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November 30, 2012

Office of Water Quality Technical Memorandum 2013.01

Subject: Guidance on Methods for Determining the Concentration of Total Nitrogen in Whole-Water Samples

Purpose

Determination of total-nitrogen (TN) concentrations in streams, lakes, and groundwater is an important component of many monitoring programs and studies. Currently, the most widely used methods for TN determination are: (1) directly by alkaline-persulfate digestion of whole-water samples, and (2) computationally as the sum of total Kjeldahl nitrogen (TKN) and nitrate-plus-nitrite. Both methods exhibited biases in a multiyear study conducted jointly by the Office of Water Quality (OWQ), the National Water Quality Assessment Program, and the National Water Quality Laboratory (NWQL). The purpose of this memo is to update OWQ Technical Memorandum 2009.03 with detailed guidance for selecting TN methods and to provide information about observed biases, which have now been thoroughly investigated and documented.

Guidance

TN in whole-water should be calculated as the sum of dissolved nitrogen (DN), determined by alkaline-persulfate digestion of a filtered-water sample, and particulate nitrogen (PN), determined by high-temperature-combustion oxidation of suspended sediment collected on a glass-fiber filter pad from a precisely measured volume of whole water. Constituents included in the determination of DN are ammonia, nitrate, nitrite, and dissolved organic nitrogen. PN includes organic and inorganic nitrogen associated with suspended organic and mineral material.

Summation of DN and PN is the only approach currently available for determination of total nitrogen in whole-water samples without measurable bias. The alkaline-persulfate digestion method is no longer recommended for whole-water samples because of a negative bias that occurs when sediment is present in the sample and increases as the sediment concentration increases. Whole-water samples from environments with very low suspended sediment and organic matter (such as groundwater or oligotrophic lakes) may still be analyzed by the alkaline-persulfate digestion method since the negative bias generally is negligible in these conditions. However, no technique is available to determine the degree of potential bias in the field, and the variations in suspended sediment that commonly occur in most surface waters will result in variable bias over time. So, the choice to use the alkaline-persulfate digestion for whole-water samples should be made only for samples from environments where conditions of low or

negligible concentrations of suspended particulate matter persist over a range of hydrologic conditions and temporal scales.

TN calculated as the sum of TKN and nitrite-plus-nitrate should be avoided in most situations because of a positive bias, most likely due to reduction of nitrate to ammonium during Kjeldahl digestion. This causes some double counting of nitrate in the analytical results for TKN and in the calculated sum of TN. Determination of Kjeldahl nitrogen, operationally defined as ammonia plus organic nitrogen, will continue to be offered by the NWQL for both whole-water and filtered samples because the method is specified in the code of federal regulations, some state regulations, and is required by some USGS cooperators. However, continued reliance on the Kjeldahl analysis of whole water samples is not recommended and should be replaced if the preferred method can be used.

Projects that need to change the approach used for determination of TN are encouraged to submit comparison samples for analysis by the former and new methods during a period of time, at least a year or more if possible, to include varying seasonal, suspended-sediment, and flow conditions. Stations with a primary or significant purpose of trend detection (for example, stations on the lower Mississippi River or its major tributaries or tributaries to Chesapeake Bay) should consider running paired samples longer than a year to definitively demonstrate the magnitude of the bias in the method being replaced. (Guidance on combining data from multiple methods for TN used for trends detection will be forthcoming.) Although an extensive field study (details provided below) has been done to compare the results from different approaches, the effects vary too much among sites and for different conditions at individual sites to make universal comparisons.

Background

Evaluation of the methods used by the NWQL for the determination of total nitrogen has taken place over a number of years and included laboratory and field phases. The guidance contained in this memo is based on the combined results from the two phases of evaluation.

The Office of Water Quality Technical Memorandum 2009.03, released in February of 2009, described the results of the laboratory study that was focused primarily on the potential for bias in alkaline-persulfate analysis of total nitrogen in whole water samples. The laboratory experiment indicated that a low bias was present across the entire range of investigated sediment concentrations and increased as sediment concentration increased (figures 1 and 2). The Kjeldahl nitrogen method was also evaluated in the laboratory study and a potential for high bias was noted (figures 1 and 2), but these results were not covered in OWQ Technical Memorandum 2009.03.

A synoptic field study was then designed not only to assess the extent of bias in the alkaline-persulfate and Kjeldahl methods, but also to evaluate the efficacy of using the sum of dissolved nitrogen plus particulate nitrogen as a more accurate alternative to direct measurement of total nitrogen in water samples (figures 2 and 3). Both studies have been documented in a Scientific Investigations Report, *Assessing Total Nitrogen in Surface-Water Samples —Precision and Bias of Analytical and Computational Methods*, by David L. Rus, Charles J. Patton, David K. Mueller, and Charles G. Crawford.

Selected graphics from the SIR are shown in Attachment 1 of this memo.

Field and laboratory information

Bottle and filter types, sample collection instructions, required treatment, and fiscal year 2013 analytical costs for the recommended total nitrogen methods, alkaline persulfate for dissolved nitrogen and high temperature combustion for particulate nitrogen, are shown in Attachment 2. Sources for the field equipment and supplies and fiscal year 2013 costs also are included. The field procedure for processing samples is the same as for total particulate carbon and can be found in the National Field Manual, Chapter 5, pages 61-71.

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This memorandum supersedes OWQ Technical Memorandum 2009.03.

Distribution: All WMA Employees

References

Wilde, F.D., Radtke, D.B., Gibs, Jacob, and Iwatsubo, R.T., eds., 2004 with updates through 2009, Processing of water samples (ver. 2.2): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A5, April 2004, accessed November 29, 2012 at http://water.usgs.gov/owq/FieldManual/chapter5/html/Ch5_contents.html

Office of Water Quality Technical Memorandum 2009.03 Potential Bias in Alkaline Persulfate Analysis of Total Nitrogen in Whole Water Samples and Recommendations for Quantifying Bias in Whole Water Samples, accessed November 29, 2012 at http://water.usgs.gov/admin/memo/QW/qw09.03.html

Rus, D.L., Patton, C.J., Mueller, D.K., Crawford, C.G. 2012, Assessing Total Nitrogen in Surface-Water Samples —Precision and Bias of Analytical and Computational Methods, U.S. Geological Survey Scientific Investigation Report 2012-xxx, xx p.

U.S. Environmental Protection Agency, 2012, Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures; Final Rule Federal Register / Vol. 77, No. 97 / Friday, May 18, 2012 / Rules and Regulations, accessed October 23, 2012 at http://www.gpo.gov/fdsys/pkg/FR-2012-05-18/html/2012-10210.htm

Attachment 1 – Selected graphics from the SIR, Assessing Total Nitrogen in Surface-Water Samples —Precision and Bias of Analytical and Computational Methods, by David L. Rus, Charles J. Patton, David K. Mueller, and Charles G. Crawford

Abbreviations used:

- TN = Total nitrogen;
- NOx = NO_2 + NO_3;
- TKN = Total Kjeldahl nitrogen;
- TN-K = TKN + NOx;
- TN-A = Total nitrogen by alkaline-persulfate digestion of whole-water samples;
- DN = Dissolved nitrogen by alkaline-persulfate digestion of filtered water samples;
- PN = Particulate nitrogen from high temperature combustion of suspended sediment; and
- TN-C = DN + PN

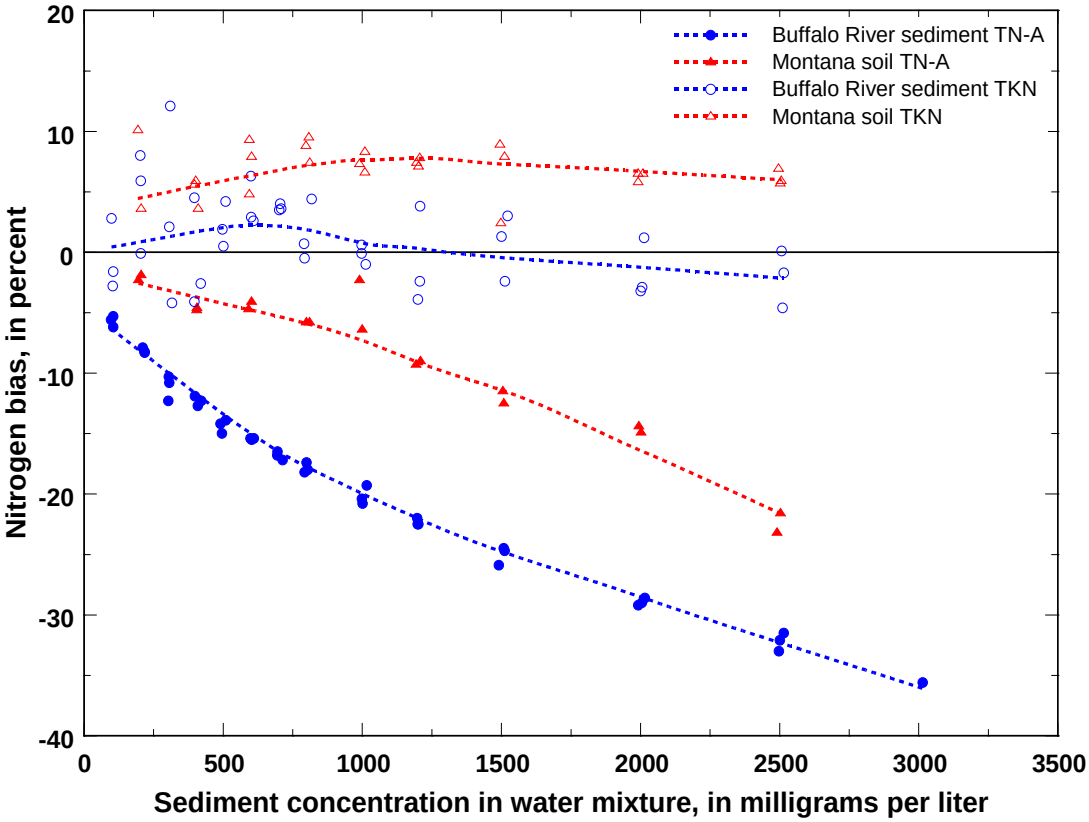

Figure 1. Percent recovery of total nitrogen by TN-A or TKN in the laboratory test using synthetic samples made from nutrient-fortified water and various concentrations of one of two sediment reference materials: Buffalo River sediment or Montana soil.

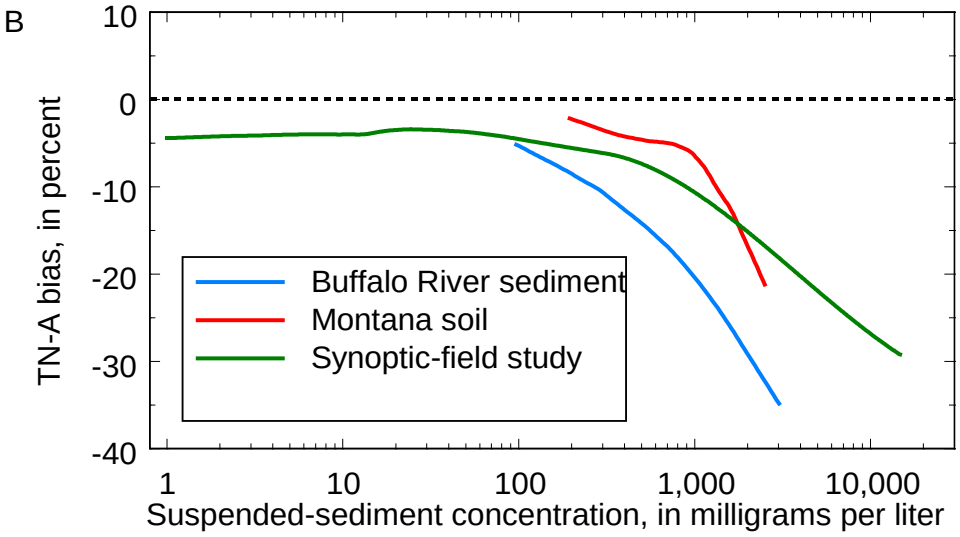

Figure 2. General trend (as represented by locally weighted regression) in TN-A percent bias of the laboratory-experiment soils and synoptic-field data.

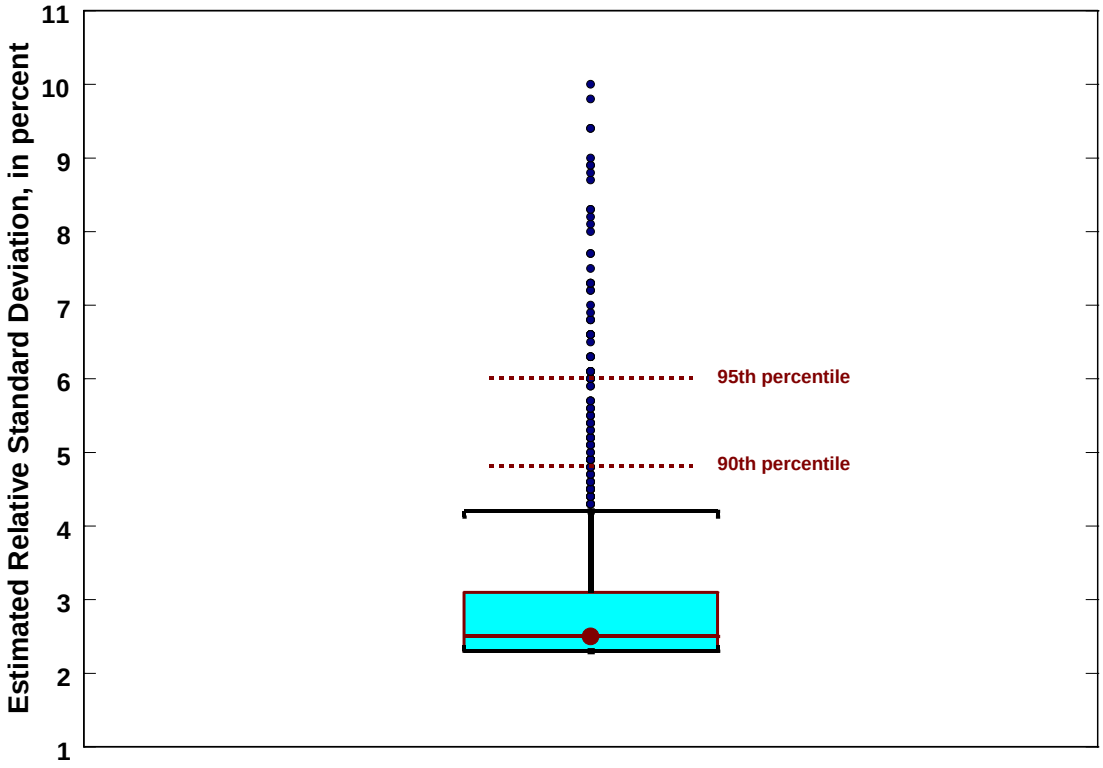

Figure 3. Relative standard deviation of 905 total N (TN-C) values based on the sum of estimated variabilities for dissolved N and particulate N results.

Attachment 2 – Lab and Field information

Sample collection requirements and costs

1. Dissolved N – Alkaline-persulfate analysis of filtered water

Description	Total Dissolved Nitrogen by alkaline persulfate digest
Lab Procedures	2754
Total Price	$21.49
Container Information	FCC 125 mL brown polyethylene bottle.
Sample Amount	Fill bottle (Minimum Amount Required: 125mL)
Filtration	Filter through 0.45 um filter. Rinse container with filtered sample.
Preservation	Chill 2^0 to 6^0C

2. Particulate N – High-temperature-combustion analysis of suspended sediment

Description	Total Particulate Nitrogen (TPN)
Lab Procedures	2607
Total Price	$47.88 (includes analysis of particulate carbon LC 2606)
Container Information	Aluminum foil package containing at least 2 (and preferably 3) 25-mm diameter, 0.7-um glass-fiber filter
Filtration	Filter with glass fiber filter. Fold filter in half and place in Al foil pouch. Place pouch in Whirlpak bag. Procedure is described in the National Field Manual, Chapter 5, Section 5.2.2.C. and online at: Particulate Carbon and Nitrogen Methods at the NWQL
Preservation	Chill 2^0 to 6^0C

Equipment & supplies

Procedure	Description	1Stop Number	Cost	Package
Lab code 2607	Foil, Aluminum, 6 in X 6 in Squares	Q443FLD	$47.80	Pg/300
Lab code 2607	25 mm baked glass fiber filter	Q441FLD	$93.98	Box/100
Lab code 2607	FEP pressure-filtration apparatus or Polypropylene filtration flask and Polysulfone filter funnel	Q444FLD Open Market Open Market	$303.37	EACH
Lab code 2607	In-line vent filter, 50 mm, 0.2µm	Q445FLD	$12.38	EACH
Lab code 2754	125 mL brown polyethylene bottle	Q33FLD	$207.60	Case/550
Lab code 2754	HDPP Bottle Cap,28MM	Q417FLD	$6.33	Bag/100
Lab code 2754	Filter, Capsule, Pall, 0.45um	Q398FLD	$19.85	EACH