Isotope Techniques for Identifying Nitrate Pollution Sources in Southern Humid Regions of China: A Case Study in Conghua District

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Abstract. Nitrates pose a significant threat to groundwater quality, with agricultural activities being the primary source. This study employs environmental isotope techniques, using stable isotopes such as D, ^{18}O, and ^{15}N as tracers, to identify and investigate the sources of nitrate pollution in groundwater and water bodies in Conghua District, Guangzhou. A total of 90 groundwater samples were collected. By utilizing hydrogen and oxygen isotopes to trace the groundwater source, the results indicate that groundwater is primarily replenished by atmospheric precipitation, with vertical replenishment dominating during the rainy season and lateral replenishment prevailing during the non-rainy season. Based on nitrogen and oxygen isotopes, along with the analysis of characteristic ranges of different forms of nitrogen, in combination with nitrogen concentrations, fertilization conditions, and land use patterns, the results reveal that nitrate in Conghua District mainly originates from manure and sewage, with contributions from fertilizers and atmospheric precipitation as well. These findings contribute crucial groundwork for groundwater resource management in southern China's humid regions.

Keywords: groundwater; nitrates; isotope techniques; source identification.

1   Introduction

Nitrates are one of the most common and serious pollutants in groundwater, which can adversely affect human health and aquatic ecosystems.[1, 2] The main source of nitrate is agricultural activities, such as fertilizer application, livestock and poultry farming, and farmland drainage, which enter the groundwater through surface runoff, soil infiltration, and groundwater recharge.[3] Therefore, the distribution, source, and process of nitrate pollution in groundwater are closely related to factors such as land use type, climatic conditions, and hydrogeological characteristics.

In China, rapid agricultural, urban, and industrial development has led to a significant environmental issue: nitrate pollution in groundwater. According to the "China

Groundwater Quality Report (2015)" published by the Chinese Ministry of Environmental Protection, 40% of groundwater samples nationwide exceeded the standard limit for nitrate nitrogen (20 mg/L). This issue is particularly severe in the northern arid and semi-arid regions due to the lack of effective rainfall recharge, resulting in slow groundwater circulation and making it difficult to dilute or remove nitrate pollution.[4]However, in the southern humid regions, due to abundant rainfall and favorable groundwater recharge conditions, nitrate pollution in groundwater has received less attention and research. In fact, in these humid regions, factors such as high soil organic matter content, strong soil microbial activity, and low dissolved oxygen levels in groundwater may lead to biological degradation or denitrification of nitrates in groundwater. These processes can alter the concentration and isotopic composition of nitrates in groundwater, thereby affecting the identification and quantification of nitrate pollution sources. Therefore, conducting research on nitrate pollution source identification in groundwater based on isotope techniques in southern humid regions holds significant theoretical and practical value.

Isotope techniques are an effective method for identifying the sources and migration processes of groundwater and its pollutants. For example, they can be used to analyze the hydrogen and oxygen isotopes to determine the sources, flow direction, mixing ratios, and exchange relationships between groundwater and surface water.[5-7] Additionally, nitrogen and oxygen isotopes can be employed to analyze the sources, migration, transformation, and fate of nitrates in groundwater, as well as differentiate between various nitrogen sources such as soil organic matter, atmospheric deposition, fertilizers, and human and animal excreta.[8-10] Furthermore, a comprehensive analysis of carbon, hydrogen, oxygen, and nitrogen isotopes can be utilized to understand the mechanisms of nitrate pollution and assess associated risks in groundwater, taking into account different geomorphic types, chemical characteristics, and hydrogeological conditions.[11]

In recent years, there have been some advances in the application of isotope techniques for identifying groundwater and its nitrate sources of pollution. For example, Ding et al.[12] and Zhang et al.[13]have shown that the concentration of nitrates in farm wastewater and rivers is directly related to the extensive use of mineral fertilizers.Jin et al.[14] used natural variations in nitrogen and oxygen stable isotopes combined with a Bayesian isotope mixing model to reveal that in the lower reaches of China's Dongtaixi River, fertilizer nitrogen contributes the most to water column nitrate-nitrogen. Zhang et al.[15]traced the pollution sources and transformation processes of nitrates in surface and groundwater in the North China Plain using nitrogen and oxygen isotopes. Their results showed that in surface waters, the main sources of nitrate-nitrogen are fertilizers and sewage, while in groundwater during dry season, soil nitrogen and sewage are the main sources of nitrate-nitrogen. However, during rainy season, nitrate-nitrogen produced by fertilizer application will be quickly leached into groundwater by precipitation.

Currently, research on the sources of nitrate pollution in the humid regions of southern China is relatively limited, especially studies that delve into the use of isotope techniques. Therefore, the initiation of this study will address this research gap,

providing crucial scientific groundwork for the protection and management of groundwater resources in the humid regions of southern China.

2   Study area

Conghua District is located in the northeast of Guangzhou City. The average annual temperature is 20.3°C, with a total annual rainfall of 2143.8mm. Precipitation is highly unevenly distributed throughout the year, primarily concentrated in the rainy season from April to September. Situated in the transitional zone from the Pearl River Delta to the mountainous regions in northern Guangdong, the topography of Conghua slopes from north to south, with higher terrain in the northeast and lower terrain in the southwest, forming a step-like landscape. The northeast is characterized by mountains and hills, the central and southern parts mainly consist of hills and valleys, and the west is dominated by hills and plateaus, as shown in Figure 1. The highest point is Tian Tang Ding in the southeast of Liangkou, with an elevation of 1210 meters, marking the boundary between eastern Conghua and Longmen County. The lowest point is in Taiping Village, Taiping Town, with an elevation of 16.2 meters.

In terms of geology, granite is distributed along the northeast edge of the research area. Lutian, Liangkou, and Wenquan are mainly composed of limestone and light gray sandstone, while the urban areas of Conghua and Aotou are dominated by granite and limestone. The southernmost part of the watershed in Taiping Town is mainly composed of granite and red sandstone.

The district covers an area of 13,600 hectares of arable land, 45,200 hectares of orchards, 114,500 hectares of forest land, 52,800 hectares of pastureland, 926.66 hectares of other agricultural land, 11,100 hectares of urban and industrial land, 3,680 hectares of transportation land, 8,960 hectares of water bodies, and 73.33 hectares of undeveloped land.

Measurements of groundwater levels were conducted in August and December of 2015, and it was found that there was little difference in water levels between the two periods. Based on the measured data from August, interpolation was performed to obtain a groundwater level distribution map of Conghua District. From the map, it can be inferred that the direction of groundwater flow in the research area is generally consistent with the direction of river flow, gradually flowing from Lutian Town to Taiping Town, as shown in Figure 2.
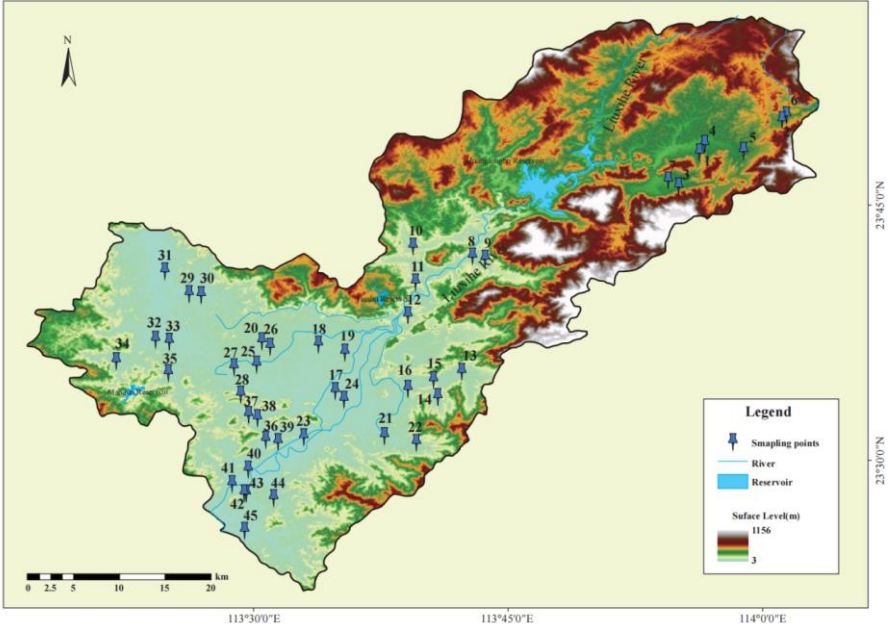
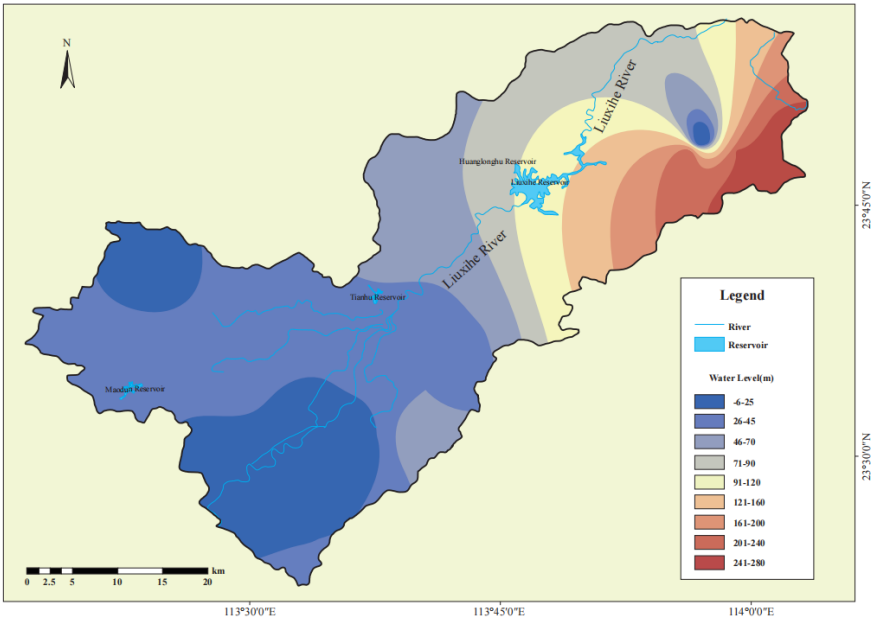**Fig. 1.** Topography of Study Area and Sampling Points Distribution

Fig. 2. Groundwater Level Distribution Map of Study Area

3   Methods

Environmental isotope methods are currently advanced techniques for quantitatively describing the water cycle and groundwater replenishment in watersheds. Isotopes are different atoms of the same element, with the same number of protons but a different number of neutrons. Stable isotopes (such as D, ^{18}O, and ^{15}N) are non-radioactive tracers with the characteristic of non-destructive integration. They can be widely used to study the interactions between plants and the biotic and abiotic environment, gradually finding extensive applications in the fields of ecology and environmental science.

The phenomenon of differences in physical and chemical properties between isotopic atoms (or molecules) of the same element due to differences in properties such as mass or spin is called the isotope effect. Isotope fractionation is generally used to measure the magnitude of this difference. Isotope fractionation is defined as the phenomenon in which different isotopes of an element in two or more phases have different isotope ratios α during physical, chemical, and biochemical processes. α represents the degree of isotope fractionation, and the further α is from 1, the greater the degree of isotope fractionation between the two phases; when α = 1, it means there is no isotope fractionation between the substances. In nature, if the difference in isotope content is too significant, relative quantities are usually used to represent the isotopic composition and isotopic ratio. This representation method does not require the accurate determination of the absolute abundance of isotopes in a substance. Internationally, the standard representation method defines δ as: $\delta X (‰) = (R_{sample}/R_{standard}-1)*1000$, where X is the name of the element, R_{sample} is the isotopic ratio of the sample, and $R_{standard}$ is the isotopic ratio of the standard substance, so δ value is the thousandth difference between the isotopic ratio of the sample and that of the standard substance.

This study focused on groundwater in Conghua District, collecting samples from 45 locations, as shown in Figure 1. In August and December of 2015, a total of 90 groundwater samples were collected to represent both the wet and dry seasons. For sample collection, we used a pump to extract the stagnant water in the wells, waiting for fresh groundwater replenishment before collection. This ensured that the samples accurately represented the flowing groundwater conditions. Water samples were collected using polyethylene bottles of 1000ml, 250ml, and 50ml capacities, each subjected to different pretreatment processes.For the 1000ml water samples, they were first filtered through a 0.8μm membrane, followed by a second filtration through a 0.22μm membrane. The filtered water samples were then stored in a refrigerator at 4°C.The 250ml water samples were filtered through a 0.22μm membrane and stored in a refrigerator at 4°C.For the 50ml water samples, they were filtered on-site through a 0.45μm membrane and preserved with the addition of two drops of 30% nitric acid.Nitrate, nitrite, and ammonium ion indicators were analyzed using the ICS-900 ion chromatograph. Stable isotopes D and ^{18}O were analyzed using a Thermo Fisher isotope mass spectrometer (Picarro L2130-i). For ^{15}N and ^{18}O in nitrate, specific denitrifying bacteria were used to convert nitrate into N_2O. This was followed by testing using TraceGas-IRMS in combination with Isoprime-100 isotope mass spectrometry.

4   Results and discussions

4.1   Identifying Groundwater Sources Based on Hydrogen and Oxygen Isotopes

The linear relationship between hydrogen and oxygen isotopes in global or specific regional atmospheric precipitation is referred to as the Global Meteoric Water Line (GMWL) or the Regional Meteoric Water Line. Utilizing the Craigh GMWL allows for the determination of modern recharge sources for groundwater and the identification of hydraulic connections between different aquifers. During rainfall, "heavier" molecules tend to condense and fall first, while during evaporation, "lighter" molecules preferentially evaporate. Consequently, water samples that have undergone significant evaporation will deviate from the GMWL, typically located to the right of the GMWL. The GMWL is primarily influenced by factors such as temperature, elevation, and seasonality, resulting in different GMWLs in various regions.

In Conghua District, the range of variation for oxygen-18 ($\delta^{18}O$) during the wet season is from -4.17‰ to -6.38‰, while in the dry season, it ranges from -4.42‰ to -6.49‰. For deuterium (δD), the range of variation during the wet season is from -29.93‰ to -40.42‰, and in the dry season, it ranges from -27.50‰ to -39.66‰. It can be observed that the seasonal variation is not significant. Figure 3 illustrates the relationship between $\delta^{18}O$ and δD during the dry season and wet season. It is evident that the points are located near the GMWL of Hong Kong, indicating that precipitation is the primary source of groundwater recharge in the study area. Due to geological influences, points during the wet season are distributed along the GMWL, with a Pearson correlation coefficient of 0.9137. In contrast, during the dry season, the distribution is more scattered, with a Pearson correlation coefficient of 0.0962. This indicates that during the wet season, groundwater is primarily sourced from precipitation recharge, predominantly through vertical replenishment. In contrast, during the dry season, lateral replenishment plays a predominant role.

From the graph, it can be observed that there is a noticeable enrichment of $\delta^{18}O$ from the recharge zone to the discharge zone during the dry season. Most points in the discharge zone are located below the GMWL, indicating significant evaporation during the process of precipitation infiltration into the groundwater. The recharge zone deviates from the GMWL and is located above it, with the recharge zone mainly composed of fractured bedrock water. This suggests the possibility of groundwater input from other areas in the recharge zone. Overall, there is still an enrichment trend from the recharge zone to the discharge zone during the wet season. However, due to the higher rainfall during this period and the dense river network in the discharge zone, where vertical replenishment prevails, the isotopic characteristics of recharge and discharge are less differentiated.
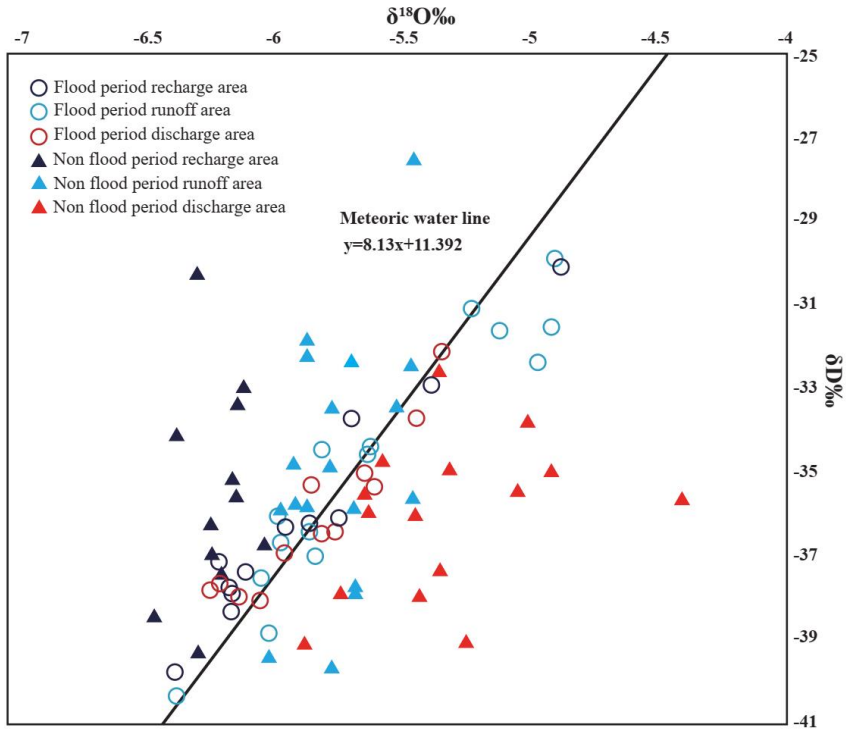
Fig. 3. The Distribution Characteristics of δD and $δ^{18}O$ in Groundwater in the Study Area

4.2   Identifying Nitrate Sources Based on Nitrogen and Oxygen Isotopes

In nature, nitrogen exists in various forms such as molecular nitrogen, inorganic nitrogen, and organic nitrogen. These different forms of nitrogen undergo transformations through biogeochemical processes (nitrogen fixation, assimilation, ammonification, nitrification, and denitrification) as well as physical and chemical reactions (ammonia volatilization, adsorption, and dissociation reactions). These transformations constitute the nitrogen cycle in nature. Nitrogen isotope fractionation occurs in each of these nitrogen cycle processes, with each process exhibiting different degrees of fractionation. This directly influences the nitrogen isotope composition of substances.

Different sources of NO_3^- have distinct ^{15}N characteristic values, providing a basis for tracing the origin of NO_3^- in the environment. Different forms of nitrogen exhibit varying fractionation effects during assimilation. Factors such as light exposure and dissolved oxygen can also influence isotope fractionation effects by affecting biogeochemical reactions. Specifically, nitrogen deposition from the atmosphere (with $δ^{15}N$ values ranging from -10‰ to -8‰) and chemical nitrogen fertilizers (with $δ^{15}N$ values ranging from 0‰ to 3‰) have lighter $δ^{15}N$ values. Nitrogen-containing organic mat-

ter in soil, after microbial nitrification, has $\delta^{15}N$ values ranging from -3‰ to -5‰, while organic fertilizers and wastewater have heavier $\delta^{15}N$ values (above 7‰ or even greater than 20‰). Due to overlapping distributions of $\delta^{15}N$ values in NO_3^- from sources like atmospheric nitrogen deposition, soil, chemical fertilizers, and organic fertilizers, the combined use of $\delta^{15}N$ and $\delta^{18}O$ stable isotope tracing techniques provides a more accurate means of determining the source of nitrates. The $\delta^{18}O$ values of NO_3^- in atmospheric nitrogen deposition range from 25‰ to 70‰, while the $\delta^{18}O$ of nitrate in chemically synthesized nitrogen fertilizers is approximately 22‰±3‰. The $\delta^{18}O$ formed from in-situ ammonium nitration in soil should be within the range of -5‰ to 5‰. Organic fertilizers and wastewater exhibit lower $\delta^{18}O$ values, below 15‰. Therefore, by using the characteristic ranges of $\delta^{15}N$ and $\delta^{18}O$ in NO_3^-, in combination with NO_3^--N concentration, ammonium nitrogen concentration, fertilization conditions, and land use, the primary sources of nitrates in water bodies can be determined.

The aforementioned variation ranges are depicted in Figure 4. Since processes like nitrification, denitrification, nitrogen volatilization, and mixing can all lead to changes in isotope composition, determining the source based solely on isotope ranges is not sufficient. Generally, the critical pH for the conversion of NH_4^+ in aqueous solution to NH_3 is 9.3. As the pH range in the study area is below this critical value, the influence of ammonia volatilization is not considered. When denitrification occurs, the remaining nitrate is simultaneously enriched in $\delta^{15}N$ and $\delta^{18}O$, with a ratio of approximately 2:1. As shown in the graph, regardless of the wet or dry season, apart from some points influenced by atmospheric precipitation, both isotopes are distributed along the denitrification line, indicating the presence of significant denitrification. During the wet season, the main processes involve the transformation of N organic compounds into ammonium, followed by plant utilization. In the dry season, due to lower precipitation, slower water recharge, and lower dissolved oxygen content in water bodies, the processes of nitrification and denitrification following the aforementioned processes become more significant. On the other hand, both mixing processes occur during the wet and dry seasons, with mixing being more pronounced during the wet season. In the recharge zone of the Hat Peak Mountain area, four points exhibit significant denitrification, while the remaining points are mostly sourced from ammonium or nitrate fertilizers.

To identify the source of nitrates, it is necessary to restore the isotopic values before denitrification. In the nitration process, two of the $\delta^{18}O$ in nitrate ions come from surrounding water molecules, while one comes from the atmosphere. Using an $\delta^{18}O$ value of 23.5‰ for O_2 in the atmosphere and an average $\delta^{18}O$ value of -5.77‰ for surrounding groundwater, the $\delta^{18}O$ value before denitrification is calculated to be 3.99‰ (indicated by the horizontal dashed line in the graph). Extending the denitrification line to intersect with the initial value, the area where they intersect indicates the source of nitrates. Consequently, in Conghua District, nitrates primarily originate from manure and sewage. Additionally, nitrate fertilizers and atmospheric precipitation contribute to nitrate pollution in local groundwater to a certain extent.
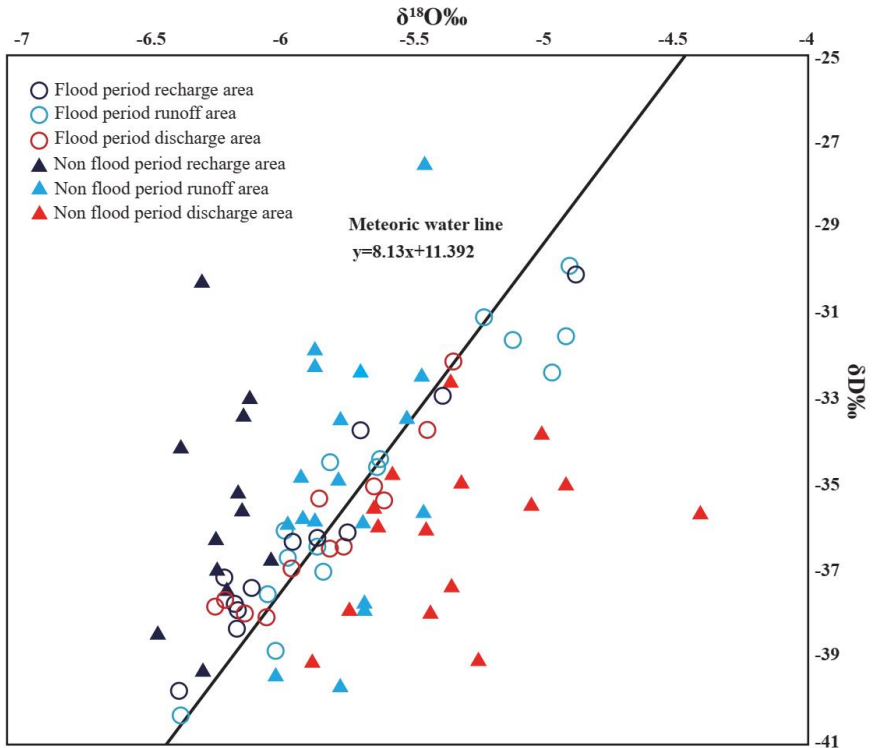
Fig. 4. The $\delta^{15}N$ and $\delta^{18}O$ Threshold Division Chart for Nitrates from Different Sources

5 Conclusions

The results of groundwater source identification based on hydrogen and oxygen isotopes indicate that in Conghua District, the range of isotopic variation in groundwater during both the wet and dry seasons remains relatively stable, suggesting that seasonal variations are not significant. The study findings reveal that groundwater primarily originates from precipitation recharge, with vertical replenishment being dominant during the wet season, and lateral replenishment being predominant during the dry season. Influenced by geological factors, groundwater points are distributed along the atmospheric precipitation line during the wet season, while the distribution is more scattered during the dry season. The results of nitrate source identification based on nitrogen and oxygen isotopes show that nitrates from different sources possess distinct nitrogen isotope characteristic values, providing a basis for tracing the origin of nitrates. By utilizing dual stable isotope tracing technology in conjunction with nitrogen and ammonia concentrations, as well as land use patterns, the primary sources of nitrates in water bodies can be accurately determined. The sources of nitrates in the study area mainly include manure and sewage, with some influence from nitrate fertilizers and atmospheric precipitation.

Given that groundwater primarily originates from precipitation recharge, especially with vertical replenishment being dominant during the wet season, it is crucial to strengthen the monitoring and protection of precipitation to ensure an ample supply of groundwater resources and stable quality.

In order to prevent further increase in groundwater NO_3^- concentration from the aforementioned pollution sources, it is recommended to implement the following management measures:

1. To address nitrate pollution sources, it is recommended to implement corresponding pollution control measures, particularly in the management and treatment of manure and sewage. Strengthening control and governance in these aspects will help reduce the pollution impact of nitrates on groundwater.

2. Regarding the use of fertilizers in agricultural activities, it is advised to apply fertilizers scientifically and utilize them judiciously, avoiding excessive use. This approach will help minimize the generation of nitrates and their impact on groundwater.

Acknowledgments

References

1. Bijay-Singh and E. Craswell. (2021) Fertilizers and nitrate pollution of surface and ground water: an increasingly pervasive global problem. SN Applied Sciences, 3(4): 518.
2. Yu, G., et al. (2020) The analysis of groundwater nitrate pollution and health risk assessment in rural areas of Yantai, China. BMC Public Health, 20(1): 437.
3. Bourke, S., et al. (2019) Sources and fate of nitrate in groundwater at agricultural operations overlying glacial sediments. Hydrology and Earth System Sciences, 23: 1355-1373.
4. Huang, T., Z. Pang, and L. Yuan. (2013) Nitrate in groundwater and the unsaturated zone in (semi)arid northern China: baseline and factors controlling its transport and fate. Environmental Earth Sciences, 70(1): 145-156.
5. Barbieri, M. (2019) Isotopes in Hydrology and Hydrogeology. Water, 11(2): 291.
6. Nayak, P.C., et al. (2017) Recharge source identification using isotope analysis and groundwater flow modeling for Puri city in India. Applied Water Science, 7(7): 3583-3598.
7. Yeh, H. and J. Lee. (2018) Stable Hydrogen and Oxygen Isotopes for Groundwater Sources of Penghu Islands, Taiwan, in Geosciences.

8. Zhang, Y., et al. (2019) Application of Nitrogen and Oxygen Isotopes for Source and Fate Identification of Nitrate Pollution in Surface Water: A Review. Applied Sciences, 9(1): 18.

9. Danielescu, S. and K.T. MacQuarrie. (2013) Nitrogen and oxygen isotopes in nitrate in the groundwater and surface water discharge from two rural catchments: implications for nitrogen loading to coastal waters. Biogeochemistry, 115(1): 111-127.

10. Wang, W., X. Song, and Y. Ma. (2016) Identification of nitrate source using isotopic and geochemical data in the lower reaches of the Yellow River irrigation district (China). Environmental Earth Sciences, 75(11): 936.

11. Gao, Y., et al. (2012) Groundwater Nitrogen Pollution and Assessment of Its Health Risks: A Case Study of a Typical Village in Rural-Urban Continuum, China. PLOS ONE, 7(4): e33982.

12. Ding, J., et al. (2014) Identifying diffused nitrate sources in a stream in an agricultural field using a dual isotopic approach. Science of The Total Environment, 484: 10-18.

13. Zhang, Y., et al. (2014) Tracing nitrate pollution sources and transformation in surface- and ground-waters using environmental isotopes. Science of The Total Environment, 490: 213-222.

14. Jin, Z., et al. (2018) Contribution of nitrate sources in surface water in multiple land use areas by combining isotopes and a Bayesian isotope mixing model. Applied Geochemistry, 93: 10-19.

15. Nakamura, K., et al. (2004) Assessment of Root Zone Nitrogen Leaching as Affected by Irrigation and Nutrient Management Practices. Vadose Zone Journal - VADOSE ZONE J, 3: 1353-1366.