Determination of Total Nitrogen and Phosphorus in Environmental Waters

Brian De Borba, Jeffrey Rohrer, and Richard Jack

Thermo Fisher Scientific, Sunnyvale, CA, USA

PITTCON™ Conference & Expo
March 4, 2014

OT70961_E 03/14S

Nutrient Pollution

- What are nutrients?
  - Chemical elements required by all living organisms to grow
  - Nitrogen and phosphorus support growth of algae and aquatic plants that provide food/habitat for fish and other aquatic life.

- Problem: excess nitrogen/phosphorus enter environment (e.g., human activity) causing increase in algae growth
  - One of America's most costly, widespread, and challenging environmental problems
  - 14,000 nutrient-related impairment listings in 49 states
  - >47% of streams have medium-high phosphorus and >53% have medium-high nitrogen
  - Impact reflects doubling of U.S. population over the past 50 years

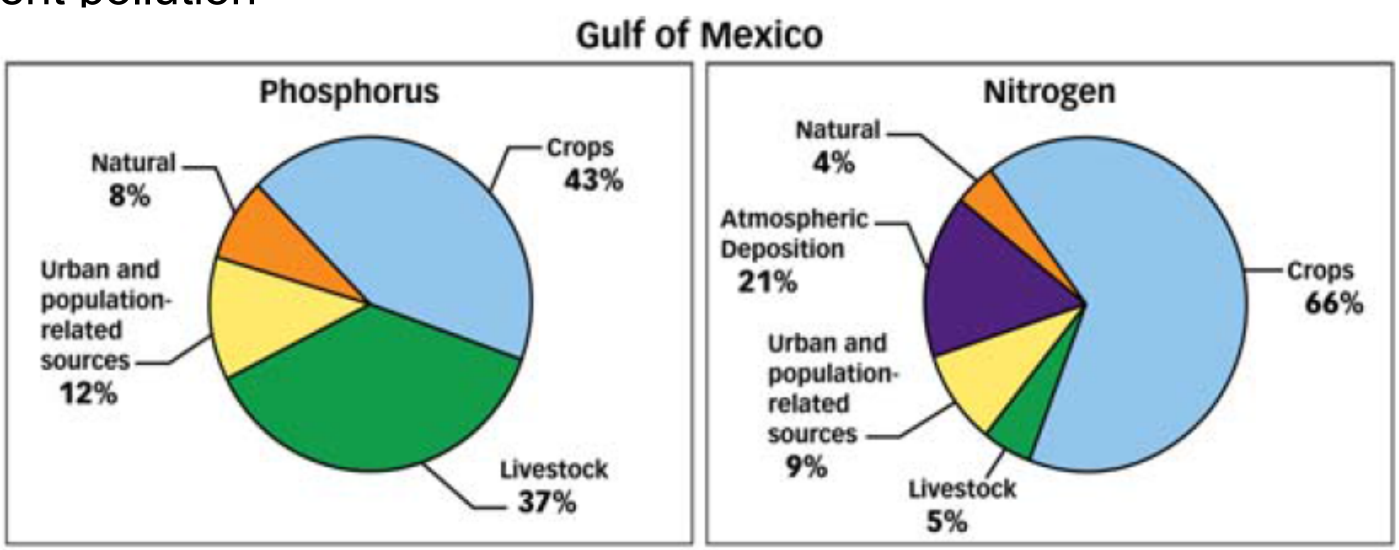
We Have a Problem!

Impact of Increased Algae Growth

- <u>Environmental</u>
  - Bad odor, increased fish deaths, waterfowl and pet deaths, decreased dissolved oxygen
- <u>Public Health</u>
  - 23 suspected illnesses, recreation/boating advisory, fish consumption advisory
- <u>Economic</u>
  - Millions in revenue lost to local businesses and public parks due to decreased tourism

Sources of Nutrient Pollution

- Primary Sources:
  - Agriculture, storm water, wastewater, fossil fuels, around the home
    - Runoff of fertilizers, animal manure, sewage treatment plant discharge, storm water runoff, car and power plant emissions, and failing septic tanks
    - Mississippi River Basin (spans 31 states) drains to Gulf of Mexico—nutrients from crops and concentrated animal feeding operations contribute the most nutrient pollution

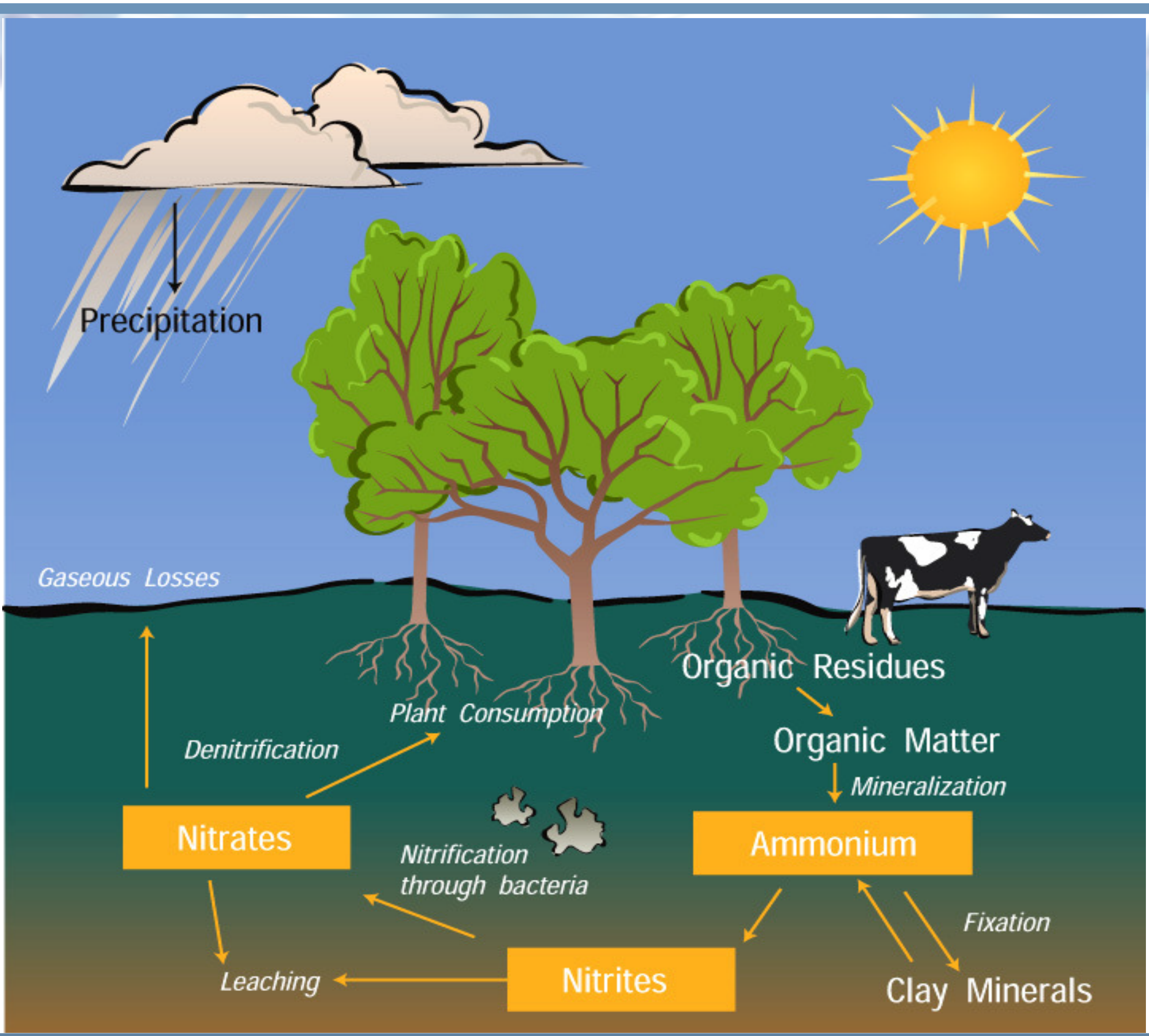


Piotrowski, J. Nutrients and Harmful Algal Blooms: A National Overview and Need for Action, *Watershed Academy Webcast*, *U.S. EPA*, **2011**.

Nitrogen Classifications

- Nitrogen
  - Ammonia (NH_3)
  - Organic
  - Inorganic (Nitrite/Nitrate)
  - Total Nitrogen (TN)
    - $TN = TKN + NO_3 + NO_2$
  - Total Kjeldahl Nitrogen (TKN)
    - $TKN = NH_3 + \text{Organic N}$

Nitrogen Cycle

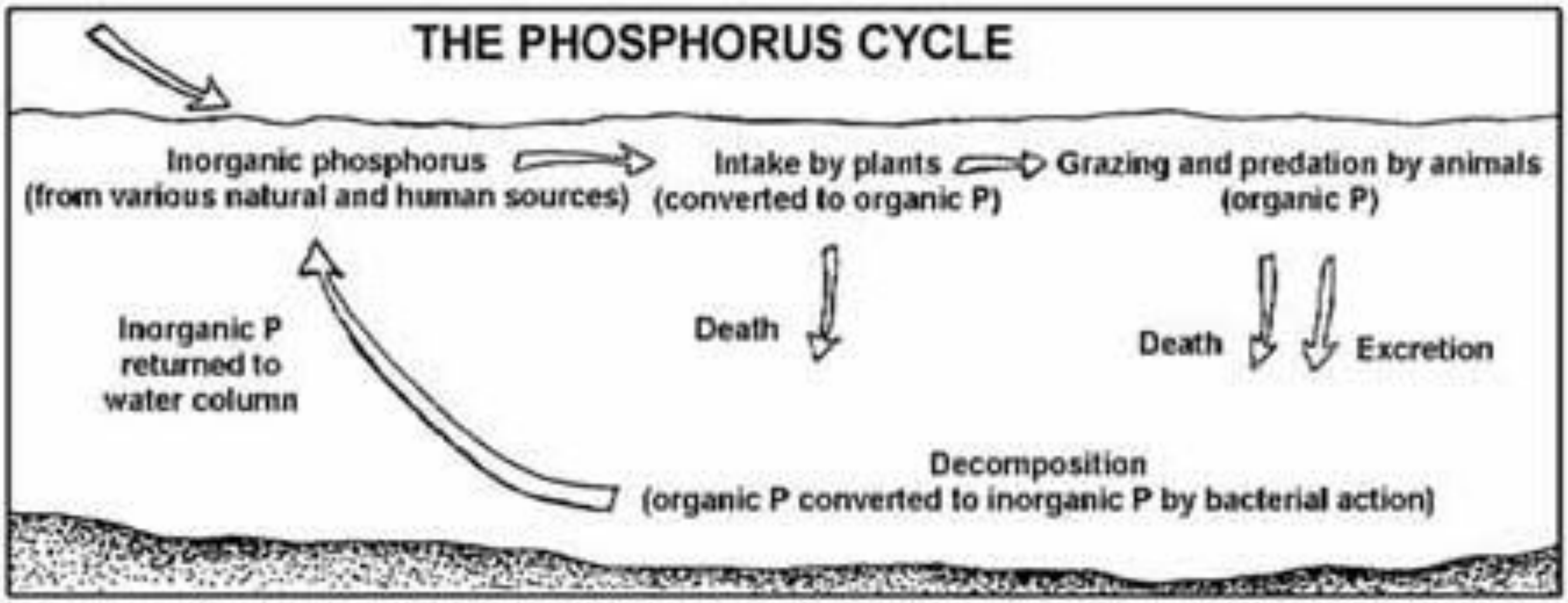


Gardiner, L. Traveling Nitrogen, Windows to the Universe. *National Earth Science Teachers Association* [Online] www.windows2universe.org/earth/Life/nitrogen_cycle.html (accessed Feb 26, 2014).

Phosphorus Classifications

- Organic Phosphorus
  - Found in plant or animal tissue
- Inorganic Phosphorus
  - Required by plants and found in animals

Phosphorus Cycle



U.S. Environmental Protection Agency, Water Monitoring and Assessment: Phosphorus [Online] http://water.epa.gov/type/rsl/monitoring/vms56.cfm (accessed Feb 26, 2014).

EPA Methods for Determining Total Nitrogen and Phosphorus

- Total Nitrogen
  - EPA Methods 351.2, 351.4, 353.2
    - EPA 351.2/351.4—TKN
    - EPA 353.2—Nitrate/nitrite (colorimetric, cadmium reduction)
- Total Phosphorus
  - EPA Methods 365.2 and 365.4
    - EPA 365.2—Phosphorus, all forms (colorimetric)
    - EPA 365.4—Total phosphorus (colorimetric)

Analysis Issues Measuring Nitrogen

- The currently accepted, EPA-approved method for total nitrogen (TN) is the sum of total kjeldahl nitrogen (TKN) and nitrate plus nitrite nitrogen (NOx).
- There is no EPA-approved method for the determination of TN as a single result.
- The determination of TN as TKN + NOx is problematic:
  - When NOx exceeds the TKN concentration, the TKN result can be low (negative bias).
  - In some matrices NOx can be partially measured as TKN (positive bias).
- States are requiring TN in permits without clearly defining what TN is.

System

- Thermo Scientific Dionex ICS-2100 integrated RFIC system including:
  - Pump
  - Degasser
  - Eluent Generator
  - Column Heater
  - Dionex AS-AP Autosampler with 250 µL syringe
- Consumables
  - Thermo Scientific™ Dionex™ IonPac™ AG19/AS19 Anion-Exchange Column set
  - Thermo Scientific Dionex EGC III KOH Eluent Generator Cartridge
  - Thermo Scientific Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
  - Thermo Scientific™ Dionex™ AERS™ Anion Electrolytically Regenerated Suppressor
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software for system control and data processing

Chromatographic Conditions

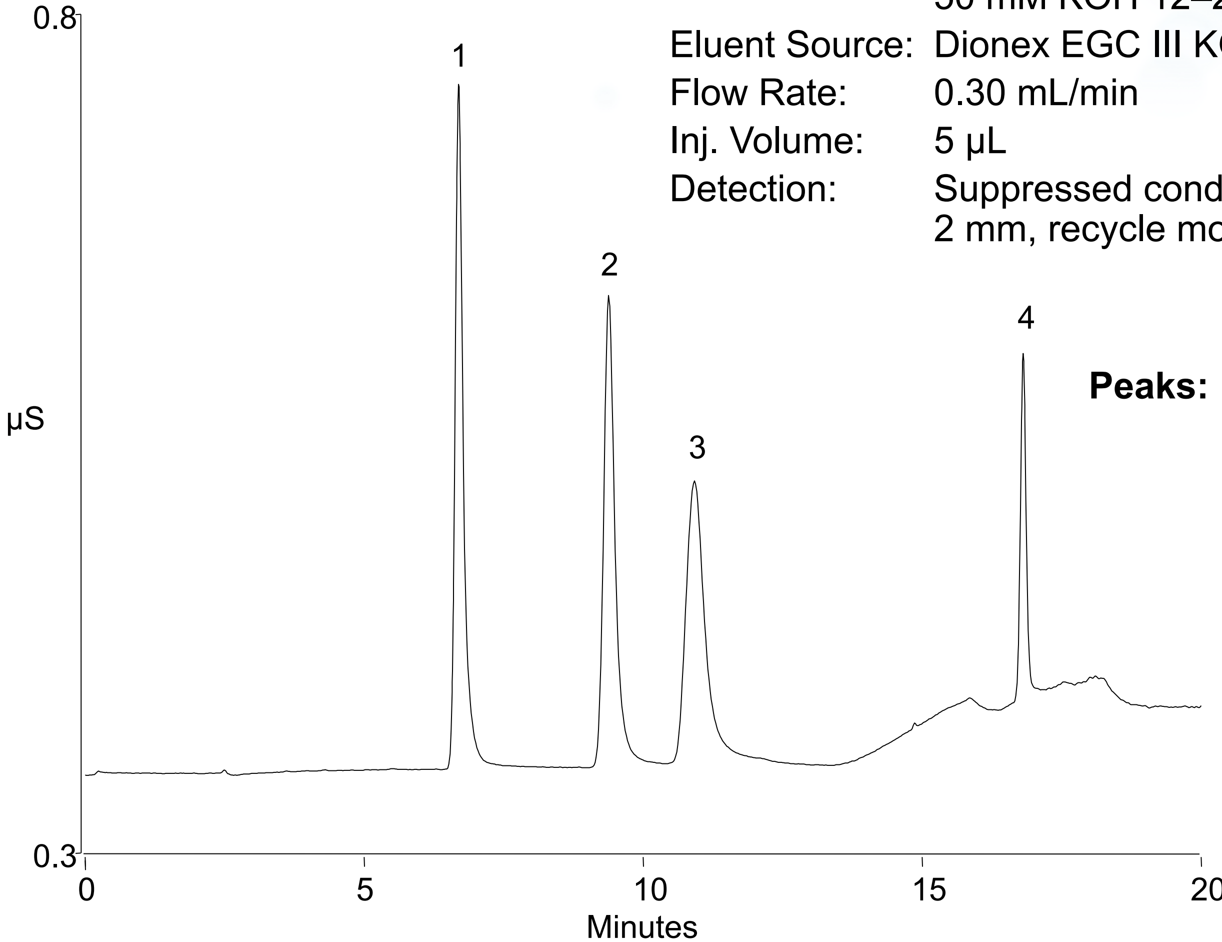
- **Columns:** Dionex IonPac AS19 Analytical, 2 x 250 mm
Dionex IonPac AG19 Guard, 2 x 50 mm
- **Eluent Source:** Dionex EGC III KOH Cartridge with Dionex CR-ATC
- **Eluent:** 20 mM KOH 0–10 min, 20–50 mM 10–12 min,
50 mM 12–20 min
- **Flow Rate:** 0.30 mL/min
- **Injection Volume:** 5 µL
- **Temperature:** 30 ^{o}C
- **Detection:** Suppressed conductivity, Dionex AERS suppressor,
2 mm, recycle mode
- **Run Time:** 20 min

Sample Preparation

1. Add 2 mL of Alkaline Persulfate to 4 mL of Sample
2. Heat Sample at 120 °C for 60 min
3. Allow Sample to Cool to Room Temperature
4. Dilute Sample in DI Water
5. Filter with 0.2 μm Filter Prior to Analysis

Standard Separation of Nitrite-N, Nitrate-N, and Phosphate-P

Column:	Dionex IonPac AG19/AS19, 2 mm
Eluent:	20 mM KOH 0–10 min, 20–50 mM KOH 10–12 min, 50 mM KOH 12–20 min
Eluent Source:	Dionex EGC III KOH with Dionex CR-ATC
Flow Rate:	0.30 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex AERS 500, 2 mm, recycle mode



Peaks:		
	1. Nitrite-N	100 µg/L
	2. Nitrate-N	100
	3. Carbonate	—
	4. Phosphate-P	100

Results

Analyte	Calibration Range (µg/L)	Linearity[1] (r^2)	System LOD[2] (µg/L)	System LOQ[3] (µg/L)	Sample LOD (µg/L)	Sample LOQ (µg/L)
Nitrite-N	2.5–300	0.9999	0.76	2.5		—
Nitrate-N	2.5–300	0.9999	1.0	3.4	—	58
Phosphate-P	2.5–300	0.9998	1.3	4.2	16.5	54

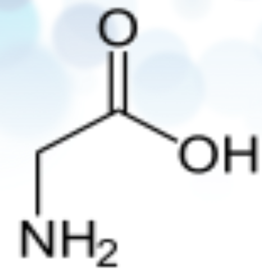
[1] Ten calibration levels, each injected in duplicate

[2] Limit of detection (LOD) calculated as 3 × S/N

[3] Limit of quantificaiton (LOQ) calculated as 10 × S/N

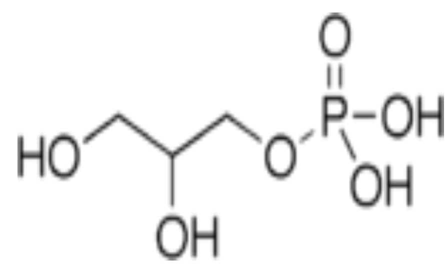
Evaluation of the N and P Recovery from Quality Control Standards

- Nitrogen-Containing Compounds
  - Nicotinic acid
  - Urea
  - Ammonium chloride
  - Glycine



Glycine

- Phosphorus-Containing Compounds
  - Glucose-1-phosphate
  - Adenosine triphosphate
  - Phytic acid
  - Glycerophosphate



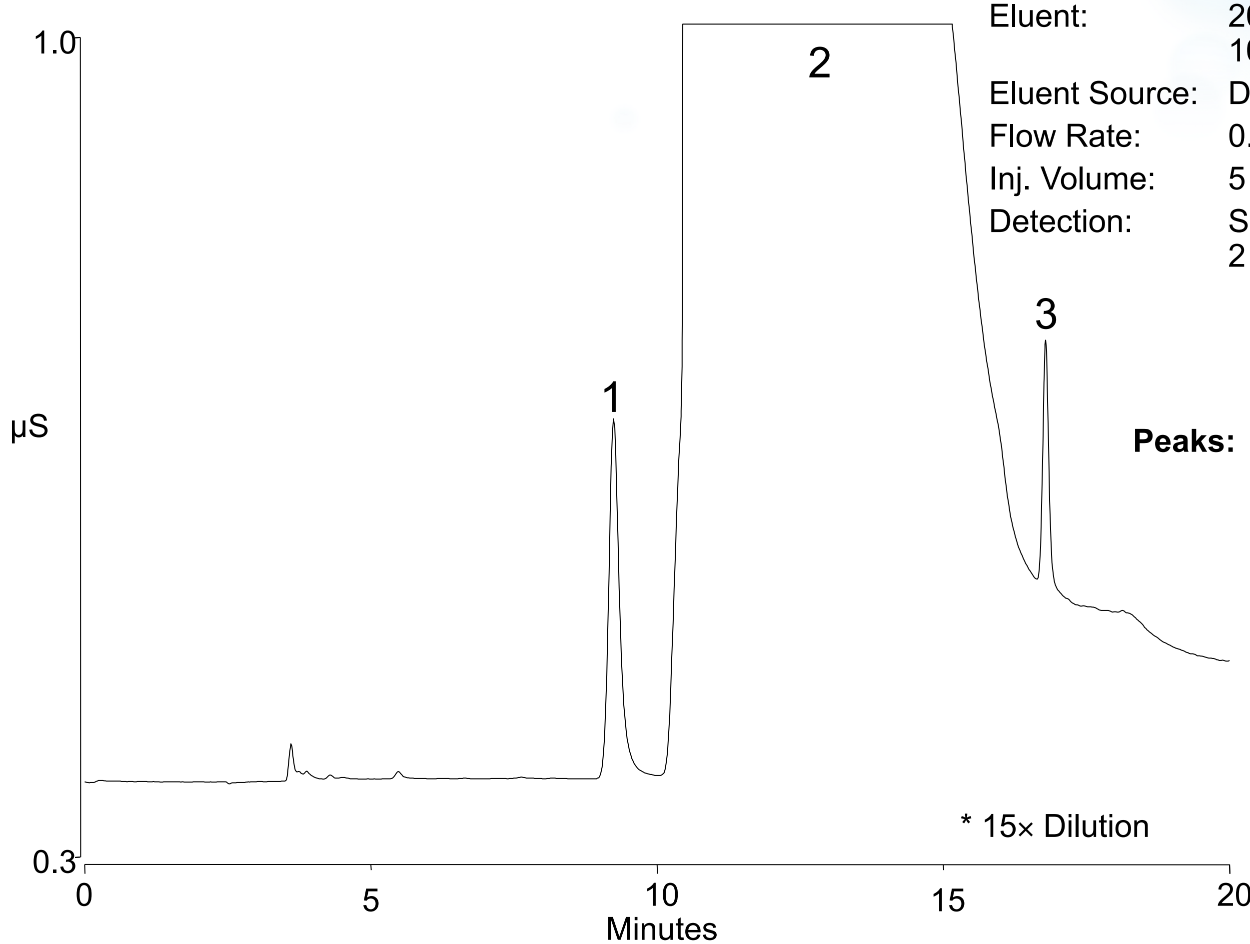
Glycerophosphate

Recovery of N and P from Quality Control Standards (Each Diluted 15×)

Nitrogen Compounds	Nominal Concn (mg N/L)	Expected Concn (mg N/L)	Found Concn (mg N/L)	% Recovery
Nicotinic Acid	1.95	0.129	0.129	100.1
Urea	2.02	0.133	0.127	95.4
Glycine	**1.49**	**0.098**	**0.094**	**95.6**
Ammonium Chloride	2.07	0.137	0.127	93.1
Phosphorus Compounds	**Nominal Concn (mg P/L)**	**Expected Concn (mg P/L)**	**Found Concn (mg P/L)**	**% Recovery**
Glucose-1-Phosphate	1.93	0.1290	0.1253	97.1
Adenosine Triphosphate	1.76	0.1162	0.099	85.2
Phytic Acid	1.86	0.1232	0.1052	85.4
Glycerophosphate	**1.63**	**0.1077**	**0.107**	**99.3**

Glycine-N and Glycerophosphate-P after Alkaline Persulfate Digestion

Column:	Dionex IonPac AG19/AS19, 2 mm
Eluent:	20 mM KOH 0–10 min, 20–50 mM KOH 10–12 min, 50 mM KOH 12–20 min
Eluent Source:	Dionex EGC III KOH with Dionex CR-ATC
Flow Rate:	0.30 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex AERS 500, 2 mm, recycle mode



Peaks:

1.	Nitrate-N	101 µg/L
2.	Sulfate	—
3.	Phosphate-P	105

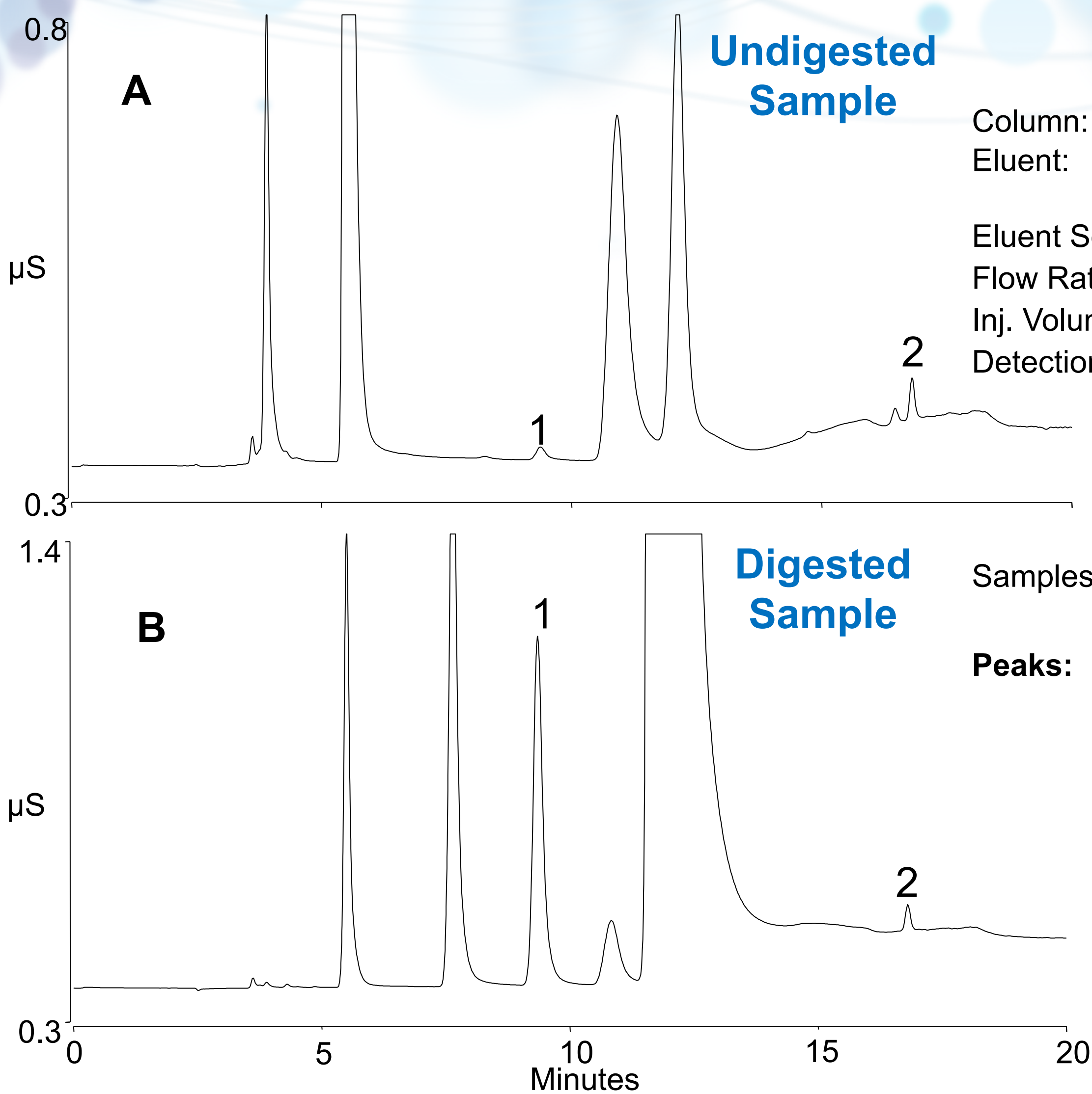
Undigested and Digested Local Wastewater Sample Results

Sample[1]	Undigested (mg N/L)	Digested (mg N/L)	Undigested (mg P/L)	Digested (mg P/L)
SVL Effluent T3	6.52	8.54	2.85	3.20
SVL Primary Effluent	0.31	36.52	2.02	3.87
SJC Filtered Effluent	11.02	13.01	0.23	0.43
SJC Final Effluent	14.77	12.66	0.33	0.40
SJC TPS	11.69	13.43	0.25	0.39
SJC Raw Sewage	0.55	39.87	2.19	4.50

All results are calculated after accounting for the dilution factor

[1]SVL = Sunnyvale, CA; SJC = San Jose, CA

Comparison Between Undigested and Digested Raw Sewage

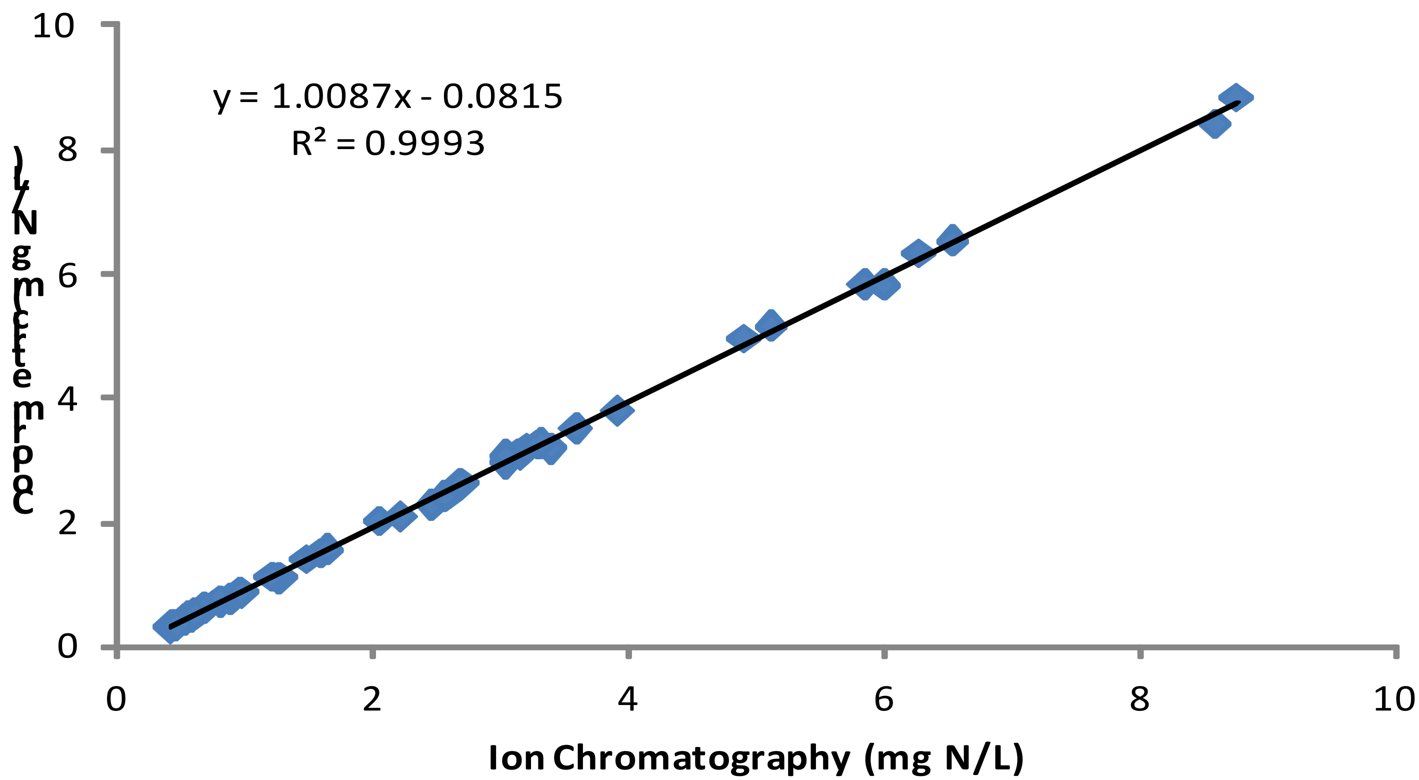


Column: Dionex IonPac AG19/AS19, 2 mm
Eluent: 20 mM KOH 0–10 min, 20–50 mM KOH 10–12 min, 50 mM KOH 12–20 min
Eluent Source: Dionex EGC III KOH with Dionex CR-ATC
Flow Rate: 0.30 mL/min
Inj. Volume: 5 µL
Detection: Suppressed conductivity, Dionex AERS 500, 2 mm, recycle mode

Samples: A. Undigested raw sewage, 100× dilution
B. Digested raw sewage, 150× dilution

Peaks:	**A**	**B**
1. Nitrate-N	5.5	262 µg/L
2. Phosphate-P	22	30

Correlation Between Ion Chromatography and Colorimetric Determination of Total N



Influence of High Chloride Concentrations on the Recovery of N and P

Injected Chloride Concn (mg/L)	Test Compound	N or P Retention Time (min)	Nominal Concn (mg N or P/L)	Dilution	% Recovery
560	Glycine-N	9.10	1.49	18	104.5
	Glycerophosphate-P	16.8	1.63	18	96.2
997	Glycine-N	8.90	1.49	10	101.2
	Glycerophosphate-P	16.7	1.63	10	94.0
1472	Glycine-N	8.70	1.49	7	87.5
	Glycerophosphate-P	16.7	1.63	7	88.9

Day-to-Day Digest Variability

Day	Sample	N	Total N (mg N/L)	Total P (mg P/L)
1	SVL Primary Effluent	6	37.27	3.88
2	SVL Primary Effluent	6	36.86	3.68
3	SVL Primary Effluent	6	36.72	3.55
4	SVL Primary Effluent	6	34.74	3.62
Average			**36.40**	**3.68**
Standard Deviation			**1.13**	**0.14**
RSD			**3.10%**	**3.92%**

Conclusions

- Demonstrated an accurate and precise IC method for determining total N and P in municipal wastewater samples.
- IC is a good alternative to TKN and other colorimetric procedures.
  - Avoids toxic and harmful chemicals
  - Provides comparable results to the colorimetric procedure
- IC enables the determination of other anions of interest to municipal water treatment plants.

Thank You!