

Nitrate Isotopes and its Applications in Hydrological Studies— A Review

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ABSTRACT: Identification of the source of nitrate and its biogeochemical histories are often of significant interest for water resources managers. Dissolved nitrate are common constituents of water and groundwater and high concentration of nitrate in rivers and lakes may lead to acidification and eutrophication of water bodies. Nitrate concentrations in public water supplies have risen above acceptable levels in many areas of world, largely as a result of overuse of fertilizers and contamination by human and animal waste. The United States of Environmental Protection Agency and the World Health Organization (WHO) recommends a maximum of 10 mg NO₃⁻ – N as an upper limit for drinking water because high-nitrate water poses a health risk, especially for children, who can contract methemoglobinemia (blue-baby disease). Hence, knowing the sources of nitrate may be essential for providing suitable and safe water for human use and consumption. Stable isotope techniques have emerged as a valuable tool in this endeavor, since the isotopic compositions of dissolved nitrate ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) in surface water and groundwater often provide information about the sources of these solutes. Nitrogen and oxygen isotope ratio is a useful technique to help in identification of sources and fate of nitrate found in the aquifers. Additionally, spatial or temporal patterns of increasing or decreasing nitrate concentrations along with changing isotope ratios may reveal biological processes governing the occurrence of nitrate in aquatic systems. The objective of the paper is to provide an overview of the analytical developments in isotopic analysis of nitrate and its applications in groundwater research and management.

Keywords: Isotopic techniques, ¹⁵NO₃⁻ – N, Stable isotopes, Applications, Analytical developments, Source Identification.

INTRODUCTION

There are two stable isotopes of nitrogen: ¹⁴N and ¹⁵N. All nitrogen compounds contain both isotopes, but because of isotopic fractionation they are incorporated into compounds in differing ratios depending on the nature of the reactions that produce the compounds. For example, as nitrogen compounds are passed up the food chain, the lighter isotopes are excreted in urine and the heavier isotopes are retained. Nitrogen in animal waste is hydrolyzed to ammonia and then converted to nitrate. During this process more of the heavy isotope is concentrated in the resulting nitrates. When various sources of nitrogen compounds are mixed together in surface runoff or in a body of water, the ratio of light to heavy nitrogen isotopes in the water can be used to estimate the relative contributions of the various sources.

Commercial fertilizers, animal or human waste, precipitation, and organic nitrogen within the soil are common sources of nitrate in groundwater. Each of these nitrate source categories has a distinguishable isotopic signature (i.e., ¹⁵N/¹⁴N ratio).

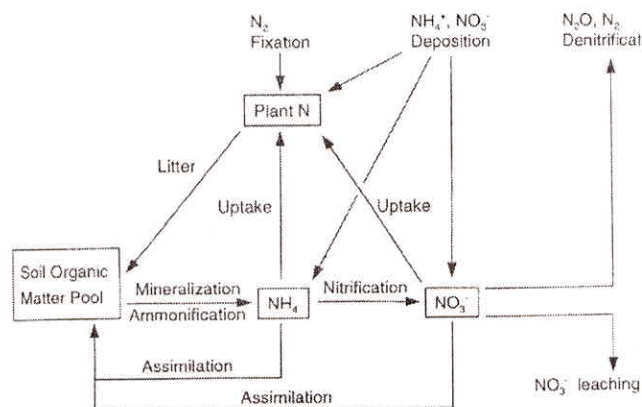


Fig. 1: Nitrogen cycle

The most important processes in the nitrogen cycle is outline in the Figure 1. The Processes are organic-N mineralization to NH₄⁺; Organic-N synthesis from inorganic NH₄⁺, NO₃⁻, and N₂; Oxidation of NH₄⁺ to NO₃⁻ (nitrification); and reduction of NO₃⁻ to N₂ (denitrification). Under the ideal circumstances, stable nitrogen isotopes offer a direct means of source identification because the two major sources of nitrate in many agricultural areas, fertilizer and manure;

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generally have isotopically distinct $\delta^{15}\text{N}$ values. Hence, under favorable conditions, the relative contributions of these two sources to groundwater or surface water can be estimated by simple mass balance. Soil-derived nitrate and fertilizer nitrate commonly have overlapping $\delta^{15}\text{N}$ values, preventing their separation using $\delta^{15}\text{N}$ alone.

Isotopic Composition of nitrate

First nitrogen isotope ratio measurements on water samples were reported in the 1950s by Hoering (1955, 1957) but it was not until the early 1970s that determinations of $\delta^{15}\text{N}$ values of dissolved ammonium and nitrate in surface water and groundwater samples gained more widespread use in hydrological studies (e.g. Edwards, 1973; Kohl *et al.*, 1971; Kreitler, 1979; Kreitler and Jones, 1975). Nitrogen has two stable isotopes, ^{14}N and ^{15}N , with approximate natural abundances of 99.6337 and 0.3663%, respectively (Junk and Svec, 1958; Rosman and Taylor, 1988). Nitrogen isotope measurements are expressed using the delta scale,

$$\delta^{15}\text{N} [\text{‰}] = \left[\frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{reference}}} - 1 \right] \cdot 1000 \quad \dots (1.1)$$

The accepted primary reference for nitrogen isotope abundance measurements is atmospheric N_2 (Mariotti, 1983). Reference materials such as IAEA-N-1 (+0.43‰) and IAEA, 1995) are available from the International Atomic Energy (IAEA) for calibration and normalization purposes. Since the later 1980s, a variety of different analytical methods have been developed also to determine the oxygen isotope ratio of NO_3^- (e.g. Amberger, 1987; Bräuer and Strauch, 2000; Casciotti *et al.*, 2002; Revesz *et al.*, 1997 silva *et al.*, 2000). The combined interpretation of nitrogen and oxygen isotope ratios has greatly facilitated the ability to trace the sources of dissolved nitrate and conclusively to identify processes that nitrate may have undergone in many hydrological settings. Oxygen isotope ratios are reported in delta units

Table 1: Reference Material for $^{15}\text{N}/^{14}\text{N}$ Analyses (IAEA, 1995; Bohlke and Coplen, 1995)

Reference Sample Name	Material	$^{15}\delta$ (‰) Rel. to Air N_2
IAEA-N1	$(\text{NH}_4)_2\text{SO}_4$	+0.43 ± 0.07
IAEA-N2	$(\text{NH}_4)_2\text{SO}_4$	+20.41 ± 0.12
IAEA-N3	KNO_3	+4.72 ± 0.13
USGS-25	$(\text{NH}_4)_2\text{SO}_4$	-30.41 ± 0.27
USGS-26	$(\text{NH}_4)_2\text{SO}_4$	+53.75 ± 0.24
USGS-32	KNO_3	+180

in permil (‰) relative to Vienna Standard Mean Ocean Water (V-SMOW) as shown in Eqn. (1.1). A sufficient number of reference materials with widely differing $\delta^{18}\text{O}_{\text{nitrate}}$ values have recently become available (Bohlke *et al.*, 2003).

Processes Affecting N isotopic Compositions

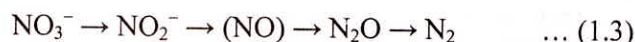
Biologically-mediated reactions (e.g., assimilation, nitrification and denitrification) strongly control nitrogen and nitrogen isotopic compositions in both soil and water. Nitrification is a chemical process that produces nitrate (NO_3^-) through the oxidation of ammonium (NH_4^+). After gaseous N_2 , nitrate is the most stable form of nitrogen and is present in most groundwater.

The nitrification reaction, below, occurs under aerobic conditions, whereas denitrification occurs under anaerobic conditions.

Nitrification, the oxidation from ammonia to nitrite and nitrate,



Denitrification, the reduction of nitrate to molecular nitrogen,



Nitrogen fixation, the conversion of dissolved molecular nitrogen to organic compounds,



A bacterium known as *Thiobacillus denitrificans* is responsible for most of the denitrification in groundwater. However, other types of bacteria can denitrify in the absence of carbon, using electron sources such as Mn^{2+} , Fe^{2+} , sulfide and methane. The resulting stable nitrogen isotope fractionation during denitrification causes the $\delta^{15}\text{N}$ of residual nitrate to increase exponentially as nitrate concentrations decrease due to fractionation. Generally, the isotopically light (^{14}N containing) molecules are reacted more easily (Letolle, 1980; Hubner, 1986). Denitrification processes can also be identified by the amount of gaseous N_2 . The N_2 produced by denitrification results in excess N_2 contents in groundwater. The total N_2 (which consists of air N_2 trapped during recharge plus N_2 produced by denitrification) can be collected, analyzed for $\delta^{15}\text{N}$, and used to estimate the extent of denitrification, initial composition of the nitrate, or the mixing history of the water.

In addition to denitrification NO_3^- may be removed from water by plant assimilation; as such plants can assist in the remediation of surface and groundwater,

for example. Riparian zones are assumed to buffer NO_3^- in surface waters. During assimilation, nutrients are incorporated in the plant, where they remain until they are released by mineralization during decay. The plants do not remove N from ecosystem, but increase the residence time of nutrients through a reduction of the mobility of N compounds. However, there are limits, and large amounts of nutrients cause increased plant growth, resulting in eutrophication and anoxia. In this way, increased nutrients lead to a shorter retention time of nutrients in the riparian buffer zone.

The effects of denitrification and assimilation can be distinguished with the use of $\delta^{15}\text{N}$ analyses combined with $\delta^{18}\text{O}$ analysis. If plant uptake alone is responsible for NO_3^- remediation, the isotopic composition of the remaining NO_3^- remains unchanged. If both denitrification and assimilation are occurring, the isotopic composition of the residual nitrate is enriched and the overlying plants reflect the isotopic composition of the NO_3^- source. The isotopic composition of the plants will remain the same and the water will become more enriched if denitrification is the only process occurring. Analysis of $\delta^{18}\text{O}$ in combination with $\delta^{15}\text{N}$ provides additional information about nitrates in water and soils, specifically on the relative contributions of fertilizers vs. soil NO_3 or manure/septic waste, and on the relative contributions of atmospheric NO_3 vs. fertilizer, soil NO_3 , or manure/septic waste.

Transformation Processes of Nitrate

Isotope tracing of nitrogen-containing compounds in the water-unsaturated and water-saturated zone is not straightforward, since numerous transformation processes in the nitrogen cycle are associated with significant but variable isotope effects. During volatilization, the conversion of NH_4^+ to NH_3 , ^{14}N is preferentially converted to NH_3 leaving the remaining NH_4^+ enriched in ^{15}N (Letolle, 1980; Heaton, 1968; Hübner, 1986). Nitrification, the conversion of NH_4^+ to NO_3^- , can also proceed with significant nitrogen isotope fractionation accumulating ^{14}N preferentially in the produced NO_3^- , provided that the substrate is not limited (Mariotti *et al.*, 1981). During the nitrification process, three new oxygen atoms are introduced into the newly formed nitrate molecule. Typically, two of these oxygens are derived from ambient water and one from O_2 (Hollocher, 1984), resulting in $\delta^{18}\text{O}_{\text{nitrate}}$ values between 0 to +15‰ depending on environmental conditions (e.g. Amberger and Schmidt, 1987; Durka *et al.*, 1994; Mayer *et al.*, 2001).

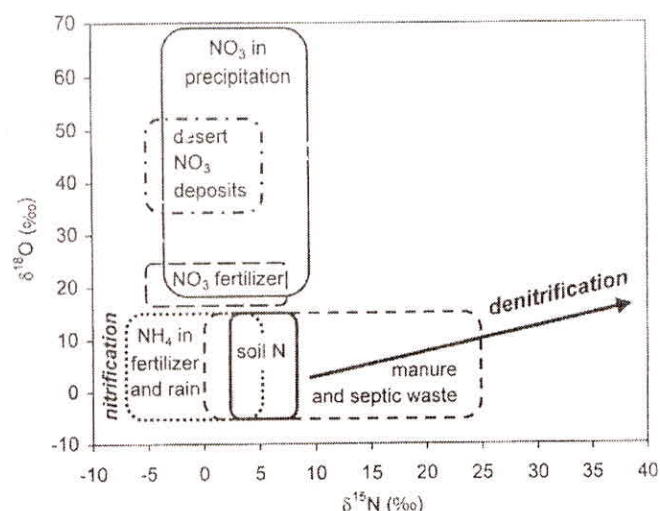


Fig. 2: Typical ranges for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate

Another process causing significant alteration of the isotopic composition of nitrate in aquatic systems is microbial denitrification, during which the lighter isotopes ^{14}N and ^{16}O are preferentially metabolized by microorganisms causing an enrichment of the heavy isotopes ^{15}N and ^{18}O in the remaining nitrate as concentrations decrease (Mariotti *et al.*, 1981; Mariotti *et al.*, 1982). The increase in $\delta^{15}\text{N}_{\text{nitrate}}$ values due to microbial denitrification appear to be about twice that of $\delta^{18}\text{O}_{\text{nitrate}}$ (e.g. Bottcher *et al.*, 1990). Hence, the remaining nitrate eventually assumes elevated $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values, which are unique for nitrate that has undergone denitrification under closed system conditions (Figure 2).

Interestingly, nitrate in surface water or groundwater rarely has the same isotopic composition as nitrate in atmospheric deposition or in synthetic fertilizers. The typically low $\delta^{18}\text{O}$ values (<15‰) of aqueous nitrate provide strong evidence that nitrate from atmospheric deposition ($\delta^{18}\text{O}_{\text{nitrate}} > +30\text{‰}$) and nitrate from synthetic fertilizers ($\delta^{18}\text{O}_{\text{nitrate}} \sim 23\text{‰}$) does not behave conservatively in the water unsaturated zone, but rather undergoes an intense immobilization mineralization cycle in the soils (e.g. Mengis *et al.*, 2001). The three original oxygen atoms of the nitrate molecule are removed during the immobilization process and hence nitrate from atmospheric deposition or from synthetic fertilizers loses its original oxygen isotope signature. Three new oxygens are acquired during the subsequent mineralization process and the $\delta^{18}\text{O}$ value of the newly formed nitrate (typically <15‰) is indicative of nitrate from soil nitrification. Mengis *et al.* (2001) demonstrated in *lysimeter* experiments that tracing of fertilizer nitrate with the dual isotope approach ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) is only possible

under conditions of very low microbial activity (e.g. winter), but fails under the biologically active conditions during the growing season because of the rapid immobilization—mineralization turnover of nitrogen in soils.

Variations in Concentrations of Nitrate Isotope

In some situations, a plot of $\delta^{15}\text{N}_{\text{nitrate}}$ and $\delta^{18}\text{O}_{\text{nitrate}}$ values may conclusively reveal the mixing of nitrate from two sources. However, simulations monitoring of spatial or temporal trends in concentration and isotopic composition of nitrate has proven to be a more effective approach for revealing nitrogen sources and transformation processes in aquatic systems. Decreasing nitrate concentrations with increasing $\delta^{15}\text{N}_{\text{nitrate}}$ (Mariotti *et al.*, 1982; Mariotti *et al.*, 1988) and $\delta^{18}\text{O}_{\text{nitrate}}$ values (Bottcher *et al.*, 1990) are indicative for denitrification. Using a combination of hydrological, chemical, and isotopic techniques, denitrification has been identified in groundwater systems (Aravena and Robertson, 1998; Bottcher *et al.*, 1990), riparian zones (Cey *et al.*, 1999; Mengis *et al.*, 1999), and surface waters (Kellman and Hillaire-Marcel, 1998).

Trends of increasing nitrate concentrations accompanied by increasing $\delta^{15}\text{N}_{\text{nitrate}}$ values are often typical for admixture of nitrate from an anthropogenic source. Nitrate derived from sewage (Aravena *et al.*, 1993) or manure (Karr *et al.*, 2001; Rock and Mayer, 2004) have been identified in surface waters and groundwater using a combination of chemical and isotopic techniques.

ANALYTICAL DEVELOPMENTS

In order to avoid inter-conversion of potentially liable nitrogen compounds such as ammonium (NH_4^+), nitrate (NO_3^-), dissolved (DON) or Particulate Organic Nitrogen (PON), it is recommended to filter water samples in the field with 0.45 μm membranes and to transport the filtered samples in cool and dark containers. Samples should be processed as soon as possible upon return to the laboratory or stored frozen until further analysis.

Determining the nitrogen isotope ratios of NH_4^+ and NO_3^- is commonly achieved in three: (1) extraction of NH_4^+ or NO_3^- from a water sample, (2) conversion of the extracted $\text{NH}_4^+\text{-N}$ or NO_3^-N into di-nitrogen gas (N_2), and (3) determination of the nitrogen isotope ratio of the produced N_2 using an Isotope Ratio Mass Spectrometer (IRMS). Nowadays, the latter two steps are usually performed by a thermal decomposition of a synthesized solid nitrogen compound in an Elemental

Analyser (EA) and subsequent sweeping of the produced N_2 with a He carrier gas into an Isotope Ratio Mass Spectrometer (IRMS) in Continuous Flow (CF) mode (Barric and Processer, 1996). Prior to the invention of CF-IRMS techniques, N_2 gas was produced off-line by hypobromite oxidation (Bremner, 1965) or by combustion in quartz ampoules (Kendall and Grim, 1990) and measured via dual inlet isotope ratio mass spectrometry.

A number of different techniques have been suggested throughout the last half century for the extraction of NH_4^+ and NO_3^- from aqueous samples. Historically, nitrogen isotope ratios of ammonium (NH_4^+) and nitrate (NO_3^-) have been determined using variations of the Kjeldahl technique, which was initially developed for soil science applications (Bremner and Keeney, 1965; and Bremner and Edwards, 1965). In a large Kjeldahl distillation apparatus that accommodates samples of up to 1000 mL, NH_4^+ in a water sample is converted to NH_3 gas by raising the pH of the solution to ~ 9.5 via addition of NaOH or MgO (e.g. Cline and Kaplan, 1975). The liberated NH_3 gas is distilled, trapped in an acidic solution, and converted to $(\text{NH}_4)_2\text{SO}_4$. Subsequent to be complete removal of NH_4^+ from the original water sample. NO_3^- can be reduced to NH_4^+ via addition of Devarda's alloy (Bremner and Keeney, 1965) and treated as described above to yield a second $(\text{NH}_4)_2\text{SO}_4$ precipitate that is representative for NO_3^-N . An alternative to the distillation method is the diffusion of the released NH_3 gas onto glass fiber filters acidified with sulphuric acid mounted in the headspace of closed containers (Books *et al.*, 1989; MacKown *et al.*, 1987)) or floating enclosed in gas-permeable but hydrophobic PTFE membranes on the water sample (Holmes *et al.*, 1998; Sigman *et al.*, 1997; Sorenson and Jensen, 1991; Stark and Hart, 1996).

These techniques have several drawbacks. They require that the water samples be preserved by chilling, acidifying, or poisoning, and transported back from the field for processing. Water with low nitrate concentrations are impractical to process because of the large volumes of sample required. The distillation process is time consuming and is subject to isotope fractionation and cross contamination (Mulvaney, 1986). Freeze-drying of large water samples requires considerable time when these samples are combusted in quartz or Vycor tubes, the tubes often fail because of reaction of alkali metals, particularly Na, with the glass. As an alternative, anion exchange resins have been used for collecting nitrate (Hoering, 1957; Morrissey, 1989; Garten, 1992; Downs *et al.*, 1999). Amberger and Schmidt (1987) developed the first

reliable method for $\delta^{18}\text{O}$ analysis using HgCN as a carbon source and nitrate in the form of KNO_3 . The drawbacks of this method are the toxicity of HgCN and the low yields of CO_2 . At that time recently developed alternative method combusts nitrate in the form of KNO_3 with graphite as a carbon source, and mathematically corrects for the fractionation caused by lower yields of CO_2 (Revesz *et al.*, 1997). Zeolites (Velinsky *et al.*, 1989) and cation exchange resin beads (Lehmann *et al.*, 2001) have also been used to recover NH_4^+ from aqueous solutions. Nitrogen contained on precipitates, filters, resin beads or zeolites is subsequently converted to N_2 typically by combustion in an elemental analyzer and the $^{15}\text{N}/^{14}\text{N}$ ratio is determined by isotope ratio mass spectrometry. All Kjeldahl distillation or diffusion techniques have the disadvantage that the nitrate-oxygen is removed during the reduction of nitrate to ammonium, making oxygen isotope measurements impossible.

Analytical techniques capable of determining the oxygen isotope ratio of dissolved nitrate initially relied on the synthesis of nitrate-containing solids and their conversion to CO_2 or CO . These gases were subsequently analysed for their $^{18}\text{O}/^{16}\text{O}$ ratios either by off-line combustion in sealed quartz tubes followed by dual inlet IRMS, or by on-line pyrolysis-CF-IRMS techniques (Revesz and Böhlke, 2002). Amberger and Schmidt (1987) generated KNO_3 using Hg (CN) $_2$ as a combustion reagent, but their procedure never gained wide-spread popularity. Revesz *et al.*, (1997) proposed a technique in which KNO_3 was reacted with catalyzed graphite to a technique in which KNO_3 was reacted with catalyzed graphite to generate CO_2 , K_2CO_3 and N_2 . Since there is oxygen isotope fractionation between CO_2 and K_2CO_3 , corrections are necessary to obtain the true $\delta^{18}\text{O}$ value for nitrate. Silva *et al.* (2000) described an analytical procedure, in which nitrate is retained on ion exchange resins, subsequently eluted, and quantitatively converted to pure AgNO_3 . Despite of relatively high costs (ion exchange resins, silver oxide, etc.), this technique has gained wide-spread popularity for the analysis of the isotopic composition of nitrate from freshwaters. However, analysis of water samples with high contents of dissolved solids remained challenging. These problems were recently overcome by the so called denitrifier technique. In this procedure, dissolved nitrate is quantitatively reduced to N_2O by denitrifying bacteria that lack N_2O reductase activity. The generated N_2O gas is subsequently used for nitrogen (Sigman *et al.*, 2001) and oxygen (Casciotti *et al.*, 2002) isotope analyses.

Matthew *et al.*, (2005) present a novel method for nitrogen and oxygen natural isotopic abundance analysis

of nitrate and nitrite of seawater and freshwater at environmental concentration. The method involves the reduction of NO_3^- to NO_2^- by spongy cadmium and further reduction to N_2O using sodium azide in an acetic acid buffer. For separate nitrite analysis, the cadmium reduction step is simply by passed. Nitrous oxide is purged from the water sample and trapped cryogenically using an automated system with subsequent release into a gas chromatography column. The isolated nitrous oxide is then analyzed on a continuous flow isotope ratio mass spectrometer via an open split. McIlvin and Altabet, (2005), and Leichter *et al.*, (2007), were following the same method for the determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- .

APPLICATIONS

Identification of Sources of Nitrate

Nitrogen and oxygen isotope ratios of nitrate provide a powerful tool to investigate nitrate sources and cycling mechanisms. The analysis of nitrate for both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ allows improved discrimination among potential sources and reaction mechanisms (Amberger and Schmidt, 1987; Boettcher *et al.*, 1990).

Numerous attempts have been made to use nitrogen isotope ratios to determine the sources of nitrate in surface water and ground water. These were particularly successful in situations where nitrate from one source with a unique $\delta^{15}\text{N}$ value (e.g. from sewage or manure) was dominant (e.g. Mariotti, 1984), but assessing the respective contributions from two or more sources remained challenging. Kohl *et al.* (1971) were among the first to utilize nitrogen isotope ratio variations for determining the sources of nitrate in surface waters. In an agriculturally used watershed in Illinois, USA, they observed a trend of decreasing $\delta^{15}\text{N}$ values (from 10 to 4‰) with increasing nitrate concentrations. Based on a simple two end number mixing model ($\delta^{15}\text{N}_{\text{soil N}} = +13\text{‰}$; $\delta^{15}\text{N}_{\text{fertilizer}} = +3\text{‰}$) they concluded that at times of peak nitrate concentration more than 50% of the nitrate-N in the surface water originated from synthetic fertilizers. This approach was widely criticized (e.g. Edwards, 1973; Hauck *et al.*, 1972) partially because there are often variable isotope effects during nitrogen transformations in the biosphere and pedosphere that need to be considered in source apportionments based on nitrogen isotope ratios. Feigin *et al.*, (1974) demonstrated that isotope effects during nitrification of anhydrous ammonia fertilizer can exceed 10‰, depending on pool size and nitrification rates. Even larger nitrogen isotope fractionation effects have been

described for volatilization of NH_3 (e.g. Heaton, 1986; Hübner, 1986) and denitrification (e.g. Wellman *et al.*, 1968), making a quantitative assessment of contributions from various nitrate sources based on nitrogen isotope ratios alone in many situations problematic.

The dual isotope approach based on the determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ value of nitrate has improved the ability of isotopes hydrologists to identify sources of nitrate in aquatic systems. Nitrate derived from manure or sewage is usually characterized by $\delta^{15}\text{N}$ value between +7 and more than +20‰ (Aravena *et al.*, 1993; Aravena and roberston, 1998; kreitler, 1979; Kreitler and Browning, 1983; Wassenaar, 1995). It is therefore distinct from nitrate in atmospheric deposition (-10 to = 8‰), nitrate in synthetic fertilizer (near 0‰), and nitrate generated via nitrification processes in soils (Kendall, 1998 and references therein). Usually, the latter three sources cannot be differentiated by nitrogen isotope analyses alone because of their overlapping range of $\delta^{15}\text{N}$ values. However, recent research has show that nitrate in atmospheric deposition has quite positive $\delta^{18}\text{O}$ value ranging from circa +30 to +80‰ (Durka *et al.*, 1994; Kendall, 1998; Voerkelius, 1990). Nitrate-containing synthetic fertilizers have $\delta^{18}\text{O}_{\text{nitrate}}$ value near $+22 \pm 3\%$ (Amberge, 1987; Voerkelius, 1990; wassenaar, 1995). Nitrate derived from nitrification processes in soils has typically $\delta^{18}\text{O}$ values of less than +15‰ (Durka *et al.*, 1994; Hollocher, 1984; Mayer *et al.*, 2001) and nitrate in manure and sewage has similarly low $\delta^{18}\text{O}$ values (Aravena *et al.*, 1993; Wassenaar, 1995). Hence, the combined determination of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of dissolved nitrate sources: (1) atmospheric nitrate deposition, (2) nitrate-containing synthetic fertilizers, (3) nitrate derived from nitrification e.g. in soils, and (4) nitrate in manure and sewage. In a study of evaluation of nitrogen isotopes as indicators of nitrate contaminationsources in an agricultural watershed, the data suggest that NO_3^- -N stable isotopes cannot be used as contaminant source indicators in the surface waters of this watershed between precipitation events, and that further evaluation of their use during storm events is required, Kellman *et al.* (2003).

Sources of Nitrate and Cycling Mechanism in Groundwater

There is a fair amount of isotopic literature on this topic, some of which will be described in detail below. Some relevant groundwater $\delta^{15}\text{N}$ include: Kreitler (1975, 1979), Vogel *et al.* (1980), Mariotti *et al.* (1988), Wells and Krothe (1989), Wilson *et al.* (1990), Smith *et al.* (1991), Spalding *et al.* (1993), Bohlke and

Denver (1995), and McMohan and Bohlke (1996). They have been some studies using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ to study nitrate sources and cycling mechanism in groundwater.

Application for Identifying the Anthropogenic Sources

Atmospheric nitrogen deposition to the ocean is significant: about twice of natural riverine input and about equal to that of river system affected by anthropogenic loadings (Fogel and Paerl, 1993; Paerl and Fogel, 1994).

There is some evidence (Kendall *et al.*, 1995b) that different anthropogenic sources of atmospheric nitrate may have characteristic $\delta^{18}\text{O}$ values that can be used to track different kinds of pollutants. $\delta^{18}\text{O}$ values might also help interpretation of seasonal variation in N deposition and $\delta^{15}\text{N}$.

Although all the potential NO_3 sources cannot be distinguished using only $\delta^{18}\text{O}/\delta^{15}\text{N}$ because of substantial overlap of compositions. This could be very useful in National Parks and other pristine areas (Lake Tahoe, for example) that are being impacted from air pollution from nearby urban areas. Also, by concentrating efforts on assessing natural variability in forested areas of major basins, one can develop estimates of atmospheric sources that can be applied to adjacent areas affected by additional N loads from agricultural and waste pollutants.

The $\delta^{15}\text{N}$ values of animal waste (human or farm) and applied fertilizers are distinctive. However, they may not have distinctive $\delta^{18}\text{O}$ values because very little of the N in either applied fertilizers or waste materials is originally in the form of nitrate. Most of the nitrate that is eventually produced by recharge of these sources is produced by nitrification from ammonium or organic N, and hence has a $\delta^{18}\text{O}$ value controlled by the $\delta^{18}\text{O}$ of ambient H_2O and O_2 . Therefore, distinctive $\delta^{18}\text{O}$ values of these two sources of N might only be achieved if the $\delta^{18}\text{O}$ of the waters utilized during nitrification are different. Situations that would promote such differences are summer evaporation of soil water in fertilized fields, use of evaporated irrigation return water on fertilized fields, and use of non-native (imported) water for home or animal use (septic waters would have the $\delta^{18}\text{O}$ value of the imported water (Aravena *et al.*, 1993).

Forensic Applications

There are some (S.R. Silva *et al.*, 2002) studies illustrate the value of nitrogen and oxygen isotopes of nitrate for

forensic applications in urban areas. Stable nitrogen and oxygen isotopes are useful in indicating sources when nitrate is present in surface or groundwater. Delta nitrate-nitrogen ($\delta\text{N-nitrate}$) and nitrate-oxygen ($\delta^{18}\text{O nitrate}$) isotope ratios in water can be used to identify nitrate sources and its fate in vadose zone soil and in groundwater, (William E. Motzer, 2006).

CONCLUSIONS

The determination value of nitrate alone does not always result in unequivocal information about the sources and processes these solutes have undergone in aquatic systems. However, if interpreted in concert with solid hydrological information and with an abundance of chemical data, it is often possible conclusively to determine the sources and the biogeochemical history of nitrate in groundwater and surface water. This is particularly true if both the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate are determined, the availability of new continuous flow techniques for the rapid determination of oxygen isotope ratios of nitrate will undoubtedly result in an increased use of this isotopic approach.

Recently, analytical techniques have been developed to determination of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate (Mellvin and Altabet, 2005) and the $^{17}\text{O}/^{16}\text{O}$ ratios nitrate (Michalski *et al.*, 2002) has been shown that atmospheric nitrate is affected by mass independent isotope fractionation. Therefore, $\delta^{17}\text{O}$ values of nitrate will likely become a useful additional parameter for tracing atmospheric nitrate in the environment.

Development of water resource management plans to preserve or improve water quality requires knowledge of the dissolved constituents and an understanding of the processes these solutes may have undergone. Determining the isotopic composition of nitrate ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) has a tremendous potential in providing this information. If the isotope ratios are interpreted in conjunction with hydrological and chemical data.

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