

## Assessment of nitrogen nutrient sources in aquatic environment of Tuyen Lam sub-catchment based on its stable isotopes ratio ( $\delta^{15}\text{N-NO}_3$ ) combined with geochemical parameters

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**Abstract:** Overloading and erosion of pollutants, especially nitrogen (nitrate and ammonium) nutrient, in catchment of multiple land use put pressure on the quality of surface water. Excess of nitrogen nutrient in the aquatic environment causes eutrophication leading to adverse effects to the habitat and ultimately it could affect the human health through the food chain. The aim of this study is to assess the sources of nitrogen nutrient that could affect the quality of surface water at a sub-catchment of Tuyen Lam reservoir in Lam Dong province by the use of water's physico-chemical parameters combined with stable isotope ratio of nitrogen in nitrate ( $\delta^{15}\text{N-NO}_3$ ) dissolved in the water. It was revealed that nitrogen nutrient in water from springs in the study area came from at least three sources: decomposition of soil organic matters, inorganic fertilizers and water waste from residential areas. In points of annual crop farming and close to residential the contribution of inorganic fertilizers to the total concentration of nitrate was estimated, based on the  $\delta^{15}\text{N-NO}_3$  values, to be as high as more than 60% and the contribution of water waste was around 35-40%. This is the first time isotopic signature of nitrogen-15 in nitrate ( $\delta^{15}\text{N-NO}_3$ ) was applied as a tool to assess and identify sources of the nutrient in aquatic environment. Results of this study, although it was initial but it would be useful for the local environmental managers in developing suitable strategies to control over nitrogen-nutrient releases in order to keep the aesthetic appeal of Tuyen Lam Reservoir.

**Keywords:** *Water quality, Nitrate, Stable isotope ratio.*

### I. INTRODUCTION

Over the years, agriculture including livestock and crop production, deforestation, mining activities cause pollution to rivers basins. This situation has become more serious in recent years due to the intensive farming, urbanization and industrialization that have led to major changes and increased pressure on soil and water resources. This is because the above mentioned activities cause erosion

which increases sedimentation rate in reservoirs and water distribution canals, reduces water quality and degrades the habitat of aquatic ecosystems. Not only, but erosion also causes surface water to be polluted in downstream areas. Among other pollutants in the aquatic environment, nitrogen nutrient in the nitrate form ( $\text{N-NO}_3$ ) and organic matters possess special concerns because of their direct impacts to human health.

The sources of nitrogen nutrient pollutant in surface water are mainly from atmospheric deposition, inorganic fertilizer used in agriculture, industrial wastewater, domestic wastewater from septic systems, animal waste, and soil organic matter degradation. Assessment and identification of nitrogen nutrient sources in surface water would help to strengthen strategies for the environment management towards improving water quality.

Sources nitrogen nutrient are related to each other in the nitrogen biochemical cycle and can be differentiated by isotopic composition of nitrogen [1, 2, 3].  $\text{N-NO}_3$  is the dominant form of N in oxidized environment, has good solubility and easily penetrates into soil [4]. Excess nitrate enters the water bodies mainly through diffuse/non-point sources, e.g. run off and soil erosion, rather than point sources. Identifying the source of nitrate causing water pollution in a multiple land-use area has been a concern over years. Analyzing the natural abundance of stable isotopes can help identify both the origin of nitrate and their distribution and modification mechanism [3].

The isotopic composition of nitrogen ( $\delta^{15}\text{N}$ ) in most terrestrial matter ranges from - 10‰ to + 25‰ vs. air. Variation in  $\delta^{15}\text{N}$ -values allows to differentiate several nitrate sources. However, there are several sources that are indistinguishable on this basis due to the overlap in the value of  $\delta^{15}\text{N}$  [5,6,7]. Moreover, the identification of nitrate pollution sources often faces other difficulties such as: heterogeneity in landscape, the spatial correlation of the study area may overlap the possible impacts in the basin, the influence of

seasonality on the sources of nitrate and on the response of the aquatic system, the presence of multiple nitrate sources in landscape [2,5,8,9].

The aim of this study is an attempt to assess and identify nitrate sources in the water body of a sub-catchment of Tuyen Lam reservoir in Lam Dong province (Vietnam) by using isotopic composition of nitrogen in nitrate combined with physic-chemical parameters of the aquatic environment.

## II. MATERIALS AND METHODS

### A. Study area

The study area was Tuyen Lam Reservoir located in the Tuyen Lam – Dinh An – Quang Hiep hydraulic system of Da Lat city, Lam Dong province. The storage capacity of the reservoir is more than 31 million  $\text{m}^3$  of water discharged from a basin of 33  $\text{km}^2$  area. Water in the reservoir is currently used for irrigation to agricultural lands, supply domestic water, for electricity generation, and tourism services.

In Da Lat city, there are two main types of landscape, mountainous and plain-mountainous ones. Rainfall in the area concentrates seasonally with high intensity so soil erosion occurs strongly, especially from sloping land, bare land and bare hills throughout the city. The landscape of Tuyen Lam Reservoir's basin has a significant impact on the water quality of the reservoir due to the multiple land-use and residences around. Figure 1 and Figure 2 depict sketches of the study area.

Table I presents meteorological data collected in Da Lat city representing for the study area.

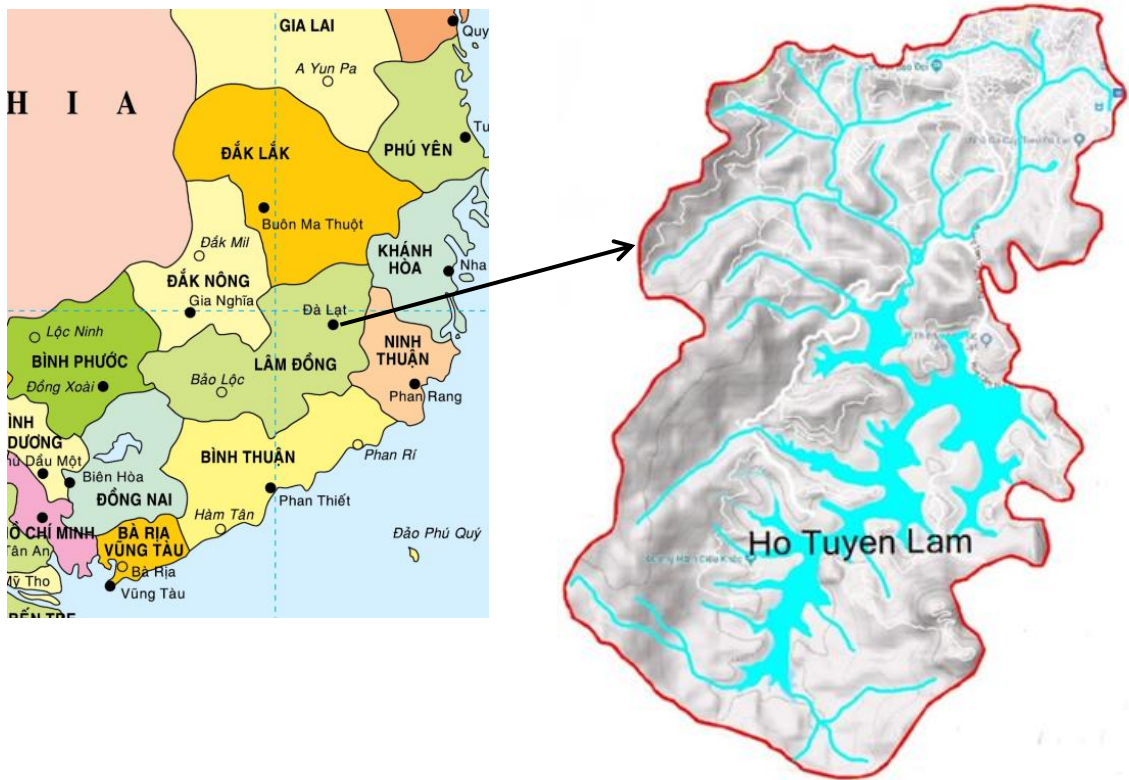


Fig. 1. A map showing the study area

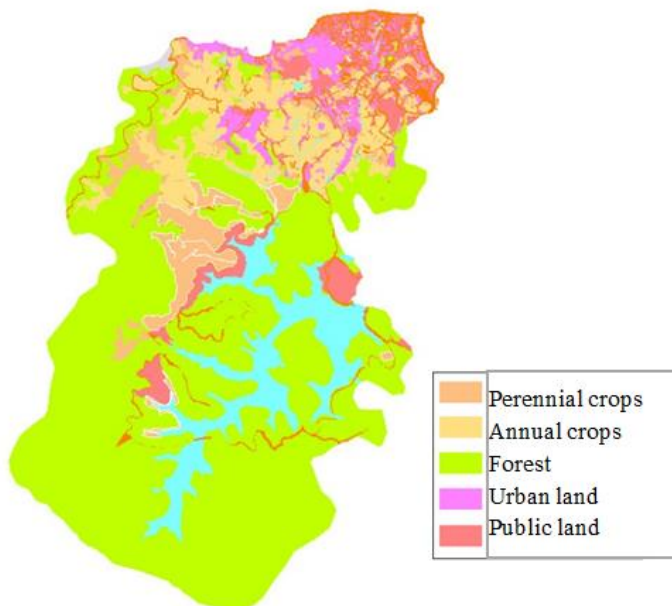


Fig. 2. The map showing land use in Tuyen Lam catchment

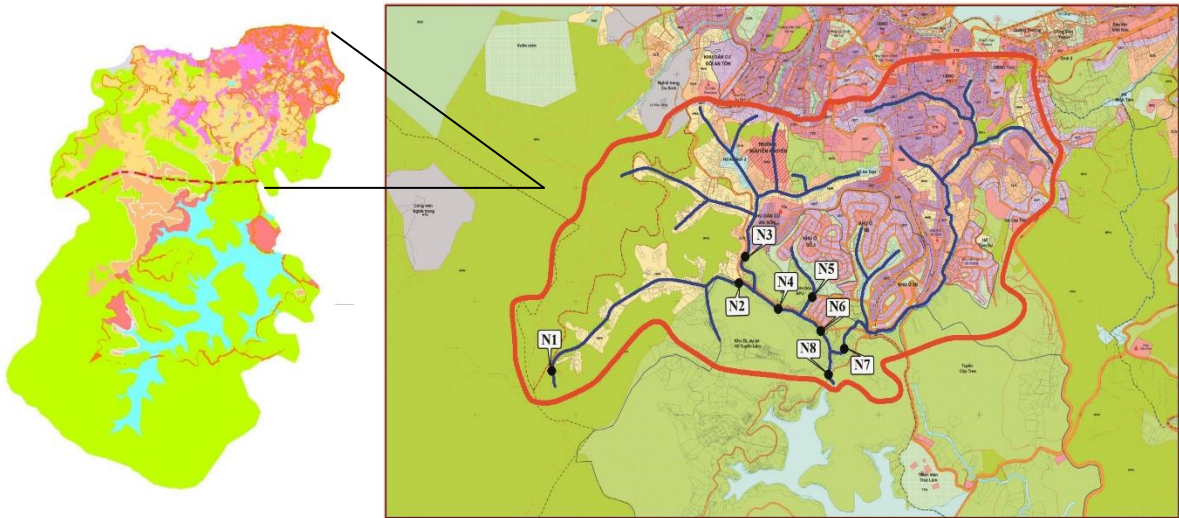
**Table I. Meteorological data in Da Lat collected for the 2019 year**

Month	1	2	3	4	5	6	7	8	9	10	11	12
Temperature, °C	16	17	19	20	21	20	20	19	19	19	18	17
Monthly rainfall, mm	7	0	100	189	538	560	560	284	655	165	168	0
Number of rainy days, day	5	0	11	16	20	13	13	20	24	22	8	0

**B. Sampling design**

The study area was selected based on the map of hydro system of Da Lat city. Water samples were taken along the main tributaries that flow through typical land-use areas: forest,

farming areas and residential areas (Figure 3). Water samples were taken 2 times, in late dry season (May 2019) and at the end of rainy season (October 2019). Table II presents sampling points and their tributaries.



**Fig. 3.** Study area (left) and sampling points (right)

**Table II. Sampling points and land-use around the points**

No.	Sample	Tributary
1	N1	Forest
2	N2	Forest and agriculture
3	N3	Agriculture and domestic waste water
4	N4	Mix N1, N2, N3
5	N5	Agriculture
6	N6	Mix N1, N2, N3, N4, N5
7	N7	Agriculture and domestic waste water
8	N8	MixN1, N2, N3, N4, N5, N6, N7

### C. Samples treatment and analyses

Physico-chemical parameters of water in the sampling points were measured directly in-situ. The water's temperature, dissolved oxygen (DO) were measured by SensoDirect Oxi200 device (Aqualytic – Germany) equipped with appropriate probes. Electric conductivity (EC), total dissolved solid (TDS) were measured by AL20Con device (Aqualytic – Germany); and turbidity was measured by Turbidity meter AL250T-IR (Aqualytic – Germany). Procedures of the measurements were complied with the Technical Requirements for Environmental Monitoring set by the Ministry of Natural Resources and Environment of Vietnam in the Circular No 24/2017/BTNMT.

Water samples were filtered through 0.45 mm mesh polycarbonate membranes and stored in bottles made of high density polyethylene (HDPE) resin and tightly capped then transported to the laboratory for chemical analysis. Concentrations of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ) in water were quantified by ion chromatography (IC) technique in the laboratory of Da Lat Nuclear Research Institute following the Vietnam National Technical Standard TCVN:6494-2-2000 (ISO10304-2 : 1995)[11]. A quality control program was applied for the ionic content determination by analyzing standard solutions supplied by the IC supplier. The standard deviation of results derived by the laboratory was within  $\pm 5\%$  from the certified value for respective constituents.

An aliquot (300 ml) of the filtered water samples were shipped to Isotope Hydrology Lab of the Institute for Nuclear Science and Technology in Hanoi for measuring isotopic composition of nitrogen

in nitrate ( $\delta^{15}\text{N}-\text{NO}_3$ ). In that laboratory nitrate was separated from water samples by anion-exchange method [12] then it was reduced to ammonium in acidic solution (pH 1) by Dewada's alloy. The ammonium formed was then converted into ammonia by raising the pH to 10 in bottles tightly capped to avoid the loss of ammonia released. The ammonia released was entrapped by  $\text{KHSO}_4$  (1M solution) that was impregnated into What man filter chips. Amount of nitrogen needed for its isotopic analysis was calculated in order to have at least 50  $\mu\text{g}$  ammonia be entrapped into  $\text{KHSO}_4$  to satisfy the sensitivity of the analytical procedure. The filter chips were encapsulated both sides with Teflon tape and allowed to float on the surface of the solution in the bottles. The content in the bottles was occasionally shaken. The ammonia was completely entrapped into the filter chips during several days, usually it took 5 days. Upon completion of the absorption, the filter chips were taken out from the bottles and the Teflon tape was removed. The filter chips contained  $\text{KNH}_4\text{SO}_4$  were pyrolyzed at  $1050^\circ\text{C}$  on the Ni-cobaltous catalyst to nitrogen gas ( $\text{N}_2$ ) in the Elemental Analyzer (Eurovector, Italy) of an Isotope Ratio Mass-Spectrometer (IR MS, IsoPrime, Micro-mass, GV, UK supplier). The  $\text{N}_2$  gas was carried by a continuous flow of helium carrier gas through chromatographic column to purify from contaminants before entering the ionization chamber of the IR MS to ionize. The continuous He-carrier gas carried the ions of masses 28 ( $^{14}\text{N}_2^+$ ) and 29 ( $^{14}\text{N}^{15}\text{N}^+$ ) into the mass-separator of the IR MS where they were separated from each other. The ions  $^{14}\text{N}_2^+$  and  $^{14}\text{N}^{15}\text{N}^+$  were collected and counted by respective Faraday cups installed on the exits from the separator.

To calculate the  $\delta^{15}\text{N}$  the Mass Lynx software supplied by the IR MS supplier was used. The  $\delta^{15}\text{N}$  notation was defined as:

$$\delta^{15}\text{N} = \left( \frac{{}^{15}\text{R}_{\text{sample}}}{{}^{15}\text{R}_{\text{Std}}} - 1 \right) \times 1000, \text{‰} \quad (1)$$

Where  ${}^{15}\text{R}_{\text{sample}}$  and  ${}^{15}\text{R}_{\text{Std}}$  are respectively the ratios of  $[{}^{29}\text{N}^+ / {}^{28}\text{N}^+]$  in samples and that in standard. The standard used in this measurement is nitrogen in the ambient air. The measurement was conducted thrice. Precision of  $\delta^{15}\text{N}\text{-NO}_3$  determination was  $\pm 0,3\%$ .

### III. RESULT AND DISCUSSION

#### A. Results

##### 1. Physical parameters

Table III presented physical parameters of water samples that were measured in situ.

##### 2. Chemical and biological parameters

Table IV presented concentration of chemical and biological parameters measured in-situ for water samples collected in May and October 2019.

**Table III.** Physical parameters of water samples

No.	Sample	Temperature, °C	DO, mg/l	EC, $\mu\text{S}/\text{cm}$	TDS, mg/l	Turbidity, NTU
May, 2019						
1	N1	21.7	4.51	24.7	18	7.03
2	N2	29.2	3.90	53.7	36	30.3
3	N3	26.2	4.40	138.9	94	28.6
4	N4	27.8	3.85	86.6	59	30.1
5	N5	26.7	3.90	124.5	84	31.2
6	N6	25.8	3.97	85.2	59	22.1
7	N7	24.6	3.57	378.0	267	7.17
8	N8	25.8	3.52	33.8	161	19.7
October, 2019						
1	N1	22.1	4.34	19.8	15	6.29
2	N2	27.2	5.26	46.1	35	18.2
3	N3	25.9	4.99	117.6	89	6.41
4	N4	26.3	4.41	79.1	60	12.0
5	N5	26.6	3.88	136.0	103	35.2
6	N6	26.0	4.04	82.5	63	14.3
7	N7	25.5	3.96	249.0	189	9.57
8	N8	25.7	3.56	166.6	126	12.5

In table III: DO, EC, TDS denote for dissolved oxygen, electrical conductivity and total dissolved solid, respectively.

**Table IV.** Chemical and biological parameters of study water samples

No	Sample	$\text{NH}_4^+$ , mg/L	$\text{PO}_4^{3-}$ , mg/L	$\text{NO}_3^-$ , mg/L	Coli form, MPN/100mL	N, mg/L	P, mg/L
May, 2019							
1	N1	0.03	0.34	0.28	-	-	-
2	N2	0.05	0.26	1.50	-	-	-
3	N3	0.12	0.24	2.43	-	-	-
4	N4	0.08	0.26	0.45	-	-	-
5	N5	1.00	0.24	1.02	-	-	-

No	Sample	NH <sub>4</sub> <sup>+</sup> , mg/L	PO <sub>4</sub> <sup>3-</sup> , mg/L	NO <sub>3</sub> <sup>-</sup> , mg/L	Coli form, MPN/100mL	N, mg/L	P, mg/L
6	N6	0.10	0.41	2.12	-	-	-
7	N7	6.30	0.39	1.24	-	-	-
8	N8	3.50	0.54	0.83	-	-	-
October, 2019							
1	N1	0.03	0.09	0.21	460	1.52	0.22
2	N2	0.17	0.16	2.49	2400	1.61	0.10
3	N3	0.35	0.08	5.55	2400	1.60	0.10
4	N4	0.17	0.14	6.66	4600	1.91	0.33
5	N5	2.70	0.20	4.80	7500	1.53	0.17
6	N6	0.03	0.14	6.41	2100	1.52	0.10
7	N7	0.25	0.73	8.48	1100	1.50	0.11
8	N8	0.11	0.39	6.45	11000	1.51	0.12
QCVN08-MT:2015/BTNMT (Column B1)		<b>0.9</b>	<b>0.3</b>	<b>10</b>	<b>7500</b>	-	-

3. *Isotopic composition of nitrogen-15 in nitrate ( $\delta^{15}N-NO_3$ ) dissolved in water samples*

Value of  $\delta^{15}N-NO_3$  in water samples were determined for October 2019 sampling campaign and shown in Table V.

**Table V.** The value of  $\delta^{15}N-NO_3$  in water samples collected in October 2019

No.	Sample	$\delta^{15}N$ vs. air N <sub>2</sub> , ‰	SD
1	N1	-1.44	± 0,14
2	N2	-1.83	± 0,16
3	N3	8.27	± 0,35
4	N4	3.08	± 0,21
5	N5	1.15	± 0,17
6	N6	6.09	± 0,11
7	N7	6.99	± 0,26
8	N8	4.02	± 0,23

## B. Discussion

Results in Table IV showed that water in site N1 came from sub-surface of the surrounding forest as water's temperature in that site was lower than that in all other sites, it was 21.7 and 22.1°C in the dry and rainy seasons, respectively. It seems that contribution of run-off water to stream in site N1 was insignificant compared to those of groundwater, so this made erosion (soil loss) around site N1 to be lowest compared to that in

all other sites. Turbidity in water from site N1 in May and October was lowest, it was around 7 NTU, however, at the same time in sites N2 and N5 where agricultural activities are conducted which probably made local soil to be disturbed frequently and soil easily eroded with run-off water. This erosion led the turbidity in water to increase up to more than 4 times higher than that observed for site N1. The turbidity in water from sites N2 and N5 was respectively 28 and 312 NTU in May and

18 and 35 NTU in October (Table I). Obviously, soil treatment (tillage for crops planting) increased rate of erosion made turbidity in water to be high.

As seen from Table IV that water flowing along all the springs was well mixed so that oxygen content in water was almost at the equilibrium at temperatures from 20 to 30°C, ranging from 3.5 to 5.56 mg/L (Table IV), meaning that water was fresh.

From Table IV it was clear a fact that water waste from human activities contributed a significant portion of dissolved substances in water. The content of total dissolved solid (TDS) in water from sites N3 and N7 where it received water waste from residential areas in May were 94 and 267 mg/L respectively, i.e. 5 and 15 times higher than TDS content in water from site N1 (forest area) or 2.6 and 7.4 times higher than TDS content in water from site N2, a composite of forest and perennial crop farming areas. Associated with TDS is electric conductivity (EC) in sites N3 and N5 in May was 138.9 and 378  $\mu\text{S}/\text{cm}$ , respectively, i.e. roughly 5 and 15 times higher than that for water in site N1 (Table IV). The similar situation was also observed in October (Table IV). In the study area, a part of waste water was not completely collected for centralized treatment therefore it could much affect to the quality of surface water.

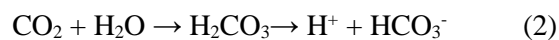
Results in Table V, for both 2 times of sampling in May and October 2019, revealed that the quality of surface water in the study region mostly met the requirements of National Technical Regulation set by the Ministry of Natural Resources and Environment of Vietnam for surface water (QCVN 08-MT: 2015/ BTNMT), excepted for  $\text{NH}_4^+$  at sites N5, N7, N8 in May and N5 (October);  $\text{PO}_4^{3-}$  at sites

N6, N7, N8 (May) and N7 (October); coli forms at sites N5 and N8 (October).

Water in sites N5 and N7 was the run-off from agricultural areas where annual crops such as strawberries, vegetables (cauliflower, cabbage, etc...) were planted but in site N8 water was a composite of water from all the sites studied (Fig. 3 and Table II). It was thought that farmers around sites N5, and N7 fertilized their annual crops with N-P-K and nitrogen fertilizer was urea. In soil and during the run-off urea would hydrolyze according to the equation:



$\text{CO}_2$  formed in water (reaction 1) would continue hydrolyze to carbonic acid following dissociation to bicarbonate ( $\text{HCO}_3^-$ ) and protonium ion ( $\text{H}^+$ ) as follows:



Ammonia formed in reaction (1) could partly escape from the aquatic environment in to the atmosphere due to evaporation and partly associate with ion protonium to stabilize in the form of ammonium (process 3):



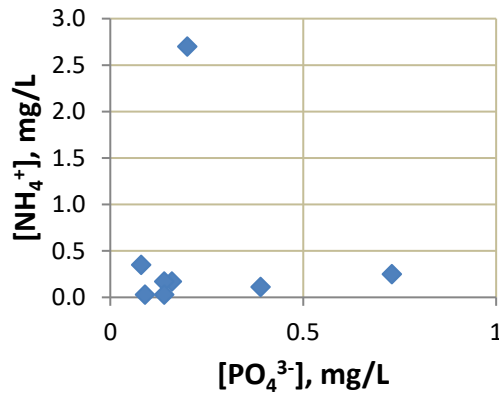
To address the fertilizer source of ammonium in water of the study region a graph of concentration of ammonium [ $\text{NH}_4^+$ ] vs. [ $\text{PO}_4^{3-}$ ] was built and depicted in Figure 4.

As seen from Fig. 4 there was no any trend of relationship between ammonium and phosphate concentrations in water samples collected. However, this could be understood from the point of view that the hydro geochemical behaviour of ammonium and phosphate in aquatic environment was quite different from each other. In aquatic environment, ammonium could be subjected to



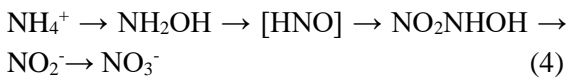
evaporation, nitrification and denitrification but phosphate would subject to precipitation in the form of hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) with calcium ion dissolved in water. Therefore, both the constituents could be lost from water by different mechanisms and as a consequence they did not closely correlated each other as it

was in the case. Unfortunately, in this work concentration of  $\text{Ca}^{2+}$  in study water samples was not available to assess whether hydroxyapatite is precipitating or not, however it believed that it is precipitating because the dissociation product of this substance is very low, it was  $10^{-115}$ [13].



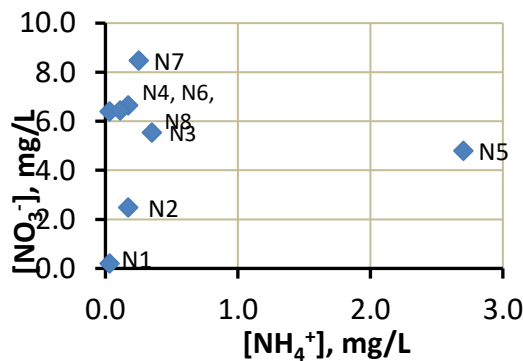
**Fig. 4.** A scatter plot of relationship between  $[\text{NH}_4^+]$  vs.  $[\text{PO}_4^{3-}]$  in surface water from Tuyen Lam sub-catchment in October 2019 to show the fertilizer source of ammonium and phosphate released into the aquatic environment of the region

The nitrification of ammonium in aquatic environment proceeds as follows [14]:



Nitrification much facilitates by the presence of oxygen as in this case when oxygen content in water was found to be

almost saturated at the temperature range of 25-30°C (Table III). The final product of the nitrification process (reaction series 4) is nitrate, therefore it expected that ammonium concentration in water would correlate well with that of nitrate. Figure 5 showed a scatter plot of the relationship between  $[\text{NH}_4^+]$  and  $[\text{NO}_3^-]$ .



**Fig. 5.** A scatter plot of  $[\text{NO}_3^-]$  vs.  $[\text{NH}_4^+]$  showing nitrate was a product of the nitrification of ammonium in aquatic environment of Tuyen Lam sub-catchment

From Fig. 5 once can see a general trend of positive and close correlation between  $[\text{NO}_3^-]$  and  $[\text{NH}_4^+]$  if site N5 excluded. The significant deviation of  $[\text{NH}_4^+]$  from the  $[\text{NO}_3^-]$  vs.  $[\text{NH}_4^+]$  relationship (Fig. 5) was not clear and needed to be studied further.

Another reason to explain the elevated (in comparison with the QCVN 08-MT: 2015/BTNMT) [15] concentration of ammonium and phosphate in water from sites N5, N6, N7 and N8 (Table V) was due to the contribution of wastewater from residential areas. The waste contains high organic matters that would decompose with the assistance of biological activity to ammonium and phosphate as it was indicated by population density of coli-forms in those sites during October, the end of rainy season (Table IV).

Results in Table V indicated that nitrate source in the area of forest and perennial crops (N1 and N2) was originated from the decomposition of soil organic matter, so that isotopic signature of N-15 in nitrate ( $\delta^{15}\text{N}-\text{NO}_3$ ) was in the range of -1.83 to -1.43‰ vs. Air. Nitrate in water from site N5 where annual crop farming is the only agricultural activity has  $\delta^{15}\text{N}-\text{NO}_3$  of 1.15‰ vs. Air, which is typical value of  $\delta^{15}\text{N}$  in inorganic nitrogen fertilizers as it was synthesized from air-nitrogen. Nitrate in water from annual crops farming and residential areas, site N3 or site N7, was enriched, up to 8.27 and 6.99‰ vs. Air, respectively. The reason for the enrichment in  $\delta^{15}\text{N}-\text{NO}_3$  in water from sites N3 and N7 could be due to the contribution of nitrate from water waste. It was reportedly that  $\delta^{15}\text{N}-\text{NO}_3$  from human water waste ranges from 10 to 30‰ vs. Air [5].

Commonly, in the study region nitrogen nutrient (nitrate) in surface water were originated from three sources, namely from

decomposition of soil organic matter, from inorganic fertilizer and from water waste of human activities.

Assume that the  $\delta^{15}\text{N}$  in inorganic fertilizer be 1‰, and that in water waste be in average of 20‰ [5, 16] one can estimate the contribution of nitrate from inorganic fertilizer and water waste in site N3, N5 and N7 based on the two end-member mixing model (Eq. 5) as follows:

$$\delta^{15}\text{N}-\text{NO}_{3, \text{ sample}} = x. \delta^{15}\text{N}-\text{NO}_{3, \text{ fer}} + (1-x). \delta^{15}\text{N}-\text{NO}_{3, \text{ ww}} \quad (5)$$

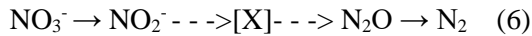
Where  $\delta^{15}\text{N}-\text{NO}_{3, \text{ sample}}$ ,  $\delta^{15}\text{N}-\text{NO}_{3, \text{ fer}}$ ,  $\delta^{15}\text{N}-\text{NO}_{3, \text{ ww}}$  are isotopic signatures of nitrogen 15 in nitrate found in study water sample, in inorganic fertilizer and in water waste, respectively;  $x$  is the contribution of nitrate from inorganic fertilizer, and  $(1-x)$  is the contribution of nitrate from water waster.

The contribution of nitrate from inorganic fertilizer in water of site N3, N5 and N7 was estimated (Eq. 5) to be as high as 61.74%; almost 100% and 68.47%, respectively. Respectively, the contribution of water waste in site N3, N5 and N7 was as high as 38.26, 0, and 31.53%.

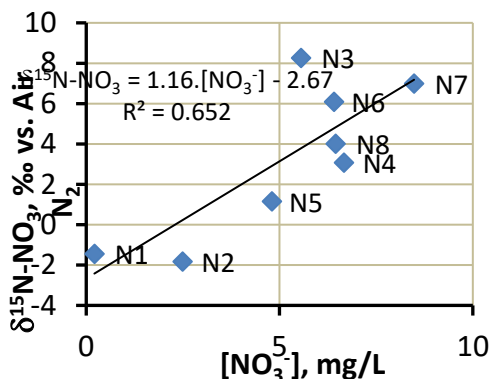
It was surprisingly a fact that in Tuyen Lam sub-catchment (Da Lat city) there is very intensive vegetable farming that it expected a lot of both inorganic fertilizer and manure be applied led to high concentration of nitrogen and phosphorus nutrient in the aquatic environment. However, as seen from Table IV the concentration of nitrate in all the sampling sites was in within the national standard (QCVN 08-MT: 2015/BTNMT) meaning that surface water in that region is clean by the nutrients substances.

The low concentration of nitrate in surface water in the study region could be

explained by the denitrification process [14]. In water, denitrifying bacteria capable of converting nitrate into nitrogen gas that easily escapes from the aquatic environment as shown in the reaction series bellows:



The denitrification made concentration of nitrate in water to decrease, and as the rule the isotopic signature of nitrogen-15 in the remaining reactant to enrich. It was well known that in any chemical or biological process composition of heavy isotopes in products will be depleted, reversely composition of heavy isotopes in initial reactants will be enriched. The denitrification in this study was evident from the correlation of  $\delta^{15}\text{N}$  in nitrate and concentrations of nitrate in water as shown in Figure 6.



**Fig. 6.** Correlation of  $\delta^{15}\text{N}\text{-NO}_3$  with nitrate concentration remained in water due to denitrification

A model of the denitrification process expressing through the  $\delta^{15}\text{N}\text{-NO}_3$  vs.  $[\text{NO}_3^-]$  correlation was as follows (Fig. 6)

$$\delta^{15}\text{N}\text{-NO}_3 = 1.16.[\text{NO}_3^-] - 2.67 \quad (\%)$$

$$R^2 = 0.652$$

## IV. CONCLUSIONS

The quality of water of sub-catchment of Tuyen Lam reservoir was studied through chemical, physical and biological parameters as well as stable isotope ratio of nitrogen-15 ( $\delta^{15}\text{N}$ ) in nitrate dissolved in water. Generally, it was revealed that in the study area, the content of nutrients contamination is still not yet at the warning level according to the National Standard (QCVN 08-MT-2015/BTNMT) of the Ministry of Natural Resources and Environment. However, if proper strategies on better use of inorganic fertilizers and public water waste management not be applied right now it would cause serious consequences to quality of the aquatic environment and ultimately to the human health of the locality.

This is the first time isotopic technique was applied to identify the source of nitrate in the aquatic environment. However, it appeared that the  $\delta^{15}\text{N}\text{-NO}_3$  value could be useful tool in assessing the sources of nitrogen nutrient released into the environment. It is advisable that isotopic technique should be continuous applied in environmental studies, particularly such a kind of study addressed to the quality of water resources in order to precisely determine the sources of nitrogen as well as phosphate nutrients in the aquatic environment of Da Lat city. In this case not only  $\delta^{15}\text{N}\text{-NO}_3$ , but also  $\delta^{18}\text{N}\text{-NO}_3$ ,  $\delta^{18}\text{O}\text{-PO}_4$  dissolved in water. The outcomes of these studies would assist the local environmental managers in developing suitable strategies for protecting water resources of the city from nutrient pollution.

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## REFERENCE

- [1]. Cravotta, C.A.I., "Use of Stable Isotopes of Carbon, Nitrogen, and Sulfur to Identify Sources of Nitrogen in Surface Waters in the Lower Susquehanna River Basin". USGS, Pennsylvania, p. 99, 1997.
- [2]. Kellman, L.M. and Hillaire-Marcel, C., "Evaluation of nitrogen isotopes as indicators of nitrate contamination sources in an agricultural watershed". *Environment*, 95(3): 87-102, 2003.
- [3]. Xue, D., Botte, J., De Baets, B., Accoe, F., Nestler, A., Taylor, P., Van Cleemput, O., Berglund, M., Boeckx, P., "Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater". *Water Research* 43 (5): 1159-1170, 2009.
- [4]. Yevenes, M. A., Soetaert, K. and Mannaerts, C. M., "Tracing nitrate-nitrogen sources and modifications in a stream impacted by various land uses, South Portugal", *Water*, 8: 385, 2016.
- [5]. Kendall, C., Elliott, E.M. & Wankel, S.D., "Tracing anthropogenic inputs of nitrogen to ecosystems". *Stable isotopes in ecology and environmental science*, pp.375-449, 2007.
- [6]. Johannsen, A., "Isotopic composition of nitrate in five German rivers discharging into the North Sea". In *General Assembly of the European-Geosciences-Union*, 2007.
- [7]. Kendall, C., McDonnell, J.J., "Isotope Tracers in Catchment Hydrology". Anonymous Elsevier Science B.V, Amsterdam, p. 839, 1998.
- [8]. Kendall, C., Caldwell, E.A., "Fundamentals of isotope geochemistry". In: Kendall, C., McDonnell, J.J. (Eds.), *Isotope Tracers in Catchment Hydrology*. Elsevier Science B.V., Amsterdam, pp. 51-86, 1998.
- [9]. Kendall, C., "Tracing nitrogen sources and cycling in catchments". In: C. Kendall, J.J. McDonnell (Eds.), *Isotope Tracers in Catchment Hydrology*. Elsevier, Amsterdam, The Netherlands, pp. 519-576, 1998.
- [10]. Fenech, C., Rock, L., Nolan, K., Tobin, J., Morrissey A., "The potential for a suite of isotope and chemical markers to differentiate source of nitrate contamination: A review". *Water Research* 46: 2023 – 2041, 2012.
- [11]. TCVN:6494-2-2000 (ISO10304-2 : 1995) Vietnam National Technical Standard on Water quality – Determination of dissolved anions by liquid chromatography of ions – Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulphate in waste water. Ministry of Science, Technology and Environment, Ha Noi, Vietnam 2000.
- [12]. Silva, S.R., Kendall, C., Wilkison, D.H., Ziegler, A. C, Chang, C.C.Y, Avanzino, R. J., "A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios". *J. Hydrol.* 228: 22-36, 2000.
- [13]. Clark, J. S., 1955. "Solubility criteria for the existence of hydroxyapatite". *Can. J. Chem.* 33: 1696-1700.
- [14]. Firestone, M.K, Davidson, E.A., "Microbiological basis of NO and N<sub>2</sub>O production and consumption in soil", p. 7-21. In: M.O. Andrea and D.S. Schimael (ed.) *Exchange of trace gases between terrestrial ecosystems and the atmosphere*. Joh Wiley & Sons. New York, 1989.
- [15]. QCVN 08-MT: 2015/BTNMT. National Technical Regulation on surface water quality. Ministry of Natural Resources and Environment. Ha Noi, Vietnam, 2015.
- [16]. Mayer. B., Boyer, E., Goodale, C., Jaworski, N., van Breemen, N., Howarth, R.W., Seitzinger, S., Billen, G., Lajtha, K., Nadelhoffer, K., van Dam, D., Hetling, L., Nosal, M., Paustian, K., "Sources of nitrate in river draining sixteen water sheds in the northeastern US: Isotopic constraints". *Biogeochem.* 57/58:171-179, 2002.