BATCH EXPERIMENTS TO UNDERSTAND PHOSPHOROUS AND FINE PARTICLE INTERACTIONS

Undergraduate Senior Research Thesis
Submitted in partial fulfillment of the requirements for the
Bachelor of Science Degree with Research Distinction
At The Ohio State University

By

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2022

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TABLE OF CONTENTS

ABSTRACT

Due to the widespread use of fertilizers in agricultural regions of the world and rising nutrient levels in many streams, it is important to understand the downstream transport of nutrients like phosphorus (P), which depends strongly on interactions with fine particles in the water column and streambed. In the last decade, new tracers have emerged to help quantify the movement of particulates, and opportunities abound to use those tracers in combination with traditional KH_2PO_4 injections in streams. I aim to improve the combined use of these tracers through a series of benchtop experiments involving KH_2PO_4, Dayglo fine particles, and the surfactant needed to mix the particles (Sodium Hexametaphosphate, or SHMP). In the first set of experiments, I measured the contribution of dissolved P from the dissociation of SHMP in deionized water and stream water. The SHMP added, on average, around 0.0040 ppb of soluble reactive phosphorous (SRP) for every ppb of SHMP added. A second set of experiments was performed to test the relationship between Dayglo particle counts from cytometry and mass concentrations. Results show a logarithmic relationship between mass concentration and particle count that can be used to relate the two units. Lastly, batch experiments were performed to determine P sorption characteristics of Dayglo particles. The data follow a Langmuir model. SRP appears to have a greater sorption affinity for Dayglo particles, compared to natural sediments. As a thermoplastic, Dayglo particles are a novel material for sorption studies. Together, these results can be used to guide future stream injection experiments and suggest that Dayglo behaves like a sorptive particle.

ACKNOWLEDGEMENTS

This project was supported with funding from the USDA-NRCS Conservation Effects Assessment Project in collaboration with USDA-Agricultural Research Service.

Funds were also provided by the McKenzie-Brecher Scholarship through the Byrd Polar and Climate Research Center. I would like to thank Michele Cook for her help administering these funds.

I would also like to thank the Joan Echols Scholarship Fund and the Ohio State School of Earth Sciences for their generous support in my pursuit of higher education.

Throughout the writing of this thesis, I have received a great deal of support and assistance from mentors and colleagues alike. I would first and foremost like to express my special appreciation to my advisor, Dr. Audrey Sawyer, whose expertise was invaluable in formulating my research topic and methodology. Your insightful feedback pushed me to sharpen my thinking and brought my work to a higher level. It has been an honor to work with you these past two years.

I would also like to thank my graduate student mentors Hannah Field and Devan Mathie for guiding me through this project. This project would not be where it is today without your guidance and knowledge. Thank you for the many hours instructing me, giving feedback, and helping with lab work.

Many thanks to Ethan Pawlowski, Dr. Gabor, Dr. Welch, Dr. Hood, Dr. Karwan, and Dr. Lyons. Your insight and suggestions greatly helped this project and allowed me to grow as a researcher and Hydrogeologist.

I would also like to extend thanks to Zackary Johnson, an associate professor in the Nicholas School of the Environment at Duke University for his assistance in completing flow cytometry at the Johnson Lab.

I would like to thank all the members of my lab group, including Hannah Field, Lauren Decker, René Castillo, Christian Roumelis, Kat Meiner, Bri Tomko, and Xiaolang Zhang who assisted and supported me in all my work the past two years.

To my mother and father, thank you for all your constant support and for raising me to be the man I am today, I couldn't have made it this far without you.

List of Figures

List of Tables

1. INTRODUCTION

Every year, hundreds of kilograms of fertilizer are applied to every acre of agricultural land across Ohio, most of which exceeds the needs of the crops (Sharpley et al. 1999). Surface and subsurface flows deliver some of the excess nutrients from fertilizers to small agricultural streams, which connect cropland to larger water bodies such as the Maumee River and Lake Erie. These nutrient loads lead to eutrophication and the formation of harmful algal blooms (HABs) (Michalak et al. 2013). Phosphorus (P) is temporarily stored in soils (Dunne et al. 2007; Powers et al. 2016) and small streams and ditches (Casillas-Ituarte et al. 2020) as "legacy P" (Osterholz et al. 2020). It is currently unclear how long it takes this P to move through the landscape to large rivers and lakes. We must understand P transport to set realistic water quality improvement goals and prioritize best management practices.

There are many factors which must be considered when determining the downstream travel time of P. While P transport is influenced by advection and dispersion, P can also sorb to sediments and can be transported as particulate P (PP) or dissolved P (DP) (Dolph et al. 2019). Metal oxides are especially effective sorption sites for P and readily dissolve under reducing conditions (Li et al. 2016), leading to the remobilization of P as dissolved P. Dissolved P is more bioavailable than particulate P and is readily taken up by microbes and plants. This P can later be mineralized upon decomposition, delaying the arrival of the P downstream. All these processes must be considered to optimally model the transportation of P in agricultural streams.

Stream injection experiments have been used for decades to understand P transport, sorption, and uptake (collectively termed "P spiralling") (Newbold et al.1981). Stream injections utilize a combination of conservative and reactive tracers to characterize these downstream processes. Relatively new advancements have been made in the study of particle transport by introducing particulate tracers such as Dayglo fluorescent particles (Drummond et al. 2016), titanium dioxide (Karwan & Saiers 2009), and dyed corn pollen and yeast (Georgian et al. 2003). Recent studies aim to combine dissolved P and particle tracers to understand how both dissolved and particulate forms of P move throughout streams, but to do so, one must understand how these tracers interact. There are several models, including OTIS (Runkel 1998) and mobile-immobile domain continuous-time random walk approaches (Boano et al. 2007; Roche et al. 2019), which serve to describe tracer movement. To use them with particle reactive solutes and particles, it is essential to parameterize the interactions between the tracers (Margolin et al. 2003). Here, I present a series of benchtop experiments to understand and quantify interactions between phosphate (PO_4^{3-}) and an increasingly used particle tracer, Dayglo fluorescent pigments. Because of the hydrophobic nature of the tracer, it must be mixed with a surfactant, sodium hexametaphosphate or SHMP ($(NaPO_3)_6$), which acts as an additional source of SRP. I begin by presenting experiments to understand the behavior of SHMP as a source of SRP. I next present experiments to understand relationships between particle mass concentrations and counts. Finally, I present batch experiments to test the sorption behavior of SRP on Dayglo particles.

2. Site Description

Stream water for batch experiments was collected from an agricultural headwater stream located in northwest Ohio that is the focus of ongoing tracer injection experiments involving particles and dissolved P. The experimental reach (41.03355°N, 84.39693°W) is approximately 200 m long and has a meandering form. The channel has a typical width of 1-2 m and depth of several cm to ~30 cm and contains pools and narrow riffles. Reed grass lines either side of the stream and causes local bifurcations in the channel. The substrate is primarily composed of unconsolidated silt- to clay-sized sediments that are easily suspended during periods of increased discharge, leading to increases in turbidity. A hog farm is located approximately 1 km upstream of the study site.

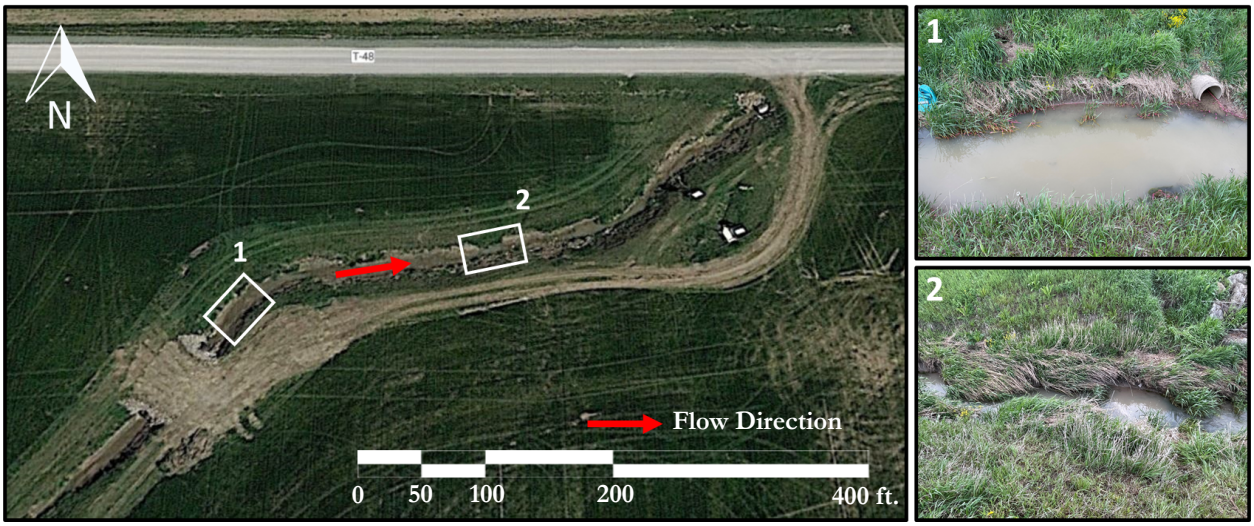

Figure 2.1: Study Area

This 200-m stretch of agricultural stream is the location of ongoing tracer injections.

3. METHODS

3.1 Stream Water Collection

Approximately 1.5 L of unfiltered stream water was collected from the study site on December 2, 2021 and transported in pre-rinsed 1 L and 250 mL Nalgene bottles back to campus for immediate refrigeration at 4 °C. Water quality (Temperature, pH, conductivity, dissolved oxygen, and ambient dissolved P) was measured with a YSI probe in the field at the time of sample collection.

3.2 SHMP Experiments

It is known that SHMP depolymerizes in aqueous solution to form sodium trimetaphosphate ($Na_3P_3O_9$) and sodium orthophosphates (Felts and Bucks, 1981) and therefore acts as an additional source of SRP. To understand this behavior, a series of batch experiments was conducted to test how varying concentrations of SHMP affect measurable SRP. Four sets of 10 mL samples were created, each containing four pairs of duplicates (8 samples per set, 32 samples total) with target SHMP concentrations of 3000, 6000, 12000, and 24000 parts per billion (ppb) to capture a wide range of concentrations used in field injections. One set included only SHMP and deionized (DI) water, the second set contained only SHMP and unfiltered stream water, the third set contained SHMP and a constant concentration of 500 ppb of KH_2PO_4 in DI water, and the final set contained SHMP and a constant concentration of 500 ppb of KH_2PO_4 in unfiltered stream water. Added concentrations in each batch sample, determined based on masses, are provided in Table B.1 (Appendix B). All sets were mixed by inversion three times before being refrigerated until they could be analyzed for soluble reactive phosphorous (SRP) using a Skalar San^{++} Continuous Flow Analyzer and 1050 Sampler. A Skalar Continuous Flow Analyzer and the Dionex ICS-2100 ion chromatograph were shown to return very similar SRP measurements for samples containing SHMP, with a regression of 0.9747, as shown in Figure B.1 (Appendix B). Given this, the Skalar produced more reproducible results, so it was chosen to obtain SRP measurements in this study.

Samples were analyzed using the molybdenum blue method. Namely, orthophosphate reacts under acidic conditions with molybdate and antimony potassium tartrate. The resultant complex is reduced with ascorbic acid, allowing color development to be measured (Nagul et al. 2015). The peaks created from the color development are then converted into the concentration of orthophosphate. While the method should solely measure orthophosphate, the degradation of SHMP in this experiment could contribute to the color signal. Instrument precision is measured on a regular basis using a standard provided by the USGS. The last test of precision was performed in October of 2015. A sample with a most probably value of 0.412 mg/L yielded a measured value of 0.403 mg/L, a -2.18 % difference.

3.3 Particle Experiments

To evaluate the relationship between Dayglo mass concentrations and particle counts, Dayglo particles and DI water were used to create a stock solution with concentration of 1.0 g/L. From this stock, 8 samples were created using a 10x dilution series to span a large range of particle concentrations. All pipetting steps were performed on a scale to calculate the final mass concentrations. The samples were then sent to the Johnson Lab at Duke University for analysis via flow cytometry to attain particle counts.

In addition to this dilution series, I also performed a small test to understand the potential to measure mass concentrations from oven drying. A set of 14 samples were prepared by mixing

unfiltered stream water with a target Dayglo concentration of 30 mg/L and SHMP concentration of 12 ppm to mimic concentrations used in field experiments. All samples were centrifuged, and excess water was removed with a syringe. For 6 samples, the mixture that remained in the centrifuge tube was rinsed into numbered, pre-weighed aluminum tins using DI water. Tins were dried in an oven at 70 °C for 72 hours. After baking, tins were removed and weighed again for dry mass of Dayglo fine particles. For the other 8 samples, the mixture that remained in the centrifuge tubes was sent for analysis using flow cytometry at Johnson Lab

3.4 Sorption Experiments

To test the sorption behavior of SRP on Dayglo particles, a series of batch experiments were created with varying concentrations of phosphate-P (from KH_2PO_4) and constant concentrations of Dayglo. An initial run was conducted with no surfactant (SHMP) in DI water to simplify potential sources of SRP. Target phosphate concentrations of 200 to 1000 ppb and a target Dayglo concentration of 500 mg/L were chosen to span the range of concentrations during stream tracer injections at the same site. A second run was conducted in unfiltered stream water using SHMP, but results were inconclusive and are provided in Figure B.2 (Appendix B). In this second run, target phosphate concentrations of 250 to 2400 ppb were used, and target Dayglo and SHMP concentrations were 30 ppm and 12 ppm, respectively. These concentrations were again chosen to mimic concentrations achieved in field experiments. Batch experiments with unfiltered stream water were conducted approximately 9 weeks after sample collection. A Standard Operating Procedure is provided in Appendix A. In brief, for the first run with DI water, two stock solutions were created for Dayglo and phosphate. The Dayglo stock solution was pipetted into pre-weighed sterile 50 mL Falcon® polypropylene centrifuge tubes. The tubes were again weighed and then filled to the desired volume for each tube with DI water. The phosphate stock solution was pipetted directly before mixing. For the second run with unfiltered stream water, a third stock solution was created for SHMP. The SMHP and Dayglo stock solutions were pipetted into pre-weighed sterile 50 mL Falcon® polypropylene centrifuge tubes. The tubes were again weighed and then filled to the desired volume for each tube with either deionized water or stream water. The phosphate stock solution was pipetted directly before mixing. Added concentrations in each batch sample, determined based on masses, are provided in Table B.2 and B.3 (Appendix B).

Samples were mixed on a ThermoFisher orbital shaker for 24 hours at room temperature. This mixing time was chosen based on equilibrium mixing times observed in previous sorption experiments (Lai & Lam 2009). After mixing, the tubes were centrifuged, and 15 mL were collected from each centrifuge tube using a sterile 30 mL syringe (SOP, Appendix A) and filtered to 0.45 μm into a second sterile 15 mL Falcon® centrifuge tube. The centrifuged, filtered samples were analyzed for SRP using a Skalar San^{++} Continuous Flow Analyzer and 1050 Sampler approximately 2 months after the conclusion of the experiments.

Dayglo particle concentrations were estimated on a mass basis from oven drying and a particle count basis from flow cytometry (see section 3.2).

4. Results

4.1 Stream Water Chemistry

The stream water had a moderate specific conductivity and was circumneutral. The concentration of dissolved oxygen was approaching saturation (Table 4.1). Dissolved P concentration was characteristic of many agricultural streams in the area.

Parameter	Injection Date: December 4, 2021
Temperature (°C)	3.8 – 7.4
pH	6.3
Oxidation reduction potential (mV)	225.4
Dissolved oxygen (mg/L)	8.2
Specific conductivity (µS/cm)	605.5
Filtered ambient dissolved P (ppb)	19.4

Table 4.1: Background water chemistry measurements of stream water collected from the field site.

4.2 SHMP Experiments

Measured SRP increases linearly and consistently with SHMP concentration, regardless of whether the solution is mixed using stream water or DI water and whether there is additional KH_2PO_4 (Figure 4.2). For every ppm of SHMP, there is approximately a 4.0 ppb increase in the SRP measured. The regressions of all series are very high, ranging from 0.9172 at the least to 0.9981 at the greatest, so these results are robust. For two series with additional KH_2PO_4, an offset of 500 ppb of DP should have presumably been observed, but these series have intercepts that are only 260 and 370 ppb greater.

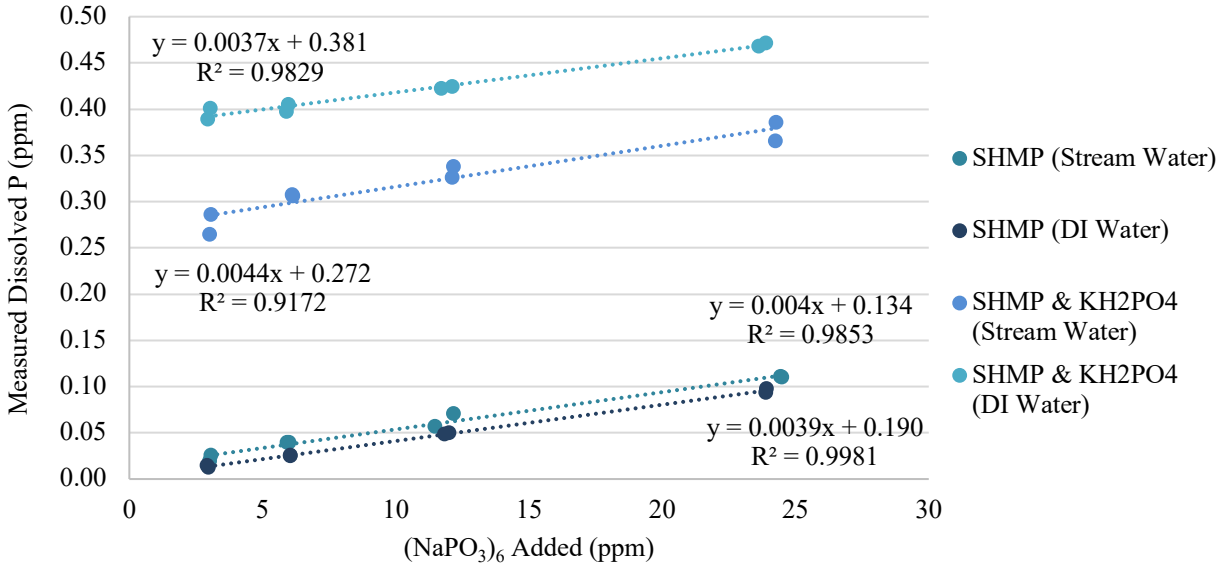

Figure 4.2: Batch experiments to assess impact of SHMP ($(NaPO_3)_6$) on measured SRP concentrations.

4.3 Particle-Mass Relationship

Over a wide range of concentrations above 10 µg/L, a logarithmic relationship exists between the mass concentration of Dayglo and particle counts using cytometry. One sample at a much smaller concentration of 1.0 µg/L does not fit the trend, likely because of limits in resolution or precision for mass-based measurements, cytometry-based measurements, or both at the most dilute concentrations. Concentrations greater than 100 mg/L were excluded due to inaccuracy from large dilutions.

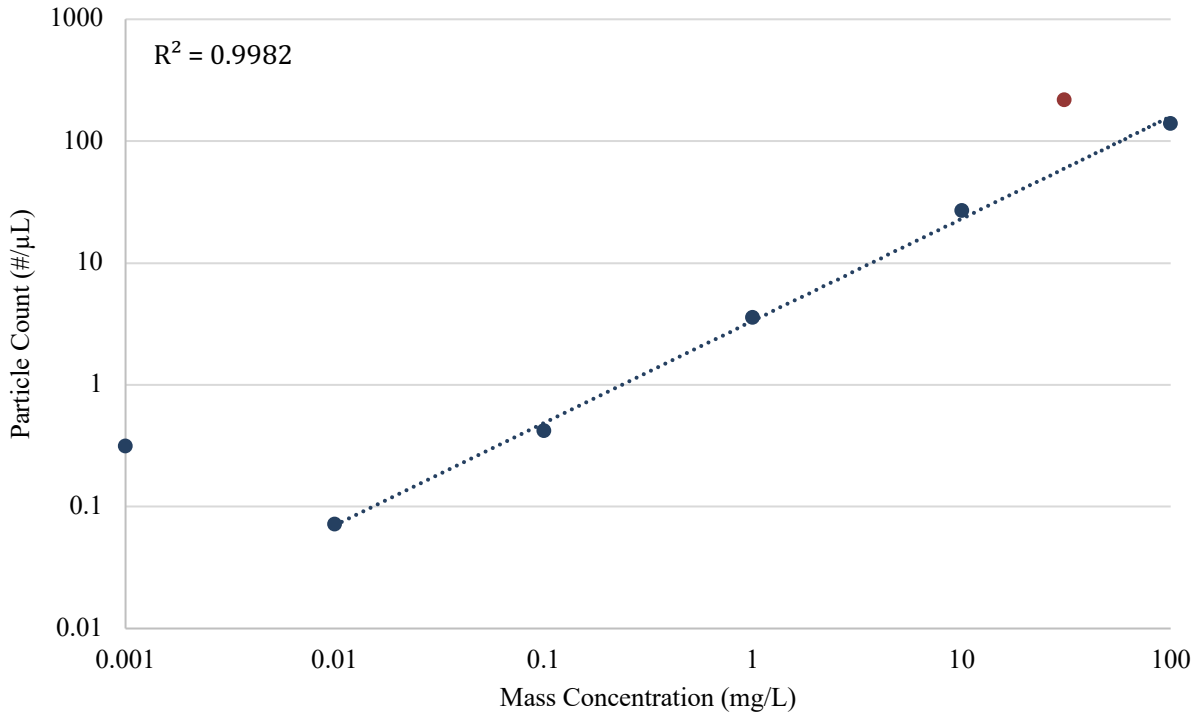

Figure 4.3.1: Dilution experiments suggests a strong logarithmic relationship between mass concentration and particle counts for Dayglo particles above approximately 0.01 mg/L, where [particle count (#/µL)] = 3.339 * [mass concentration (mg/L)]$^{0.839}$. The orange point shows the average mass concentration and particle count from the replicate samples (Figure 4.3.2).

Examining many replicate samples with the same concentration (Figure 4.3.2), particle concentrations on a mass basis have a mean of 30.9 mg/L and a standard deviation of 0.87 mg/L. Particle counts based on cytometry have a mean of 218 particles per microliter and a standard deviation of 34.41 particles. The average concentration for these replicates also loosely follows the trend shown in the dilution experiments (Figure 4.3.1).

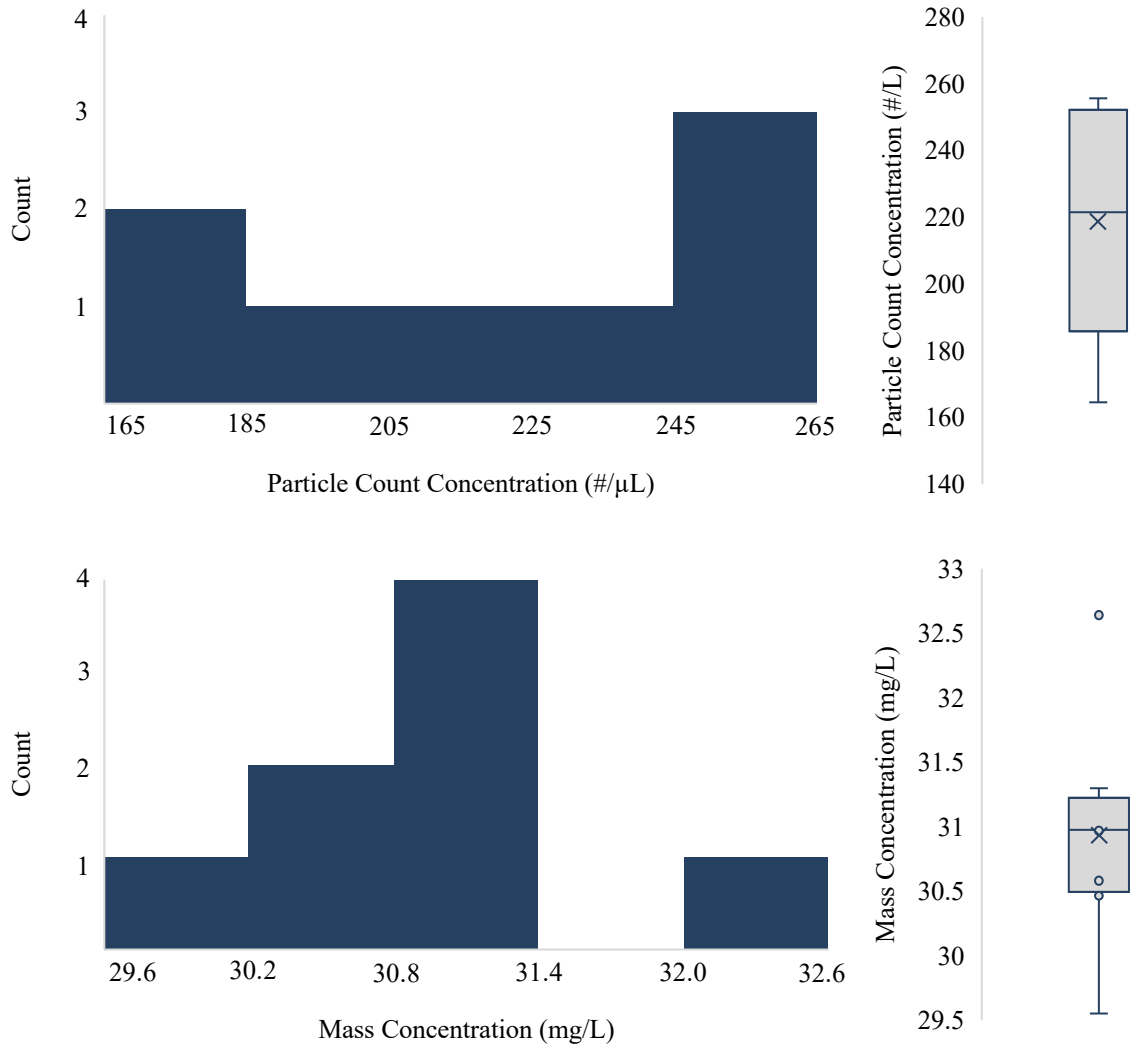

Figure 4.3.2: (Top) Distribution of Dayglo particle counts obtained from flow cytometry. (Bottom) Distribution of Dayglo particle concentration based on mass.

4.4 Sorption Isotherms

The isotherm created using DI water was visually fit with a Langmuir isotherm model (Figure 4.4) with a sorption maximum (S_{max}) of 28 mg/g and a bonding energy (k) of 0.004 L/µg, where:

$$S = S_{max} * kC/[1 + kC]$$

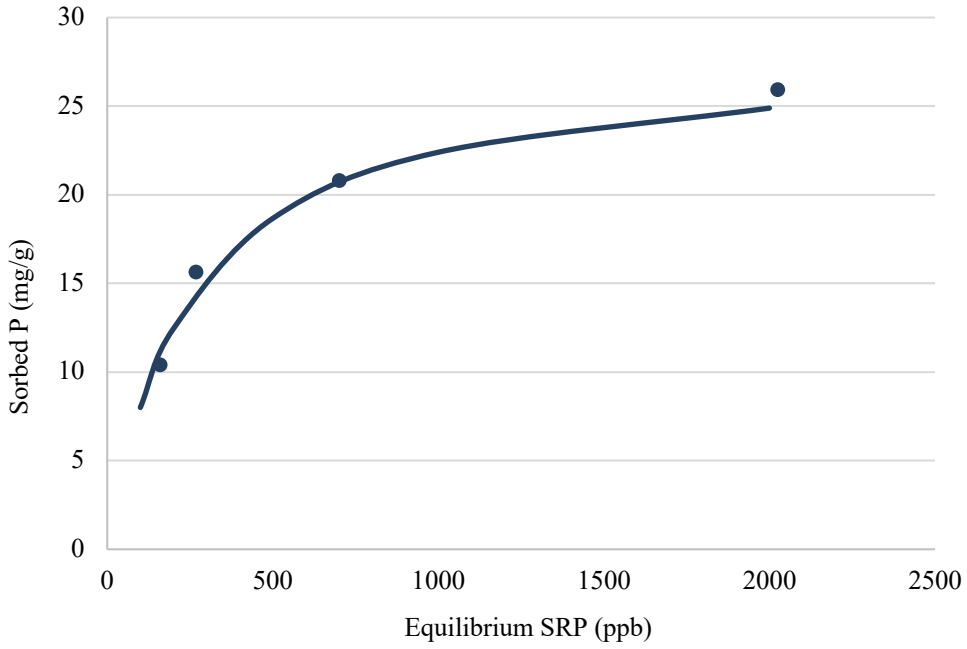

Figure 4.4: Batch experiments to assess sorption of SRP on Dayglo fine particles follow a Langmuir isotherm.

The isotherm created using stream water shows a linear behavior and suggests there is an unknown but constant source of SRP to the samples (Appendix B). Because of this unaccounted source, no isotherm model was fitted to the data.

Discussion

Dayglo fluorescent pigments are an emerging tracer for studying particle transport in streams that has great potential to be combined with other nutrients like SRP. My experiments reveal several important considerations for combining these tracers: 1) the surfactant that is commonly used with Dayglo, SHMP, acts as a substantial additional source of SRP into water, 2) particle mass concentrations and counts are well correlated, but cytometry appears to be a more accurate measurement approach with lower detection limits, and 3) SRP appears to have some sorption affinity for Dayglo particles following a Langmuir model analysis.

First, the surfactant SHMP acts as a substantial source of SRP in water (Figure 4.1). The chemical structure of SHMP is a ring of six phosphates, meaning that approximately 30% of the SHMP molecule, by mass, is P. SHMP could thus add up to 0.3 ppb of SRP for every ppb of SHMP if complete dissociation occurred. I instead observed about 0.004 ppb of SRP measured per ppb of SHMP added. This represents approximately 1.33% of the available P in the molecular formula, so there is still a large proportion of P that does not readily dissociate from the phosphate ring. Without further testing, it is unclear what conditions cause the bonds between these phosphates to break down and depolymerize from one another. It is known that more SHMP dissociates at a greater pH value (McMcullough et al. 1956). Thus, adding SHMP to de-ionized water should raise the pH, which should in turn cause additional SHMP dissociation. Measuring the pH of samples would help clarify this behavior, but I did not measure pH at the time the samples were analyzed. Running additional experiments with stream water of differing pH and chemistry would also help clarify the contribution of SHMP to the SRP measured. This contribution of SHMP to SRP at least partially explains excessive concentrations of SRP measured in the other batch experiments and would also contribute to excess SRP in field experiments. While the exact behavior of SHMP in water of different chemistries requires more testing, the approximate trends (Figure 4.2) are helpful for planning SHMP and potassium phosphate additions in field experiments.

Second, Dayglo concentrations can be measured on a mass basis or using counts with cytometry, but the latter appears to be more precise, especially at low concentrations (Figures 4.3.1 and 4.3.2). Though cytometry is more costly and time-intensive, it is the optimal approach for concentrations on the order of 0.01 mg/L or less. At much greater concentrations, mass-based estimates are cheaper and less time-intensive. One additional approach for measuring Dayglo concentration is fluorescence, as Dayglo fluoresces with an emission peak at 365 nm. This approach is particularly attractive due to the rapidness and affordability of fluorescence measurements. The relationship between fluorescence and concentrations is an important area for future work.

Third, the data from sorption experiments appears to follow a Langmuir isotherm model. The sorption of P to natural sediments has been fitted with Langmuir, Freundlich, and linear isotherm models. The Langmuir isotherm assumes the occurrence of surface adsorption while the Freundlich isotherm also accounts for chemosorption (Lai & Lam 2009). Linear trends have been seen before on some natural sediments in agricultural streams in the Midwest. In the study by McDaniel et al. (2009) conducted in Illinois, there is a strong linear relationship between the P adsorbed and equilibrium P concentrations for sediments in stream water and deionized water. However, many studies (Lai & Lam 2009; Badiou et al. 2018; McDonald 2018) have applied Langmuir models to SRP-sediment sorption data. Here, the isotherm for P sorption on Dayglo reflects a greater sorption affinity with P. Specifically, S_{max} tends to be greater than the range seen in several studies of agricultural and wetland types of streams throughout North America, precisely the Midwest region, and Hong Kong, and k also tends to be greater (Lai & Lam 2009; Badiou et al. 2018; McDonald

2018). These results do not consider the effects of stream water chemistry (for example, pH) on isotherm behavior. The second run of batch experiments in stream water would have revealed any differences in isotherm behavior, if any, but results did not behave in an interpretable way. This could be due to the uncertainties involved with using unfiltered stream water. Biologic growth and natural particles could have served as additional sources and sinks of SRP in the samples. The measured concentration of SRP at equilibrium was greater than what I aimed to introduce to the samples, so there was an unexpected source of P in the samples (Appendix B). It is possible that Dayglo acted as a source of P. Dayglo particles are composed of a thermoplastic resin which have been shown to have high amounts of sorption with heavy metals and organic chemicals because of their high surface area-to-volume ratio (Verla et al. 2019). Perhaps some P was already sorbed on the Dayglo particles. Future experiments will isolate for all potential P sources.

In general, there were some challenges associated with the use of Dayglo particles. Like most thermoplastics (Zilio et al 2014), they are hydrophobic, necessitating the use of SHMP to keep the Dayglo from sticking during field injection experiments and the benchtop experiments here. SHMP helps to suspend the particles but creates uncertainties with P sources in nutrient studies. The "stickiness" of the particles also makes it difficult to accurately add precise concentrations of the particles to the batch experiment samples. Because of their light weight, a stock was created to add the Dayglo to the centrifuge tubes. The pipetting of the stock could cause errors from Dayglo particles adhering to the walls of the disposable pipette tips.

Conclusions

Together, the presented results can be used to guide future stream injection experiments and suggest that SRP sorbs readily to Dayglo and follows a Langmuir model. To work with this hydrophobic particle, a surfactant is required that acts as an additional source of P, releasing around 0.0040 ppb of SRP for every ppb of SHMP added. Knowing this can help scientists plan for and achieve target concentrations of P in field injection experiments. Finally, a consistent relationship exists between particle mass concentrations and counts, suggesting the two can be related interchangeably through a power-law relationship. Cytometry is highly accurate for analyzing Dayglo particle concentrations at concentrations ranging from 0.01-100 mg/L, while mass concentrations become impractical to measure in the lower end of this range.

RECOMMENDATIONS FOR FUTURE WORK

These batch experiments are an important initial step in understanding P interactions with particle tracers, but many questions remain. First, the dissociation of SHMP into orthophosphate and pyrophosphate is dependent on the pH of the solution (McCullough et al. 1956). Sorption characteristics are also known to vary with pH. While the stream water used for the batch experiments was fairly neutral (6.3), the pH of natural streams varies widely, and the results of these experiments may not be applicable to other streams or even the same stream under different seasons and flow conditions. Second, many other tracers have been used in stream injection studies, including dyed yeast, dyed corn pollen (Shogren et al. 2019), and titanium dioxide (Karwan & Saiers 2009). These tracers have various advantages and disadvantages as model stream particles (Karwan & Saiers 2009). One of the major drawbacks of using Dayglo fluorescent particles as a tracer is that they are a microplastic that, when released into the environment, contribute to the rapidly expanding load of microplastics to aquatic systems. While these particles have been shown to be safe to aquatic and terrestrial species (Grayson & Roe 2009), research on the toxicology of microplastics is in an early stage. Future work should repeat similar batch experiments with a collection of model stream particles, including yeast and corn pollen, as the use of these alternative model particles may increase over time. Other solutes besides SRP also have mild to strong sorption affinity and are commonly used in stream tracer studies. Future work should also repeat batch experiments for Rhodamine dye, which has been used as a conservative solute in stream injection studies for decades (Garklavs & Toler 1985) even though it weakly sorbs (Kausar et al 2021), and ammonium, which is commonly used to understand nitrogen transport in stream injection studies (Ashkenas et al. 2004). Expanding these batch experiments will allow the scientific community to employ novel mixtures of stream tracers for understanding a variety of solute and particulate transport processes in streams.

Lastly, sorption batch experiments should be repeated for Dayglo particles and P using stream water and surfactant in order to replicate tracer injection conditions in the field. In my initial attempt (Appendix B), the stream water was unfiltered, and biological processes may have contributed to the unexpectedly large equilibrium SRP concentrations. To address this inconsistency, future studies should filter stream water at the time of collection or before experiments begin. Another option is to create a simulated stream water using a calcium chloride matrix as other sorption studies have done previously (King et al. 2021).

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APPENDIX A

Standard Operating Procedure for Dayglo Particle Sorption and Isotherm Experiments

*Prior to running sorption and isotherm experiments, the nutrient samples collected at each site must be analyzed for the ambient phosphorous concentration.

-See the Soluble Reactive Phosphorous Protocol for guidance.

-Keep what is left of the secondary standard (100 μM Phosphorous) from the analysis in a labeled centrifuge tube and store in the refrigerator until needed.

*It is recommended that the Centrifuge and Shaker Table Protocol is read prior to carrying out these experiments.

Experimental Set-Up:

The day before the experiments are to be run, prepare the experiment tubes:

1. Prepare and label the experimental 50 mL centrifuge tubes and 15 mL sample tubes.
   a. Use the respective Excel document to create labels.

      -Two identical sets of labels will be needed for each sample; one set is for the centrifuge tubes; the other set is for the sample tubes that the centrifuge tube samples will be filtered into.

   b. For the centrifuge tubes:
      -Wrap the labels for the isotherm experiments around the top of the tube.
      -Place the labels for the time point experiments lengthwise along the side of the tube with the time point (in minutes) closest to the lid.
      -Use an empty scintillation vial box to organize and hold the centrifuge tubes upright.
   c. Keep the labeled sample tubes organized in their respective groups in their box until needed.

Prepare the stock standards:

Primary Phosphate Standard – 0.5 g/L

1. Calculate the mass of Potassium Phosphate and volume of DI Water needed to create a 1 g/L standard.
   (0.1 g) + (200 mL) = 0.5 g/L
   a. Triple rinse and partially fill a 200 mL volumetric flask with deionized water.
   b. Open the balance chamber and place a new, small weigh boat onto the balance platform.
   c. Close the door and zero out the weight of the boat.
   d. Open the door to the chamber and using a small spatula weight out 0.100 g of Potassium Phosphate.

e. Record the actual mass of Potassium Phosphate in the data table
f. After weighing out 0.100 g of Potassium Phosphate, remove the weigh boat from the balance chamber.
    a. Do not zero the balance
g. Using a squeeze bottle filled with deionized water, **gently** spray deionized water into the weigh boat creating a slurry of the crystals in the weigh boat with the deionized water.
h. Transfer the slurry to the flask by gently squeezing the sides of the weigh boat, creating a spout over one of the points and pouring off the slurry into the flask. (A stream of water from the squeeze bottle into the boat may be necessary to help move the slurry into the flask.)
i. Rinse the weigh boat with copious amounts of deionized water, each time pouring the rinse into the flask to ensure that all the powder was transferred into the flask.
j. Bring the flask over to the water system and swirl the liquid in a flask a few times to mix.
k. Fill the flask to the base of the neck with deionized water.
l. Use the deionized water squeeze bottle to finish filling the flask to the final volume. (Bottom of the meniscus is at the top of the line.)
m. Place a ground glass stopper in the neck of the flask and gently invert the flask a few times to mix.
n. Confirm that the bottom of the meniscus is still at the line. If not, add water until it is.
    a. Record the final mass of the volumetric flask

Transfer the Potassium Phosphate Solution to bottle for storage and repeat the above process if needed to create enough solution for the experiment.

Secondary Phosphorous Standard – 0.05 g/L

1. To prepare the 0.05 g/L secondary standard - use the 0.5 g/L Potassium Phosphate stock solution.
2. Calculate the volume of primary standard needed to create a 0.05 g/L standard using a dilution.
    a. We are doing a 10 times dilution, meaning we will use one part 0.5 g/L stock to 9 parts DI water. Since we are using a 200 mL volumetric flask, we will use 20 mL stock and 180 mL DI water.
3. Put a clean, dry 200 mL volumetric flask on the precision balance and zero out the weight of the flask. (Place the ground glass stopper for the flask on a KimWipe on the benchtop.)
4. Using the 5 mL pipette, add 20 mL of the primary stock standard to flask.
    – Record the weight on the datasheet.
5. Remove the flask from the balance chamber, **do not** zero out the weight.
6. Fill the flask most of the way with deionized water directly from the water system. Using a squeeze bottle filled with deionized water, gradually bring the water volume up so the bottom of the meniscus is at line on the neck of the flask.
7. Place the flask on the balance platform.

-Record the weight on the datasheet.

Repeat the process used to create the primary phosphate stock and repeat this process to create the SHMP and Dayglo stock.

Starting the Experiments:

1. Bring the 0.05 g/L Phosphate solution, SHMP solution, and Dayglo solution appropriate pipettes/tips, and the shaker table, 'Roy Orbitson,' to the environmental room on the lab cart.
2. See separate Centrifuge and Shaker Table Protocol for details on safely operating the shaker table.
   -Make sure that Roy in on a stable and flat surface in the environmental room shelf and that he is a safe distance away from the power outlet.
   -Also make sure that the Zip ties are in place to use the scintillation box shelf.
3. Prepare the particle pipette
4. Bring the experimental centrifuge tubes out onto the lab cart.

Isotherm experiment: This runs for 24 hours, so start this at a time that will be convenient 24 hrs. later.

1. Start the shaker table: Move the timer dial all the way to 'constant' so that Roy will stay on indefinitely, then gradually increase the speed knob until the indicator is around 3 o'clock.
2. Add the correct volumes of SHMP stock and Dayglo stock to the centrifuge tubes according to the datasheet.
3. Using the 0.1 g/L Phosphate standard, spike the isotherm tubes to their appropriate concentrations.

Concentration of Spike	Volume of 0.05 g/L Solution Added
0 g/L	0
0.00025 g/L	200
0.0005 g/L	400
0.0008 g/L	640
0.0012 g/L	960
0.0018 g/L	1440
0.0024 g/L	1920

4. Cap the tubes and give them a small shake as you move them into the milk crate on top of the shaker table.
5. Note the start time of the 24 hours shake on the datasheet.

6. **End the experiment:** When the 24 hours of shaking are finished, take the tubes out of the shaker table, and place them into the benchtop centrifuge.
    a. Spin at 4000 rpm for 10 minutes to clarify.
    b. Insert a 20 mL syringe into the centrifuge and draw up ~15 mL of liquid.
    c. Place a 0.45 μM syringe filter onto the end of the syringe.
    d. Filter the liquid from the syringe into the corresponding labeled sample tube.
    e. Remove the filter from the end of the syringe and draw up some air in the syringe.
    f. Replace the filter and push the air from the syringe through the filter to get all the liquid from the syringe into the vial.
    g. Freeze the scintillation vial standing upright. Once frozen, transfer to a Ziploc bag labeled with the Date and Sed Exp.
    h. Re-cap the centrifuge tube and set aside for later processing.

Processing the Sediment Experiment Tubes for Sediment Dry Weight and AFDM

-The calculations from the sorption experiments to find sorption rates and the EPC value all need to be normalized to the amount of sediment that was in the tube.

Supplies needed:

-Pre-combusted 70 mL aluminum weigh dishes (Fisher # 08-732-103)

-Diamond tip pencil.

-Open the Excel sheet and fill in the correct information.

1. **Prepare the tins:** Unwrap the tins and separate out as many tins that are needed for the set of centrifuged tubes to be processed.
    a. Place a paper towel on the benchtop next to the precision balance. Place a tin upside down onto the paper towel and scribe a number from the datasheet into the bottom on the tin using the diamond tip pencil. (Be sure to press hard. Place a line underneath numbers that can get confusing, like 6 and 9, to show orientation.)
    b. Weigh the numbered tins on the precision balance and record the weight in grams on the 'Tin Weight' column of the datasheet.
    *Note: Always weigh the tins after scribing the numbers to obtain the most accurate tin weight.
2. **Fill the tins:** Shake the remaining supernatant and sediment in the centrifuge tube and gently pour into tin. *Pouring too vigorously can cause liquid to spill over the edge of the tin.
    -Use a squeeze bottle filled with deionized water to carefully rinse remaining liquid and sediment from the centrifuge tube into the tin.
    -Gently rinse the lid of the centrifuge tube into the tin as well.
    -Once no trace of sediment is left in the centrifuge tube and the lid, dispose of the empty tube in the trash.
3. Carefully transfer the tin into drying oven, starting with the upper racks first.

Notes: The drying oven should be powered off and be at room temperature prior to starting to fill it with sediment tins. Keep the door open during the entire filling process. It may be easier to do the filling of the tins close to the oven so that the tin does not need to be moved too far while full of liquid.

4. Once all samples are placed in oven, close the door, and turn the oven on. Verify that it is set to 70°C.
5. Allow the samples to dry for at least 48 hours.
6. When the sediment tins are dry, carefully transfer them to shelves of the desiccator and allow them to come to room temperature. Always keep the tins level to keep any loose sediment in the tins.
7. Re-weigh the tins on the precision balance and record the weight in the 'Wt. Tin w/sed dry' column of the datasheet.
    a. It is important to keep the tins as dry as possible prior to weighing. Use the small Tupperware container with desiccant in the bottom to bring a few tins at a time to the balance area. Always keep the lid on the container unless actively accessing the tins.

Keeping the tin level, lift it above your head so that you can see the number on the bottom of the tin.

This SOP is modified from previous protocols defined in King et al. and Walbridge et al. for the purposes of these experiments.

APPENDIX B

Sample ID	SHMP Addition (ppb)	P Addition (ppb)
stream_blank1	-	-
stream_blank2	-	-
sh_st_1	3001	-
sh_st_2	3050	-
sh_st_3	5914	-
sh_st_4	5981	-
sh_st_5	11468	-
sh_st_6	12155	-
sh_st_7	24481	-
sh_st_8	24441	-
sh_di_1	2926	-
sh_di_2	2963	-
sh_di_3	6038	-
sh_di_4	6045	-
sh_di_5	11985	-
sh_di_6	11837	-
sh_di_7	23900	-
sh_di_8	23874	-
shdp_st_1	3016	499.2
shdp_st_2	3054	505.8
shdp_st_3	6120	505.0
shdp_st_4	6128	503.8
shdp_st_5	12107	503.4
shdp_st_6	12155	505.7
shdp_st_7	24237	499.7
shdp_st_8	24260	501.1
shdp_st_9	24060	996.9
shdp_di_1	3037	498.5
shdp_di_2	2945	488.2
shdp_di_3	5963	485.6
shdp_di_4	5896	486.8
shdp_di_5	12108	487.2
shdp_di_6	11708	486.1
shdp_di_7	23873	487.2
shdp_di_8	23612	488.7
shdp_di_9	23977	998.5
iso_1	11965	82.1
iso_2	11970	81.9
iso_3	12016	166.3
iso_4	12007	164.5

iso_5	11928	267.0
iso_6	11990	264.6
iso_7	11871	399.6
iso_8	11917	391.7
iso_9	12034	598.6
iso_10	12086	595.5
iso_11	11931	799.6
iso_12	12018	793.8

Table B.1: Mass-corrected concentrations of SHMP experiment solutions.

Sample ID	SHMP (g/L)	Dayglo (g/L)	Phosphate (g/L)
SHST1-M	-	0.5	0.2
SHST1-C	-	0.5	0.4
SHST2-M	-	0.5	0.6
SHST2-C1	-	0.5	0.8

Table B.2: Mass-corrected concentrations of DI water isotherm samples.

Sample ID	SHMP (g/L)	Dayglo (g/L)	Phosphate (g/L)
SHST1-M	0.01303	0.03026	0.00024
SHST1-C	0.01269	0.03096	0.00025
SHST2-M	0.01270	0.03151	0.00051
SHST2-C1	0.01267	0.03127	0.00049
SHST2-C2	0.01299	0.03046	0.00052
SHST3-M	0.01262	0.03029	0.00082
SHST3-C1	0.01272	0.03059	0.00084
SHST3-C2	0.01270	0.03094	0.00078
SHST4-M	0.01269	0.03054	0.00117
SHST4-C1	0.01279	0.03099	0.00126
SHST4-C2	0.01263	0.02952	0.00121
SHST5-M	0.01270	0.02978	0.00186
SHST5-C	0.01230	0.03256	0.00178
SHST6-M	0.01319	0.03012	0.00244
SHST6-C	0.01267	0.03050	0.00241
BLANK	-	-	-

Table B.3: Mass-corrected concentrations of stream water isotherm samples.

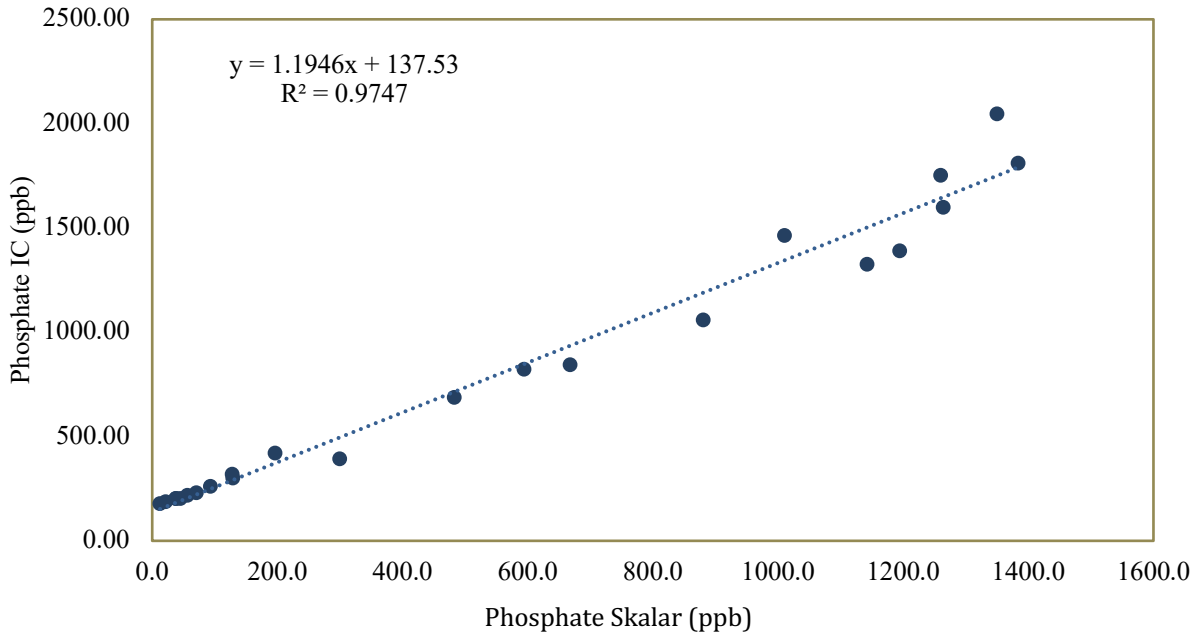

Figure B.1: Regression plot of dissolved P measurements for water samples run on the Ion Chromatograph and the Continuous Flow Analyzer, showing very similar measurements. There is a higher regression at lower concentrations of dissolved P.

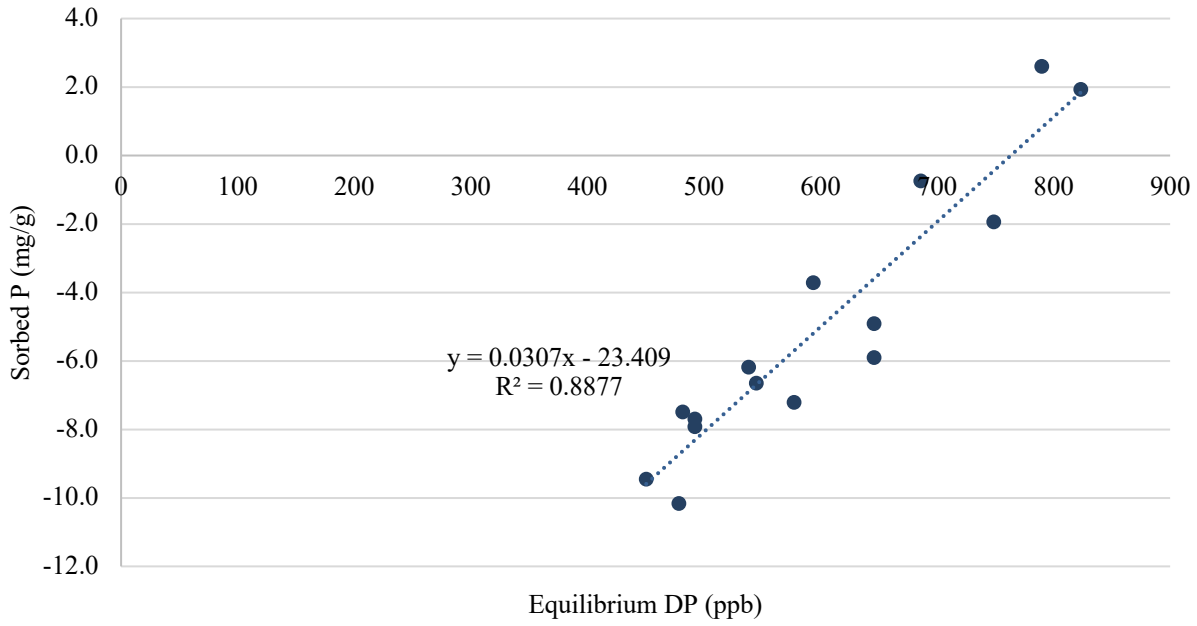

Figure B.2: Batch experiments using stream water to assess the sorption relationship between Dayglo fine particles and SRP show a linear trend but an accounted for source of SRP (measured equilibrium SRP concentrations exceed the concentrations of SRP that were thought to be added to samples, leading to apparently negative concentrations of sorbed P).