In Reply Refer To:
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Office of Water Quality Technical Memorandum 2008.04

Subject: Nitrogen Isotope Analyses – Information and Revised Field Procedures

PURPOSE:

The purpose of this technical memorandum is to describe changes in the standard field procedures used to collect water samples to be analyzed for $^{15}N/^{14}N$ of nitrate, $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of nitrate, and $^{15}N/^{14}N$ of ammonium. This memorandum supersedes Office of Water Quality Technical Memorandum 2003.07, but incorporates from that memorandum relevant information on sampling procedures (Attachment 1), nitrogen isotopic variation (Attachment 2), and reporting units and references (Attachment 3).

BACKGROUND:

The standard procedure for the collection of water samples for nitrogen isotope analyses has been, until now, to filter samples through 0.45-µm filter media and ship the untreated, chilled samples to the Reston Stable Isotope Laboratory (RSIL) for analysis. It has come to the attention of the RSIL that bacteria passing through a 0.45-µm filter can cause sample degradation and interference with the method of isotopic analysis being used by the RSIL.

SYNOPSIS OF CHANGES TO THE FIELD PROCEDURE:

The following bullets summarize the major changes to be implemented for collecting water samples for analysis of nitrogen isotopes. Detailed instructions, including type and use of sample containers and filtration and preservation methods for nitrogen isotope analyses in water and solids are provided in Attachment 1. Refer to the RSIL website for these and future updates (http://isotopes/usgs.gov).

- Effective immediately, every water sample to be analyzed for nitrogen isotopes of nitrate, and nitrogen isotopes of ammonium, are to be processed through a pre-rinsed 0.2-µm filter.
- $^{15}N/^{14}N$ in ammonium samples are to be preserved by adding 4.5*N* reagent-grade sulfuric acid (H_2SO_4) to achieve a sample pH of ≤ 2.
- The preservation procedure for $^{15}N/^{14}N$ or $^{15}N/^{14}N$ and $^{18}O/^{16}O$ in nitrate samples has not changed and requires maintaining the sample at or below 4 degrees Celsius (≤ 4˚C) from the time of collection to analysis; however, if keeping the sample chilled at ≤ 4˚C or frozen is not an option or may not be successful, or if samples will be archived, the sample can be preserved by adding reagent-grade sodium hydroxide (NaOH) or potassium hydroxide (KOH) to achieve a pH greater than 10 but less than 12.

Questions about these nitrogen isotope laboratory methods and associated field procedures should be directed to Tyler Coplen (tbcoplen@usgs.gov).

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This memorandum supersedes OWQ Technical Memorandum 2003.07.

Distribution: All WRD employees

Attachment 1. Sampling requirements for nitrogen-isotope analyses in water and solids
Attachment 2. Explanation of nitrogen isotopic variation, with figure
Attachment 3. Reporting units in ‰, and References

ATTACHMENT 1. Sampling Requirements for Nitrogen Isotope Analyses in Water and Solids[1]

[LC, laboratory (lab) code; N, nitrogen; oz, ounce(s); mL, milliliter; µm, micrometer; ˚ C, degrees Celsius; DIW, distilled/deionized water; NaOH, sodium hydroxide; KOH, potassium hydroxide; O, oxygen; >, greater than; <, less than; RSIL, Reston Stable Isotope Laboratory; NWQL, National Water Quality Laboratory; L, liter; *N*, normal; H_2SO_4, sulfuric acid; NH_4, ammonium; C, carbon; HDPE, high-density polyethylene]

Lab Code	Description	Bottle	Treatment, Preservation, and Shipping
LC2899 *and* **LC2900**	$^{15}N/^{14}N$ of nitrate dissolved in water *and* $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of nitrate dissolved in water	4-oz (125 mL) amber polyethylene bottle, untreated (Q33FLD or Q405FLD in One-Stop Shopping). Field-rinse the sample bottle three times with a small volume of sample filtrate. Fill bottle to the shoulder and cap securely. **Use a polyseal cap with conical insert** (Q419FLD).	**Samples must be filtered through 0.2-µm media[2] and chilled or preserved with reagent-grade hydroxide onsite.** 1) In advance of sampling, pre-rinse the 0.2-µm-pore filter media and housing with DIW and sample water. 2) Filter sample(s), filling the sample bottle three-quarters full. Do not overfill bottle; be sure to use the polyseal cap with conical insert. Use electrical tape to secure the cap. 3) Chill sample(s) immediately and maintain sample(s) chilled at or below 4˚C or frozen. 4) Ship chilled or frozen sample(s) to the RSIL after receiving NWQL analysis of nitrate concentration in the sample (NWQL LC 1975 or LC 1990). **If sample(s) cannot be kept chilled or frozen, or if samples are to be archived:** 1) Preserve the filtered sample(s) by adding reagent-grade NaOH or KOH to raise the sample pH to 11; sample pH must be >10 but <12. Generally, addition of one hydroxide tablet is sufficient; however, check that the pH will not exceed 12 on a 100-mL test sample that is collected for this purpose alone. 2) Label sample bottle(s) to indicate that hydroxide was added. 3) Ship sample(s) to the RSIL for isotope analysis after the nitrate concentration in the sample(s) is known. (Do not send the hydroxide addition test sample to the RSIL.)
LC2898	$^{15}N/^{14}N$ of ammonium dissolved in water	32-oz (1 L) polyethylene bottle, untreated (Q35FLD in One-Stop Shopping). Field-rinse the sample bottle three times with a small volume of sample filtrate. Fill bottle to the shoulder and cap securely, using a polyseal cap.	**Samples must be filtered thru 0.2-µm media and preserved with reagent-grade 4.5*N* H_2SO_4 at the field site.** 1) In advance of sampling, pre-rinse the filter media and housing with DIW and sample water. 2) Filter sample(s), filling the sample bottle to the shoulder. 3) Add a 1-mL ampoule of H_2SO_4 to achieve a pH ≤2 on a 100-mL volume of sample. One ampoule should be sufficient, but this can be tested using a check sample. 4) Label the sample bottle(s) to indicate that H_2SO_4 was added. 5) Ship sample(s) to the RSIL for isotope analysis after receiving NWQL analysis of ammonium concentration in the sample (LC 1991). (Do not send the acid test sample to the RSIL.)
LC2893 **Schedule 1832**	$^{15}N/^{14}N$ and N concentration of solids. $^{15}N/^{14}N$, $^{13}C/^{12}C$, N concentration, and C concentration of solids.	HDPE liquid scintillation vial with polyseal cap, Wheaton 986706, available from RSIL.	Sample should be dried and powdered to 100 to 200 µm to ensure isotopic homogeneity. There are no treatment, preservation, or special shipping requirements.

[1] Questions about these nitrogen isotope laboratory methods and associated field procedures should be directed to Tyler Coplen (tbcoplen@usgs.gov).

[2] Filters with 0.2-*µ*m pore size currently are not supplied by the USGS National Field Supply Service ("One-Stop Shopping") and therefore are to be purchased on the open market. Many types of filters and filter-containing units are available; personnel should select that which best meets their data-collection needs. An example of a 0.2-*µ*m filter unit that is similar to the 0.45-*µ*m disposable unit that is in common use is the Whatman Polydisc (http://vwrsp.com/catalog/product/index.cgi?catalog_number=28137-862&inE=1&highlight=28137-862).

ATTACHMENT 2. Explanation of nitrogen isotopic variation

Molecules, atoms, and ions in their natural occurrences contain isotopes in varying proportions, whereby they possess slightly different physical and chemical properties. This gives rise to partitioning of isotopes (isotope fractionation) during physical or chemical processes, and these fractionations commonly are proportional to differences in their relevant isotope masses. Nitrogen has two isotopes, ^{14}N and ^{15}N, with mole fractions of approximately 0.9963 and 0.0037, respectively. The compounds of nitrogen possess isotopic variability, and this variation in isotopic composition (see figure) allows researchers to "tag" nitrogen-bearing sources and investigate the fate of nitrogen in the environment.

Users should be aware that atmospheric nitrate is enriched in ^{17}O by mass-independent processes and that this new method for nitrate isotope measurements may overestimate the $\delta^{15}N$ values of atmospheric nitrate samples by as much as 1 to 2 per mil (Sigman and others, 2001; Coplen and others, 2004). For samples that users suspect may contain more than about 20 percent atmospheric nitrate, users should contact RSIL about methods to resolve this problem.

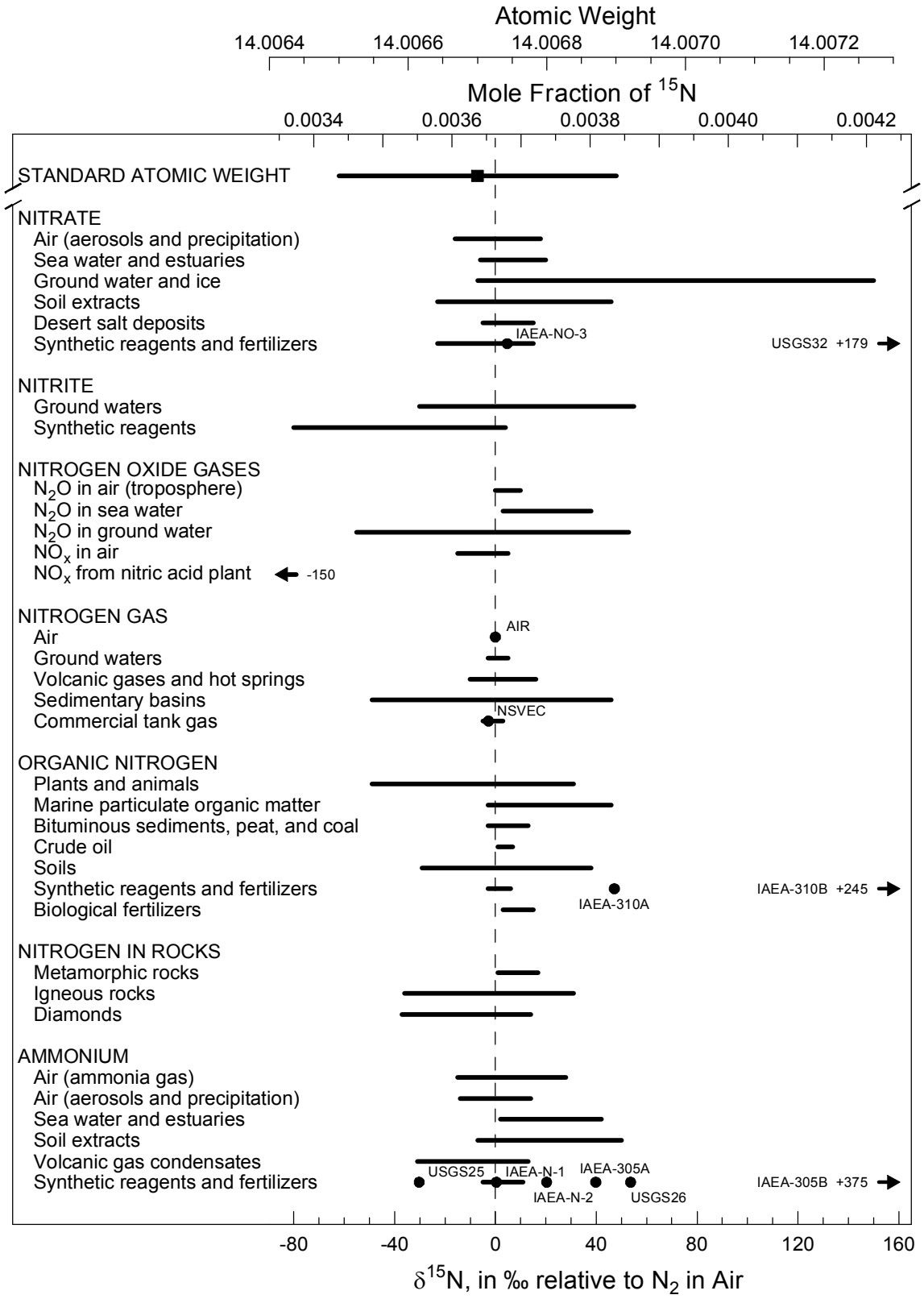

Figure 1. Nitrogen isotopic composition and atomic weight of selected nitrogen-bearing materials (Coplen and others, 2002).

ATTACHMENT 3. Reporting units, ‰

Variations in stable isotope abundance ratios typically are small. Nitrogen stable isotope ratios commonly are expressed as relative isotope ratios in δ^{15}N notation (pronounced delta) according to the relation

$$\delta^{15}\mathrm{N} = \left[\frac{n_{\mathrm{X}}\left(^{15}\mathrm{N}\right) / n_{\mathrm{X}}\left(^{14}\mathrm{N}\right)}{n_{\mathrm{air}}\left(^{15}\mathrm{N}\right) / n_{\mathrm{air}}\left(^{14}\mathrm{N}\right)} - 1 \right],$$

where δ^{15}N refers to the delta value of sample X relative to N_2 in air, and $n_X(^{15}N)/n_X(^{14}N)$ and $n_{air}(^{15}N)/n_{air}(^{14}N)$ are the ratios of the isotope amounts in unknown X and in N_2 in air (Böhlke and Coplen, 1995). A positive δ^{15}N value indicates that the unknown is more enriched in ^{15}N than is the reference. A negative δ^{15}N value indicates that the unknown is depleted in ^{15}N relative to the reference. In the literature, δ^{15}N values commonly are reported in parts per thousand (‰ or per mil).

Correspondingly, for oxygen isotope ratios (for example, in nitrate) the expression is

$$\delta^{18}\mathrm{O} = \left[\frac{n_{\mathrm{X}}\left(^{18}\mathrm{O}\right) / n_{\mathrm{X}}\left(^{16}\mathrm{O}\right)}{n_{\mathrm{VSMOW}}\left(^{18}\mathrm{O}\right) / n_{\mathrm{VSMOW}}\left(^{16}\mathrm{O}\right)} - 1 \right]$$

and the reference is now VSMOW reference water instead of nitrogen in air. Nitrogen and oxygen isotope data are calibrated by analyzing isotopic reference materials. Descriptions of reference materials used in the analyses listed below are given in Böhlke and Coplen (1995) and Böhlke and others (2003).

Variations in stable isotope abundance ratios typically are small. Nitrogen stable isotope ratios commonly are expressed as relative isotope ratios in δ^{15}N notation (pronounced delta) according to the relation.

SELECTED REFERENCES

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