Evaluation of the origin of nitrate influencing the Ključ groundwater source, Serbia

Nada Miljević, Djulija Boreli-Zdravković, Vesna Obradović, Dušan Golobočanin and Bernhard Mayer

ABSTRACT

This paper describes the use of the dual isotope method involving $\delta^{15}$N and $\delta^{18}$O measurements of dissolved nitrates to assess the origin and fate of groundwater nitrate at the Ključ groundwater source, Serbia. A sampling campaign was conducted in September 2007 during flow conditions obtaining groundwater from observation wells and river water fed by a shallow aquifer hosted in alluvial (sandy-gravel) sediments. Nitrate isotope ratios ranged from $-5.3$ to $+16.9\%$ and $\delta^{18}$O$_{NO_3}$ values varied from $-2.3$ to $+5.0\%$. Two major contamination sources were identified with isotopic compositions characteristic for nitrate derived from nitrification of soil organic nitrogen ($\delta^{15}$N to $+5.3$ to $+7.8\%$ for $\delta^{15}$N) resulting in nitrate concentrations of 33.6 and 78.8 mg/L and nitrate derived from animal wastes or human sewage, e.g. via septic systems, yielding $\delta^{15}$N values of $-9.9$ to $-11.9\%$ and elevated nitrate concentrations of 31.2–245.8 mg/L. The occurrence of nitrification and denitrification was also revealed based on concentration and isotope data for dissolved nitrate.

Key words | BARTs testers, groundwater, nitrate, nitrification, Serbia, stable isotopes

INTRODUCTION

It is believed that the natural background concentrations of nitrates in groundwater, resulting from infiltration of precipitation and mineralization of organic plant and animal matter are generally far below 10 mg NO$_3$/L (WHO 2004). Concentrations above this value usually reflect the impact of human activity on water quality. High nitrate in drinking water poses a health risk because high nitrate ingestion can cause methemoglobinemia in infants and young children (Comly 1945) which is commonly known as ‘blue baby syndrome’. As a by-product of agriculture (inorganic fertilizer and animal manure), human waste disposal (septic and sewage effluents), industrial activities, and emissions from combustion engines (airborne nitrogen compounds) nitrates represent one of the most common contaminants in groundwater world-wide. In recent decades, nitrate pollution has become a major threat to groundwater quality as the threshold value for drinking water of 50 mg/L NO$_3$ (corresponding to approximately 10 mg/L NO$_3$ as N) (EC 1998) is being reached in most of the local and regional aquifers in Europe. The European Groundwater Directive (EC 2006) considered nitrate as one of the main contaminants that can hamper the achievement of the goals of the Water Framework Directive (EC 2000).

Conventional nitrate analysis only provides quantitative concentration data and does not discriminate between sources of nitrate. The combined determination of the isotopic ratios of nitrogen ($^{15}$N/$^{14}$N) and oxygen ($^{18}$O/$^{16}$O) of dissolved nitrate provides a tool for distinguishing among four major nitrate sources: (1) nitrate derived from nitrification in soils, (2) nitrate from manure and sewage, (3) nitrate-containing synthetic fertilizers, and (4) atmospheric nitrate deposition (Kendall 1998; Kendall et al. 2007). This dual isotope approach can be used to: (1) evaluate the source of nitrate in groundwater and, (2) elucidate the processes of nitrate attenuation in groundwater or riparian zones due to groundwater-surface water interaction (Fukada et al. 2003).

The aim of this investigation was: (1) to demonstrate how the dual isotope approach ($\delta^{15}$N$_{NO_3}$ and $\delta^{18}$O$_{NO_3}$) can be used for determining the dominant nitrate sources
in the investigated shallow groundwater in Serbia, and (2) to assess the fate of nitrate within the aquifer.

MATERIALS AND METHODS

Study area

The catchment of the Velika Morava River (VMR) covers about 38,000 km² located between the Dinaric Alps and the Macedonian-Serbian and Rio-Rhodope Mountains ranging in elevations between 100 and 1,800 m. The study area (35 km²) is situated near Pozarevac city central Serbia, and located close to the confluence of the VMR with the Danube River, and comprises a shallow aquifer formed in alluvial (sandy-gravel) sediments with high permeability (hydraulic conductivity in the order of $10^{-3}$ m/s) (Boreli-Zdravković & Damjanović 2007). The total thickness of the Quaternary sediments is ~15 m, and the water table is 7–8 m below the ground surface. Silty-clayey roof layers are deposited over water-bearing sand horizons with variable thicknesses (0.5–5 m). This area represents an important resource for the municipal drinking water supply of Pozarevac city (~43,000 inhabitants) with an abstraction rate of ca. 240 L/s from the so-called Kljuc groundwater source. The water source is recharged from the direction of the VMR and its hinterland which contains human settlements without access to sewer systems. The majority of the catchment is under agricultural land use.

The nitrate concentration in the Kljuc groundwater source has continuously increased from 2000 to 2006. The average annual nitrate concentrations in groundwater during the period 2003–2006 increased from 35.8 to 51.9 mg/L. At individual sites in the hinterland nitrate concentrations of up to 100 mg/L were observed. However, in the area closer to the VMR, the nitrate concentrations in the groundwater were lower (5–20 mg/L) due to lower nitrate concentrations in river water (less than 10 mg/L) suggesting dilution in the hyporheic zone.

In order to protect groundwater, an infiltration-protective system was put into operation in September 2006 (Radenković et al. 2007). The system consists of nine abstraction wells adjacent to the VMR, a pipeline for delivering abstracted water to infiltration basins, and five infiltration ponds built at the water source location. The formation of a hydraulic barrier enabled the usage of the entire existing water supply infrastructure. The construction of wells near the VMR was designed as a temporary solution prior to the construction of river water intake and pretreatment of river water for subsequent infiltration.

Sampling and analysis

A sampling campaign was carried out during low flow conditions in September 2007. Groundwater samples were collected for chemical and isotopic analyses from 13 observation wells (samples N2–N14) completed in the aquifer, and operating wells (N1 − domestic well in Lucica settlement, N15 − water from the wells close to the VMR, N16 − water from all wells at the Kljuc groundwater source). Surface water was obtained from the VMR (N17) (Figure 1). In situ measurements of temperature (T), pH, electrical conductivity (EC), dissolved oxygen (DO), and redox potential (Eh) were carried out using a digital multiparameter probe MPS-D system (SEBA, Hydrometrie GmbH). Chemical parameters ($\text{Cl}^-$, $\text{NO}_3^-$, $\text{SO}_4^{2-}$, $\text{HCO}_3^-$, $\text{Cu}^{2+}$, $\text{Mg}^{2+}$, $\text{Na}^+$, $\text{K}^+$) were determined by standard analytical techniques outlined in Standard Methods (APHA 2005).

Samples for stable isotope analyses of hydrogen, oxygen and nitrogen were stored following standard procedures (Clark & Fritz 1997). Laser absorption spectroscopy (LGR DLT-100, at Jaroslav Cerni Institute for the Development of Water Resources, Serbia) was used for isotope analyses of $^2$H and $^{18}$O in water (Lis et al. 2008). $\delta^{15}$N and $\delta^{18}$O in $\text{NO}_3^-$ were determined at the University of Calgary (Alberta, Canada) using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002). All stable isotope results are reported

![Figure 1](https://iwaponline.com/wst/article-pdf/66/3/472/443056/472.pdf)
in the usual delta (\(\delta\)) notation in per mil (‰) relative to V-SMOW (Vienna Standard Mean Oceanic Water for \(\delta^2H\), \(\delta^{18}O\), \(\delta^{18}O-NO_3\)/C0), or AIR (\(\delta^{15}N\)). The precision of measurements was usually better than \(\pm 1\)% for \(\delta^2H\) and \(\pm 0.2\)% for \(\delta^{18}O\). For \(NO_3\) analytical precisions were \(\pm 0.5\)% for \(\delta^{15}N-NO_3\)/C0 and \(\pm 0.5\)% for \(\delta^{18}O-NO_3\).

Biological Activity Reaction Tests (BART, Droycone Bioconcepts Inc. Canada) were used to estimate bacterial populations (Strosnider et al. 2011) of denitrifying bacteria (DN-BART™) and nitrifying bacteria (N-BART™) based on bacterial biochemical activity in a selective culture medium by the time lag (TL). If the TL (the number of days between start-up of a test and producing a complete reaction) is longer, the bacteria are less active, normally with a low population in that particular sample.

The groundwater regime of the alluvial aquifer was analyzed using software for mathematical simulation of groundwater flow and hydrodynamic calculations. The Groundwater Vistas interface, Version 4, developed by the US Company Environmental Simulations, Inc., was used to interpret water level measurement results. The calculations were aided by MODFLOW 2000 software. Transient simulations were conducted and contour lines and path lines were analyzed.

**RESULTS AND DISCUSSION**

The groundwaters were either HCO_3-Ca or HCO_3-Mg type with pH values close to neutral (6.8–7.3) at shallow depths (5.7–9.5 m bgl) with relatively high redox potential (339–371 mV). A strong positive correlation between Cl\(^-\) and NO_3 concentrations (NO_3-N = (0.95 ± 0.07)\times[Cl\(^-\)]−6 ± 3, R = 0.96, n = 17) suggests that Cl\(^-\) and NO_3 may have a common source, which contaminates the groundwater in the study area.

Two compositional groups were identified plotting groundwater NO_3 and SO_4_2\(^-\) concentrations against EC (Figure 2) for samples (Table 1) obtained from the Ključ groundwater source.

Groundwaters in Zone 1 have low water quality characterized by both high nitrate (79–246 mg/L) and sulfate (138–165 mg/L) concentrations. According to the groundwater modeling results, Zone 1 mainly covers the part of the aquifer under the settlements Lucica and Prugovo with non-regulated public water utilities and without a sanitary sewer system. Every house has a septic tank and some livestock (cows, chickens, pigs), poultry breeds and pets. Groundwaters in Zone 2 have lower concentrations of sulfate (<140 mg/L) and nitrate (<80 mg/L). Zone 2 represents the part of the aquifer around the mentioned

<table>
<thead>
<tr>
<th>Zone</th>
<th>Sampling points</th>
<th>Value</th>
<th>T [C]</th>
<th>pH</th>
<th>EC [(\mu)S/cm]</th>
<th>Eh [mV]</th>
<th>DO [mg/L]</th>
<th>NO_3</th>
<th>SO_4_2</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>N1, N2, N3, N6, N7, N8, N9, N10</td>
<td>Average</td>
<td>13.1</td>
<td>7.0</td>
<td>1,356</td>
<td>363</td>
<td>7.4</td>
<td>173.7</td>
<td>148.1</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>13.0</td>
<td>6.8</td>
<td>1,165</td>
<td>339</td>
<td>5.2</td>
<td>62.5</td>
<td>137.5</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>14.3</td>
<td>7.3</td>
<td>1,785</td>
<td>371</td>
<td>8.3</td>
<td>245.9</td>
<td>165.0</td>
<td>71.6</td>
</tr>
<tr>
<td>Zone 2</td>
<td>N4, N5, N11, N12, N13, N14, N15, N16</td>
<td>Average</td>
<td>13.1</td>
<td>6.9</td>
<td>997</td>
<td>360</td>
<td>7.1</td>
<td>38.2</td>
<td>113.9</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>12.6</td>
<td>6.9</td>
<td>917</td>
<td>346</td>
<td>6.9</td>
<td>3.4</td>
<td>56.2</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>13.3</td>
<td>7.0</td>
<td>1,127</td>
<td>369</td>
<td>7.2</td>
<td>78.9</td>
<td>162.5</td>
<td>30.3</td>
</tr>
</tbody>
</table>
human settlements in Zone 1. The influence of the upstream sector of the Velika Morava valley is dominant in this zone, although agricultural field-activities and meadows are present.

The δ15N of nitrate ranged from +5.3 to +16.9‰ and δ18O values of nitrate varied between −2.3 and +5.0‰ (Figure 3). The highest δ15N (+16.9‰) and δ18O values (+5.0‰) were observed in the VMR water.

Three synthetic fertilizers commonly used in Serbia have been analyzed for nitrogen isotope ratio (Table 2). Their N content ranged between 21.5 and 48.9% and δ15N values between −0.8 and +1.0‰, which is typical for fertilizers derived from atmospheric nitrogen.

The application of the dual isotope method of measuring both the δ15N and δ18O values of dissolved NO3− in this study identified two major contamination sources (Aravena et al. 1993): nitrate derived from soil organic nitrogen (+5.3 to +7.8‰ for δ15N, Zone 2, Figure 3) resulting in concentrations between 33.6 and 78.8 mg/L, and nitrate derived from animal wastes or human sewage, e.g. via septic systems (+9.9 to +11.9‰ for δ15N, Zone 1, Figure 3) resulting in elevated nitrate concentrations of 31.2–245.8 mg/L. The δ15N values of nitrate derived from manure are often in the range of +10 to +20‰ or higher (Karr et al. 2001). In Zone 1, the mean δ15N value of +10.6 ± 0.6‰ (n = 8) is consistent within the range expected for animal waste-impacted groundwater. The results of δ15N-NO3 revealed an absence of nitrate originating from mineral fertilizers in the investigated groundwaters due to either little use of mineral fertilizers or the proper use of fertilizers according to crop needs.

A narrow range of δ18O values of nitrate from −2.3 to +3.4‰ suggests that organic nitrogen and NH4+ from manure is nitrified to NO3−. Nitrate generated by soil nitrification is reported (Mayer et al. 2001; Kaown et al. 2009) to contain two-thirds of the oxygen from soil water and one-third of the oxygen from atmospheric oxygen (Equation (1)):

\[
\delta^{18}O_{NO_3} = \frac{2}{3}\delta^{18}O_{H_2O} + \frac{1}{3}\delta^{18}O_{O_2}
\]  

Assuming the oxygen from these sources is incorporated without isotope fractionation, and using the δ18O values of groundwater in the study area ranging from −10.1 to −9.3‰ and δ18O=O = +23.5‰ for molecular oxygen, the expected δ18O-NO3 values in the groundwater typical for nitrate derived from nitrification would be around +1.1 to +1.6‰. This figure is close to the observed δ18O-NO3 values in the groundwater of the study area. Therefore, we conclude that nitrate reaching groundwater is the result of biological processes associated with the decomposition of organic matter often referred to as nitrogen mineralization (Boreli Zdravković & Miljević 2008). As ammonia is the first reaction product of organic matter decomposition, this biological conversion is called ammonification. The cation ammonium can be adsorbed by negatively charged soil particles such as clay minerals, or be taken up by plants. Under aerobic soil conditions, the remaining ammonium undergoes nitrification, and the formed nitrates can be leached into the aquifer during recharge events.

The groundwater samples in Zone 1 had slightly lower δ18O-NO3 (−1.9 to +1.0‰, mean −0.8‰, n = 8) values than those from Zone 2 (−2.3 to +3.4‰, mean 0.0‰, n = 6). δ18O-NO3 values of water cannot explain the difference in δ18O-NO3 values between the two zones because the mean δ18O values of local groundwater differed by less than 0.2‰. It is possible that the lower δ18O-NO3 values in Zone 1 indicate more intensive nitrification than in Zone 2. Sample N11 had a δ18O-NO3 value of +5.4‰ and a δ15N-NO3 value of +10.1‰ possibly indicating the

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**Table 2** | Nitrogen isotope composition of synthetic fertilizers used in Serbia

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>N [%]</th>
<th>δ15N [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-18 urea</td>
<td>48.9</td>
<td>−0.8</td>
</tr>
<tr>
<td>N-19 KAN</td>
<td>27.6</td>
<td>1.0</td>
</tr>
<tr>
<td>N-20 NPK</td>
<td>21.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
occurrence of denitrification facilitated by the local hydrogeological conditions. Measured DO values were >2 mg/L but it is possible that parts of the aquifer or the riparian zone were under reducing conditions.

Denitrification is an important nitrate attenuation process in the subsurface. This process is traditionally viewed as the predominant microbial anaerobic process that reduces nitrate to NO, N₂O and N₂. Based on measured chemical parameters related to redox conditions (359–371 mV, 5–8 mg/L of DO), oxic conditions exist at all observation wells and denitrification is unlikely. On the other hand, isotope results may indicate that denitrification occurs in micro-anaerobic environments where DO has been completely removed (Moncaster et al. 2000) in an otherwise oxic aquifer. Our data indicate an enrichment of both ¹⁵N and ¹⁸O in nitrate accompanied by a decrease of nitrate concentrations in Zone 1 that is characteristic for denitrification (Figure 4). The increase in δ¹⁵N and δ¹⁸O values of the residual nitrate in concert with the down-gradient decrease of nitrate concentrations result in a straight line fit of δ¹⁵N = (−4.3 ± 1.0)ln(NO₃⁻N) + 26.6 ± 3.8 (n = 6) with a highly significant correlation coefficient (R = −0.90, at the 99.9% confidence interval). The sampling spots N1 and N9 are not taken into consideration since N1 is located in Lucica settlement and hence affected by new septic effluent, while N9 is under the influence of newly infiltrated water.

The isotopic enrichment factor (ε) is calculated from the following Equation (2):

\[ \delta_R = \delta_{R0} + \epsilon \ln C/C_0 \]  

where \( \delta_R \) is the \( \delta^{15}\)N value of the reactant nitrate at time \( t \), \( \delta_{R0} \) is the initial \( \delta^{15}\)N value of the nitrate, \( C \) is the NO₃ concentration at time \( t \), and \( C_0 \) is the initial NO₃ concentration.

The obtained nitrogen isotope enrichment factor of −4.3‰ (εₕ) is at the low end of enrichment factors reported for denitrification in various aquatic systems (between −40‰ and −5‰, Kendall 1998). For oxygen isotope ratios, the increasing trend in \( 18O_{NO3} \) content is less significant (Figure 4(b)). The \( \delta^{18}O\)-NO₃ values in Zone 1 had a narrow range from −2.3 to +5.4‰. The best-fit equation (\( \delta^{18}O = (−4.2 ± 3.0)\ln(NO₃⁻N) + 15.1 ± 11.3, n = 6 \)) has a less significant correlation coefficient (\( R = −0.57 \)). The estimated isotopic enrichment factor for oxygen (\( \epsilon_O \)) from the slope of the \( \delta^{18}O \) versus ln(NO₃⁻N) plot is −4.2‰, which is lower than oxygen isotope enrichment factors reported for denitrification in other studies (\( \epsilon_O = −18.3‰ \) (Mengis et al. 1999) to −8.0‰ (Bottcher et al. 1990)).

During denitrification, residual nitrate becomes enriched in \( ^{15}\)N and \( ^{18}\)O with a reported \( \epsilon_{N/\epsilon_O} \) ratio that ranges from 1.3 (Fukada et al. 2003) to 2.1 (Bottcher et al. 1990). In our samples, a positive correlation (\( R = 0.81, n = 6 \)) between \( \delta^{15}N_{NO3} \) and \( \delta^{18}O_{NO3} \) values with a slope of 1.3 was observed. This indicates that the enrichment of \( ^{15}\)N is higher by a factor of 0.8 than for \( ^{18}\)O. Denitrification should be linked either to organic matter oxidation or to sulfide oxidation. Therefore, an increase in \( \delta^{15}N_{NO3} \) and \( \delta^{18}O_{NO3} \) values should be coupled with a decrease in carbon isotope ratios of dissolved inorganic carbon (\( \delta^{13}C_{DIC} \)) (Otero et al. 2009). Unfortunately we do not have enough data for \( \delta^{13}C_{DIC} \) (−13.5 to −12.2‰, n = 3) to conclusively study the role of organic matter oxidation in the denitrification. Pyrite could also act as a reducing agent and the evidence for pyrite oxidation has been reported by Miljević et al. (2012) in the study area. Aerobic and anaerobic patches or conditions may occur concurrently or alternately e.g. after heavy rain showers. It seems that both nitrification and denitrification occur in the watershed with nitrification likely most happening in the soils and water-unsaturated zone, and denitrification most likely in the aquifer or riparian zones. This finding is supported by BART test results that showed a medium level of activity of denitrifying bacteria.

![Figure 4](https://example.com/figure4.png)

**Figure 4** Cross-plot of the (a) δ¹⁵N and (b) δ¹⁸O values of dissolved NO₃⁻ versus the natural log of N-NO₃ concentrations (in mg/L) of samples from Zone 1 of the Kljuć groundwater source (without taking into consideration samples N1 and N9).
(DN-BART™) and a medium to low activity of nitrifying bacteria (N-BART™).

Nitrate concentrations for river water at the profile Ljubičevski (N17) between 1989 and 2008 ranged between 0.4 and 33.2 mg/L (RHSS 2008). High δ\(^{15}\)N and δ\(^{18}\)O values of nitrate indicated that denitrification (Dimkic et al. 2011) must have occurred along the flow path from the source of pollution to the river. The elevated δ\(^{15}\)N\(_{\text{NO3}}\) value in the Velika Mora River is in agreement with values for surface water affected by point sources during low flow periods (Showers et al. 1990) indicating the dominant origin of nitrate contamination present is due to waste water influence.

**CONCLUSION**

The application of the dual isotope method of both δ\(^{15}\)N and δ\(^{18}\)O measurements of dissolved nitrates at the Ključ groundwater source identified two major contamination sources with values characteristic for nitrate derived from nitrification of soil organic nitrogen (+5.3 to +7.8‰ for δ\(^{15}\)N) and nitrate derived from animal wastes or human sewage, e.g. via septic systems (+9.9 to +11.9‰ for δ\(^{15}\)N) in two distinct zones. In spite of agricultural activities in the study area, we found no evidence of nitrate originating in two major contamination sources of synthetic fertilizers in the groundwater. Elevated δ\(^{15}\)N values of dissolved nitrate were associated with higher nitrate concentrations. In the zone with higher nitrate contamination, a pronounced trend of 15N enrichment with a decrease in residual nitrate fraction suggested that some denitrification was occurring. The riparian zones around the river are a likely site for denitrification. BART results indicated the presence of both nitrifying and denitrifying bacteria at medium to low levels.

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**REFERENCES**


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