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USDA LTAR Common Experiment measurement: Dissolved nitrate (NO_3^-) concentration

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Abstract

Nitrate (NO_3^-) is an ionized form of nitrogen (N) that is of interest in surface and groundwater. Nitrate often occurs in trace amounts (<0.01 mg/L) in rivers and lakes but can reach high levels in some surface water and groundwater. In excessive amounts, it can elicit an illness known as methemoglobinemia in infants, and the drinking water standard is limited to 10 mg NO_3^--N/L to prevent this illness. In addition, NO_3^- is an essential nutrient for growth and photosynthesis in aquatic autotrophs, and elevated NO_3^- loading contributes to eutrophication and hypoxia of surface waters, particularly in coastal marine ecosystems. The recommended methods for measuring NO_3^- concentration in water include the colorimetric cadmium reduction method, whereby NO_3^- is reduced quantitatively to nitrite (NO_2^-) in the presence of cadmium. Following the addition of appropriate reagents, a highly colored azo dye is produced and measured colorimetrically. An alternative enzymatic method utilizes "greener" chemistry by replacing the toxic copperized cadmium with nitrate reductase (specifically, AtNaR2) in combination with two coenzymes (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate) to reduce NO_3^- to NO_2^-, followed by the identical colorimetric quantification reaction used in the cadmium method. This enzyme reduction method overcomes interferences from phenolics and humic substances noted in earlier enzymatic methods. The method detection levels (or limits; MDL) for standard and low NO_3^- + NO_2^- concentrations with enzyme reduction are comparable to the long-term MDLs of the continuous-flow analyzer method with cadmium reduction. In addition, NO_3^- concentration differences were statistically equivalent to zero or analytically equivalent to zero (i.e., concentration differences were less than MDLs [Patton and Kryskalla, 2011]). Recent advances in field sensor technology have afforded commercial sensors that allow for in situ NO_3^- measurement using an optical sensor. These sensors can record values over short durations (seconds to minutes) and use an ultraviolet spectrophotometer (Johnson and Coletti, 2002). Although these sensors provide high-resolution data, their accuracy can degrade because of background interference from colored dissolved organic matter (DOM) and high turbidity. Thus, sensor readings must be validated at least monthly by conducting water sample collection and lab analyses.

Safety warnings

Follow appropriate safety, health, and environmental precautions based on the selected methods, instrumentation, and workflow. Laboratory supervisors are responsible for knowledge of these precautions and their implementation.

Sample collection and filtration

1 Return samples to the laboratory On ice and filter them on collection day if possible.

2 For NO_3^- measurements, filter water samples through a 0.45 µm pore-size filter to minimize interference from particulates before chemical analysis. If desired, remove the microbial community using a 0.22 µm filter. Filters can be membrane or glass fiber, and may require pre-washing to prevent contamination.

3 Occasional checks of filter blanks by filtering deionized water with the same equipment are always prudent. The use of a blank is described in the *USDA LTAR Common Experiment measurement: Best practices for collection, handling, and analyses of water quality samples* protocol (Pisani et al., 2024b).

Sample storage and preservation

4 Analyze samples as soon as possible after collection.

5 If storage is required, maintain filtered samples at 4 °C (not frozen) for up to 48 hours (US EPA, 1993). If lengthier storage is required, prepare samples with concentrated sulfuric acid to a pH < 2, and maintain them at 4 °C .

6 Hold acidified samples for up to 28 days in clean (N-free) plastic bottles. For acidified samples, NO_3^- and NO_2^- cannot be determined as individual species but only as total inorganic N.

Archiving

7 The common practice is to store water samples for NO_3^- analysis at 4 °C (not frozen) until data certification (QA/QC verification).

8 Storage of preserved water samples should not exceed 28 days.

Sample analysis

9 Using the cadmium reduction method, determine NO_3^- in water by passing a filtered sample through a column containing granulated copper–cadmium to reduce NO_3^- to NO_2^-.

10 The sum of NO_2^- initially present plus the reduced NO_3^- is measured colorimetrically (APHA, 2005; USEPA, 1993).

11 The sum of NO_3^- + NO_2^- is sometimes called NO_x^-.

12 To obtain separate NO_3^- and NO_2^- values, perform the procedure with the reduction step (total NO_x^-) and then without the reduction step (NO_2^- only), and calculate NO_3^- by difference.

13 The NO_2^- is usually, but not always, a relatively small component of the total.

14 To reduce NO_3^- to NO_2^- using the enzymatic reduction method, nitrate reductase (specifically, AtNaR2) is used in combination with two coenzymes (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate). Enzymatic reduction is an alternate method to cadmium reduction.

15 This effort is followed by the identical colorimetric quantification reaction used in the cadmium method (ASTM, 2014; Patton and Kryskalla, 2011), as described in Step 10.

Covariate metrics to be sampled concurrently

16 Dissolved ammonia (NH_3), total dissolved N (TDN), total dissolved phosphorus (TDP), and total suspended solids (TSS). These metrics are described in the following protocols:

- USDA LTAR Common Experiment measurement: Dissolved ammonia (NH_3) concentration
- USDA LTAR Common Experiment measurement: Total nitrogen (TN) and total dissolved nitrogen (TDN) concentration
- USDA LTAR Common Experiment measurement: Total phosphorus (TP) and total dissolved phosphorus (TDP) concentration
- USDA LTAR Common Experiment measurement: Total suspended solids (TSS)

Calculations

17 Prepare a calibration curve by plotting the blank-corrected area of each standard peak against its respective NO_3^- or NO_x^- (NO_3^- + NO_2^-) concentration. The laboratory blank is used for this step.

18 Compute the NO_3^- or NO_x^- concentration of the sample by comparing the sample peak area to the calibration curve. Report the concentration(s) as mg NO_x^--N/L.

Recommendations for data collection

19 Table 1. Summary of recommendations for the collection and measurement of dissolved NO_3^- concentration.

	A	B	C	D
	Attribute	**Preferred**	**Minimum**	**Comments**
	Spatial scale	Field	Plot	
	Frequency	Event-driven	Event-driven	More frequent (weekly) measurements may be preferred when the flow regime varies seasonally or following precipitation events. Sampling in this protocol is event-driven to enable cross-site comparisons
	Covariate metrics	NH3-N, TDN, TDP, TSS	NH3-N, TDN, TDP	
	Sample preservation and storage	Filter with a 0.45 µm pore-size filter (membrane or glass fiber), refrigerate as soon as possible, and analyze within 48 hours	Filter with a 0.45 µm pore-size filter (membrane or glass fiber), freeze as soon as possible, and analyze within 28 days	Alternatively, use a 0.7 µm GF/F. Use a 0.2 µm pore-size filter to exclude bacteria from samples but at much slower flow rates
	Sample analysis	Nitrate reductase with colorimetric analysis	Cadmium reduction with colorimetric analysis	Manually set up reduction columns or use them as part of an auto-analyzer system
	Water quantity	Discharge or flow rate	Discharge or flow rate	Calculate NO3-N loads by linking this metric to the

	A	B	C	D
				water quantity metric "flow"

Covariate metrics = NH_3-N = ammonia-N; TDN = total dissolved N; TDP = total dissolved phosphorus; TSS = total suspended solids.

Protocol references

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