

5. Analyze for P by colorimetry or inductively coupled plasma emission spectroscopy using a blank and standards prepared in the Olsen P extracting solution.

Calculations:

Olsen Extractable P (mg P/kg soil) =
[Concentration of P in Olsen extract, mg/L] x [0.020 L extract ÷ 0.001 kg soil]

Colorimetric Test [7]

Procedure:

1. Prepare calibration standards. Use a Mohr pipette to add the following volumes of the standard phosphate solution to 250 mL volumetric flasks. Fill to the mark with deionized water.

Standard 1: 0.0 mL (0 µg/250 mL = 0.00 mg/L PO_4^{3-} -P)

Standard 2: 0.5 mL (25 μg/250 mL = 0.10 mg/L PO_4^{3-} -P)
Standard 3: 1.0 mL (50 μg/250 mL = 0.20 mg/L PO_4^{3-} -P)
Standard 4: 2.0 mL (100 μg/250 mL = 0.40 mg/L PO_4^{3-} -P)
Standard 5: 4.0 mL (200 μg/250 mL = 0.80 mg/L PO_4^{3-} -P)

2. Remove 100mL of each phosphate standard from the volumetric flask and place in an Erlenmeyer flask. Label these flasks as "Standard" with the μg P, group name, and date.

3. Measure 100mL of each water sample to be analyzed and place in an Erlenmeyer flask.

4. To each flask, add 2 drops of phenolphthalein indicator. If a red color develops, add strong acid dropwise to discharge the color.

5. Add, with thorough mixing after each addition, 4.0mL of molybdate reagent and 10 drops of stannous chloride reagent.

6. After 10 minutes, but before 12 minutes, using the same interval for each sample, measure the color spectrophotometrically at 690nm. To zero the reading for the old instruments, use the left hand knob to set the transmittance to zero when no sample is in the instrument. Then place a sample of the blank (Standard 1) into the instrument and set the transmittance to 100% using the knob on the front-right side of the instrument. Change the mode to "Absorbance" and measure your sample. The new instruments are zeroed using the blank by pressing the "0 ABS" button. Always run the blank on a sample that includes the reagents.

APPENDIX B: EXPERIMENT DATA

Trial 1:

Table 3: Trial 1, [P] over Time

	Time	Hr	Concentration of Phosphorus (mg/L) in Supernatant					
			L1	L2	L3	R1	R2	R3
2/16/2014	11:33	0	0.000	0.000	0.000	0.000	0.000	0.000
	12:33	1	0.274	0.408	0.209	0.206	0.190	0.188
	13:33	2	0.429	0.538	0.294	0.294	0.239	0.262
	14:33	3	0.508	0.656	0.392	0.362	0.322	0.341
	15:33	4	0.596	0.712	0.461	0.408	0.401	0.366
	16:33	5	0.642	0.804	0.535	0.533	0.431	0.487
	17:33	6	0.693	0.839	0.591	0.510	0.508	0.505
2/17/2014	10:00	16.45	0.221	0.240	0.148	0.744	0.498	0.553
	12:00	18.45	0.234	0.381	0.154	0.769	0.529	0.609
	14:00	20.45	0.387	0.403	0.197	0.608	0.410	0.507
	16:00	22.45	0.422	0.499	0.252	0.596	0.407	0.519
	17:00	23.45	0.465	0.573	0.310	0.577	0.414	0.550
2/18/2014	09:45	40.2	0.494	0.561	0.467	0.568	0.378	0.636
	12:30	42.95	0.550	0.782	0.490	0.475	0.467	0.509
	16:45	47.2	1.253	1.507	1.141	0.464	0.288	0.550

Trial 2:

Table 4: Trial 2, [P] over Time

	Time	Hr	Concentration of Phosphorus (mg/L) in Supernatant						T (°C)
			L1	L2	L3	R1	R2	R3	
2/21/2014	17:30	0	0.000	0.000	0.000	0.000	0.000	0.000	24
	18:30	1	1.174	2.551	0.568	2.407	3.424	1.345	24
	19:30	2	3.059	3.479	2.675	3.627	5.554	2.694	24
	20:30	3	4.307	4.406	3.495	4.763	7.891	3.515	24
	21:30	4	4.574	4.515	3.852	4.921	8.149	3.951	24
	22:30	5	4.664	5.159	4.406	4.753	9.119	4.119	24
	23:30	6	5.436	5.188	5.159	5.436	9.495	4.178	24
2/22/2014	0:30	7	5.984	6.048	6.086	5.668	10.412	5.099	24
	1:30	8	6.377	6.035	6.351	6.149	11.235	5.023	24
	2:30	9	6.490	6.250	6.554	6.161	11.716	5.314	24
	3:30	10	6.414	6.541	6.743	6.554	12.045	5.238	24
	4:30	11	6.916	7.501	7.778	6.962	13.169	5.714	24
	5:30	12	7.717	7.593	7.747	6.962	13.446	6.053	24
	6:30	13	7.070	7.624	8.240	7.424	13.600	5.437	23.5
	7:30	14	7.578	7.932	8.348	7.408	15.110	5.699	23.5
	8:30	15	8.132	7.994	8.810	7.562	14.001	6.192	23.5
	9:30	16	7.840	7.994	8.702	7.593	14.078	6.407	23.5

Table 5: Trial 2, pH over Time

Time	pH of supernatant						Vol. of supernatant (mL)
	L1	L2	L3	R1	R2	R3	
17:30	7.67	8.27	6.99	6.61	6.70	7.14	900
18:30	7.68	8.15	7.07	6.68	6.73	7.03	895
19:30	7.52	7.55	7.34	7.09	6.98	7.45	890
20:30	7.60	7.43	7.31	7.17	7.07	7.38	888
21:30	7.55	7.28	7.28	7.25	7.09	7.38	886
22:30	7.50	7.29	7.26	7.28	7.14	7.40	884
23:30	7.49	7.25	7.19	7.37	7.17	7.41	882
0:30	7.46	7.22	7.16	7.33	7.18	7.43	880
1:30	7.41	7.19	7.14	7.35	7.17	7.43	878
2:30	7.43	7.18	7.13	7.41	7.19	7.44	876
3:30	7.41	7.16	7.14	7.40	7.20	7.36	874
4:30	7.45	7.18	7.16	7.44	7.22	7.44	872
5:30	7.42	7.19	7.17	7.43	7.24	7.44	870
6:30	7.43	7.19	7.18	7.43	7.24	7.44	868
7:30	7.42	7.2	7.16	7.43	7.23	7.41	866
8:30	7.43	7.21	7.2	7.47	7.24	7.43	864

Trial 3:

Table 6: Trial 3, [P] over Time

		Hr	Concentration of Phosphorus (mg/L) in Supernatant					
			L1	L2	L3	R1	R2	R3
2/28/2014		0	0.000	L2 not ready for testing yet	0.000	0.000	0.000	0.000
		1	2.867		3.626	1.213	3.342	6.907
		2	3.403		4.440	1.518	3.715	8.026
		3	4.802		5.894	1.771	4.979	9.970
		4	5.235		6.140	1.870	5.087	10.071
3/1/2014		5	5.235		6.583	2.076	5.441	10.976
		6	5.294		6.838	2.056	5.441	11.366
		7	5.274		5.982	2.115	5.687	11.227
		8	5.294		6.474	2.302	5.618	12.133
	Hr (L2)	9	6.002		7.537	2.175	6.081	12.145
	0	10	6.110	0.000	7.468	2.155	5.677	12.082
	1	11	7.330	9.140	8.021	2.540	6.236	12.812
	2	12	6.664	12.585	8.059	2.339	6.173	13.063
	3	13	7.913	15.046	8.923	2.342	6.719	13.699
	4	14	7.974	17.311	8.740	3.321	6.597	13.607
	5	15		21.135				
	6	16	8.954	30.758	9.474	2.372	7.408	14.479
	7.5	17.5		23.261				
	8	18	9.321	22.936	9.857	2.480	7.760	15.046
	9	19		24.028				
	10	20	8.724	24.294	10.071	2.617	7.928	16.285

Table 7: Trial 3, pH and Temperature over Time

Time	pH of Supernatant						Vol. of supernatant (mL)	T (°C)
	L1	L2	L3	R1	R2	R3		
19:00	-	-	-	-	-	-	900	24.5
20:00	7.52	L2 not ready for testing yet	7.26	7.05	6.95	6.86	895	24
21:00	7.61		7.36	7.22	7.10	6.96	890	23.5
22:00	7.61		7.39	7.28	7.16	7.02	888	23.5
23:00	7.61		7.39	7.30	7.20	7.03	886	23.5
00:00	7.58		7.41	7.28	7.19	7.04	884	23.5
01:00	7.59		7.41	7.25	7.20	7.06	882	23.5
02:00	7.58		7.42	7.20	7.18	7.06	880	23.5
03:00	7.55		7.42	7.09	7.13	7.08	878	23.5
04:00	7.54		7.39	7.06	7.11	7.09	876	23.5
05:00	7.53		7.39	6.99	7.07	7.07	874	23.5
06:00	7.56		7.38	7.02	7.09	7.07	872	23.5
07:00	7.51	6.90	7.36	6.92	7.03	7.09	870	23.5
08:00	7.50	6.92	7.34	6.89	7.01	7.02	868	23.5
09:00	7.44	6.93	7.32	6.89	7.03	7.03	866	23.5
10:00		6.94						23.5
11:00	7.50	6.95	7.28	6.93	7.07	7.09	864	23.5
12:00		6.97						23.5
13:00	7.46	6.97	7.27	6.98	7.13	7.09	862	23.5
14:00		6.94						23.5
15:00	7.49	6.97	7.25	7.07	7.19	7.07	860.00	23.5

Trial 4:

Table 8: Trial 4, [P] over Time

	Time	Hr	Concentration of Phosphorus (mg/L) in Supernatant					
			L1	L2	L3	R1	R2	R3
3/9/2014	8:00	0	0.000	0.000	0.000	0.000	0.000	0.000
	9:00	1	2.430	5.989	3.582	0.923	2.762	3.100
	10:00	2	3.022	6.532	4.765	1.659	3.558	3.812
	11:00	3	3.164	7.710	5.404	1.867	4.695	4.107
	12:00	4	3.537	8.970	6.263	2.147	4.396	4.630
	13:00	5	3.453	9.175	6.450	2.529	4.415	4.490
	14:00	6	3.817	10.057	8.026	2.688	4.826	4.919
	15:00	7	3.855	10.370	8.306	2.931	4.956	4.779
	16:00	8	4.041	10.767	8.703	2.996	4.966	5.255
	17:00	9	4.032	9.578	9.082	2.903	5.367	5.339
	18:00	10	4.312	11.543	10.073	3.229	5.283	5.544
	19:00	11	4.210	11.923	10.156	4.178	5.879	5.488
	20:00	12	4.079	11.923	9.644	3.323	5.264	4.928
	21:00	13	3.985	11.477	10.205	3.313	5.143	4.891
	22:00	14	4.434	12.286	10.948	3.416	5.647	5.852
	23:00	15	4.443	12.286	11.130	3.472	5.619	5.722
3/10/2014	0:00	16	4.172	11.758	10.899	3.621	5.348	5.852
	1:00	17	4.079	10.915	11.444	3.715	5.498	5.386
	2:00	18	4.662	10.748	9.280	3.722	5.767	6.281
	3:00	19	4.816	12.088	10.882	3.873	5.862	6.310
	4:00	20	5.470	13.640	12.930	4.041	6.282	6.758
	5:00	21	5.572	14.086	13.376	4.126	6.254	6.860

Table 9: Trial 4, pH and Temperature over Time

Time	Hr	pH of Supernatants						Vol. of supernatant (mL)	T (°C)
		L1	L2	L3	R1	R2	R3		
8:00	0	-	-	-	-	-	6.68	900	26.5
9:00	1	7.36	7.32	7.72	7.84	7.30	6.88	895	26
10:00	2	7.47	7.48	7.79	7.74	7.30	7.03	890	25.5
11:00	3	7.45	7.50	7.71	7.61	7.29	6.95	888	25
12:00	4	7.43	7.53	7.65	7.49	7.35	6.87	886	24
13:00	5	7.35	7.52	7.63	7.36	7.29	7.01	884	24
14:00	6	7.30	7.51	7.60	7.25	7.26	6.94	882	24
15:00	7	7.26	7.52	7.59	7.19	7.27	7.00	880	24
16:00	8	7.31	7.52	7.58	7.15	7.29	7.07	878	24
17:00	9	7.28	7.52	7.56	7.11	7.32	7.10	876	23.5
18:00	10	7.26	7.53	7.57	7.09	7.30	7.11	874	24
19:00	11	7.22	7.53	7.55	7.11	7.28	7.12	872	23.5
20:00	12	7.10	7.45	7.54	7.18	7.29	7.10	870	23.5
21:00	13	7.21	7.54	7.57	7.21	7.28	7.17	868	23.5
22:00	14	7.19	7.55	7.55	7.25	7.30	7.19	866	24
23:00	15	7.22	7.54	7.54	7.28	7.30	7.16	864	23.5
0:00	16	7.26	7.60	7.55	7.31	7.25	7.09	862	23.5
1:00	17	7.21	7.55	7.56	7.33	7.28	7.14	860	24
2:00	18	7.31	7.57	7.55	7.35	7.28	7.04	858	24
3:00	19	7.32	7.56	7.54	7.35	7.27	7.08	856	24
4:00	20	7.29	7.57	7.56	7.31	7.27	7.10	854	24
5:00	21	7.29	7.54	7.56	7.29	7.30	7.12	852	24

APPENDIX C: TREND-LINES FOR TRIAL 3 & 4

Trial 3, Left ('L'):

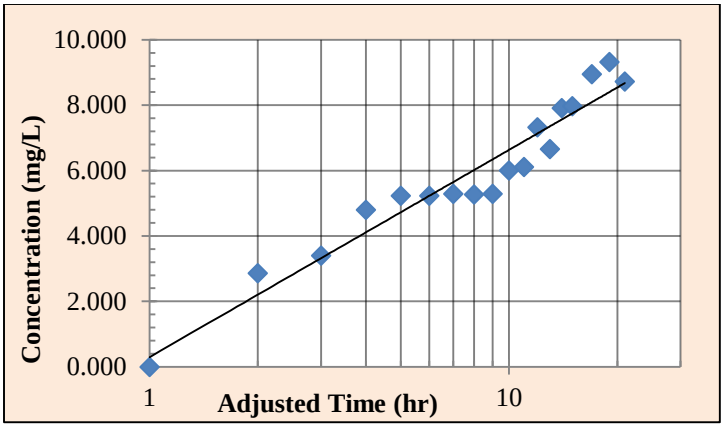


Figure 12: Trial 3, L1, Shifted

Trend-line:

$$[P] = 2.753 \ln(t + 1) + 0.2955$$
$$R^2 = 0.932$$

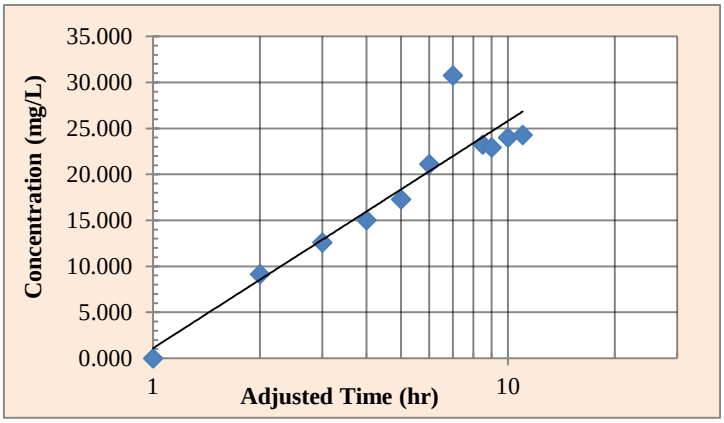


Figure 13: Trial 3, L2, Shifted

Trend-line:

$$[P] = 10.73 \ln(t + 1) + 1.095$$
$$R^2 = 0.872$$

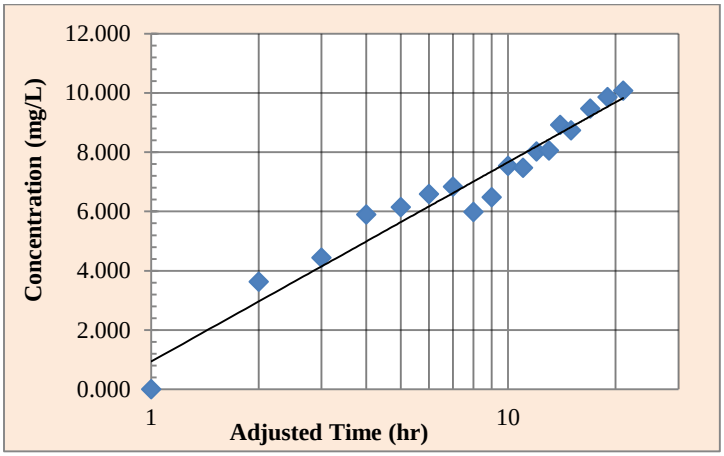


Figure 14: Trail3, L3, Shifted

Trend-line:

$$[P] = 2.920 \ln(t + 1) + 0.939$$
$$R^2 = 0.949$$

Trial 3, Right ('R'):

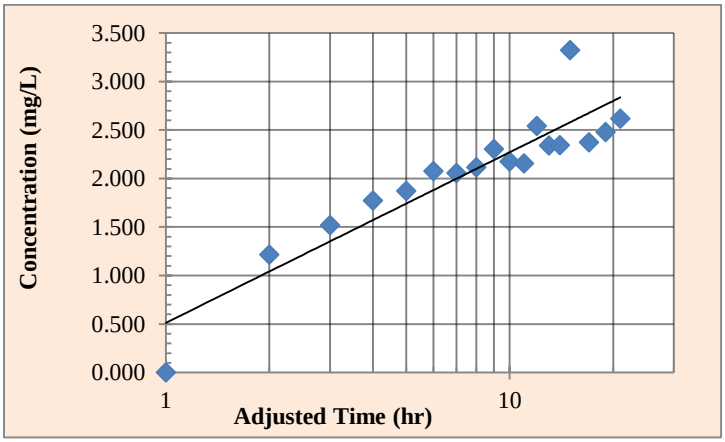


Figure 15: Trial 3, R1, Shifted

Trend-line:

$$[P] = 0.764 \ln(t + 1) + 0.513$$
$$R^2 = 0.836$$

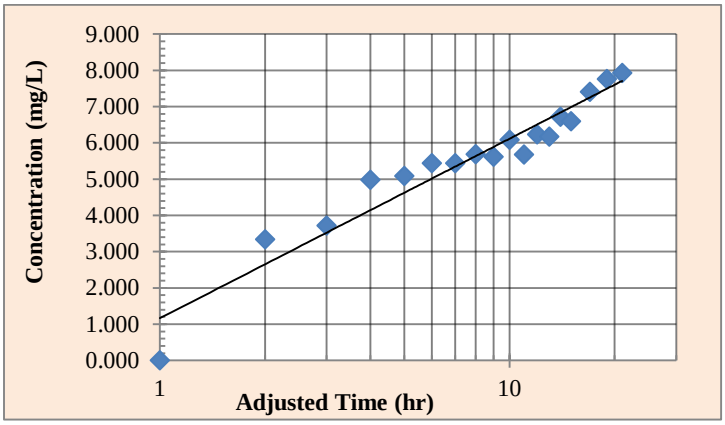


Figure 16: Trial 3, R2, Shifted

Trend-line:

$$[P] = 2.150 \ln(t + 1) + 1.164$$
$$R^2 = 0.929$$

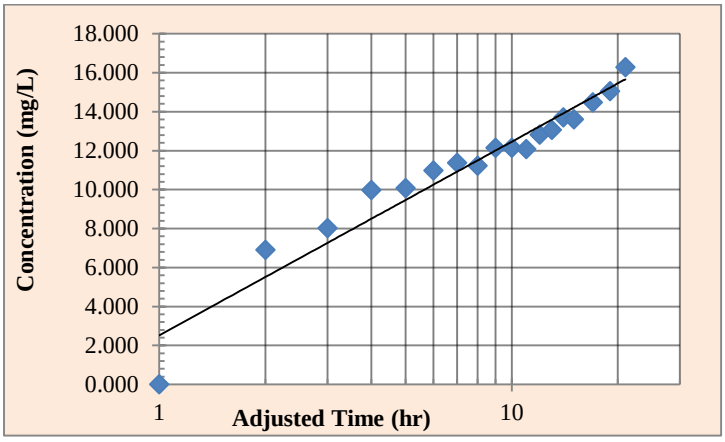


Figure 17: Trial 3, R3, Shifted

Trend-line:

$$[P] = 4.316 \ln(t + 1) + 2.523$$
$$R^2 = 0.938$$

Trial 4, Left ('L'):

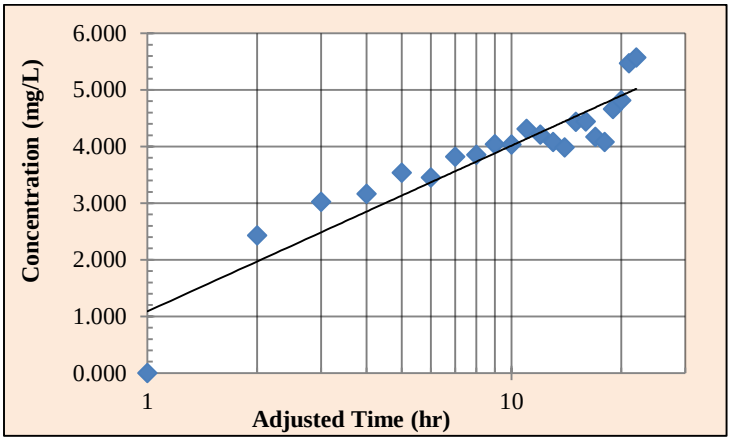


Figure 18: Trial 4, L1, Shifted

Trend-line:

$$[P] = 1.272\ln(t+1) + 1.087$$
$$R^2 = 0.860$$

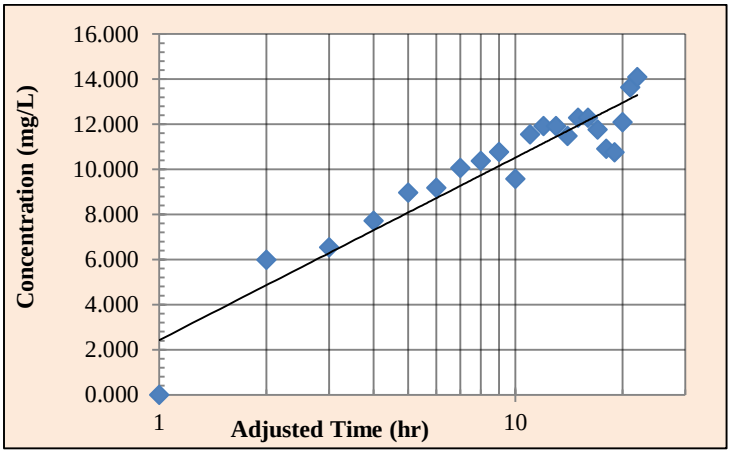


Figure 19: Trial 4, L2, Shifted

Trend-line:

$$[P] = 3.516\ln(t+1) + 2.427$$
$$R^2 = 0.893$$

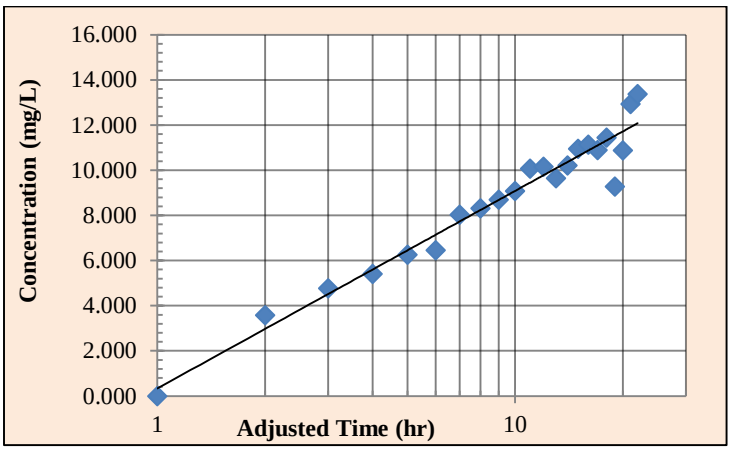
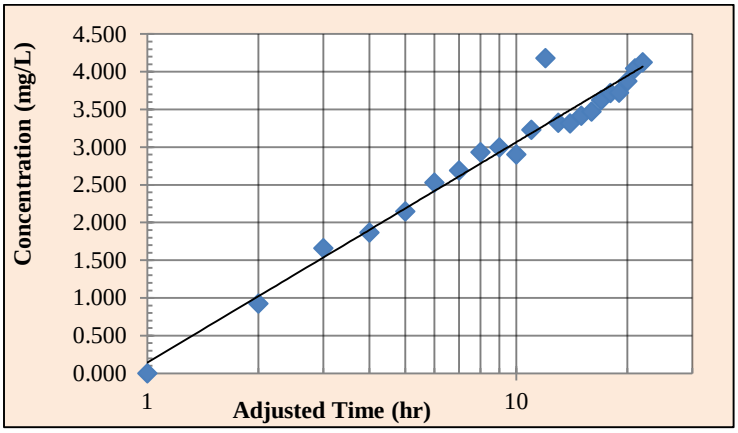


Figure 20: Trial 4, L3, Shifted

Trend-line:

$$[P] = 3.797\ln(t+1) + 0.340$$
$$R^2 = 0.951$$

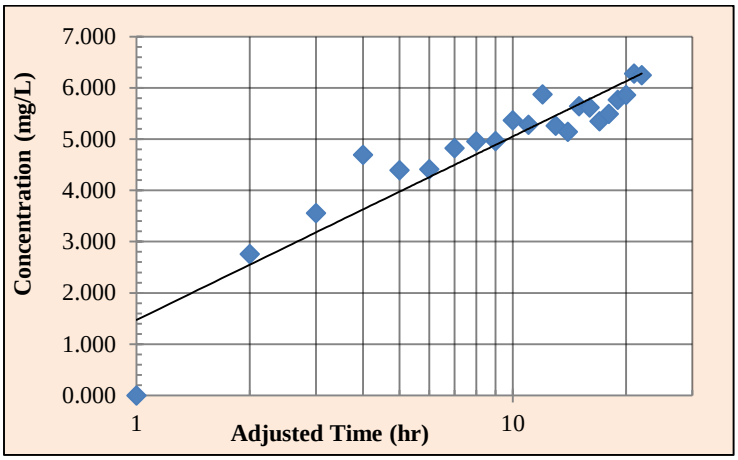
Trial 4, Right ('R'):



Trend-line:

$$[P] = 1.270 \ln(t + 1) + 0.142$$
$$R^2 = 0.956$$

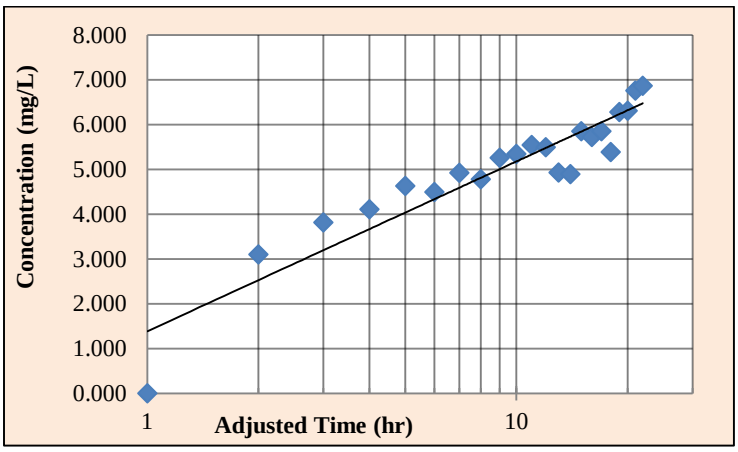
Figure 21: Trial 4, R1, Shifted



Trend-line:

$$[P] = 1.555 \ln(t + 1) + 1.474$$
$$R^2 = 0.869$$

Figure 22: Trial 4, R2, Shifted



Trend-line:

$$[P] = 1.646 \ln(t + 1) + 1.387$$
$$R^2 = 0.874$$

Figure 23: Trial 4, R3, Shifted

APPENDIX D: INITIAL/FINAL CONDITION APPROACH

General form of trend-lines:

$$[P] = A \cdot \ln(t + 1) + B$$

Table 10: Initial [P] and Constants of Trend-lines

	Trial 3			Trial 4		
	$[P]_{initial}$ (ppm)	A	B	$[P]_{initial}$ (ppm)	A	B
L1	162.21	2.753	0.296	146.69	1.272	1.087
L2	116.54	10.73	1.095	173.15	3.516	2.427
L3	148.09	2.92	0.939	108.59	3.797	0.34
R1	64.29	0.764	0.513	249.83	1.27	0.142
R2	95.77	2.15	1.164	241.4	1.555	1.474
R3	147.91	4.316	2.523	171.75	1.646	1.387

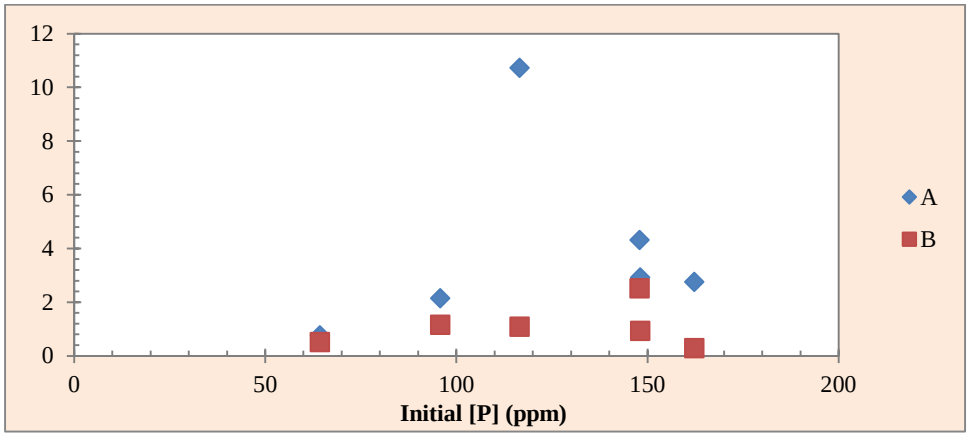


Figure 24: Trial 3, Initial [P] and Coefficients of Trend-line

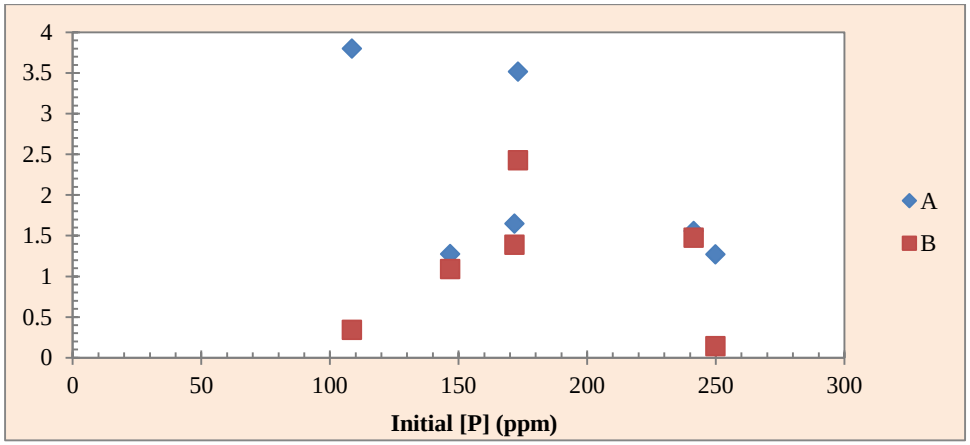


Figure 25: Trial 4, Initial [P] and Coefficients of Trend-line

APPENDIX E: ARITHMETRIC MEAN APPROACH

Table 11: L1, Arithmetic Mean of [P] over Time

Hr	[P] in supernatant (mg/L)				Coefficient of Variation (%)
	Trial 3	Trial 4	Avg	Stdev	
0	0.000	0.000	0.000	0.000	-
1	2.867	2.430	2.649	0.309	11.7%
2	3.403	3.022	3.212	0.270	8.4%
3	4.802	3.164	3.983	1.158	29.1%
4	5.235	3.537	4.386	1.200	27.4%
5	5.235	3.453	4.344	1.259	29.0%
6	5.294	3.817	4.556	1.044	22.9%
7	5.274	3.855	4.564	1.003	22.0%
8	5.294	4.041	4.668	0.885	19.0%
9	6.002	4.032	5.017	1.393	27.8%
10	6.110	4.312	5.211	1.271	24.4%
11	7.330	4.210	5.770	2.206	38.2%
12	6.664	4.079	5.371	1.828	34.0%
13	7.913	3.985	5.949	2.777	46.7%
14	7.974	4.434	6.204	2.504	40.4%
16	8.954	4.172	6.563	3.381	51.5%
18	9.321	4.662	6.992	3.294	47.1%
20	8.724	5.470	7.097	2.301	32.4%

Table 12: L2, Arithmetic Mean of [P] over Time

Hr	[P] in supernatant (mg/L)				Coefficient of Variation (%)
	Trial 3	Trial 4	Avg	Stdev	
0	0.000	0.000	0.000	0.000	
1	9.140	5.989	7.565	2.228	29.5%
2	12.585	6.532	9.558	4.281	44.8%
3	15.046	7.710	11.378	5.187	45.6%
4	17.311	8.970	13.140	5.898	44.9%
5	21.135	9.175	15.155	8.457	55.8%
6	30.758	10.057	20.408	14.638	71.7%
7	23.261	10.370	16.816	9.115	54.2%
8	22.936	10.767	16.851	8.605	51.1%
9	24.028	9.578	16.803	10.218	60.8%
10	24.294	11.543	17.918	9.016	50.3%

Table 13: L3, Arithmetic Mean of [P] over Time

Hr	[P] in supernatant (mg/L)				Coefficient of Variation (%)
	Trial 3	Trial 4	Avg	Stdev	
0	0.000	0.000	0.000	0.000	-
1	3.626	3.582	3.604	0.031	0.9%
2	4.440	4.765	4.602	0.230	5.0%
3	5.894	5.404	5.649	0.346	6.1%
4	6.140	6.263	6.201	0.087	1.4%
5	6.583	6.450	6.516	0.094	1.4%
6	6.838	8.026	7.432	0.839	11.3%
7	5.982	8.306	7.144	1.643	23.0%
8	6.474	8.703	7.589	1.576	20.8%
9	7.537	9.082	8.310	1.093	13.2%
10	7.468	10.073	8.771	1.842	21.0%
11	8.021	10.156	9.089	1.509	16.6%
12	8.059	9.644	8.851	1.121	12.7%
13	8.923	10.205	9.564	0.907	9.5%
14	8.740	10.948	9.844	1.562	15.9%
16	9.474	10.899	10.187	1.007	9.9%
18	9.857	9.280	9.568	0.408	4.3%
20	10.071	12.930	11.501	2.021	17.6%

Table 14: R1, Arithmetic Mean of [P] over Time

Hr	[P] in supernatant (mg/L)				Coefficient of Variation (%)
	Trial 3	Trial 4	Avg	Stdev	
0	0.000	0.000	0.000	0.000	-
1	1.213	0.923	1.068	0.205	19.2%
2	1.518	1.659	1.588	0.099	6.2%
3	1.771	1.867	1.819	0.068	3.7%
4	1.870	2.147	2.008	0.196	9.8%
5	2.076	2.529	2.303	0.321	13.9%
6	2.056	2.688	2.372	0.447	18.8%
7	2.115	2.931	2.523	0.576	22.8%
8	2.302	2.996	2.649	0.491	18.5%
9	2.175	2.903	2.539	0.515	20.3%
10	2.155	3.229	2.692	0.760	28.2%
11	2.540	4.178	3.359	1.158	34.5%
12	2.339	3.323	2.831	0.696	24.6%
13	2.342	3.313	2.828	0.687	24.3%
14	3.321	3.416	3.369	0.067	2.0%
16	2.372	3.621	2.997	0.883	29.5%
18	2.480	3.722	3.101	0.879	28.3%
20	2.617	4.041	3.329	1.007	30.2%

Table 15: R2, Arithmetic Mean of [P] over Time

Hr	[P] in supernatant (mg/L)				Coefficient of Variation (%)
	Trial 3	Trial 4	Avg	Stdev	
0	0.000	0.000	0.000	0.000	-
1	3.342	2.762	3.052	0.410	13.4%
2	3.715	3.558	3.636	0.110	3.0%
3	4.979	4.695	4.837	0.201	4.2%
4	5.087	4.396	4.742	0.489	10.3%
5	5.441	4.415	4.928	0.726	14.7%
6	5.441	4.826	5.133	0.435	8.5%
7	5.687	4.956	5.322	0.517	9.7%
8	5.618	4.966	5.292	0.462	8.7%
9	6.081	5.367	5.724	0.505	8.8%
10	5.677	5.283	5.480	0.279	5.1%
11	6.236	5.879	6.057	0.253	4.2%
12	6.173	5.264	5.719	0.643	11.2%
13	6.719	5.143	5.931	1.115	18.8%
14	6.597	5.647	6.122	0.672	11.0%
16	7.408	5.348	6.378	1.457	22.8%
18	7.760	5.767	6.764	1.409	20.8%
20	7.928	6.282	7.105	1.165	16.4%

Table 16: R3, Arithmetic Mean of [P] over Time

Hr	[P] in supernatant (mg/L)				Coefficient of Variation (%)
	Trial 3	Trial 4	Avg	Stdev	
0	0.000	0.000	0.000	0.000	-
1	6.907	3.100	5.004	2.692	53.8%
2	8.026	3.812	5.919	2.980	50.3%
3	9.970	4.107	7.038	4.146	58.9%
4	10.071	4.630	7.350	3.848	52.3%
5	10.976	4.490	7.733	4.587	59.3%
6	11.366	4.919	8.142	4.559	56.0%
7	11.227	4.779	8.003	4.560	57.0%
8	12.133	5.255	8.694	4.863	55.9%
9	12.145	5.339	8.742	4.813	55.1%
10	12.082	5.544	8.813	4.623	52.5%
11	12.812	5.488	9.150	5.178	56.6%
12	13.063	4.928	8.996	5.752	63.9%
13	13.699	4.891	9.295	6.228	67.0%
14	13.607	5.852	9.730	5.483	56.4%
16	14.479	5.852	10.166	6.100	60.0%
18	15.046	6.281	10.663	6.198	58.1%
20	16.285	6.758	11.522	6.737	58.5%

Plots of Arithmetic Means with Standard Deviation Bars (L1, L2, L3)

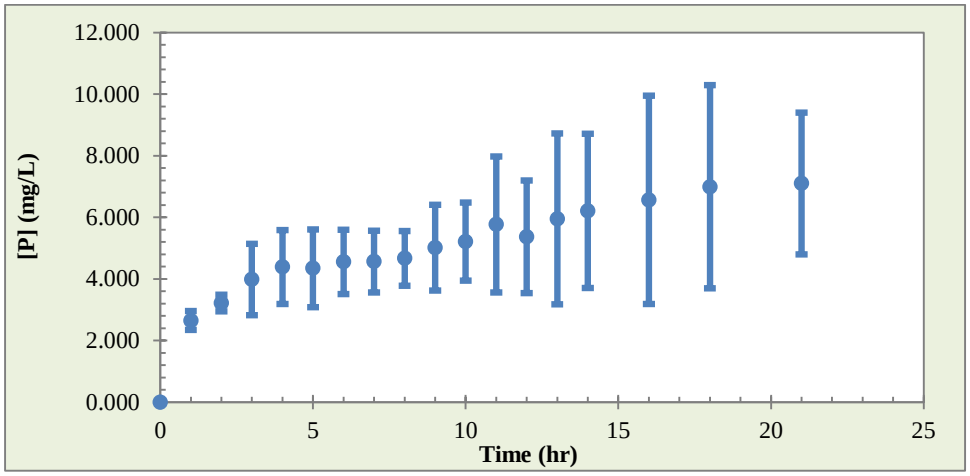


Figure 26: L1, Arithmetic Mean

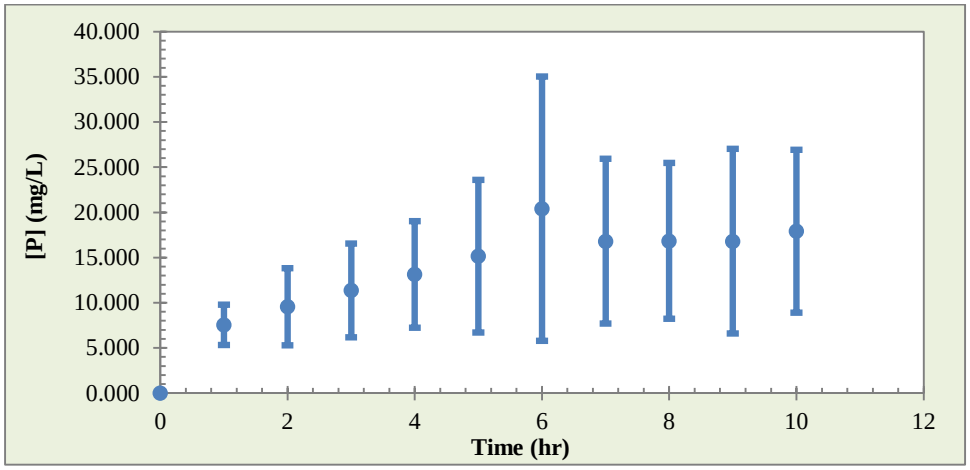


Figure 27: L2, Arithmetic Mean

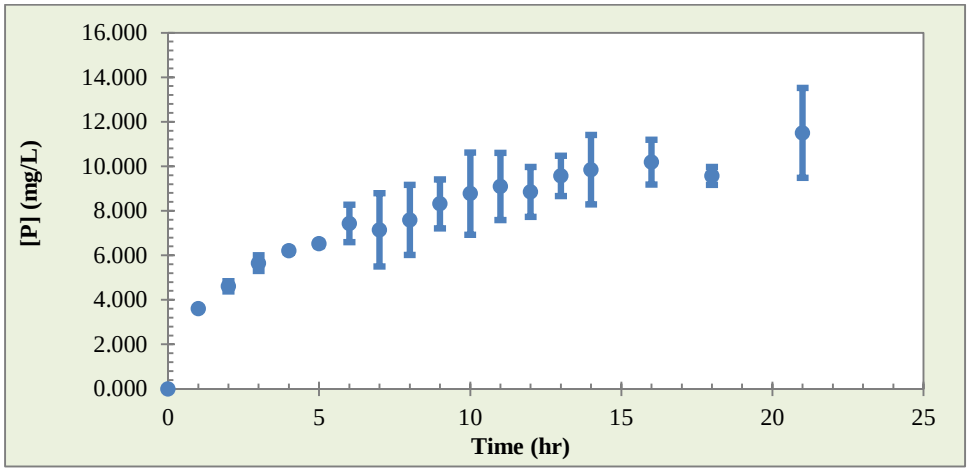


Figure 28: L3, Arithmetic Mean

Plots of Arithmetic Means with Standard Deviation Bars (R1, R2, R3)

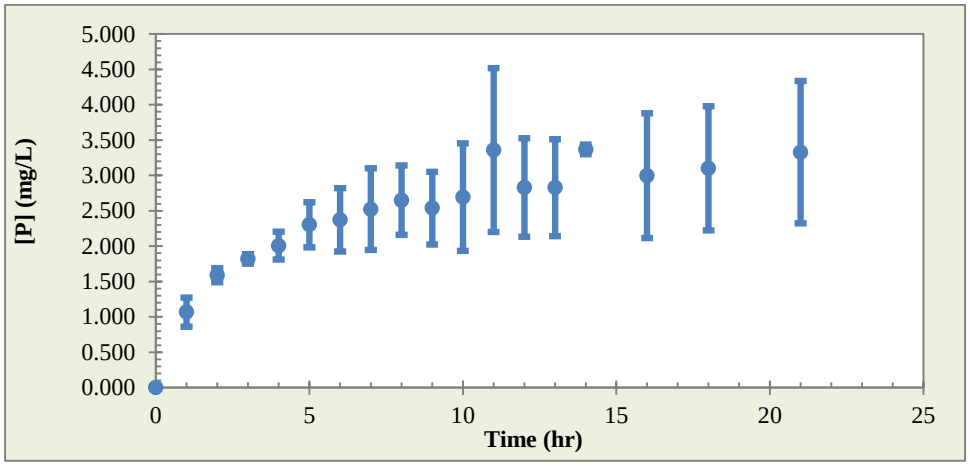


Figure 29: R1, Arithmetic Mean

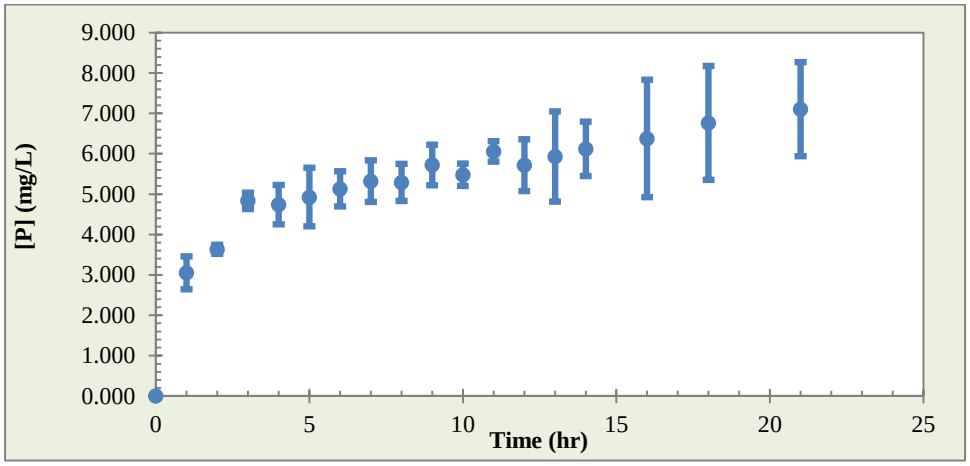


Figure 30: R2, Arithmetic Mean

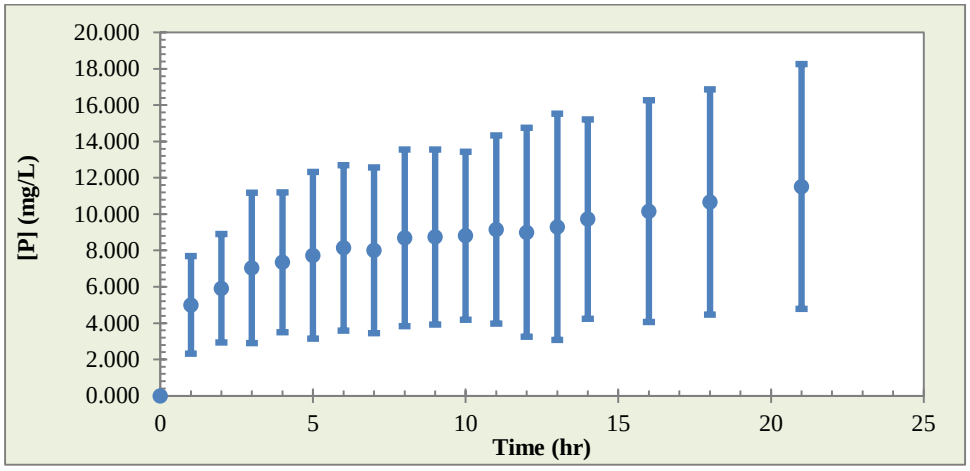


Figure 31: R3, Arithmetic Mean

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