TOTAL PHOSPHORUS

METHODS OF ANALYSIS, WASTEWATER TREATMENT AND EFFECTS ON NATURE

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WHAT IS PHOSPHORUS?

- Phosphorus (P) is a nutrient that is vital to human, animal, and plant growth.
- It's one of the most common substances found in nature.
- It's found in our water, our food, and our bodies.





WHAT IS PHOSPHORUS?

 It's a highly reactive, nonmetallic element existing in three allotropic forms, white (or sometimes yellow), red, and black. In its pure form, it is toxic.





WHERE DOES IT COME FROM?

- Phosphorus occurs *naturally* at low levels in water, plants, and animals.
- Phosphorus occurs *unnaturally* in fertilizers (used in agriculture), cleaners (used in industry) and wastewater (from household sewage).
- Phosphorus is found in water, solids (detritus), and in the bodies of biological organisms.



SO WHY IS IT IMPORTANT?

- We need it to survive in low levels!
- However, high levels of Phosphorus in waterways causes eutrophication, an excess richness of nutrients, which leads to *algal blooms* and/or dense plant growth.
- This process decreases sunlight and oxygen levels (hypoxia) thus affecting fish and other aquatic life.



SO WHY IS IT IMPORTANT?





ALGAE – LAKE ERIE





ALGAL BLOOM - POND





FORMS OF PHOSPHORUS

Phosphorus (P) occurs
 Phosphates (PO4).

• These are classified as

Orthophosphates



(reactive phosphates), *Condensed Phosphates* (pyro, meta, and polyphosphates) and *Organic Phosphates*.



ORTHOPHOSPHATES

 Orthophosphates, also known as Reactive Phosphates, are a main constituent in fertilizers used for agriculture and residential purposes.

 Orthophosphates can be carried into streams and lakes through run-off.



CONDENSED PHOSPHATES

- Condensed (inorganic) phosphates are phosphorus compounds that contain salts and/or metals such as sodium, potassium, and calcium in various structures and chains.
- Condensed phosphates are used in industry and as food additives.





DID YOU KNOW?

• Phosphoric acid was (and still is in dark varieties) added to pop to enhance flavor and fizz.

• However, phosphoric acid can leach calcium from bones and teeth.

• High levels of phosphorus in the body can cause premature aging and cancer.



ORGANIC PHOSPHATES

 Organic Phosphates are formed primarily by biological processes (ex: ATP – Adenosine Triphosphate). ATP is a chemical compound that breaks down to release energy in the body.

Organic phosphates enter sewage via human waste and food residues.

 Organic phosphates can be formed from orthophosphates in biological treatment processes or by receiving water biota.



PHOSPHORUS ANALYSIS

- Collection, Preservation and Holding Times
- Ortho vs Total Phosphorus
- Dissolved Phosphorus
- Different Methods of Analysis
- Digestion of Total Phosphorus
- Quality Control (QC) samples
- Interferences



COLLECTION AND PRESERVATION

• <u>Collection:</u> Plastic or glass

• <u>Hold Time:</u> Total Phosphorus: 28 days Orthophosphate: 48 hours

<u>Preservation</u>: Total Phosphorus: pH<2 H₂SO₄
 Orthophosphate: No Preservation

• <u>Storage:</u> Refrigerate, <4°C



ORTHOPHOSPHATE VS. TOTAL PHOSPHORUS

- Orthophosphate (reactive) is analyzed directly on an unpreserved sample within 48 hours of sampling.
- *Total Phosphorus* (all forms) is analyzed on an acid preserved sample within 28 days of sampling following an acid digestion.



DISSOLVED PHOSPHORUS

- Dissolved Reactive Phosphorus samples are field filtered, within 15 minutes of sampling, through a 0.45-µm filter and analyzed within 48 hours.
- Low Level Dissolved Reactive Phosphorus samples are treated the same as above, except the filter size is $0.2-\mu m$.
- Dissolved Total Phosphorus samples are filtered through a 0.45-μm filter prior to preservation. The sample is then digested and analyzed within 28 days of sampling.



PHOSPHORUS METHODS

- Colorimetric EPA Method 365.2 Manual Spectrophotometer
- Semi-Automated Colorimetric EPA Method 365.1 Automated Spectrophotometer (Flow injection Analysis –FIA)
- Standard Methods 4500-P
- Ion Chromatography (IC) EPA Method 300.0
- Discrete Analyzers



DISCRETE ANALYZER

- A instrument that employs robotics and syringes to aspirate, dispense, and mix appropriate amounts of sample and reagents into reaction wells.
- The sample/reagent mixture then incubates in the reaction well for a pre-programmed time.
- A single aliquot of the mixture is then transferred to a cuvette for spectrophotometric analysis.
- Absorbance is plotted against ppm in a linear regression curve to calculate concentration.
- Can analyze the various forms of phosphorus.



DISCRETE ANALYZER

- Advantages:
 - Reduces manual labor
 - Reduces reagents
 - Reduces sample volume
 - Reduces waste
- Disadvantages:
 - Instrument cost



• Anions are separated based on their affinities toward the stationary phase in the column. A suppressor cartridge chemically suppresses the background conductance of the eluent (dilute KOH solution) and converts the anions into species of higher conductance.



- Using pressure and eluent, the sample is "pushed" through a stationary phase column. The anions are separated by size and then measured using a conductivity detector.
- The electronic signal is converted to peak area. Concentration is determined using a linear regression curve (plotting area vs. ppm).



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- Analyzes Reactive (Ortho) Phosphate only
- Linear Range 0.25- 5.0mg/L for Phosphate
- Calibrate monthly
- Minimum of 4 Standards covering the linear range, from which a linear regression curve is created.
- <u>></u>0.995 Correlation Coefficient
- Method Detection Limits (MDL) performed following new MDL rules with quarterly checks



LINEAR REGRESSION CURVE ION CHROMATOGRAPHY

- +	A23 Standard 1											
	Α	В	С	D	E	F	G	Н	1			
6 7	Calibration Summary											
8 10	Peak Name	Eval.Type	Cal.Type	Points	Offset (C0)	Slope (C1)	Curve (C2)	Coeff.Det. %				
11	Fluoride	Area	Lin, WithOffset, 1/A	5.000	-0.003	0.442	0.000	99.8606				
12	Chloride	Area	Lin, WithOffset	5.000	-0.176	0.287	0.000	99.9959				
13	Nitrite	Height	Lin, WithOffset	5.000	0.035	1.092	0.000	99.9531				
14	Bromide	Area	Lin, WithOffset, 1/A	4.000	-0.006	0.120	0.000	99.9330				
15	Nitrate	Height	Lin, WithOffset, 1/A	4.000	-0.041	0.681	0.000	99.9985				
16	Sulfate	Area	Lin, WithOffset	5.000	-0.009	0.216	0.000	99.9990				
17	Phosphate	Height	Lin, WithOffset, 1/A	4.000	-0.030	0.694	0.000	99.9463				
18						_						
19	Injection Name	Amnt.Dev.	Area	Height	Amount	9.00 Fluoride	External	ECD_1				
20		%	µS*min	μS		μS*min						
21	Fluoride	Fluoride	Fluoride	Fluoride	Fluoride]≮						
22		ECD_1	ECD_1	ECD_1	ECD_1	7.50						
23	Standard 1	2.1937	0.0418	0.499	0.102	6 25						
24	Standard 2	-3.3326	0.1034	1.225	0.242							
25	Standard 3	-5.4123	0.2056	2.357	0.473	5.00						
26	Standard 4	7.3591	0.4708	5.116	1.074	3.75						
27	Standard 7	n.a.	8.2045	60.134	18.585	2.50		+				
28	Standard 6	n.a.	4.2537	37.186	9.639	1.25						
29 30	Standard 5	-0.8078	2.1871	21.494	4.960	0.00	2.50	3.75 5.00 6.	.			



IC ANALYSIS QC

- Initial Calibration Verification (ICV) and Initial Calibration Blank (ICB) analyzed daily
- Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) analyzed initially before samples, after every 10 samples and again at the end of the sequence
- Reporting Limit Check (RLC) analyzed with each batch containing Drinking Water samples



IC BATCH QC

- Laboratory Control Blank (LCB) and Laboratory Control Sample (LCS) prepared fresh daily per batch of 20 samples
- Duplicate/Matrix Spike or MS/MSD per 10 samples.
 Percent Recovery and Relative Percent Difference (RPD) are calculated



IC SAMPLE PREPARATION

• All samples and batch QC (LCB, LCS, Duplicates, MS/MSD) are filtered through a 0.20-mm syringe filter to remove all particulate.

• MS/MSD's are spiked as to be within the analysis range.



IC INTERFERENCES

• All solids must be filtered out. Any particulate in the system will cause problems and plug up the system.

• High concentrations of some ions will cause false readings of other ions.

 Peak shifting: as the column gets older, peaks may shift causing misidentification.



IC

ADVANTAGES/DISADVANTAGES

- Analyzes up to 7 Anions in less than 15 minutes
- Can be very stable
- Can run overnight (load and go!)
- No hazardous waste
- Small sample volume needed
- May have to do multiple dilutions per sample
- Each injection takes up to 30 minutes to run



PHOSPHORUS 365.1/365.2

- Phosphorus is analyzed using colorimetric (spectrophotometric) analysis. This can be performed manually or automated.
- Orthophosphate (Reactive Phosphorus) is analyzed directly.
- Total Phosphorus (as P) is analyzed after going through an acid digestion to convert all forms of phosphorus to orthophosphate.



METHOD SUMMARY

 Ammonium molybdate and antimony potassium tartrate react in a acid medium with orthophosphate to form a phosphomolybdic acid, which is then reduced to an intense molybdenum blue color by ascorbic acid. The resultant color and read spectrophotometrically at 880-nm.

• This can be performed on a manual spectrophotometer or on an automated FIA spectrophotometer.



ORTHOPHOSPHATE (REACTIVE)

- Orthophosphate (reactive) is analyzed directly on an unpreserved sample within 48 hours of sampling.
- No preparation needed for Manual Spectrophotometer
- For Automated Spectrophotometer, samples must be filtered though Whatman 41 filter paper to remove solids
- Dissolved Reactive Phosphorus and Low Level Dissolved Reactive Phosphorus filtered through 0.45-mm and 0.2-mm syringe filter, respectively, IN THE FIELD



ORTHOPHOSPHATE (REACTIVE)

- Calibrate Daily
- Linear Range 0.01-2 mg/L
- Minimum of 5 Standards covering the linear range, from which a linear regression is created.
- <u>>0.995</u> Correlation Coefficient
- Method Detection Limits (MDL) performed following new MDL rules with quarterly checks.



ORTHOPHOSPHATE

• Final results are obtained from the linear regression curve created with the known standard concentrations.

Linear Regression Curve

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		1									
		Known Conc. (mg/L)	Rep.	Peak Area (V.s)	Peak Height (V)	% RSD	% Resid.	Unused	Det. Conc (mg/L)	Date	Time
	1	1.000	1	39.04	1.729	0.0	0.0		1.000	4/12/2018	8:44:57 AM
	2	0.5000	1	19.50	0.8616	0.0	-0.1		0.5004	4/12/2018	8:47:41 AM
	$\left\ \frac{3}{3} \right\ $	0.2500	1	9.682	0.4260	0.0	0.4		0.2491	4/12/2018	8:50:24 AM
	4	0.1000	1	3.822	0.1666	0.0	0.9		0.09912	4/12/2018	8:53:06 AM
	5	0.05000	1	1.912	0.08289	0.0	-0.5		0.05026	4/12/2018	8:55:47 AM
	6	0.02500	1	0.9380	0.03976	0.0	-1.4		0.02534	4/12/2018	9:00:33 AM
No. of Contraction		0.01000	1	0.3634	0.01534	0.0	-7.4		0.01064	4/12/2018	9:05:15 AM
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ANALYSIS QC

 Initial Calibration Verification (ICV) and Initial Calibration Blank (ICB) analyzed daily

 Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) analyzed initially before samples, after every 10 samples and again at the end of the sequence



BATCH QC

 Laboratory Control Blank (LCB) and Laboratory Control Sample (LCS) prepared fresh daily per batch of 20 samples

 Duplicate/Matrix Spike or MS/MSD per 10 samples.
 Percent Recovery and Relative Percent Difference (RPD) are calculated



TOTAL PHOSPHORUS

- Method Summary: Total Phosphorus analysis has two procedural steps:
 - ONE convert all phosphorus forms to orthophosphate using acid hydrolysis.
 - *TWO* analyze orthophosphate colorimetrically, either manually or automated.



TOTAL PHOSPHORUS DIGESTION

- In the acid hydrolysis step, sulfuric acid and ammonium persulfate (peroxydisulfate) are added to a known volume of sample. It is then digested on a hotplate to near dryness. The sample is cooled and diluted back to the original volume. Sample is then filtered to remove any solids for Auto-Analysis.
- Alternatively, an autoclave may be used for digestion (30 min at 121°C, 15-20 psi) or hotblock. Both are a closed digestion, therefore no evaporation nor dilution required (30 min at 150°C).



TOTAL PHOSPHORUS ANALYSIS

- The colorimetric step, is the same as the orthophosphate in that, Ammonium molybdate and antimony potassium tartrate react in a acid medium with orthophosphate to form a phosphomolybdic acid, which is then reduced to an intense molybdenum blue color by ascorbic acid. The resultant color and read spectrophotometrically at 880-nm
- The colorimetric step may be performed on a manual spectrophotometer or on an automated analyzer.



TOTAL PHOSPHORUS ANALYSIS

- Calibrate Daily
- Linear Range 0.01-2 mg/L (ours 0.02-1 mg/L)
- Minimum of 5 Standards covering the linear range from which a linear regression is created.
- >0.995 Correlation Coefficient
- Method Detection Limits (MDL) performed following new MDL rules with quarterly checks



TOTAL PHOSPHORUS

• Final results are obtained from the linear regression curve created with the known standard concentrations.

Linear Regression Curve

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	<u> </u>	Known Lonc. (mg/L)	Hep.	Peak Area (V.s)	Peak Height (V)	% RSD 0.0	% Resid.		Uet. Lonc (mg/L)	Date Art 2/2010	Lime
		0.000	- 1	33.04	1.723	0.0	0.0		0.5004	4/12/2018	8:44:57 AM
	- 2	0.3000	1	9,692	0.0010	0.0	-0.1		0.0004	4/12/2018	8:50:24 AM
		0.2300	1	3.822	0.4200	0.0	0.4		0.2431	4/12/2010	8:53:06 AM
	$\frac{7}{5}$	0.1000	1	1 912	0.1000	0.0	-0.5		0.05026	4/12/2018	8:55:47 AM
	6	0.02500	1	0.9380	0.03976	0.0	-1.4	Ē	0.02534	4/12/2018	9:00:33 AM
	7	0.01000	1	0.3634	0.01534	0.0	-7.4		0.01064	4/12/2018	9:05:15 AM
	8	0.000	1	-0.04809	-0.001239				1.126e-4	4/12/2018	9:06:40 AM
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ANALYSIS QC

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 Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) analyzed initially before samples, after every 10 samples and again at the end of the sequence



BATCH QC

- Laboratory Control Blank (LCB) and Laboratory Control Sample (LCS) digested fresh daily per batch of 20 samples
- Duplicate/Matrix Spike or MS/MSD per every 10 samples. Percent Recovery and Relative Percent Difference (RPD) are calculated



QC FAILURE & CORRECTIVE ACTIONS

- Calibration Criteria, such as residuals or correlation coefficient out of range, results in recalibrating the instrument.
- Analysis QC criteria not met, results in re-analyzing the initial or continuing QC. Repeated failure results in re-calibrating the instrument.
- Batch QC results out of acceptable range may result in redigestion and/or re-analysis of QC and samples.



PHOSPHORUS INTERFERENCES

• As with any colorimetric analysis, *turbidity* (solids) can be a positive interference but can be removed with filtration. Filtration must occur prior to analysis, not prior to digestion (unless the sample is dissolved) on total phosphorus samples.

 Sample color may also interfere. Background correction can be performed in this case.



PHOSPHORUS INTERFERENCES

 High levels of arsenic can cause a positive interference if the As concentrations are higher than the phosphorus concentration.

• High levels of iron may cause the precipitation of, and subsequently the loss of phosphorus.



TROUBLESHOOTING

- Contamination!! Contamination!! Contamination!!
- All glassware must be washed with hot water and phosphate free soap, rinsed with dilute hydrochloric acid and rinsed with copious amounts of de-ionized water.
- All instrument components and reagents must be phosphorus free.



TROUBLESHOOTING

- FIA Auto-Spectrophotometers: *Plugs* and *leaks* are the most common problems.
- Routine Maintenance helps prevent problems:

 -Changing pump and tygon tubing
 -Rinsing and/or cleaning manifold tubing
 -Keeping area clean and dust free
 -Keeping reagents covered when in use



TREATMENT OF PHOSPHORUS

 Some of the Phosphorus entering the treatment plant is removed by primary (settling) and secondary treatment. However, phosphorus remains in the solids.

 Additional phosphorus is removed by adding Ferric Chloride, Ferrous Sulfate, Alum, or Lime during the waste treatment process.



TREATMENT OF PHOSPHORUS

Chemical removal

- Ferric Chloride
 - Fe^{3+} + $(H_nPO4)^{3-n}$ \longleftrightarrow $FePO_4$ + nH^+
- Aluminum Sulfate (Alum)
 - Al^{3+} + $(H_nPO4)^{3-n}$ \leftrightarrow $AlPO_4$ + nH^+





PHOSPHORUS

Any Questions??



PHOSPHORUS

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