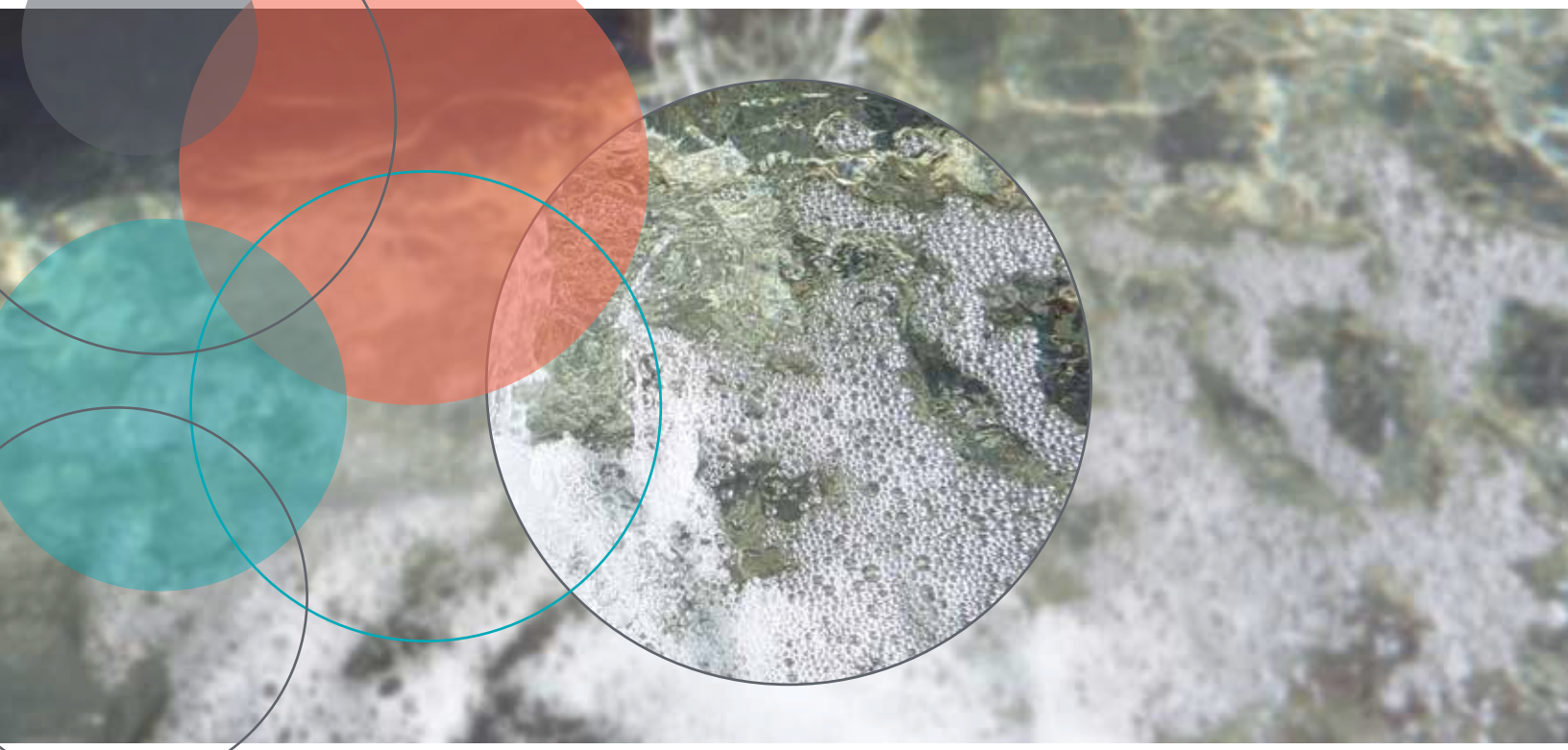

Phosphorus Analysis in Wastewater: Best Practices

2015

Increasingly stringent phosphorus requirements are driving the need for methods that reliably and accurately measure phosphorus in wastewater at lower levels. *Phosphorus Analysis in Wastewater: Best Practices* was prepared as part of the Nutrient Removal Challenge to assess if lessons learned from freshwater analysis can be applied to wastewater, or if wastewater has unique characteristics that confound low-level phosphorus measurement.

THE Water Research FOUNDATION

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ABSTRACT AND BENEFITS

Abstract

Phosphorus (P) monitoring at wastewater treatment plants is essential as phosphorus (total phosphorus) is an important main constituent regulated in treatment plant effluents. Recent trends are toward increasingly lower phosphorus limits, requiring reliable lower and lower phosphorus measurements. There is a long history of P analysis in dilute matrices; i.e., river and lake water, and best practices have been developed. These best practices for surface waters are reported herein. Potential issues in wastewater P analysis by colorimetry include pH, proton to molybdenum ratio, color development time, and digestion method. Of equal importance are the QA/QC measurement protocols implemented by wastewater analysis labs; demonstrably well performing examples from Coeur d'Alene, Spokane, and the City of Las Vegas are presented. Total reactive phosphorus is an ambiguous analytical measurement because the quantitative results depend strongly on color development time. For low-level analysis, long path lengths have advantages in more precisely resolving low concentrations. Replicate measurements are essential, especially for low-level P samples, in order to capture the true value of the sample within variability. When dealing with low concentrations, even a small absolute error is a large relative error; thus, replicate measurements are essential to estimate true concentrations for dilute phosphorus samples.

Benefits

- Presents best practices from surface water and wastewater as a practical tool and quick reference for wastewater analytical labs.
- Presents example QA/QC procedures to ensure reliable low-level P analysis.
- Presents the background theory for the molybdate blue method, because in order to debug/optimize an analytical procedure, it is essential to understand the theoretical basis.
- Presents potential issues with phosphorus analysis (pH, reagent ratios, time, matrix effects, digestion method) and how to detect (multi-laboratory/multi-method comparisons) and potentially correct the issues.
- Presents future research and method development priorities.

DEFINITIONS AND ACRONYMS

AHP	Acid Hydrolyzable Phosphorus
AMP	Andenosine-5-MonoPhosphate
ATP	Andenosine-5-TriPhosphate
CCV	Continuing Calibration Verification
CDA	City of Coeur d'Alene
CNA	Cellulose-nitrate-acetate (filters)
CRM	Certified Reference Material
EPA	United States Environmental Protection Agency
FIA	Flow Injection Analysis
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICV	Initial Calibration Verification
LCS	Laboratory Control Sample
MS	Matrix Spike
MSD	Matrix Spike Duplicate
P	Phosphorus
ppb	Concentration in parts per billion (phosphorus corresponds to µg P/L)
PPE	Personal Protective Equipment
RDL	Reporting Detection Limit
RP	Reactive Phosphorus
RPD	Relative Percent Difference
SAHP	Soluble Acid Hydrolyzable Phosphorus
SM	Standard Methods
SOP	Standard Operating Procedure
SRP	Soluble Reactive Phosphorus
TAHP	Total Acid Hydrolyzable Phosphorus
TP	Total Phosphorus
TRP	Total Reactive Phosphorus
TSP	Total Soluble Phosphorus
USGS	U.S. Geological Survey

CHAPTER 1

INTRODUCTION

There is a long history of phosphorus (P) analysis in dilute matrices; i.e., river and lake water (Maher and Woo 1998, Worsfold et al. 2005, Jarvie et al. 2002). Phosphorus monitoring at wastewater treatment plants is essential as phosphorus (as total phosphorus) is one of the main constituents regulated in treatment plant effluents. Recent trends are toward increasingly lower phosphorus limits, requiring lower and lower phosphorus measurements. This document attempts to assess if the lessons learned from freshwater analysis apply to wastewater, or if wastewater has unique characteristics that confound low-level phosphorus measurements. There is a need to assess best practices for low-level phosphorus analysis in wastewater.

In earlier work, Eleuterio and Neethling (WERF 2009), showed that orthophosphate in clean water solutions responds very well and reliably (in terms of variability) to measurement. The measurements become more variable when performed on wastewater effluent and when total phosphorus is measured. This project is a continuation of that work and attempts to point out some of the potential confounding factors in wastewater, while demonstrating that it is possible to achieve reliable low-level analysis.

The first section of this document (Chapter 2) presents a theoretical overview of phosphorus analytical methods with a particular emphasis on colorimetry. Colorimetry is the main technique discussed in the remainder of the document and it is important for the reader to have a thorough chemical understanding of the basis for this important analytical method. Chapter 2 also includes a list of best practices based on literature review of surface water phosphorus analysis experience.

Subsequent sections focus on kinetics of phosphorus colorimetry (Chapter 3) and digestion methods (Chapter 4) used to determine total phosphorus (TP).

The remainder of the white paper focuses on case studies where literature, plant, and unpublished Wilfrid Laurier Laboratory data are presented to highlight specific issues around phosphorus analysis in wastewater. Chapter 5 presents a specific example of when inter-laboratory comparisons and multi-measurement techniques allowed for the identification and eventual correction of a systematic error in TP measurement by one of the labs. Chapters 6, 7, and 8 demonstrate how by using careful technique and good QA/QC it is possible to demonstrate reproducible and accurate phosphorus measurements in wastewater for the City of Coeur d'Alene, Spokane, and Las Vegas, respectively. The final case study in Chapter 9 demonstrates a year's worth of data from two labs monitoring TP and total reactive phosphorus (TRP) for the same pilot study.

Finally, conclusions based on the literature review and case studies are presented in Chapter 10 and some suggestions are given for future work in Chapter 11.

CHAPTER 2

PHOSPHORUS ANALYSIS

The most common class of methods for determining aqueous phosphorus concentrations, especially in routine wastewater analysis, are colorimetry-based methods; those methods in which a colored species is formed with absorbance (*A*) proportional to the phosphorus concentration (*c*) according to Beer's Law ($A=ecl$, where (e) is the molar absorptivity coefficient and (l) is the path length for the measurement cell). More details of colorimetry are discussed below (see Colorimetry). Advantages of colorimetry include:

- Relatively inexpensive
- Does not require specialized equipment
- Does not require complicated operator training
- Can be automated
- Several instrument suppliers specifically support Standard Methods (i.e., flow injection analysis equipment)
- Can be used to measure phosphorus speciation (see Digestion)

The main alternatives to colorimetric analysis for phosphorus analysis are ion chromatography (IC) and inductively coupled plasma with a mass spectrometer (ICP-MS) or optical emission spectrometer (ICP-OES) detector. There are more specialized research methods such as ion-pair, fluorescence methods, or electrochemical methods (Estela and Cerdà 2005); but such methods are not yet used for routine analysis because they do not have corresponding standard methods (SM) status. IC analysis tends to only focus on phosphate species and in fact cannot analyze neutral P compounds so total phosphorus analysis is not really possible by IC. ICP shows potential as a method of the future as technology improves and detection limits decrease (Estela and Cerdà 2005). A disadvantage of ICP techniques is that they cannot measure speciation without being coupled to chromatographic separation or parallel colorimetric analysis (Manzoori et al. 1990). In addition, ICP is more expensive in terms of infrastructure, operation and operator training.

Standard Methods for ICP can be used for wastewater monitoring if phosphorus is treated like a metal (even though technically phosphorus is a nonmetallic element); for example, by following SM 3020 (quality assurance/quality control), SM 3030 B (filtration for dissolved and suspended metals), SM 3030 D (digestion for metals), 3030 E (nitric acid digestion) and finally SM 3120 (metals by plasma emission spectroscopy). Some regional and municipal laboratories do use ICP for analysis; for example, the Region of Waterloo analytical facility in Southern Ontario. The major method used for phosphorus analysis at wastewater treatment plants in North America and Europe is colorimetry and that will be the focus of the remainder of this document.

Colorimetry

Most colorimetry methods are based on the classic papers of Riley and Murphy including Riley and Murphy (1962). These methods have been intensely studied and standardized for adoption in water analysis. The result is that several variations exist as methods approved for wastewater analysis; i.e. Standard Methods for the Examination of Water and Wastewater, 21st Edition, and United States Environmental Protection Agency (EPA) Test Methods Collections. Specific methods will be indicated in the text below as SM or EPA for Standard Methods or EPA protocols. Standardized colorimetric methods available for phosphate analysis in wastewater are summarized in Eleuterio and Neethling (WERF 2009).

In Riley and Murphy (1962) based methods, a phosphomolybdate complex is formed and then reduced to form an intensely blue species. In modern practice, several options exist for the reduction step including stannous chloride (SM 4500-P D) or ascorbic acid (SM 4500-PE/PF, EPA 365.1, 365.3). Since the basis of P colorimetry is the molydate complex the chemistry of this process is discussed further below. To avoid a reducing agent the vanadate method (SM 4500-P C) would be used and generates the yellow colored phosphomolybdate complex but with a higher detection limit.

The principle behind the colorimetry method of phosphorus determination involves the production of the phosphomolybdenum blue complex (McKelvie et al. 1995). The initial reaction forms a heteropolyacid, 12-phosphomolybdic acid, formed from the reaction of orthophosphate with acidified molybdate, which comes from ammonium molybdate:

Equation 1

$$PO_4^{3-} + 12MoO_4^{2-} + 27\ H^+ \rightarrow\ H_3PO_4(MoO_3)_{12} + 12\ H_2O$$

The acid is then reduced to phosphomolybdenum blue. The reduction converts some molybdenum (VI) to molybdenum (V) (Barrows et al. 1985, Worsfold et al. 2005).

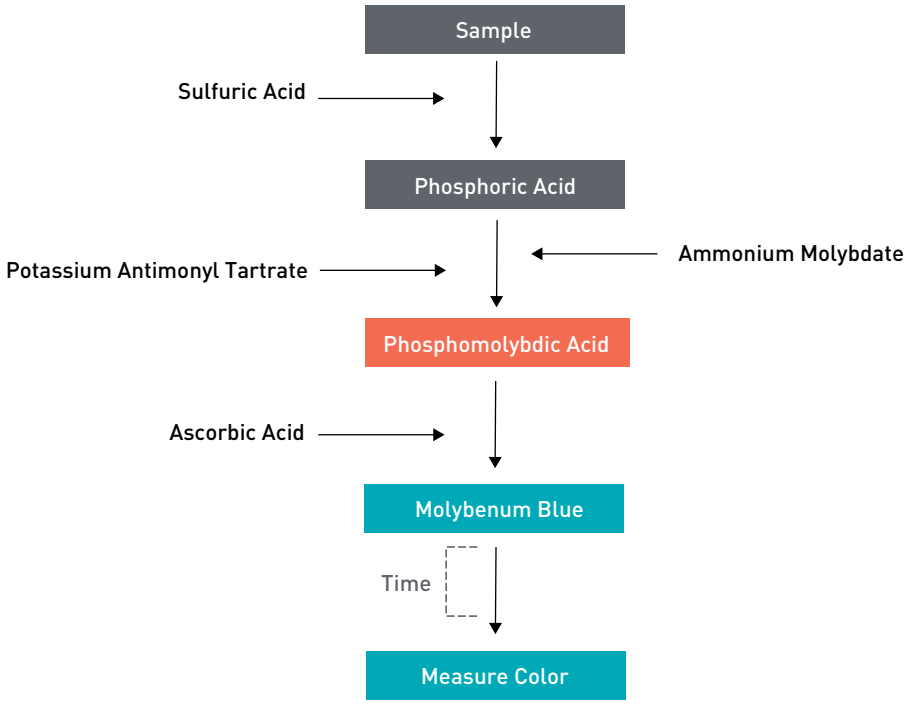
Equation 2

$$H_3PO_4\ (Mo(VI)O_3)_{12} \rightarrow H_7PO_4(Mo(V)O_3)_4(Mo(VI)O_3)_8$$

The overall steps in phosphorus colorimetery are shown in Figure 2-1. The first step is acidification because the colored species are only formed at acidic pH. At this stage it is important to not under or over acidify the sample. At too low pH molybdenum species form that are nonreactive with phosphate. At too high pH self-reduction of the molybdate can occur. This self-reduction creates blue color that is not proportional to the phosphate concentration and can lead to overestimation of

phosphate concentrations (Chapter 5). Some work suggests that for low level analysis, optimal pH should be in the range 0.57 to 0.88 and a $[H^+]/[MoO_4^{2-}]$ ratio of 70 (Drummond and Maher 1995).

Figure 2-1 - Schematic of Phosphate Colorimetric Analysis



Once the phosphoric acid has been formed, antimony and molybdate salts are added (Figure 2-1). The molybdate is added to form molybdenum phosphate species. The purpose of the antimony is to accelerate the color forming reaction. The final colored species is actually a 2:1 complex of the antimony with the molybdophosphoric acid complex ($PSb_2Mo_{12}O_{40}$).

The next step is to reduce the complex, unless the vanadate method is being used, in which case absorbance would be measured at the red box in Figure 2-1. Figure 2-1 shows ascorbic acid as the reducing agent but potentially different ways in which the heteropolyacid can be reduced. Stannous chloride ($SnCl_2$) or ascorbic acid are the most common reducing agents (Standard Methods 1998). In most commercial and municipal labs the ascorbic acid method is used and that method will be the focus of this review. Ascorbic acid donates two electrons in the reduction process and is more commonly used due to the fact that the process is less sensitive to salt (Worsfold et al. 2005). The main drawback to the ascorbic acid method is the slow color development time, but this problem has been resolved by the addition of the potassium antimonyl tartrate (see above).

Once the phosphomolybdenum blue complex has been formed, the absorbance can be measured. Typically, the spectrophotometer should be set to measure the absorbance at 650 or 880 nanometers.

The 880 nm wavelength has higher molar absorptivity and is the more sensitive choice per Standard Methods (1998). For vanadate related methods, 470 nm is used for absorbance measurements corresponding to the yellow colored non-reduced phosphomolybdate complex.

The final step indicated in Figure 2-1 is color development. Chapter 3 focuses on color development time, but wide variation in literature recommendations and some versions of Standard Methods are vague on the time. For example, Drummond and Maher (1995) use color development times of less than 10 minutes and Eaton et al. (2005) state that the sample's absorbance must be measured between 10 and 30 minutes after addition of color forming reagents. A single color development time recommendation is difficult because kinetics of reactions depend on all the reagent concentrations, yet for different methods the reagent concentrations are quantitatively different (Chapter 3).

Digestion

Phosphorus can exist in many different forms; only one of which is detected by molybdate-based colorimetric methods. Phosphorus colorimetry is actually more appropriately referred to as phosphate colorimetry; it is phosphate that causes the formation of blue colored solutions. To determine different forms of phosphorus a digestion step is necessary to convert all of the desired forms of phosphorus to phosphate prior to analysis. Complete digestion results in determination of total phosphorus while incomplete digestion can be used to determine concentrations of intermediate forms.

This sequential digestion potentially allows for the determination of phosphorus speciation. Phosphorus speciation has been reviewed by several authors Spivakov et al. (1999), Eleuterio and Neethling (2009), Jarvie et al. (2002), Worsfold et al. (2005) often with different nomenclature. In brief phosphorus can occur as protonated and deprotonated forms of orthophosphate (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}), polyphosphates such as triphosphoric acid ($H_5P_3O_{10}$), organic phosphorus such as organic phosphates (i.e., C-O-PO_3 bonds) as well as organic phosphonates (i.e., C-P bonds) and phospholipids. Specific phosphorus forms present in a water sample depend on the nature of the sample but due to its importance as a nutrient, phosphorus is associated with biological molecules and their breakdown products. Worsfold et al. (2005) lists several model compounds that represent the type of molecules found in natural and sewage-derived sources.

Phosphorus speciation is measured as three operationally defined fractions. The first fraction is measured without digestion and includes orthophosphate. The second fraction is measured after an acid digestion step and measured species include inorganic polyphosphates in addition to orthophosphate species. The third fraction is measured after an oxidizing digestion and converts all forms of phosphorus to orthophosphate. The normal definitions include reactive phosphorus (RP) as the first measurement with acid hydrolyzable phosphorus (AHP) as the second

measured value minus RP. Total phosphorus (TP) is determined from the third measured value. The major fraction's identities depend on if the sample was filtered or not before analysis–Soluble Reactive Phosphorus (SRP), Soluble Acid Hydrolyzable Phosphorus (SAHP), and Total Soluble Phosphorus (TSP). Similarly, fractions for unfiltered samples include Total Reactive Phosphorus (TRP), Total Acid Hydrolyzable Phosphorus (TAHP), and TP. Many different fractions of phosphorus such as organic or particulate forms can be determined by difference (see Maher and Woo (1998) for a complete list).

Wastewater treatment plants tend to have discharge limits based on TP which tends to be the most important measurement required for permits. SRP is often measured as a specific species if speciation information is required. The other fractions of phosphorus tend not be considered for routine monitoring. Potentially, phosphorus speciation can contribute useful information in understanding how different treatment processes work (WERF 2014).

A number of different digestion techniques are used for TP determination and discussed more in Chapter 4. The simplest digestion method is the acidic persulfate oxidation method (i.e., EPA 365.3, SM 4500-P B5). In this method the persulfate anion is reduced to the sulfate anion. The two electrons being supplied to the persulfate come from the broken bonds of the oxidized compound. The reaction for this reduction is given by Equation 3.

Equation 3

$$S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-}$$

It should be noted that for organic phosphates, phosphorus retains oxidation state V. The reduction reaction is actually converting carbon in the organic molecule to oxidized forms and thus releasing the associated phosphorus as phosphate. For phosphonates, the oxidation state on the phosphorus is more likely III (Quin 2000) and the phosphorus itself can be oxidized to P(V) during the TP oxidation step.

Flow Injection Analysis

Estela and Cerdà (2005) provide a summary of the state-of-the-art in phosphorus flow injection analysis (FIA). Many commercial and municipal labs utilize flow analysis for determination of phosphorus species (TP and possibly SRP). Digestion can occur online while sample is being pumped towards the detector or in autoclave or hotplate batches prior to colorimetry. There are Standard Methods utilizing flow injection analysis including: Flow Injection Analysis for Orthophosphate (SM 4500-P G), Manual Digestion and Flow Injection Analysis for Total Phosphorus (SM 4500-P H), Inline UV/Persulfate Digestion and Flow Injection Analysis for Total Phosphorus (SM 4500-P I), and Methods of Analysis by the U.S. Geological Survey (USGS) National Water Quality Laboratory-Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water (USGS Test Method I-4650-03).

In general, flow injection analysis is preferred over batch analysis. Flow techniques tend to be more reproducible because reaction time is consistent, but it is essential that reactions proceed to completion (see Chapter 3). Additionally, flow injection techniques tend to be associated with an auto-sampler and the automation allows for greater productivity and throughput of samples. For good quality FIA data, it is essential to include initial calibration verification (ICV) and continuing calibration verification (CCV) samples as well as blanks. Details of QA/QC protocols for FIA used by Coeur d'Alene and Spokane labs are presented in Chapters 6 and 7.

Method Comparisons

Three methods for batch analysis of phosphate are compared in terms of reagent concentrations, contact time, and reported concentration ranges. For this comparison batch, methods are used because it is possible to readily calculate the molar reagent concentrations in the measured solution. For flow through methods, the concentrations are determined by stock reagent concentrations, flow rates, and tubing diameter, making comparisons more difficult. Flow methods are modified versions of the manual/static methods; the comparisons below highlight that although the methods are based on the same chemical principles and reactions, the specifics of implementation are different.

The three methods selected for comparison represent Standard Methods SM 4500-P E and EPA 365.3 and a "research" method specifically used for low phosphorus concentrations in oligotrophic lakes (Drummond and Maher 1995). These methods are for orthophosphate but represent the measurement step for determination of SM and EPA phosphorus species and TP after partial or complete sample digestion respectively.

Table 2-1 demonstrates that there is substantial variability even in methods commonly used for phosphorus and all nominally based on the original work of Riley and Murphy (Riley and Murphy 1962).

Table 2-1 - Final Solution Conditions for Three Phosphate Analysis Methods

	SM 4500-P E	EPA 365.3	D and M (1995)[a]	Ratio[b]
H_2SO_4 (M)	0.17	0.16[f]	0.13	0.9 / 0.8
pH[c]	0.73	0.97	0.76	1.4 / 1.0
Sb[d] (µM)	57	42	58	0.7 / 1.0
Mo (mM)[e]	65	45	35	0.7 / 0.5
H:Mo	39	34	72	0.9 / 1.8
Ascorbic (mM)	4.1	12.0	9.6	3 / 2.3
P Range (ppb)	10-2000[g]	10-1200	<100[h]	
Time (min)	10-30	5-60	0.8-1	

[a]Drummond and Maher (1995). [b]See text. [c]pH calculated assuming pKa value of 1.92 for HSO_4^-. [d]Antimony added as K(SbO) $C_4H_4O_6 \cdot (0.5)H_2O$. [e]Molybdenum added as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. [f]This value was calculated here from the stated pH of the optimized method. [g]For the full range different path lengths must be used. [h]Has been demonstrated not to work for values ≥ 800 ppb

The final column in Table 2-1 is calculated as the ratio of the EPA and Drummond and Maher methods to the SM method. For example the first entry in the ratio column corresponding to H_2SO_4 concentration indicates that EPA is 90% as concentrated as SM and Drummond and Maher's method is 80% as concentrated. Looking at the ratios reveals that there are factors from 0.5 to 3 variation in the methods using SM as a reference point. The largest differences are in the H:Mo ratio and ascorbic acid concentration.

In terms of practice, the largest difference between the methods is the measurement times. Both EPA and SM have much longer color development times than the method of Drummond and Maher (1995); i.e., tens of minutes compared to a minute or less. The concentration ranges for the methods have similar lower limits, but the Drummond and Maher method only works for low concentration samples, generally less than 100 µg P/L but certainly less than 800, whereas the SM and EPA methods are reported to work on samples greater than 1000 µg P/L. The kinetics of these methods are investigated further in Chapter 3.

Best Practices Based on Literature Review

Several papers review P analysis methods for surface waters (Maher and Woo 1998, Worsfold et al. 2005, Jarvie et al. 2002). These papers include some recommendations for storage and digestion of samples. The key recommendations relevant to wastewater matrices are summarized below in Tables 2-2 and 2-3.

Tables 2-2 and 2-3 repeat suggestions from the literature regarding best practices for washing of labware. There is no question that proper washing of labware is essential for good quality analytical results, especially for low-level analysis. It should be noted that some acids can include significant amounts of phosphorus and can potentially contaminate low-level analysis methods. For example, the laboratory at Wilfrid Laurier University stopped using nitric acid for their cleaning acid baths due to significant P contamination (as observed in elevated blanks during analysis). The laboratory now uses sulfuric acid in the cleaning baths. Most reagent quality analysis reported by manufacturers do not specifically include phosphorus. However, the more commonly reported ignition residues and as concentrations can be used to evaluate optimal acids for acid baths; higher residues and as would suggest, the likelihood of greater P concentrations. Arsenic has analogous chemistry to P, as it is from the same group of the periodic table. In terms of good practice, laboratories should analyze their acid baths regularly to avoid systematic errors from their cleaning protocols.

Table 2-2 - Phosphorus Handling Recommendations as Reported in Indicated Citations

Aspect	Recommendation	Citation
Filtration	Field filtration of samples for SRP using 0.45 µm CNA (cellulose-nitrate-acetate) filters (45 mm diameter)	Jarvie et al. 2002
Filtration	Filters should be pre-flushed with 30 mL of sample prior to sample collection	Jarvie et al. 2002
Storage	Slow freezing of filtered and turbid samples appear to be satisfactory for long-term storage (years) of a wide variety of samples for TP analysis	Maher and Woo 1998
Storage	SRP analysis should be performed on the same day as the sample collection	Jarvie et al. 2002
Storage	Storage TP analysis should be carried out within 24 hours of sample collection	Jarvie et al. 2002
Storage	Addition of acid may be needed to prevent flocculation and formation of precipitates in water samples, especially in samples containing calcite	Maher and Woo 1998
Storage	Acid washed low density polyethylene containers appear to be universally suitable for the storage of water samples	Maher and Woo 1998
Storage	Recommended that containers be cleaned overnight with a nutrient-free detergent, rinsed with ultrapure water, soaked in 10% HCl overnight, then rinsed again in ultrapure water	Worsfold et al. 2005
Storage	Adsorption of phosphorus to containers may be significant at low concentrations < 20 µg P/L and low ionic strength	Maher and Woo 1998
Digestion	For ease, simplicity, and precision, batch digestion of samples with alkaline or acid persulfate using autoclave or microwave heating is recommended	Maher and Woo 1998
Digestion	For turbid samples caution must be exercised to ensure carbon or suspended solids concentration does not exceed the capacity of the digestion procedure	Maher and Woo 1998
Digestion	Advisable to test the efficiency of any digestion method using a range of model phosphorus containing compounds that reflect different chemical bond and stabilities and represent the naturally occurring compounds in the sample	Worsfold et al. 2005
Analysis	Test for matrix interferences (As, F, SiO_2) by making up calibration solutions containing various interfering chemical species and determine if there are differences in the absorbance from regular calibration solutions	Jarvie et al. 2002
Analysis	Eliminate sulfide interferences by aeration until no hydrogen sulfide odor can be detected	Jarvie et al. 2002
Analysis	Certified reference materials (CRM) are the most efficient to measure and control accuracy. *Unfortunately there are limited CRMs for P and no wastewater standards.*	Worsfold et al. 2005
Analysis	Regular testing is necessary to assure the quality of environmental data submitted since the performance of many laboratories does not remain constant	Worsfold et al. 2005
Analysis	Use a 10 cm cuvette for spectroscopy	M. Brett[a]
Analysis	Autoclave glassware in acid-wash between runs	M. Brett
Analysis	Never share glassware with other labs or between projects within a lab; it is essential to always know what glassware has been used for last and how it was cleaned	M. Brett

[a] The citation to M. Brett corresponds to personal communication with Dr. Michael Brett, University of Washington, Seattle, Washington.

CHAPTER 3

PHOSPHORUS COLORIMETRY KINETICS

As mentioned in Chapter 2, the color development time is potentially a significant variable in phosphorus analysis. Jarvie et al. (2002) highlights the importance of color development time in her review of phosphorus analysis in river water. Color development kinetics depends on reagent concentrations, pH, and temperature. To maximize sensitivity, sufficient color development time must be allowed for complete reaction to form the colored complex. Systematic bias in results can be introduced even with a fixed color development time (such as in flow injection analysis). This is because different concentrations of phosphate will react at different rates; higher concentrations react faster than lower concentrations (Sjösten and Blomqvist 1997).

Reaction kinetics are temperature dependent; for example, Sjösten and Blomqvist (1997) show that, for the phosphomolybdate method they used, at 10°C 5 ppb P takes eight times longer than 500 ppb P, while at 21°C only takes twice as long to reach complete color formation. EPA and SM methods do not recommend different color development times for different concentrations of analyte.

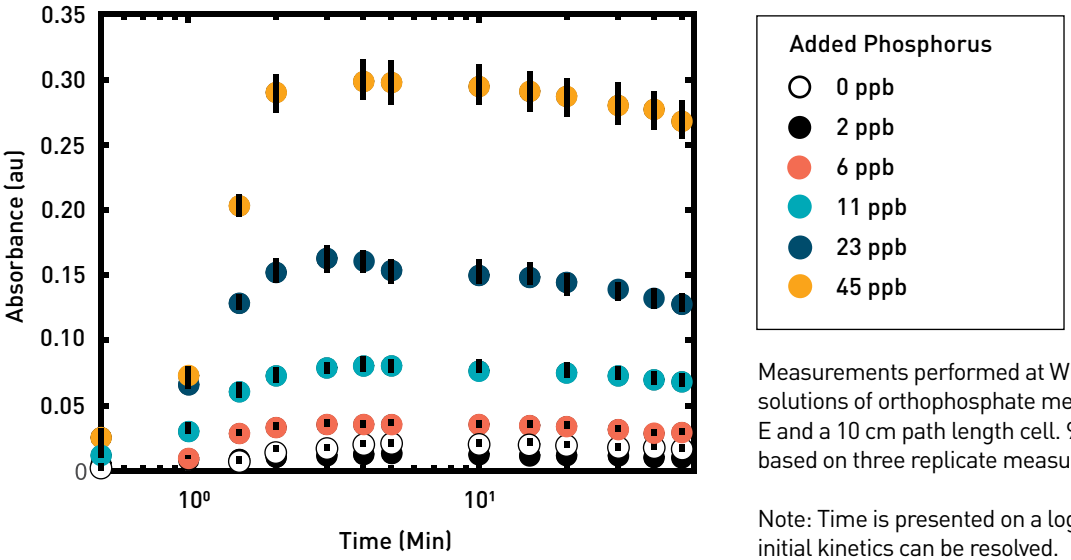
Table 2-1 compares SM, EPA, and Drummond and Maher methods for phosphate analysis. For Standard Methods, the sample's absorbance must be measured between 10 and 30 minutes (Eaton et al. 2005); similarly, EPA 365.3 specifies 5 to 60 minute color development time. Drummond and Maher (1995) present a method that has complete color development in a minute or less. In other methods color development is fairly short compare to SM and EPA; for example, Sjösten and Blomqvist (1997) at 21°C reactions are complete at all P concentrations tested within three minutes.

Figure 3-1 demonstrates data for SM 4500-P E where absorbance is monitored versus time for P concentrations 2 to 45 µg P/L. A trend versus time is evident with maximum color within the first 20 minutes and a decrease in absorbance after 30 minutes especially at the higher concentrations tested. The implication of these results is that there are potentially optimal lower and upper time limits when absorbance should be measured to maximize sensitivity. In addition, as already mentioned, a constant time for absorbance measurement should be used in analysis to prevent bias in the analytical results.

To put the data in Figure 3-1 into context, the same data is plotted after assuming an optimal reaction time of 10 minutes and using Beer's Law to calculate the corresponding phosphate concentrations. This analysis is presented in Figure 3-2. It can be seen that the systematic error is essentially independent of concentration as the symbols corresponding to different concentrations significantly overlap. Figure 3-2 implies that a systematic underestimation of concentration will result if too little color development time is used. Similarly too long a development time can also bias the results low.

If the systematic error tolerance is 10%, as shown by the dashed lines on Figure 3-2, 3 to 30 minutes represent a reasonable window of opportunity for P determination using SM 4500-P E. The window of 10 to 30 minutes recommended in Standard Methods overlaps with the window demonstrated by the data in Figure 3-2.

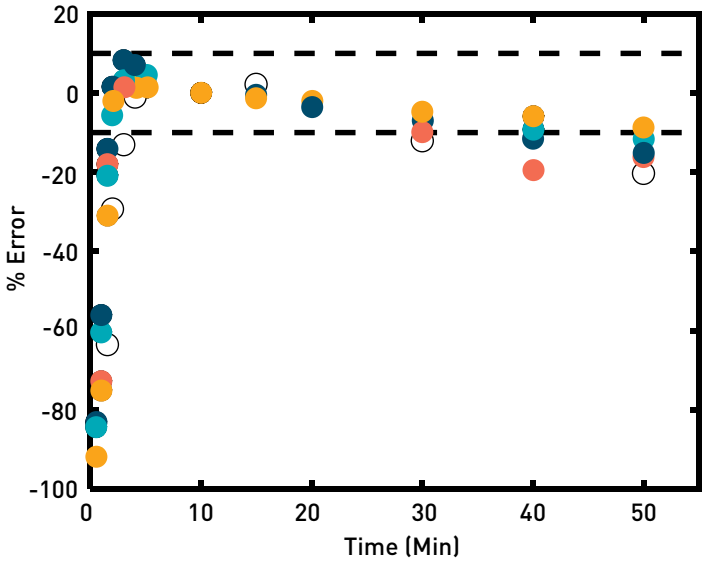
Figure 3-1 - Phosphomolybdate Blue Color Formation Reaction Kinetics



Measurements performed at Wilfrid Laurier University for solutions of orthophosphate measured using SM 4500-P E and a 10 cm path length cell. 95% confidence intervals based on three replicate measurements are shown.

Note: Time is presented on a logarithmic scale so that initial kinetics can be resolved.

Figure 3-2 - Phosphorus Measurement Error Versus Time



Same data as Figure 3-1 plotted as concentration assuming Beer's Law relationship.

For each data point calibrated using the absorbance at 10 minutes. Color code of symbols corresponds to Figure 3-1. The dashed lines represent ± 10% systematic error.

All data points are on top of each other for the 10-minute point because that is the point that was used to calibrate each time series against.

CHAPTER 4

TOTAL PHOSPHORUS DIGESTION

Several authors have summarized literature on TP digestion methods (Maher and Woo 1998, Worsfold et al. 2005, McKelvie et al. 1995). There are extensive options for digestion including thermal methods, photochemical methods, microwave methods, and enzymatic and chemical methods. For this report the focus is on the commonly used methods as presented by SM and EPA utilizing chemical oxidants and acids and hotplates or an autoclave for heating.

Model Compounds

For this white paper, several model compounds were tested with the acidic persulfate and the nitric-sulfuric acid methods for digestion. The model compounds utilized are as shown in Table 4-1 and Figure 4-1. A final effluent sample was also analyzed as part of this testing to see if different TP concentration would be determined using a more vigorous digestion protocol.

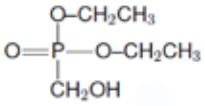
Table 4-1 - Model Compounds Used for Digestion Efficiency Study

Type	Compound[a]	Purity
Polyphosphate	Sodium Triphosphate Pentabasic	98%
Organic Phosphate	Adenosine 5´- Monophosphate Sodium Salt	>99%
Organic Phosphate	Adenosine 5´ - Triphosphate Sodium Salt	>99%
Phospholipid	1,2-Dipalmitoyl-Rac-Glycerol-3-Phosphocholine	>99%
Organic Phosphonate	Diethyl (Hydroxymethyl) Phosphonate	>97%
Organic Phosphate	Sodium Phenyl Phosphate Dibasic Dihydrate	>95%
Final Effluent	Unfiltered Sample, Blue Plains, Washington, DC	

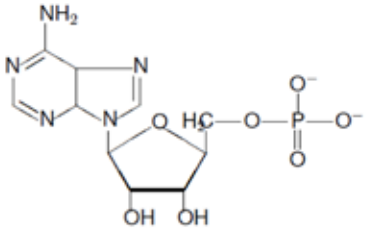
[a]All chemicals obtained from Sigma Aldrich.

Figure 4-1 - Structures of Model Compounds Used in Digestion Experiments

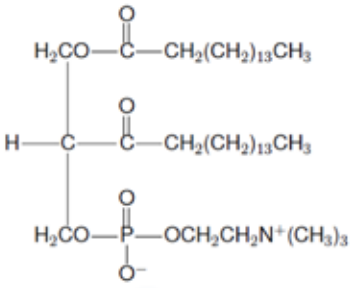
A. Diethyl (Hydroxymethyl) Phosphonate



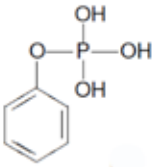
B. Andenosine-5-Monophosphate (AMP)



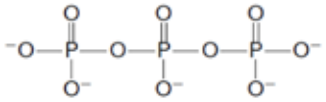
C. 1,2-Dipalmitoylrac-Glycero-3-Phosphocholine (Phospholipid)



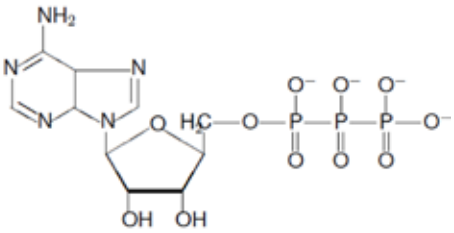
D. Phenylphosphate (Organic Phosphate)



E. Triphosphate (Inorganic Phosphate)



F. Andenosine-5-Triphosphate (ATP



Results of digestion tests are reported in Table 4-2 and for the Andenosine-5-MonoPhosphate (AMP) series shown in Figure 4-2 and for the phospholipid series shown in Figure 4-3. In general recoveries are at least 60% or greater except for the phospholipid compound. The phospholipid compound had the best recovery only with sulfuric and nitric acid digestion (yielding 82% recovery). This suggests that for samples rich in bacterial cell wall remnants should be digested using the sulfuric and nitric acid method; bacterial cell walls are made up of lipid bilayers. AMP recoveries also improved using the sulfuric and nitric acid method compared to the acidic persulfate method (70.4% versus 57.2%) although longer autoclave times did improve AMP recoveries by a few percent. Polyphosphate and organic phosphate compounds were well recovered (98.4% to 99.4%). The one phosphonate sample tested actually had greater than 100% recovery, likely due to contamination by other phosphorus compounds.

Table 4-2 - Digestion Experimental Results (Reporting as ± One Standard Deviation on Three Replicate Measures)

Method	Time (Min)	Compound	Recovery (%)	Label
Persulfate	30	Polyphosphate (500 ppb)	98.4 ± 1.6	
Persulfate	30	Polyphosphate (100 ppb)	99.1 ± 1.5	
Persulfate	30	AMP (300 ppb)	51.2 ± 0.2	1
Persulfate	30	AMP (50 ppb)	57.2 ± 2.7	2
Persulfate	60	AMP (500 ppb)	67.0 ± 1.7	3
Persulfate	75	AMP (500 ppb)	62.8 ± 6.4	4
Sulfuric-Nitric	30	AMP (50 ppb)	70.4 ± 0.8	5
Persulfate	30	ATP (100 ppb)	97.2 ± 2.3	
Persulfate	60	Phospholipid (500 ppb)	15.3 ± 0.8	1
Persulfate	75	Phospholipid (500 ppb)	18.9 ± 0.9	2
Persulfate	30	Phospholipid (300 ppb)	3.2 ± 0.6	3
Persulfate	30	Phospholipid (50 ppb)	29.4 ± 2.6	4
Sulfuric-Nitric	30	Phospholipid (50 ppb)	82.0 ± 0.4	5
Persulfate	30	Phenylphosphate (100 ppb)	99.4 ± 1.5	
Persulfate	30	Phosphonate (100 ppb)	126.0 ± 1.4	
			Concentration (ppb)	
Persulfate	30	Blue Plains	92 ± 2	
Sulfuric-Nitric	30	Blue Plains	98 ± 1	

Figure 4-2 - Digestion Recoveries for Andenosine-5-Monophosphate (AMP)

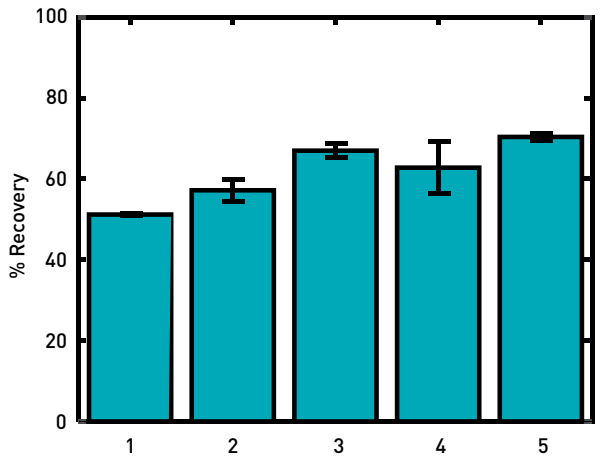
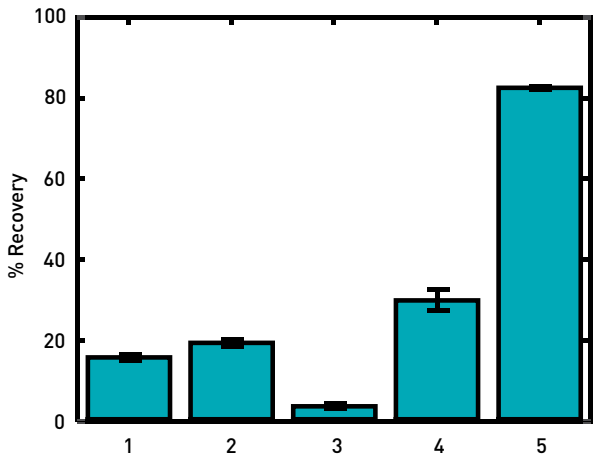


Figure 4-3 - Digestion Recoveries for Phospholipid



Digestion method indicated as the corresponding label number in Table 4-2.

Analysis of the final effluent sample demonstrated little difference between the two digestion methods. There is a slightly higher concentration of phosphorus determined with the more aggressive method (92 versus 98 µg P/L) but not really significant within reproducibility of the measurements. Differential recovery results for model compounds highlight the possibility of systematic bias in different wastewaters if poorly recovered components represent a large fraction of the total phosphorus. The specific composition of TP, non-reactive phosphorus in particular, at different plants, or in different seasons, is not known though and this represents a possible area for research. The Blue Plains results (Table 4-2) suggest that, for that sample at least, difficult to digest fractions of TP were negligible.

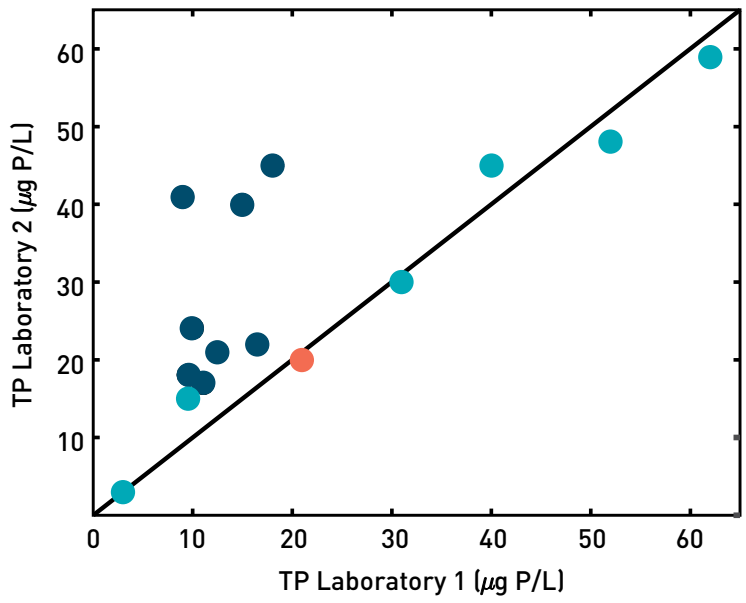
CHAPTER 5

CASE STUDY: IMPORTANCE OF INTERLAB/MULTI-METHOD COMPARISONS

During an anonymous pilot study two labs were measuring phosphorus species (TP, TRP, SRP) on parallel samples. Detailed comparisons between the analytical results of these two labs are shown in Chapter 9. Samples from this study were analyzed at an anonymous analytical facility (referred to as Laboratory 1) and at Wilfrid Laurier University (Laboratory 2). Both laboratories utilized ascorbic acid colorimetry for phosphorus analysis but Laboratory 1 was equipped for continuous flow-through analysis (Standard Methods 4500-P H) and Laboratory 2 did the analysis using a batch method (Standard Methods 4500-PE). Both methods utilized persulfate digestion to convert total phosphorus into colorimetrically detectable phosphorus. A final major difference between the two labs is that Laboratory 1 used a 1 cm light path for absorbance measurements while Laboratory 2 used a longer 10 cm path. According to Beer's Law, longer path lengths result in greater sensitivity for the colorimetric analysis.

This case study highlights the advantages of inter-laboratory comparisons because at the initial phase of the study a systematic error was found that Laboratory 2 consistently overestimated Laboratory 1. Secondary effluent samples were measured; these data are shown as blue dots in Figure 5-1, with Laboratory 2 having results more than two times greater than Laboratory 1. In the absence of additional data it was not possible to determine which laboratory analysis was correct.

Figure 5-1 - Comparison Between TP Measured by Two Different Laboratories



Laboratory 2 corresponds to Wilfrid Laurier University and measurements are shown as blue circles using the initial ascorbic acid method, red circles using ICP-OES, or aqua circles using the improved ascorbic acid method.

All measurements for anonymous commercial Laboratory 1 were done using the ascorbic acid method Manual Digestion and Flow Injection Analysis for Total Phosphorus (SM 4500-P H).

To determine which laboratory was correct in their analytical results, an identical sample was run for TP by ICP-OES at Laboratory 2 and by SM 4500-P H at Laboratory 1. The result is the red data point on Figure 5-1 demonstrating that Laboratory 2 was consistently overestimating TP in the initial comparison data set.

Thus the cross lab comparison using multiple methods revealed that the implementation of the ascorbic acid method at Laboratory 2 was systematically overestimating TP. To achieve accurate analytical performance it became necessary to investigate the source of the systematic error. It was found that the sulfuric acid stock solution concentration used at Laboratory 2 was low compared to the recommendations of SM 4500-P E. As mentioned in Chapter 2, too high a pH can cause self reduction of the molybdenum and a "false" blue that will be incorrectly be attributed to phosphorus.

The erroneous sulfuric acid solution was replaced and the subsequent cross lab validation (aqua points in Figure 5-1) demonstrate that the two labs are performing very similar across the low range of TP that was tested (less than 100 ppb). Further comparisons of the performance of these two labs are given in Chapter 9.

CHAPTER 6

CASE STUDY: CITY OF COEUR D'ALENE

The City of Coeur d'Alene (CDA) has an excellent QA/QC system that can serve as a role model for other jurisdictions. Performance results are given below and demonstrate that Coeur d'Alene has very reproducible and accurate performance even at very low levels of phosphorus. The method used by CDA is the Lachat method 10-115-01-1 F for total phosphorus. This method is EPA certified as an acceptable version of the approved EPA method 365.1. For TP analysis, after persulfate digestion, samples are run on a Lachat QuikChem 8500 Series 2 flow injection analyzer. For orthophosphate (SRP) CDA uses Lachat method 10-115-01-1 M, which is essentially the same as the TP method but without digestion. To ensure quality of data CDA adhere to the QC acceptance criteria in Table 6-1.

Table 6-1 - Coeur d'Alene QC Acceptance Criteria for Phosphorus Analysis

Criteria	Acceptance Level
Initial Calibration Verification/Continuing Calibration Verification/Laboratory Control Sample/Blank Spike	90-110% Recovery
Digested TP/Ortho Blanks	< 5 ppb
Matrix Spike/Matrix Spike Duplicate	80-120% Recovery
Relative Percent Difference	< 20%

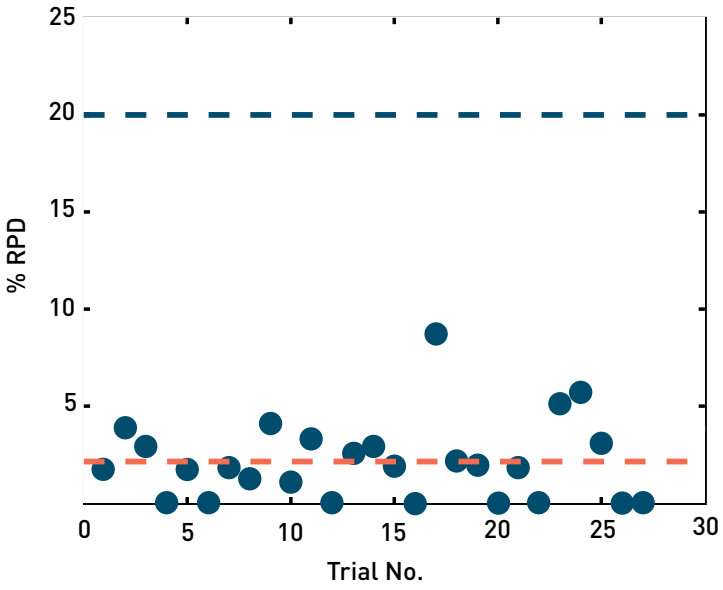
To assess reproducibility of measurements, relative percent difference (RPD) can be used. RPD is defined for two values of TP (TP_1 and TP_2) measured on the same sample as shown in Equation 6.

Equation 6

$$RPD = \left| \frac{TP_1 - TP_2}{(TP_1 - TP_2)/2} \right| \times 100$$

RPD results demonstrate that performance was well within the control criteria (Figure 6-1).

Figure 6-1 - CDA Results of Duplicate Analysis of TP in Effluent Samples

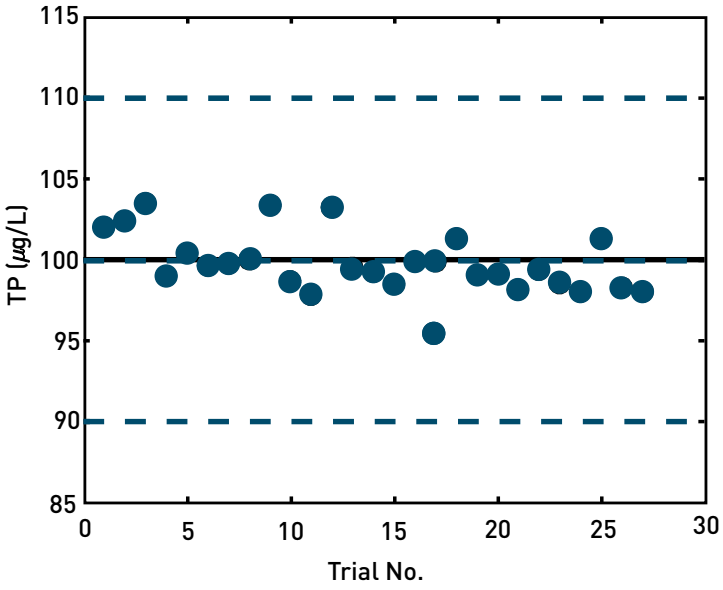


Effluent sample concentrations in the range 0.30 mg P/L to 1.1 mg P/L. Relative percent difference (RPD) calculated as in Equation 4. Data were monitored January to April 2013. Mean of the RPD data is shown as a red line.

The data does not exceed the control criteria is shown as a dashed blue line.

To assess accuracy ICV/CCV and LCS samples are used. It is important that these samples are at a level similar to the samples that are being measured. In the case of CDA 100 ppb ICV/CCV samples are used and even lower 50 ppb LCS samples (Figures 6-2 and 6-3 respectively). It can be seen that in terms of accuracy CDA is well within their control criteria.

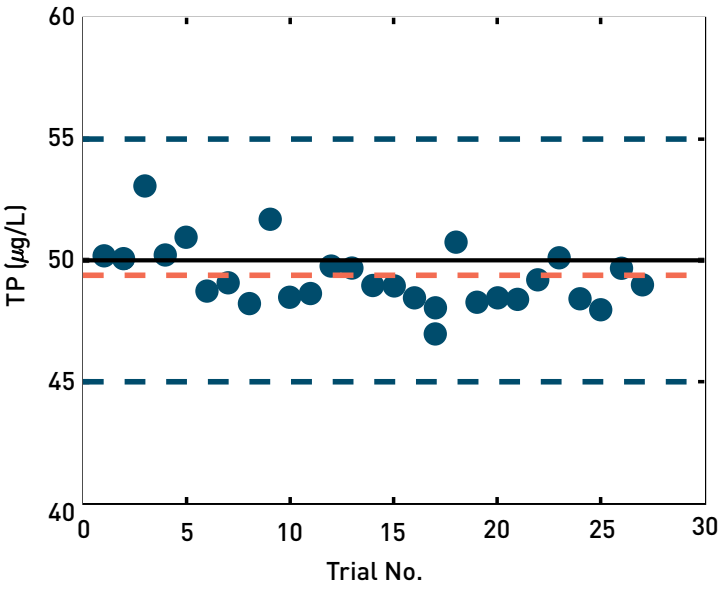
Figure 6-2 - ICV/CCV Samples Monitored January to April 2013



The calibration sample had a phosphorus concentration of 100 ppb. The mean of all 27 data points over this period is 100.

The blue dashed lines indicate ± 10% as stipulated by the CDA control criteria (Table 6-1).

Figure 6-3 - LCS Monitoring by CDA January to April 2013



The LCS sample was prepared at 50 ppb phosphorus. The mean value is shown as a red dashed line and the blue dashed lines represent the control criteria limits (Table 6-1).

Summary

CDA have excellent reproducibility and accuracy at low levels of TP analysis. The fact that true effluent samples were used for RPD determination will take into account any potential matrix effects of the wastewater being analyzed. CDA demonstrates what is possible with careful adherence to an analysis protocol (in this case a Lachat protocol) and with careful QA/QC program in place with specific control criteria.

CHAPTER 7

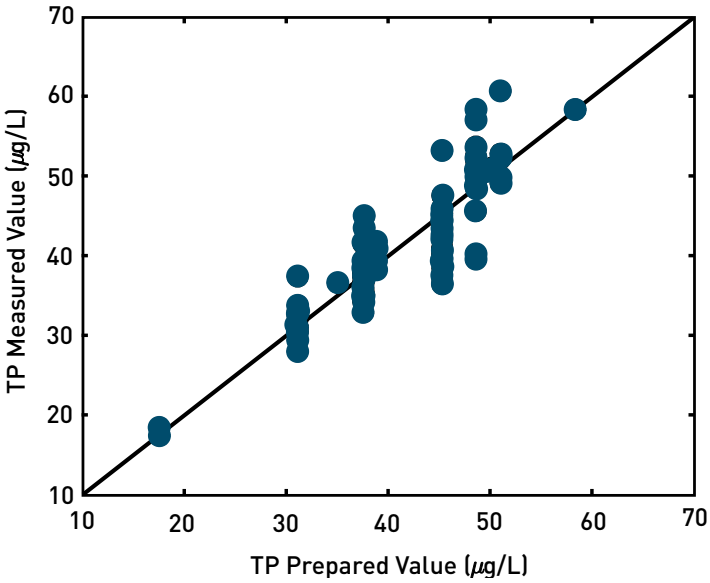
CASE STUDY: CITY OF SPOKANE

The City of Spokane uses EPA method 365.3 to analyze for low-level phosphorus (typically samples that are below 0.05 mg/L PO4-P). For higher range samples, Spokane uses the Hach brand Test in Tube method which is equivalent to SM 4500-P E. This report is focused on low-level analysis so only the EPA 365.3 QA/QC results are reported here.

In terms of QC, the lab for the City of Spokane analyzes certified reference check standards, blanks, and duplicate samples (to assess reproducibility). Spokane approaches things differently from CDA (Chapter 6). Both municipalities utilize very reasonable methods to validate their low-level phosphorus analysis. This highlights the flexibility that laboratories have in developing their own QA/QC protocols. What is required is some regular QA/QC assessment to ensure consistent quality in laboratory data (see recommendations in Tables 2-2 and 2-3).

The City of Spokane has had projects involving low-level TP and SRP measurements. To support these projects, QC data as RPD and LCS were analyzed to check for precision and accuracy of analysis respectively. The results show very good agreement between prepared and observed phosphorus concentration in LCS samples (Figure 7-1). In particular there are no systematic trends in the data about the 1:1 line between prepared and measured values. Maximum deviations are approximately 10 µg P/L. This highlights the essential of replicate measurements on samples. The mean value is centered on the true value, but in this case, any one measurement may be off by 10 µg P/L from the true value. Replicate measurements are the only way to obtain estimates of the true mean.

Figure 7-1 - City of Spokane Low-Level Phosphorus Testing

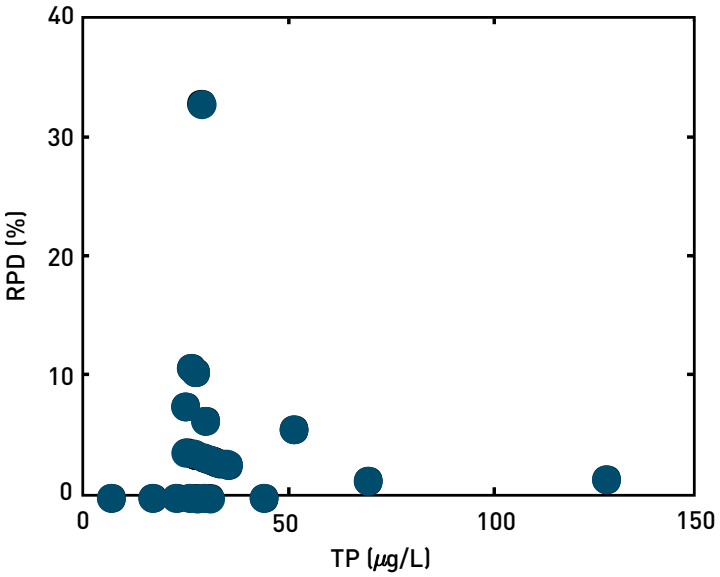


LCS for pilot project and various other low-level testing projects from September 2009 to July 2013.

The 1:1 line for prepared versus measured is shown as a solid diagonal line.

Interestingly, RPD testing shows a trend in reduced quality of performance at lower TP measurements (Figure 7-2). This makes sense in that error from colorimetry tends to be constant (i.e., ± 0.002 absorbance units on the actual absorbance measurement step); the small amount of error becomes more significant as concentrations, and associated absorbance measurements, are lower. All the data is less than the CDA reported control criteria (20%, Table 6-1) except for one (outlier) point at almost 35% deviation at about 30 ppb P.

Figure 7-2 - City of Spokane Low-Level Phosphorus RPD Data



Data shown for actual water samples from pilot project and various other low-level testing projects from September 2009 to July 2013. RPD is defined as Equation 6-1.

CHAPTER 8

CASE STUDY: CITY OF LAS VEGAS

The City of Las Vegas utilizes SM 4500-P methods including B5 (Persulfate Digest) for total phosphorus sample preparation and E (ascorbic acid) for measurement of total phosphorus (TP) and ortho-phosphate (TRP and SRP) measurements. The quality control chart for Las Vegas is very extensive (Table 8-1). An example of the excellent performance achieved is shown in Figure 8-1. Even with the very low LCS at 20 µg P/L the analysis is within the 85%-115% recovery criteria (see Table 8-1) 360 out of 365 times (98.6% of measurements). Thus, the Las Vegas analytical lab is very accurate for phosphorus analysis. The reproducibility of the laboratory (see Figure 8-2) is demonstrated by the fact that there were only two measurements out of 334 that exceeded the 10% RPD control criteria (99.4% of measurements were within the criteria).

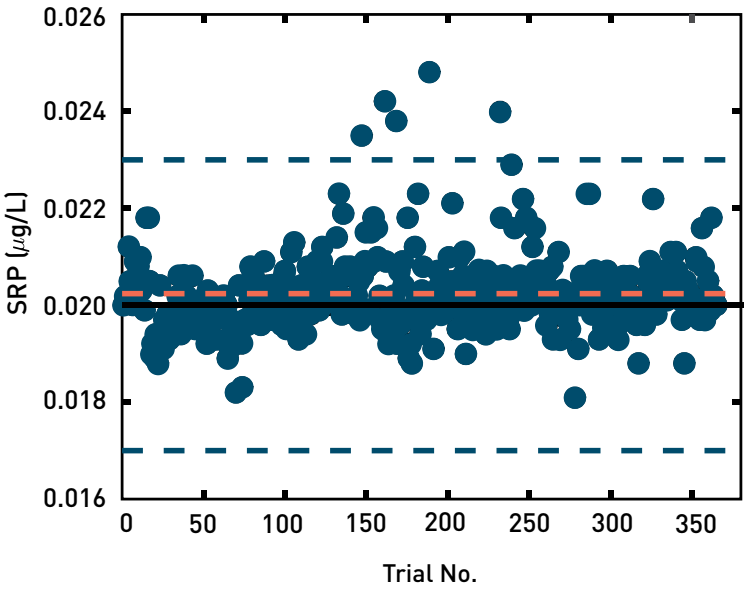
Table 8-1 - Las Vegas Quality Control Chart: Criteria and Corrective Action

QC Check	Frequency	Acceptance Criteria	Corrective Action
Calibration (6 Stds.)	Each Batch	R ⩾ 0.995	Recalibrate
Method Blank Result	Each Batch	|Result| ⩽ RDL	Correct Problem, Recalibrate
Initial Control Recovery	Pre Batch	90%–110%	Reanalyze Entire Batch
Matrix Dup. Precision	1 per Batch	RPD ⩽ 10%*	Qualify Result, Investigate
Matrix Spike Rec.	1 per Batch	90%–110%	Qualify Result, Investigate
Matrix Spike Duplicate Rec.	1 per Batch	90%–110%	Qualify Result, Investigate
Matrix Spike/Matrix Spike Duplicate Precision	1 per Batch	RPD ⩽ 10%	Qualify Result, Investigate
Continuing Calibration Blank Result	Every 10 Samples and Ending	|Result| ⩽ RDL	Reanalyze Affected Samples (less than 10x blank result)
Continuing Calibration Verification Recovery	Every 10 Samples and Ending	90%-110%	Reanalyze Affected Samples
Final Control Recovery	Post Batch	90%–110%	Reanalyze Entire Batch
Laboratory Control Sample Recovery	1 per Batch	85%–115%	Qualify Result, Investigate

RPD=relative percent difference RDL=reporting detection limit RDL=0.02 mg P/L

* For samples wherein the concentration is less than 10 times the RDL, RPD is not applicable.

Figure 8-1 - Las Vegas Water Pollution Control Facility Results for Low-Level P

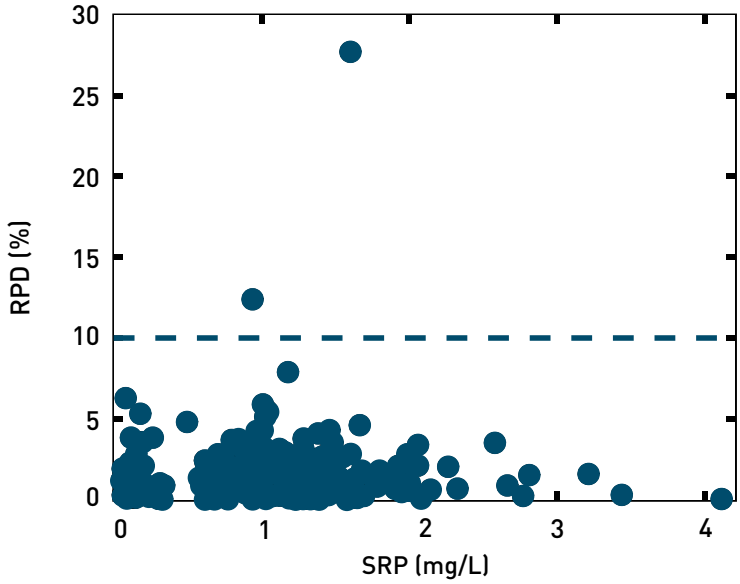


Samples prepared at 20 µg P/L as orthophosphate sample. Samples measured during 2012.

Dashed blue lines indicate acceptance criteria (± 15% recovery).

Red line indicates the mean of the measured values and black line represents the "true" value of the LCS.

Figure 8-2 - Las Vegas Water Pollution Control Facility Replicate Analysis Results



Samples measured for orthophosphate during 2012.

Dashed blue lines indicate acceptance criteria (≤ 10% RPD).

Specific Recommendations from Standard Operating Procedures (SOP)

Table 8-2 presents specific recommendations from the Las Vegas SOP for total phosphorus analysis unless otherwise indicated. The format of Table 8-2 is the same as Tables 2-2 and 2-3 with each aspect of the analytical process highlighted with separate recommendations. Additional aspects are highlighted including safety and waste disposal not directly influencing quality of analytical results but very important for operation of any analytical facility.

Table 8-2 - Specific Recommendations from Las Vegas Environmental Division Laboratory SOP

Aspect	Recommendation
Storage	Analyze samples immediately after receipt in the laboratory or preserve to pH < 2 with H_2SO_4, (generally 1 mL/L of sample) and keep at < 6°C. The maximum hold time is 28 days.
Storage	Do not store samples containing low concentration of phosphorus in plastic bottles unless kept in a frozen state due to adsorption of phosphates on the walls of plastic bottles.
Storage	For orthophosphate analysis samples are kept under refrigeration at < 6°C with a maximum hold time of 48 hours unless frozen.
Filtration	For dissolved orthophosphate, samples are filtered using 0.45 micron filter immediately after collection.
Analysis	Arsenic, at concentrations as low as 0.1 mg/L, reacts with molybdate reagent to produce a blue color, which interferes with phosphorus determination. Hexavalent chromium and nitrite also interfere.
Analysis	Wash all glassware according to the Glassware Cleaning SOP.
Safety	Sampling and analysis procedures require handling of raw wastewater samples and corrosives. Use appropriate personal protective equipment (PPE). The minimum PPE with over 100 mL concentrated acid requires the use of gloves, lab coat, fume hood with sash drawn low enough to avoid face being splashed or goggles.
Safety	Digestion of samples must be done in a fully operational fume hood.
Waste Disposal	Neutralize acids before disposing. Use proper PPE for handling of samples and reagents.

CHAPTER 9

CASE STUDY: IMPORTANCE OF INTER-LABORATORY COMPARISONS

For a period of approximately one year (May 2012 to April 2013) a pilot study was conducted to test water treatment technologies on phosphorus removal (details of location and technology are confidential and thus not included as part of this document). Samples from this study were analyzed at a commercial analytical facility (referred to as Laboratory 1) and at Wilfrid Laurier University laboratory (Laboratory 2). During initial phases of monitoring systematic differences between the labs were noted and resolved as discussed in Chapter 5. Once the analytical issues were resolved for Laboratory 2 analysis proceeded at both labs on samples from the pilot facility.

Samples for both labs were taken from the same points in the treatment stream at approximately the same time but analysis was not performed on the same samples. During pilot plant phosphorus monitoring, both laboratories utilized ascorbic acid colorimetry for phosphorus analysis but Laboratory 1 was equipped with continuous flow-through analysis (SM 4500-PG) and Laboratory 2 did the analysis using a batch method (SM 4500-PE). Both methods utilized persulfate digestion to convert total phosphorus into colorimetrically detectable phosphate. A significant difference between the two labs is that Laboratory 1 used a less sensitive 1 cm light path for absorbance measurements while Laboratory 2 used a more sensitive 10 cm path.

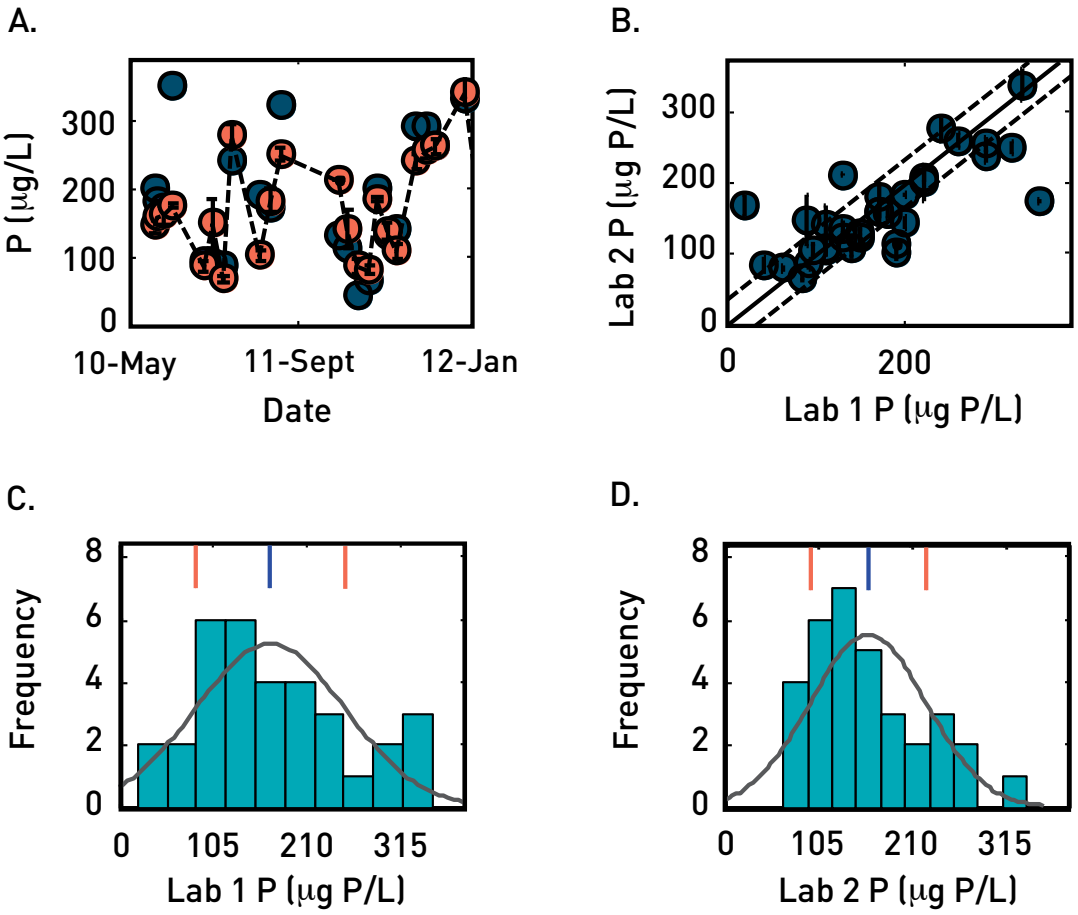
Both labs utilized QA/QC protocols similar to the City of Coeur d'Alene (Chapter 6) and satisfied control conditions within the tolerances of Table 6-1.

Thus, the comparisons in performance between the two analytical facilities are comparisons of facilities that perform extremely well on laboratory prepared samples. Variability between the labs then should not be due to analytically methods but due to sample variability. Overall, the results presented below demonstrate that the analytical labs gave the same average behavior for the pilot facility but with some random variation, likely because the samples were not split analysis but actually collected as separate samples. The random variations highlight that for low-level analysis to get the true value it is necessary to measure as many replicate samples as possible (see conclusions about Figure 7-1). The central tendency should approach the true value.

TP Comparisons: Pilot Plant Influent

Analysis of influent samples to the pilot facility is shown in Figure 9-1. The influent to the pilot facility was actually secondary effluent from a nearby municipal treatment works (identity withheld). For the most part the data from the two laboratories agree very well with most results within 10% of the one to one comparison line (Figure 9-1(a)). To assess the performance of the labs, it is possible to compare the average concentrations (Figure 9-1(c,d)). This comparison demonstrates that over the course of one year of monitoring on average the measurements matched very well and have a very similar distribution width (compare blue and red lines in Figure 9-1(c,d)).

Figure 9-1 - Total Phosphorus (TP) Analysis of Pilot Plant Influent

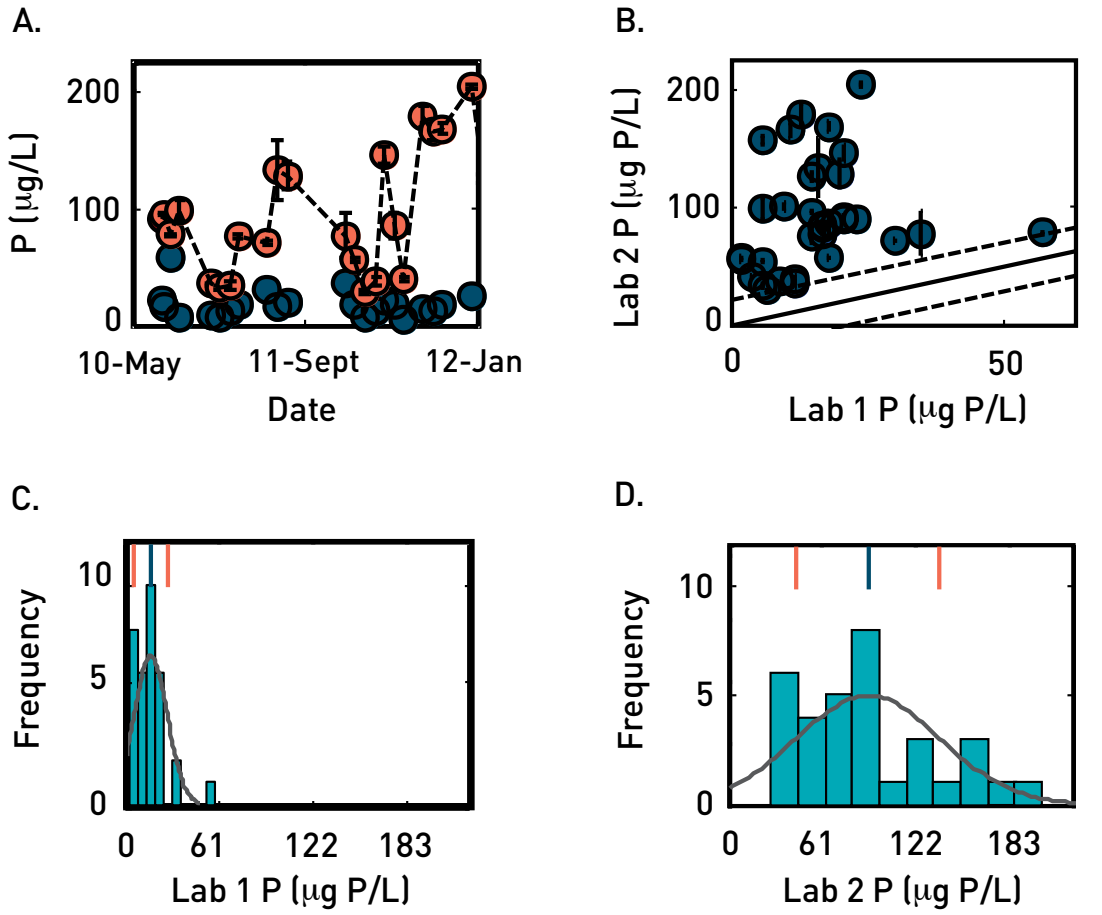


Samples measured by two analytical labs. Laboratory 2 did three replicate measures on each sample and corresponding 95% confidence intervals are shown.
(a) Blue points correspond to Laboratory 1 and red points correspond to Laboratory 2.
(b) Solid line indicates the one to one line and the two dashed lines are equivalent to 10% deviation from the maximum measured value.
(c) and (d) Histograms of the results are shown for each indicated laboratory with a solid blue "tick" line indicating the mean value and two red lines at plus and minus one standard deviation.

TRP Comparison: Pilot-Plant Influent

When TRP was measured in influent samples the results do not agree nearly as well between the two laboratories (Figure 9-2). Consistently Laboratory 9-2 had higher TRP results than Laboratory 1. This is very clear in Figure 9-2(b) where only a few data points are within 10% of the one to one comparison line. Laboratory 2 often has values almost an order of magnitude greater than Laboratory 1. The potential for this overestimation is discussed in (McKelvie et al. 1995) and attributed to the potential for hydrolysis of labile P-compounds in the acidic color developing media.

Figure 9-2 - Total Reactive Phosphorus (TRP) Analysis of Pilot-Plant Influent



Samples measured by two analytical labs. Laboratory 2 did three replicate measures on each sample and corresponding 95% confidence intervals are shown.
(a) Blue points correspond to Laboratory 1 and red points correspond to Laboratory 2.
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(c) and (d) Histograms of the results are shown for each indicated laboratory with a solid blue "tick" line indicating the mean value and two red lines at plus and minus one standard deviation.

If the ubiquitous presence of iron and aluminum oxides in wastewater is considered, an additional ambiguity in the determination of TRP can be proposed. TRP is proportional to the blue color developed when an unfiltered sample is treated with the color forming reagents. Figure 2-1 clearly shows that this measurement step acidifies the sample (to pH <1.0, Table 2-1). In the presence of acidic conditions surface bound phosphate will dissociate from mineral surfaces or potentially the minerals themselves will dissolve (Smith et al. 2008). The dissolution or desorption processes are time dependent, therefore, depending on the exposure time to the color forming reagents there can be higher or lower blue color development.

For this study, the influent to the pilot facility was secondary effluent from a municipal treatment works that uses alum for phosphorus removal. Thus, the unfiltered samples would have residual particulate aluminum oxide and associated phosphorus. Laboratory 1 utilizes a flow through

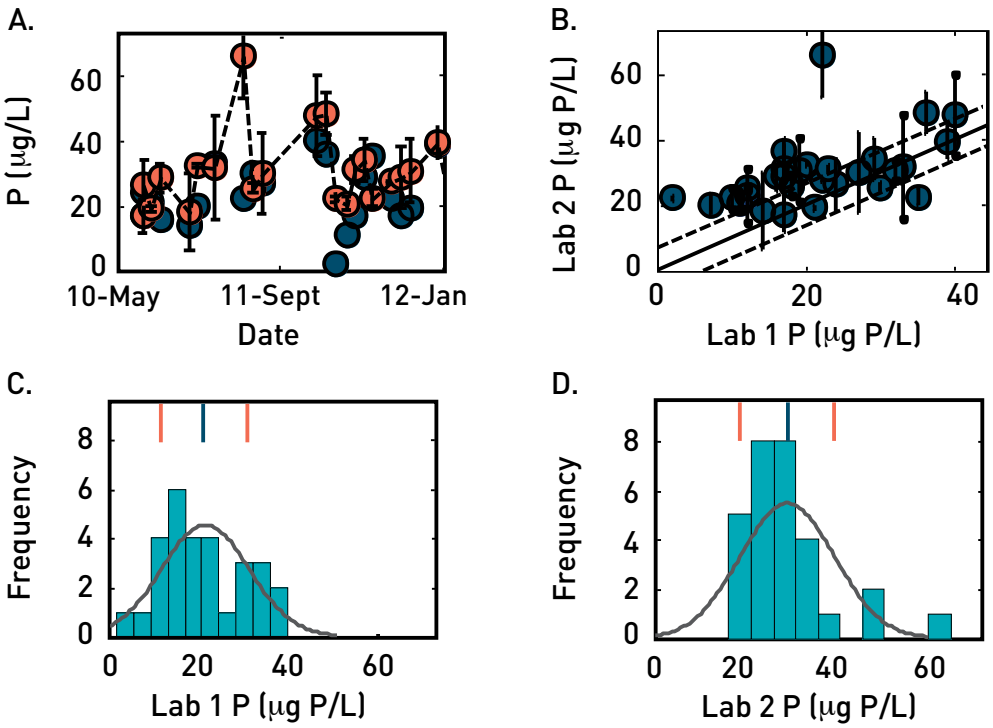
method and the acidic solutions are in contact with the sample for approximately five minutes before the measurement step. For Laboratory 2 a color development time of 10 to 30 minutes was utilized (SM 4500-P E). Potentially this discrepancy explains the difference in the results; Laboratory 2 determines systematically higher values because Laboratory 2 has a significantly longer contact time between the acidic reagent and the sample.

Thus, the measurement of TRP is ambiguous and operationally defined. This has been reported from the context of color development time as well as storage Gu et al. (WERF 2014). The question remains though if TRP is a desirable analytical quantity, what is the best method to measure it? Likely the definition of TRP will depend on the specific use. If the purpose is a proxy number for bioavailability, then likely a short contact time is appropriate. If the purpose is to estimate long-term transport and loading of phosphorus, then a longer contact time is most appropriate.

TP Comparisons: Membrane Permeate

Within the pilot-plant facility there was a membrane filtration step. Results of membrane permeate analysis for TP represented in Figure 9-3 demonstrate that even at very low levels of

Figure 9-3 - Total Phosphorus (TP) Analysis of Pilot-Plant Membrane Permeates



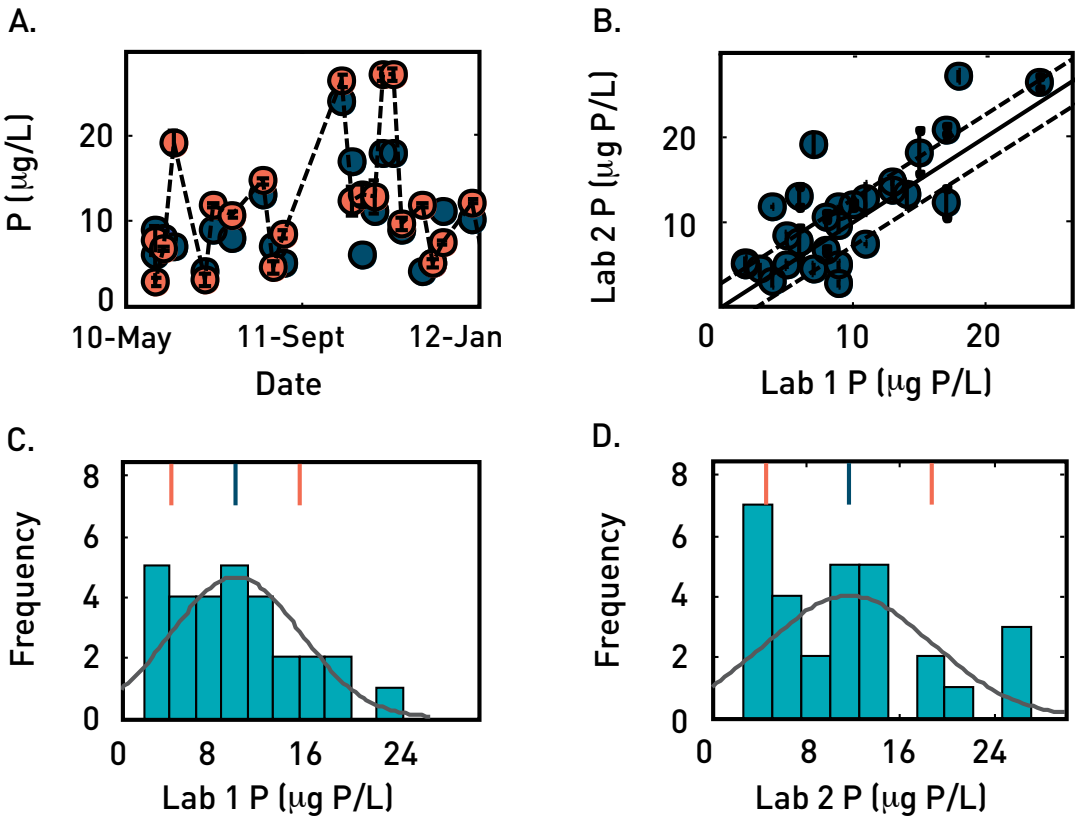
Samples measured by two different analytical laboratories. Laboratory 2 did three replicate measures on each sample and corresponding 95% confidence intervals are shown.
(a) Blue points correspond to Laboratory 1 and red points correspond to Laboratory 2.
(b) Solid line indicates the one to one line and the two dashed lines are equivalent to 10% deviation from the maximum measured value.
(c) and (d) Histograms of the results are shown for each indicated laboratory with a solid blue "tick" line indicating the mean value and two red lines at plus and minus one standard deviation.

total phosphorus (10s of ppb) the two laboratories agree very well. Figure 9-3(b) does have one point where Laboratory 2 measured 65 ppb and Laboratory 1 measured closer to 20 ppb but overall the measurement results are very close to the one to one line. Similar to Figure 9-3(c,d) the means and standard deviations over the monitoring period are very consistent.

TRP Comparisons: Membrane Permeate

Measurement of TRP in membrane permeate allow for testing of the P-associated-oxide particle hypothesis. If particles are fundamentally causing the systematic error between the two laboratories, then differences should be removed when filtered samples are compared. This is in fact the case for the samples analyzed here. This is shown in Figure 9-4 which presents TRP measurements on samples collected within the pilot facility after a membrane filtration. Figure 9-4(b) in particular demonstrates that the majority of samples are within 10% of the one to one comparison line. These are for very low levels of TRP in the range 5 to 25 µg P/L so the absolute differences between the samples are small.

Figure 9-4 - Total Reactive Phosphorus (TRP) Analysis of Pilot-Plant Membrane Permeates



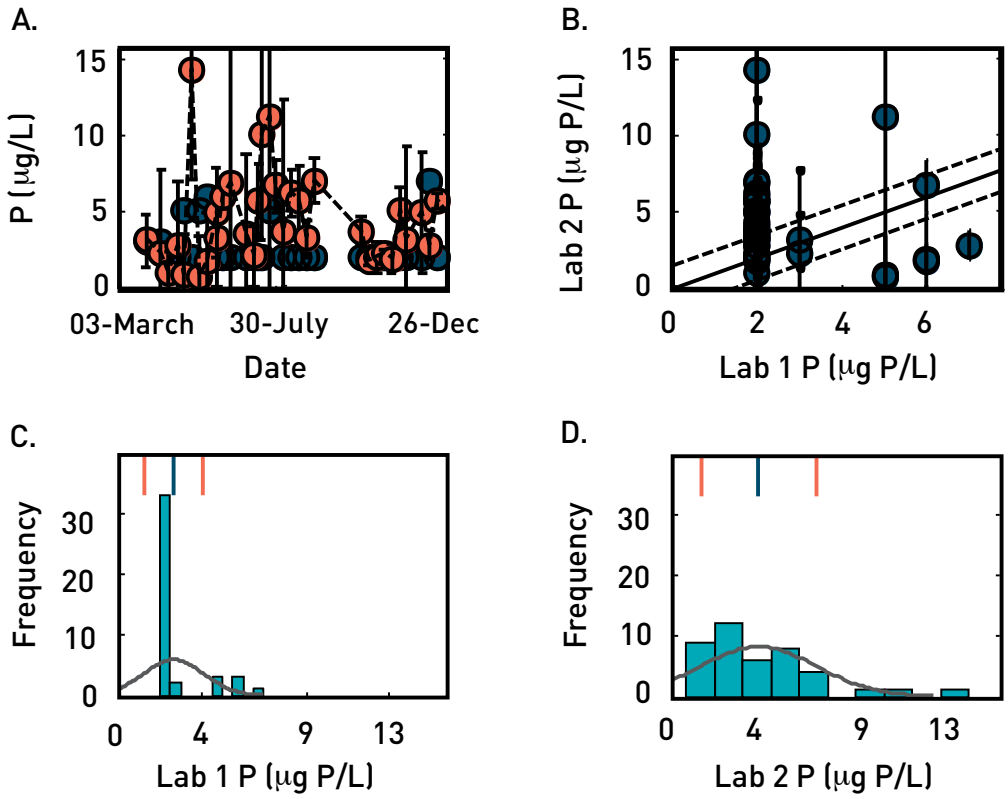
Samples measured by two analytical laboratories. Laboratory 2 did three replicate measures on each sample and corresponding 95% confidence intervals are shown.
(a) Blue points correspond to Laboratory 1 and red points correspond to Laboratory 2.
(b) Solid line indicates the one to one line and the two dashed lines are equivalent to 10% deviation from the maximum measured value.
(c) and (d) Histograms of the results are shown for each indicated laboratory with a solid blue "tick" line indicating the mean value and two red lines at plus and minus one standard deviation.

TP Comparisons: Final Pilot Effluent

Low concentrations represent a challenge for phosphorus determinations. The lowest concentrations in this study were observed for the final pilot plant effluent. Figure 9-5 presents comparison plots between the two laboratories performing the analysis. The mean values agree between the two laboratories (see Figure 9-5(c,d) but Laboratory 2 had dramatically greater width to the measurements.

To understand why Laboratory 2 has a wider distribution of TP results, it is necessary to consider the difference between a 1 cm and a 10 cm path length for absorbance measurements. Laboratory 2 (10 cm path) reports concentrations often above values reported by Laboratory 1 (1 cm path). For a 1 cm light path, samples at the detection limit of the spectrometer need to measure 0.001 absorbance units. For that same sample with a 10 cm light path, the measured absorbance would be approximately 0.010 units. Thus, Laboratory 2 has an added digit of precision compared to Laboratory 1. Samples detected at or below the detection limit by Laboratory 1 compress the range of numbers measured, but Laboratory 2 can resolve concentrations below detection by Laboratory 1. This additional resolution allows for greater differences to be observed between samples and thus a greater variability in measured results.

Figure 9-5 - Total Phosphorus (TP) Analysis of Final Pilot-Plant Effluent



Samples measured by two different analytical laboratories. Laboratory 2 did three replicate measures on each sample and corresponding 95% confidence intervals are shown.
(a) Blue points correspond to Laboratory 1 and red points correspond to Laboratory 2.
(b) Solid line indicates the one to one line and the two dashed lines are equivalent to 10% deviation from the maximum measured value.
(c) and (d) Histograms of the results are shown for each indicated laboratory with a solid blue "tick" line indicating the mean value and two red lines at plus and minus one standard deviation.

Statistical Comparison Test

A simple t-test was utilized to compare data from the two labs over the period of operation of the pilot facility.

Test used for two sets of results x and y:

Equation 7

$$t_{calc} = \frac{|\bar{x} - \bar{y}|}{\sqrt{\frac{s_x^2}{n_x} + \frac{s_y^2}{n_y}}}$$

Degrees of freedom are calculated as:

Equation 8

$$df = \frac{(s_x^2/n_x + s_y^2/n_y)^2}{\frac{(s_x^2/n_x)^2}{n_{x-1}} + \frac{(s_y^2/n_y)^2}{n_{y-1}}}$$

If t_{calc} is less than t_{table}, the conclusion is that at 95% the results have been sampled from the same underlying population. If t_{table} is greater than t_{calc}, the results are not the same (at 95% confidence).

The result of the t-test is presented in Table 9-1 and it can be seen that statistically over the year of monitoring, both labs statistically represent the mean behavior of the pilot plant the same except for the ambiguous measurement of TRP.

Table 9-1 - Summary of TP and TRP Results from Two Laboratories

	Lab 1 Mean	Lab 2 Mean	Lab 1 sd	Lab 2 sd	df	t_{calc}	t_{table}	Same?
Secondary Effluent								
TP	208.89	184.83	125.74	72.89	25.78	0.91	2.06	yes
TRP	22.95	111.22	16.06	63.12	11.28	4.81	2.19	no
Membrane Effluent								
TP	27.52	31.01	11.58	8.94	27.96	0.99	2.05	yes
TRP	13.65	16.97	6.34	9.70	16.04	1.07	2.12	yes
Final Effluent								
TP	2.60	3.13	2.59	1.21	32.53	1.03	2.04	yes

The final column indicates the result of application of the t-test given by equations 5 and 6.

CHAPTER 10

CONCLUSIONS

The first section of this report (Chapter 2) presented a theoretical overview of phosphorus analytical methods with a particular emphasis on colorimetry. The recommendations gleaned from the literature and presented in Tables 2-2 and 2-3 can be used in wastewater analytical labs. From Table 2-2 it becomes clear that certified reference materials should be part of the QA/QC protocol used by laboratories doing wastewater analysis. This is not currently being done because no existing CRMs exist for wastewater. It is possible to purchase certified orthophosphate solutions for RP or TP determination, but organic phosphorus standards do not exist. It is suggested that until such time as wastewater-specific CRM is available that alternative proxy CRMs could be used (Worsfold et al. 2005, see Table 4-1).

Chapter 2 highlighted the importance of pH, and the associated H:Mo ratio in the color forming step of phosphate analysis. The case study presented in Chapter 5 highlighted how this issue can be present in laboratory analysis as a systematic bias and not detected until an inter-laboratory validation study was performed. Analysts should be aware if their samples have sufficient acid neutralizing capacity to impact the pH and H:Mo ratio of the final sample (Jarvie et al 2002).

The chapter on reaction kinetics for phosphorus colorimetry (Chapter 3) highlights the potential systematic errors that can result if reactions do not progress to completion, or if samples are left too long to develop color. The section highlighting digestion methods (Chapter 4) for TP determination highlights the fact that all digestion methods are not created equally and model compounds should be assessed as well as comparisons between mild and aggressive digestion techniques should be performed on relevant samples to ensure there is no systematic bias in the results.

Excellent reproducible and accurate results for low-level phosphorus is achievable in wastewater as demonstrated for Coeur d'Alene (Chapter 6), Spokane (Chapter 7), and the City of Las Vegas (Chapter 8). The QA/QC methods presented in these chapters, and the SOP for Las Vegas in particular (Table 8-2), represent excellent operating procedures that wastewater analysis facilities could consider adopting for their own purposes.

The final case study in Chapter 9 demonstrated the ambiguity of TRP as an analytical measurement and clearly showed the advantages of longer path length colorimetry. For low-level analysis, replicate measurements are essential in order to capture the true value of the sample within variability. When dealing with low concentrations even a small absolute error is a large relative error; thus, replicate measurements are essential to estimate true concentrations for dilute phosphorus samples.

CHAPTER 11

FUTURE RESEARCH

It is interesting that with all the years spent measuring phosphate using molybdate colorimetry that no standard conditions have been agreed on (see Table 2-1). Yet despite the method differences, good analytical results are being generated by laboratories in the wastewater industry. There are likely still possible improvements for wastewater phosphorus analysis. Some possibilities include:

- Drummond and Maher (1995) represent an interesting avenue for future work. By tweaking reagent ratios (see Table 2-1) it might be possible to create new Standard Methods specifically for low-level analysis with fast reaction times and good accuracy.
- New Standard Methods for low-level analysis will also need to consider reaction time (Chapter 3). In particular, the potential different reaction times for high and low phosphorus concentrations.
- Certified Reference Materials specific to the wastewater industry are essential for truly robust QA/QC analysis. To put this in context, part of normal QA/QC is re-analysis of calibration samples to verify that concentrations are recovered. If there is a systematic error (i.e., pipetter consistently over or under delivering) the reanalysis will not detect it. Yet as an absolute standard CRM analysis would verify quality of analytical results.
- Explore ICP as a viable alternative to colorimetry for TP analysis. ICP is more expensive but because it is truly and elemental analysis technique there is much less ambiguity to what is being measured and any potential issues with sample digestion are removed.

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