

# **MYTHS, LIES & FAIRYTALES - UNRAVELLING THE MYSTERY OF TESTING FOR PHOSPHORUS.**

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## **ABSTRACT**

Human activities throughout Australia's natural landscape have resulted in stream health degradation. This degradation includes elevation of phosphorus in our waterways. Elevated phosphorus levels can result in increased algae and cyanobacterial blooms that, in turn, produce human health and aesthetic impacts, odour problems, reduced water clarity, and oxygen deficiency (Cassidy, 2003). For this reason, many federal, state, regional and local organisations select phosphorus as an indicator for measuring aquatic ecosystem health and as an indicator for ecological risk.

Determining phosphorus concentrations in a water sample is not straightforward. A range of factors can present challenges and are they often the source of much confusion and debate. Common questions that arise include:

- Which fraction (or part) of phosphorus should I be measuring?
- What are the differences between the phosphorus test equipment types?
- What chemical methodology should I be using?
- How do the chemicals found in my water sample interfere with the chemical methodology I am using?
- Does the colour of the water interfere with the equipment type or chemical methodology used?
- What digestion method should I use for my total phosphorus analysis?
- What are the major sources of contamination and how can I minimise this error?
- Should I express my results as the weight per litre of orthophosphate or elemental phosphorus alone?

In Queensland the State Community Monitoring Team is developing a suite of technical manuals and tools for the planning, monitoring and communication of stream health. Each module within the technical manual will be prepared and compiled to help guide monitoring coordinators through the specific considerations for each indicator. This paper provides a technical summary of the phosphorus topic in order to "demystify" some of the myths, lies & fairytales surrounding testing for phosphorus.

## **KEYWORDS**

Phosphorus, Orthophosphate, Analytical Digestion, Total Phosphorus, Filterable Reactive Phosphorus, Total Reactive Phosphorus, Colorimetry, Colour Comparators, Photometers, Spectrophotometers, Phosphorus Chemical Methodologies, Phosphorus-Orthophosphate conversion.

## WHAT IS PHOSPHORUS?

Phosphorus, (with chemical symbol P), is one of some 116+ pure elements that exist on earth and is widely distributed in many rocks, soils and as a trace element in the aquatic environment (Croke, 2002). Its scarcity in the aquatic environment is a result of phosphorus's low solubility and the tendency for dissolved phosphorus to bind with soil particles suspended in the water column. When the particles settle, the phosphorus bound to this suspended soil is buried in bottom sediments, leaving the water relatively devoid of phosphorus. This scarcity of phosphorus in many natural waterways tends to limit the growth of algae and other biological organisms that require phosphorus for function and growth (McKelvie, 2000). Unlike nitrogen and carbon, phosphorus has no gaseous form present in the atmosphere (Horne and Goldman, 1994). Therefore, entry of all phosphorus in the water column is a result of phosphorus-bearing sediment, animal waste and decomposing organic material falling, washing or blowing into the waterway or being re-released from bottom sediments under high flow (Croke, 2002) or anoxic (Spiro & Stigliani, 1996) (no or low oxygen) conditions. Once phosphorus enters a waterway it is transported with the flow regime until it reaches the endpoint of this flow or settles back into the bottom sediments.

## WHICH FRACTION (OR PART) OF PHOSPHORUS SHOULD I BE MEASURING?

Before deciding what to measure, it is good to be aware of some common terms used. Phosphorus can occur as a range of salts and other compounds. Appendix 1 defines commonly used terms relating to phosphorus.

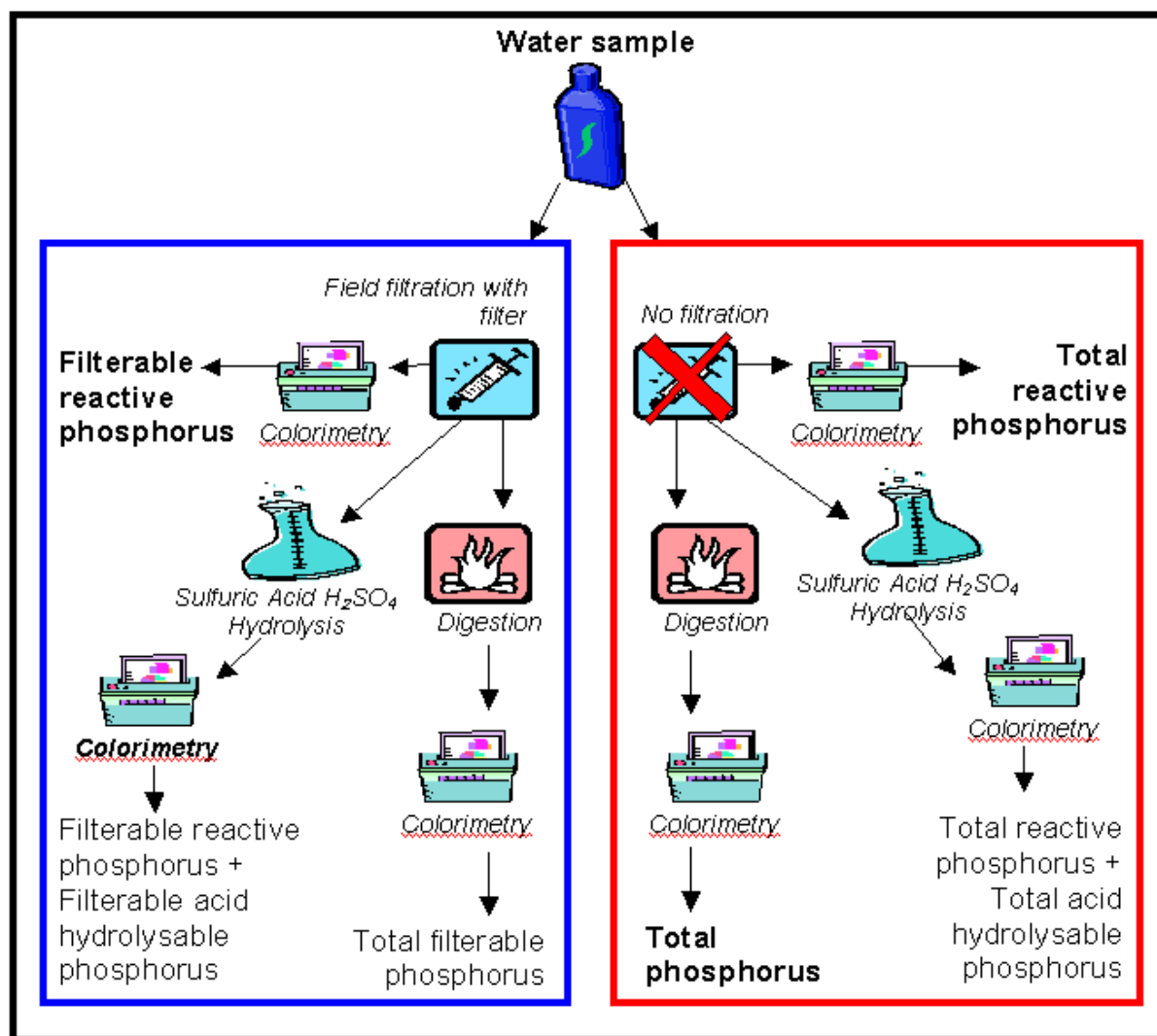
Orthophosphate ( $\text{PO}_4^{3-}$ ) is formed by the combination of one atom of phosphorus and four atoms of oxygen and is the molecule that **all** phosphorus tests react with to produce a coloured reaction that can be measured (colorimetry). To measure any other form of phosphorus, extra steps have been developed to break the phosphorus bound in larger compounds down into orthophosphate and/or to filter the larger chemical compounds containing phosphorus from a sample. The various testing methods will separate out and measure a certain part or fraction of the phosphorus that is present in the sample collected. The procedures for determining the more common fractions of phosphorus are indicated in Figure 1.

### Commonly Used Measures of Phosphorus

Of the six common fractions of phosphorus illustrated in Figure 1, the scientific community makes use of three on a regular basis. These fractions (marked in bold in Figure 1) include; total phosphorus, filterable reactive phosphorus and total reactive phosphorus.

### Total Phosphorus

Undertaking a total phosphorus test provides a result on the total amount of phosphorus in a water sample including the inorganic and organic fractions both suspended and dissolved in the water (Clesceri *et al.*, 1998), which is useful for characterising the water body and assessing catchment condition. This measure can be used in conjunction with flow rates to provide load-based estimates of phosphorus being transported downstream but does not indicate the fraction of phosphorus that is available for direct biological uptake, thus limiting its value as an indicator for ecological risk. As indicated in Figure 1, the sample must be digested to measure total phosphorus. Analytical digestion is discussed in detail later.



**Figure 1. Steps required in determining different phosphate fractions. Adapted from Standard Methods, 1998, Figure 4500-P:1.**

### **Total Reactive Phosphorus & Filterable Reactive Phosphorus**

Undertaking a total reactive phosphorus test will provide a measure of the amount of orthophosphate present in a water sample. However, due to the chemicals used to produce the colorimetric reaction, a small fraction of condensed phosphates and phosphates bound to complex inorganic and organic compounds are hydrolysed (broken down) during the reaction. What this means is that the result for total reactive phosphorus is an overestimation of the orthophosphate in a water sample.

Filtering the water sample before analysis drastically reduces this overestimation with the results expressed as filterable reactive phosphorus. The filters used and recommended in the filterable reactive phosphorus test are 0.45µm pore size (Horne and Goldman, 1994), however filters with differing pore sizes may be used as long as the pore size and methods used are described with the results.

Another advantage of the filtration process is that the filtered sample is left virtually colourless (except in the case of tannin stained waters). So colorimetric instrument readings are less hampered by background colour readings.

Both total reactive phosphorus and filterable reactive phosphorus may be used as an estimate of phosphate concentrations available for biological uptake, but to obtain more accurate results filterable reactive phosphorus is recommended.

## **WHAT ARE THE DIFFERENCES BETWEEN THE PHOSPHORUS EQUIPMENT TYPES?**

### **Colorimetry**

The concentration of orthophosphate in a water sample is proportional to the intensity of colour produced by phosphorus test chemicals. This photochemical reaction, called colorimetry, can be measured using various grades of equipment, which includes comparators, photometers and spectrophotometers. All three types operate on the principal of measuring the intensity of colour development at a certain colour range (wavelength). However, there are some important differences between each equipment type and these are summarised below:

### ***Comparators***

Comparators utilise the operator's visual perception to determine the intensity of colour produced. The concentration of orthophosphate is estimated by best matching the colour produced to a colour wheel or colour chart for predetermined concentrations. This kind of measurement can be rather subjective, considering many people have varying degrees of colour-blindness. Another shortcoming of colour comparators is the choice of only one chemical methodology to test for orthophosphate and the fact that comparators are designed to test for one chemical. The advantage of a comparator is their reasonably low equipment cost compared to photometers and spectrophotometers. When used correctly, comparators provide a simple and low cost means of roughly indicating phosphorus levels (Module 4 Waterwatch Australia, 2002).

### ***Photometers***

Photometers operate by focussing light through a lens, a coloured filter and the water sample, then onto a detector. The detector converts light intensity into an electrical current, which is displayed as the concentration of chemical in solution (Radojevic & Bashkin, 1999). The photometer removes human error associated with a comparator and usually comes with filters capable of measuring many wavelengths. This means that photometers usually have the capacity to test a sample using more than one chemical methodology and may cover a range of physico-chemical parameters. The ability to measure at different wavelengths is limited by the types of filters provided by the manufacturer. Instrument specifications should be consulted before purchasing a photometer to determine if the filters provided will cover the wavelengths required to measure a particular chemical. The cost of a photometer falls between that of a comparator and a spectrophotometer. When used correctly, photometers provide a simple and low cost means of indicating phosphorus levels.

### ***Spectrophotometers***

Spectrophotometers are essentially the same as photometers with the following exception, spectrophotometers employ a glass prism or grating (rather than filters) which can produce and measure across the full light spectrum. The required wavelength is obtained using a

moveable slit between the prism and the sample. What this means is, that a spectrophotometer has the flexibility to measure a range of physico-chemical parameters using more than one chemical methodology (Radojevic & Bashkin, 1999). The downside of a spectrophotometer is that it is more expensive than comparators and photometers. Spectrophotometers are usually less robust than comparators and photometers, and for this reason portable field spectrophotometers should be handled carefully. Spectrophotometers are generally used in analytical laboratories.

## **WHAT CHEMICAL METHODOLOGIES SHOULD I BE USING?**

As stated earlier the concentration of orthophosphate in a water sample is proportional to the intensity of colour produced by the phosphorus test chemicals. These chemicals bind to all available orthophosphate creating a new chemical complex. This complex is then reacted with another chemical to produce a colour intensity that is proportional to the amount of orthophosphate in the sample ([www.geocities.com/RainForest/5161/lab2.htm](http://www.geocities.com/RainForest/5161/lab2.htm)).

There are three major chemical methodologies used for measuring orthophosphate in a water sample (Clesceri *et al.*, 1998)

- Vanadomolybdophosphoric Acid Colorimetric Method,
- Stannous Chloride Method, and
- Ascorbic Acid Method.

Test kit manufacturers will often modify these chemical methodologies slightly and each product should be examined carefully to gain a better understanding of the specific reactions and associated interferences of the kit. A summary of each chemical methodology has been provided in Appendix 2. It is important to understand the typical range for each different chemical methodology. This will ensure the right test can be chosen taking into account the natural conditions of your local waterway.

## **HOW DO THE CHEMICALS FOUND IN MY WATER SAMPLE INTERFERE WITH THE CHEMICAL METHODOLOGY I AM USING?**

Appendix 2 also shows the way different chemical methodologies interfere with your water sample. Many ions, including salts, interfere with the vanadomolybdophosphoric acid colorimetric method and stannous chloride method. The ascorbic acid chemical methodology is recommended as the test of choice for most situations. However, be aware of the low range restrictions and colour interferences for this test.

## **DOES THE COLOUR OF THE WATER INTERFERE WITH THE EQUIPMENT TYPE OR CHEMICAL METHODOLOGY USED?**

All phosphorus tests use colorimetry to measure the proportion of phosphorus in a water sample. Therefore, turbid or coloured water has the potential to affect the accuracy of results. The effect of turbid waters can be minimised by filtering the sample before analysis as with the filterable reactive phosphorus test. To minimise error associated with coloured water that passes through the filter (e.g. tannins), a blank can be prepared with filtered sample water minus the reagents. Once the results are known, subtract the blank's result from the sample's result.

## **WHAT DIGESTION METHOD SHOULD I USE FOR MY TOTAL PHOSPHORUS ANALYSIS?**

Digestion is required to measure total phosphorus. Digestion describes the process where heat and acids are used to breakdown all forms of phosphorus to orthophosphate (Clesceri *et al.*, 1998). There are five common digestion methods. Although total phosphorus is usually analysed in a laboratory and these laboratories complete the digestion step, it may be useful to be aware of each digestion method in regard to the type of water sample and the data requirements. Appendix 3 provides an overview of each method of digestion.

## **WHAT ARE THE MAJOR SOURCES OF CONTAMINATION AND HOW CAN I MINIMISE THIS ERROR?**

Many everyday items such as food and laundry detergent contain high concentrations of orthophosphates. When collecting and testing waters for phosphorus analysis it is important to be aware of potential contamination from your hands and fingers, and care should be taken not to transfer contaminants to a test sample. Never directly touch the water and use buckets, bottles and vials that are both cleaned and rinsed. Never store food or fish products near samples that are destined for phosphorus analysis (Wruck & Ferris, 1997).

When cleaning buckets, bottles and vials, never use detergents as they contain large amounts of phosphorus, which will readily adhere to the side of a container.

Here are some more hints (Ferris, 1992):

- Sample buckets should be rinsed with the sample water at least three times before taking a grab sample.
- Containers used to store water samples ready for laboratory analysis, should be cleaned and rinsed as per the laboratory's specifications.
- Glassware that is used for *in situ* colorimetric analysis must be rinsed with tap water three times, rinsed with 1M or 2M hydrochloric acid then rinsed with sample water three times before being filled with the water sample being tested. This process must be done before every new sample is tested (Cassidy, 2003).

## **SHOULD I EXPRESS MY RESULTS AS THE WEIGHT PER LITRE OF ORTHOPHOSPHATE OR ELEMENTAL PHOSPHORUS?**

Different colorimeters report results in different units, either the weight per litre of orthophosphate ( $\text{PO}_4^{3-}$ ) or elemental phosphorus (P). Most orthophosphate test kits provide results as orthophosphate, however some test kits and most scientific reports and papers report results as the weight per litre of the elemental phosphorus component of the sample. It is important to record the units that the test equipment displays.

If the phosphorus test kit displays the result as orthophosphate and a conversion to elemental phosphorus is necessary, simply multiply the result by 0.33. If the phosphorus test kit displays the result as elemental phosphorus and a conversion to orthophosphate is necessary, simply multiply the result by 3.066.

## Examples

### Conversion from orthophosphate to elemental phosphorus

<i>Test Kit Result - orthophosphate</i>		<i>Conversion Factor</i>		<i>Conversion to elemental phosphorus</i>
10mg PO <sub>4</sub> <sup>3-</sup> /L	X	0.33	=	3.3mg P /L

### Conversion from elemental phosphorus to orthophosphate

<i>Test Kit Result – Phosphorous</i>		<i>Conversion Factor</i>		<i>Conversion to orthophosphate</i>
10mg P/L	X	3.066	=	30.66mg PO <sub>4</sub> <sup>3-</sup> /L

### Explanation of Conversion Factors

The 0.33 factor is calculated by dividing the atomic weight of phosphorus (30.97) by the molecular weight of orthophosphate (PO<sub>4</sub><sup>3-</sup>) (which is 1 x phosphorus atomic weight (30.97) = 30.97, plus 4 x oxygen atomic weight (16.00) = 64.00. Therefore, 30.97 + 64.00 = 94.97). Therefore, 30.97(P) ÷ 94.97 (PO<sub>4</sub><sup>3-</sup>) = 0.326 (or rounded up to 0.33) (Cassidy, 2003).

## ACKNOWLEDGEMENTS

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## **Appendix 1.** Definition of commonly used terms relating to phosphorus

### **Phosphorus**

Elemental phosphorus, defined as P.

### **Phosphate**

The phosphate ion,  $\text{PO}_4^{3-}$  (also known as orthophosphate) and/or any compound containing the phosphate ion (eg. fertilisers).

### **Orthophosphate (Inorganic Phosphate)**

Orthophosphate is the simplest form of phosphate with a chemical symbol of  $\text{PO}_4^{3-}$ . As orthophosphate is not bound to any carbon molecule/s and not bound to other phosphates in the form of a condensed phosphate, it is immediately available for biological uptake (McKelvie, 2000).

### **Condensed Phosphate**

Condensed phosphates are chains of two or more orthophosphate groups that are linked together. Forms of condensed phosphates include metaphosphate, pyrophosphate and polyphosphates. Condensed phosphates can be rapidly hydrolysed (broken up) into orthophosphate, which is then available for immediate biological uptake.

### **Organic phosphate**

Organic phosphates are phosphates that are bound to carbon molecule/s. As a result of being bound to carbon molecule/s, organic phosphates are not directly available for biological uptake.

#### ***Dissolved Organic Phosphorus***

Organic phosphate dissolved in the watercolumn that has the potential to become readily available for biological uptake. This fraction usually passes through filters used in the Filterable Reactive Phosphorus test.

#### ***Particulate Organic Phosphorus***

Organic phosphorus bound to particulate matter suspended in the watercolumn. It is not usually available for biological uptake. Filters used in the Filterable Reactive Phosphorus test usually trap this fraction.

**Appendix 2.** Summary of the different chemical methodologies used to determine the orthophosphate concentration of a water sample taken from Standard Methods for Water and Wastewater Treatment (1998).

Chemical methodology	General comments	Interferences
<p><b>Vanadomolybdophosphoric Acid Colorimetric Method</b></p> <p><b>Reaction</b> Ammonium molybdate reacts under acid conditions to form a heteropolyacid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed, the intensity of which indicates the amount of orthophosphate present</p>	<p><b>Colour development</b> Yellow</p> <p><b>Wavelength</b> 400-490 nm</p> <p><b>Typical Range</b> High range – 1-20 mg/L as P</p>	<p><b>Blue Colour</b> – Blue colour is caused by ferrous iron but doesn't affect results if concentration is less than 100 mg/L</p> <p><b>Negative</b> – Arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, excess molybdate.</p> <p><b>Only interfere in concentrations over 1000 mg/L</b> - <math>\text{Al}^{3+}</math>, <math>\text{Fe}^{3+}</math>, <math>\text{Mg}^{2+}</math>, <math>\text{Ca}^{2+}</math>, <math>\text{Ba}^{2+}</math>, <math>\text{Sr}^{2+}</math>, <math>\text{Li}</math>, <math>\text{Na}^+</math>, <math>\text{K}</math>, <math>\text{NH}_4^+</math>, <math>\text{Cd}^{2+}</math>, <math>\text{Mn}^{2+}</math>, <math>\text{Pb}^{2+}</math>, <math>\text{Hg}^+</math>, <math>\text{Hg}^{2+}</math>, <math>\text{Sn}^{2+}</math>, <math>\text{Cu}^{2+}</math>, <math>\text{Ni}^{2+}</math>, <math>\text{Ag}^+</math>, <math>\text{U}^{4+}</math>, <math>\text{Zr}^{4+}</math>, <math>\text{AsO}_3^-</math>, <math>\text{Br}^-</math>, <math>\text{CO}_3^{2-}</math>, <math>\text{ClO}_4^-</math>, <math>\text{CN}^-</math>, <math>\text{IO}_3^-</math>, <math>\text{SiO}_4^{4-}</math>, <math>\text{NO}_3^-</math>, <math>\text{NO}_2^-</math>, <math>\text{SO}_4^{2-}</math>, <math>\text{SO}_3^{2-}</math>, pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate &amp; salicylate. Thus if salinity is more than 1ppt, this method may be unsuitable)</p> <p><b>If HNO<sub>3</sub> is used in the test</b> – Cl interferes at 75mg/L</p>
<p><b>Stannous Chloride Method</b></p> <p><b>Reaction</b> Molybdophosphoric acid is formed and reduced by stannous chloride, forming an intensely colored molybdenum blue.</p>	<p><b>Colour development</b> Blue (Molybdenum)</p> <p><b>Wavelength</b> 650 nm. Can be measured at 690 nm with reduced sensitivity and precision.</p> <p><b>Typical Range</b> Low Range - 0.01-6 mg/L as P</p>	<p><b>Temperature</b> – Temperature range should be 20-30°C. Each 1°C increase in temperature equals 1% increase in colour intensity.</p> <p><b>Blue Colour</b> – Blue colour is caused by ferrous iron but doesn't affect results if concentration is less than 100 mg/L</p> <p><b>Negative</b> – Arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, excess molybdate.</p> <p><b>Only interfere in concentrations over 1000 mg/L</b> - <math>\text{Al}^{3+}</math>, <math>\text{Fe}^{3+}</math>, <math>\text{Mg}^{2+}</math>, <math>\text{Ca}^{2+}</math>, <math>\text{Ba}^{2+}</math>, <math>\text{Sr}^{2+}</math>, <math>\text{Li}</math>, <math>\text{Na}^+</math>, <math>\text{K}</math>, <math>\text{NH}_4^+</math>, <math>\text{Cd}^{2+}</math>, <math>\text{Mn}^{2+}</math>, <math>\text{Pb}^{2+}</math>, <math>\text{Hg}^+</math>, <math>\text{Hg}^{2+}</math>, <math>\text{Sn}^{2+}</math>, <math>\text{Cu}^{2+}</math>, <math>\text{Ni}^{2+}</math>, <math>\text{Ag}^+</math>, <math>\text{U}^{4+}</math>, <math>\text{Zr}^{4+}</math>, <math>\text{AsO}_3^-</math>, <math>\text{Br}^-</math>, <math>\text{CO}_3^{2-}</math>, <math>\text{ClO}_4^-</math>, <math>\text{CN}^-</math>, <math>\text{IO}_3^-</math>, <math>\text{SiO}_4^{4-}</math>, <math>\text{NO}_3^-</math>, <math>\text{NO}_2^-</math>, <math>\text{SO}_4^{2-}</math>, <math>\text{SO}_3^{2-}</math>, pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate &amp; salicylate. (Thus if salinity is more than 1ppt, this method may be unsuitable)</p> <p><b>If HNO<sub>3</sub> is used in the test</b> – Cl interferes at 75mg/L</p>
<p><b>Ascorbic Acid Method</b></p> <p><b>Reaction</b> Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with orthophosphate to form an antimony phosphomolybdate complex. This can be reduced to molybdenum blue using ascorbic acid.</p>	<p><b>Colour development</b> Blue (Molybdenum)</p> <p><b>Wavelength</b> 880nm (Spectrophotometer, With infrared phototube for use at 880 nm as long as the light path is at least 2.5cm Photometer, With a red colour filter and a light path of 0.5cm or longer)</p> <p><b>Typical Range</b> Low Range - 0.01-6 mg/L as P</p>	<p><b>Arsenates</b> – Arsenates react with the molybdate reagent to produce a blue colour similar to that formed with orthophosphate. Concentrations as low as 0.1 mg As/L interfere with the orthophosphate determination.</p> <p><b>Negative</b> - Hexavalent chromium and <math>\text{NO}_2^-</math> interfere to give a result about 3% less at concentrations of 1 mg/L and results 10-15% lower at 10mg/L</p> <p><b>Sulfide and silicate</b> – Do not interfere at concentrations of 1.0 and 10 mg/L respectively Turbid and coloured waters – Natural coloured waters don't interfere. In highly coloured or turbid waters, prepare a blank with all of the reagents except ascorbic acid and potassium antimonyl tartrate. Subtract the blank's result from the sample's result.</p>

**Appendix 3.** Overview of digestion methods used to reduce complex phosphates into orthophosphate, taken from Standard Methods for Water and Wastewater Treatment (1998).

Method	Summary	Apparatus & Reagents
Preliminary Acid Hydrolysis	Hydrolyses condensed phosphates into orthophosphate, but not complex organic and inorganic phosphates.	Autoclave or pressure cooker Phenolphthalein indicator aqueous solution, Strong acid solution H <sub>2</sub> SO <sub>4</sub> , Sodium hydroxide
Perchloric Acid Digestion	Most drastic and time-consuming method. Recommended for samples with very high sediment concentrations. Breaks complex organic and inorganic phosphates and condensed phosphates down into orthophosphate. May explode – not used often.	Hotplate, safety shield, safety goggles, Erlenmeyer flasks, Nitric acid, Perchloric acid, Sodium Hydroxide, Methyl orange indicator solution, Phenolphthalein indicator aqueous solution
Kjedahl Procedure	Recommended for most samples. High temperatures and strong acids.	Kjeldahl distillation apparatus, Micro-kjeldahl flasks, H <sub>2</sub> SO <sub>4</sub> / K <sub>2</sub> SO <sub>4</sub> /Catalyst
Sulfuric Acid-Nitric Acid Digestion	Recommended for most samples. Breaks complex organic and inorganic phosphates and condensed phosphates down into orthophosphate.	Digestion Rack, Micro-kjeldahl flasks, Sulfuric acid, Nitric acid, Phenolphthalein indicator aqueous solution, Sodium hydroxide
Persulfate Digestion	Simplest method. Breaks complex organic and inorganic phosphates and condensed phosphates down to orthophosphate. Will not break down refractory compounds	Hotplate, Autoclave, Glass scoop, Phenolphthalein indicator aqueous solution, sulfuric acid solution, Ammonium persulfate, Sodium hydroxide.