CHARACTERISATION OF NITRATE STABLE ISOTOPES TO IDENTIFY NITRATE SOURCES IN BUKIT MERAH RESERVOIR, PERAK

by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BMR	Bukit Merah Reservoir
C	Carbon
Chl a	Chlorophyll a
DO	Dissolved oxygen
LMWL	Local meteoric water line
N	Nitrogen
N_2	Nitrogen gas
NO	Nitric oxide
NO_2	Nitrogen dioxide
N_2O	Nitrous oxide
NO_3^-	Nitrate
NO_2^-	Nitrite
NH_4^+	Ammonium
NH_3	Ammonia
NO_3^--N	Nitrate-nitrogen
NO_2^--N	Nitrite-nitrogen
NH_3-N	Ammonia-nitrogen
NAHRIM	National Hydraulic Institute Malaysia
O	Oxygen
O_2	Oxygen gas
P	Phosphorus
PO_4^{3-}	Phosphates

SDGs	Sustainable development goals
TN	Total Nitrogen
TP	Total Phosphorus

LIST OF UNITS

ºC	Degree celcius
µm	Micrometer
µg/ L	Microgram per liter
cm	Centimeter
g	Gram
ha	Hectare
kg	Kilogram
L	Liter
m	Meter
m^3	Cubic meter
ml	Milliliter
mg/ L	Milligram per liter
Tg N yr^{-1}	Teragram of Nitrogen per year

PENCIRIAN ISOTOP STABIL NITRAT UNTUK MENGENALPASTI SUMBER NITRAT DALAM TAKUNGAN BUKIT MERAH, PERAK

ABSTRAK

Takungan Bukit Merah (BMR) dikenali sebagai tasik buatan manusia tertua di Malaysia telah mengalami masalah sedimentasi yang mengganngu kapasiti simpanan tasik dan menyebabkan masalah berkaitan seperti eutrofikasi. Input nitrogen dalam jumlah yang berlebihan ke dalam kawasan tadahan air boleh menyebabkan kemerosotan kualiti air takungan. Oleh itu, kajian ini bertujuan untuk mengenalpasti sumber nitrat dalam sampel air di BMR menggunakan isotop stabil nitrat, menentukan proses transformasi nitrogen yang terlibat dan menentukan nilai iotop stabil untuk sumber nitrat tempatan termasuk tinja kambing, sampel tanah, air hujan dan baja, serta nilai isotop stabil C dan N dalam tanah. Kepekatan spesies nitrogen terlarut bukan organik (NO_3^--N, NO_2^--N, NH_3-N), jumlah nitrogen (TN), fosfat (PO_4^{3-}), jumlah fosforus (TP) dan klorofil-a telah dianalisa menggunakan spektrofotometer HACH DR2800. Isotop stabil nitrat ($\delta^{15}N$-NO_3^- dan $\delta^{18}O$-NO_3^-) untuk sampel air dan sumber nitrat tempatan yang dikenalpasti telah dikumpulkan sewaktu musim hujan dan kering dan dianalisa dengan menggunakan alat Trace Gas Pre-concentrator digabungkan dengan isotopic ratio mass spectrophotometry (IRMS), Finnigan MAT-253. Isotop stabil air ($\delta^{2}H$-H_2O dan $\delta^{18}O$-H_2O) dalam air permukaan telah dianalisa menggunakan penganalisis isotop air, Picarro L2140-I. Manakala, isotop stabil karbon dan nitrogen ($\delta^{13}C$ dan $\delta^{15}N$) ditentukan dengan menggunakan EA-IRMS dan C/ N juga ditentukan menggunakan penganalisis CHN dalam kajian ini. Kajian ini mendapati bahawa biplot $\delta^{15}N\text{-}NO_3^-$ dan $\delta^{18}O\text{-}NO_3^-$ menunjukkan input dari hujan, nitrogen dalam tanah, baja dan tinja sebagai sumber nitrat dalam sampel air BMR. Hasil kajian menunjukkan bahawa BMR menjalani proses nitrifikasi dan ‘mixing process’ dalam kolam nitrogen (N) tempatan. Keputusan kajian juga mendapati kepekatan nitrogen bukan organik yang rendah menunjukkan penguasaan nitrogen terlarut organik dalam air permukaan di BMR yang datang dari pelbagai sumber yang telah dinyatakan dan mungkin turut dipengaruhi oleh proses yang telah dikenalpasti. Untuk pertama kalinya, nilai isotop daripada sumber nitrat setempat, telah diekstrapolasi dan menunjukkan bahawa ia sepadan dengan nilai teori sumber-sumber nitrat sedia ada dalam tadahan air yang telah direkodkan. Oleh itu, dapatan kajian mengenalpasti input nitrat dari hujan, nitrogen dalam tanah, baja dan tinja sebagai sumber nitrat selain daripada input sedimentasi dari Sungai Kurau seperti yang dilaporkan oleh kajian terdahulu.

CHARACTERISATION OF NITRATE STABLE ISOTOPES TO IDENTIFY NITRATE SOURCES IN BUKIT MERAH RESERVOIR, PERAK

ABSTRACT

Bukit Merah Reservoir (BMR) is known as the oldest man-made lake in Malaysia has experienced sedimentation which threatens the storage capacity and may lead to other associated problems such as eutrophication. Excessive inputs of nitrogen in water catchment may cause deterioration of the water quality. Hence, this study aims to determine the nitrate sources in water samples from BMR catchment by using nitrate stable isotopes, to assess the processes of nitrogen in the water samples and determine the local sources of goat manure, rain water, sediment and fertilizer for nitrate isotopic signature and C and N stable isotopes in sediment. The concentration of dissolved inorganic nitrogen species (NO_3^--N, NO_2^--N, NH_3-N), total nitrogen (TN), phosphate (PO_4^{3-}), total phosphorus (TP) and chlorophyll-a (Chl-a) were analysed using HACH DR2800 spectrophotometer. The nitrate stable isotopes ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) of water and the potential local sources of goat manure, sediments, rain water and fertilizer had been collected from BMR during wet and dry season and analyzed using a Trace Gas Pre-concentrator unit coupled to an isotope ratio mass spectrophotometry, (IRMS), Finnigan MAT-253. Water stable isotopes ($\delta^{2}H$-H_2O and $\delta^{18}O$-H_2O) in the water samples were analyzed using Picarro L2140-I isotope water analyzer. Meanwhile, carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were determined using EA-IRMS and C/ N ratio were also determined using CHN analyser in this study. This study found out that biplots of

$\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ showed the contribution of atmospheric deposition input, fertilizer, soil nitrogen and manure as the sources of nitrate in the water samples. The results suggested that BMR undergoes nitrification and mixing processes within the local nitrogen (N) pool. From the results, the low inorganic nitrogen concentration reflected the domination of dissolved organic nitrogen in water samples of BMR which may come from all the possible sources that had been recognized and the other processes that may influenced the current condition. The isotopic value of the local nitrate sources for the first time, were extrapolated and showed that it falls in agreement to the existing theoretical values of nitrate sources in water catchment. Therefore, this study highlights the contribution of atmospheric deposition, soil nitrogen, fertilizer and manure as the nitrate sources apart from the sedimentation input from Sg Kurau as reported from previous studies.

CHAPTER 1: INTRODUCTION

1.1 Background of Study

Surface water plays important ecosystem functioning and services including as drinking water supply for human population. Globally, about one third of water requirement for the world communities are managed through the surface water sources including lakes, reservoirs and tributaries (Das & Acharya, 2003; Gasim et al., 2006). In Malaysia, this surface water resources either natural or man-made is comprise of over 100,000 hectares with about 30 billion m^3 of water (Sharip et al., 2013). Lakes in general are water catchments that are naturally formed through natural processes while reservoir in contrast, is man-made and constructed with certain purposes. These freshwater lakes and reservoirs play a vital role as an important component of national water resource by providing domestic water supply, including supplying for irrigation, agricultural, recreational and various industrial activities. However, the growth of human population and urbanization in the freshwater lakes and reservoirs trigger the expansion of land-use activities within these waterbodies. The existence of reservoirs has increased access to domestic water supply, irrigation and flood control but at the same time, were exposed to dynamic changes of organic or inorganic inputs and nutrient loading which can deteriorate the water quality (Huang et al., 2015). These excessive inputs can be either from point sources like waste from industrial factories and sewage plant, or non-point sources including excess fertilizer from agricultural land, manure and sediment runoff and atmospheric deposition. The uncontrolled land-use activities and unsustainable development could increase the sediment and nutrient fluxes which will eventually affect the water quality of the catchment.

The point sources are usually more manageable than the non-point sources due to the direct connection of the pollutant to its sources where the pollution control and implementation steps can be taken immediately. On the other hand, the presence of non-point sources that are hard to be traced or detected, threaten the health quality and biodiversity of aquatic ecosystem. The fertilizer runoff is known among the ecologists and environmentalists as one of the major issues in developing country due to the nutrients nourishment effect in the water column. As in recent decades, the rapid development in agriculture and industrial sectors have increased the accumulation of nitrogen in main water bodies (McIsaac et al., 2001; Galloway et al., 2004; Zeng and Wu, 2015).

In Malaysia, eutrophication is reported as one of the most prevalent issue that threatens the lakes and reservoirs functioning (Sharip, 2007). Bukit Merah Reservoir (BMR) is categorized as an eutrophic lake based on the trophic status together with the other 55 lakes in Malaysia by National Hydraulic Research Institute of Malaysia (NAHRIM, 2009). An excessive input of the essential nutrients, nitrogen and phosphorus that essential for plant growth will cause deterioration of water quality. Nitrogen in the ecosystem is available in many forms; nitrogen (N_2), nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), ammonium (NH_4^+) which have an important role in biogeochemical cycles. An excess amount of nitrate may heighten the population of phytoplankton that cause algal blooming and increasing the risk of nitrogen pollution. High nitrate concentration in water causes ‘baby-blue syndrome’ or Methemoglobinemia in infants and increase in stomach cancer problem in others (Norman, 2011; Zeng & Wu, 2015). Considering the importance of nitrate as one of the essential factor that affect the productivity and trophic status, and their various point and non-point sources in a reservoir, we are focusing on tracing nitrate sources using nitrate dual-stable isotopes, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ which provides the first baseline data using BMR as a case study in this thesis.

BMR provides various ecosystem services through supplying domestic water to locals, providing main water supply for irrigation of paddy fields and drinking water, regulating nutrient cycles, supporting fishing, agricultural and recreational activities (Hidzrami, 2010). As the oldest man-made lake in Malaysia, it faces plausible risks from natural eutrophication and sediment filling up the reservoir due to aging process. Several issues were addressed throughout the years inclusive of increasing land use activities within the water catchment; expansion of agriculture and small-scale farming, and sand mining activities at Sungai Kurau which flows to the reservoir. Previous researchers mentioned that Sungai Kurau contributed about 93% of sediment input into BMR than the other three rivers; Merah, Jelutong and Selarong (Ismail and Najib, 2011). This nutrient input includes phosphate, ammoniacal nitrogen, nitrate and nitrite in which the system act as sink for sediment, nitrite and nitrate ,while phosphate and ammonia were not retained in the catchment (Talib et al., 2016). Previous existing studies conducted in BMR were focused on water quality (Zakeyuddin et al., 2016), suspended sediments and trophic status (Najib et al., 2017), sand mining (Sharip & Zaki, 2014) and heavy metals (Akinbile et al., 2013) in order to observe the productivity and current condition of the ecosystem of BMR. However, the studies on tracing potential sources of nitrate is not conducted by any other researchers.

Since nitrate can be derived from different possible point and non-point sources, it is quite challenging for freshwater system to trace the source of nitrate that includes atmospheric deposition, synthetic nitrogenous fertilizer, animal manure, domestic sewage and soil nitrogen. Ecologists and environmentalists are currently focusing on the N cycle due to the increases in the existing of N reactive compounds that alter the cycle of nitrogen in water system that are driven by human activities (Galloway et al., 2004; Mayer et al., 2004). Stable isotopes techniques is used to trace the origins of nitrate and understand the nitrogen processes that involved in aquatic ecosystem (Mayer et al., 2004; Tobari et al., 2010; Yue et al., 2014). Specifically, the dual-stable isotopes of nitrate, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ give a specific isotopic signature or ranges, which helps specify the various potential sources of nitrate in the reservoir initiated by Kendall and colleagues (Kendall, 1998; Kendall et al., 2007). Kendall et al. (2007) biplot diagram, showed a wide range of $\delta^{18}O\text{-}NO_3^-$ is helpful in differentiating the sources of nitrate from soil nitrogen, atmospheric deposition and nitrate fertilizers (Deutsch et al., 2006; Mayer et al., 2004).

The utilization of nitrate dual-stable isotopes to trace the sources of nitrate in lakes and reservoirs can aid the existing work in water quality and environmental monitoring programs by understanding the current lake condition for better management and implementation steps. This effort is in line with the sustainable management and protection of lake waters through the formation of national water quality criteria and standard by NAHRIM for inland water resources in Malaysia (Sharip, 2007) and the sustainable development goals (SDGs) 6 and 14; covering clean water and sanitation and also life below water.

The scope of this research project covers the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ analyses along with the water quality and nutrient analyses of the surface river water which flows to BMR and the surface water from the mid area of BMR. Apart from that, the trophic status, background information on nutrient and sediment quality and $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values of potential nitrate sources within BMR were also analyzed. Our study also focused on non-point sources which are normally overlooked. Overall, this analysis was carried out to identify the sources of nitrate in surface water of BMR by using the application of nitrate dual-stable isotopes, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$.

1.2 Problem Statement

Considering the ecosystem services that it holds, the age and dynamic productivity of the reservoir which had been reported to be in mesotrophic (Zakeyuddin et al., 2016) and eutrophic condition (Sharip et al., 2014), it is important for current research to update the condition of the water catchment. Over the years, sedimentation problem had been reported as one of the major problems that occurred in the reservoir by Hasan (2011); Hidzrami (2010); Ismail et al. (2010); Najib et al. (2017) and Sharip et al. (2014). Thus, sedimentation is indirectly known as the major contributor of nutrient loading in BMR. However, in order to tackle the nutrient issue wholly, the presence of non-point sources of nutrient should also be taken into account. The potential of nitrate from non-point sources including the atmospheric deposition drew this research to be conducted during the wet and dry season in Malaysia. Various potential nitrate sources and the dynamic processes involving nitrate in water catchment results in difficulty to identify the sources and the interaction of nitrogen-cycling in environment. Therefore, the identification of nitrate sources using a dual isotopes approach of both $\delta^{15}N$ and $\delta^{18}O$ of NO^{3-} may complement the other water quality through analysis which is extensively used in current studies on aquatic ecosystem (Yue et al., 2014).

Thus, this study aims to address these following questions;

1.3 Research Questions

a) What are the non-point sources of nitrate in surface water of BMR water catchment? And, is it affected by wet and dry seasons?

b) What are the processes affecting nitrate sources in surface water of BMR?

c) Does the local isotopic signature from the potential sources of nitrate falls in the range of the isotopic source composition developed by Kendall (1998)?

1.4 Objectives of Study

Monitoring the water quality is an important step to ensure the lakes and reservoirs are sustainably managed. The nonpoint sources of nitrate which contributed into BMR can be identified by adapting the plot by Kendall, (1998) to determine whether the isotopic ranges fall within the value of the Kendall plot. Thus, the main interest is to determine the nitrate dual-stable isotopes in water samples of BMR catchment through tracing the nitrate sources utilizing the $\delta^{15}N–NO_3^-$ and $\delta^{18}O$ -NO_3^-. Therefore, the objectives of this study are

a) To determine the nitrate isotopic ranges in water samples in BMR and Sg Kurau using nitrate dual stable isotopes, $\delta^{15}N–NO_3^-$ and $\delta^{18}O$ -NO_3^-

b) To assess the processes of nitrogen in the water samples

c) To determine the local sources range of goat manure, rain water, sediment and fertilizer for nitrate isotopic signature and C and N stable isotopes in sediment.

1.5 Hypothesis

Theoretically, atmospheric deposition, animal manure and sewage, nitrogenous fertilizer and soil organic nitrogen are the potential sources of nitrate in water catchment. Based on the previous studies that highlighted on the sedimentation problem and sand mining activities into their concerned, we hypothesized that sedimentation and soil nitrogen as the major contribution of nitrate source in BMR. Apart from that, we also hypothesized that atmospheric nitrate might contribute as nitrate sources in BMR as one of the significant source of nitrogen in surface waters is through the atmospheric deposition (Howarth et al., 1996). Hence, we were expecting larger contribution of atmospheric deposition as one of nitrate sources during wet season compare to dry season.

1.6 Significance of Study

Generally, this application of nitrate stable isotope in water samples to trace the sources of nitrate in order to understand the local nitrogen cycle, is the first study that had been conducted as such in Malaysia. In near future, all the findings will act as a baseline data and as reference to other potential studies on the isotopic application in Malaysia’s waterbodies.

CHAPTER 2: LITERATURE REVIEW

2.1 Sources of nitrogen in freshwater

In freshwater system, nitrogen (N) and phosphorus (P) are known as the main nutrients that influence the waterbodies (Paerl et al., 2010), while uncontrolled or over enrichment of N may affect the biodiversity by triggering the growth of alien species (Carpenter et al., 1998). Other than P as the main focus in aquatic ecosystem, increasing N loading in many watersheds triggers the blooms of non-nitrogen fixers (Zeng & Wu, 2015) and a study by Wang et al. (2018) showed that N supply stimulates the presence of two non-nitrogen fixers. The rising of deposited nitrogen in worldwide ecosystem was recorded in 1860s and 1995, with 34 Tg N yr^{-1} and 100 Tg N yr^{-1} with expectation on increment during 2050 with 200 Tg N yr^{-1} as predicted by Vitousek et al. (1997) and Galloway et al. (2008). In nitrogen cycle, nitrate is known as the dominant form of N causing eutrophication, algal blooms, hypoxia and water quality impairment in water catchment (Paerl et al., 2010; Kozaki et al., 2014; Zeng & Wu, 2015). There are different potential sources of nitrate to surface water including soil nitrate, sewage, manure, nitrate and ammonia fertilizer from agriculture and the atmospheric deposition. (Lijklema 1994, Ma et al., 2011; Yang et al., 2017). These researchers discussed on the non-point sources of nitrogen which are the atmospheric deposition, fertilizers, runoff and leaching, while the point source is being categorised as discharge of sewage and industrial wastes. Point sources can be monitored by identifying the source and periodically measuring the discharge and chemical concentration at the allocated source (Carpenter et al., 1998; Ain Nihla et al., 2011). Contrary to that, the non-point source is characterised by multiple discharge point that are hard to manage (Kamarudzaman et al., 2011) which can be linked to irregular events such as heavy precipitation, potentially transporting non-point inputs into surface water or groundwater of water catchment (Carpenter et al., 1998) as characterized in Table 2.1 below.

Table 2.1 The characterization of both non-point and point sources into water catchment as adapted from Carpenter et al. (1998).

Non-point sources	Point sources
Agriculture runoff	Wastewater effluent
Leachate from septic tank and runoff from failed septic system	Leachate runoff in dumping sites
Urban runoff	Overflows of combined storm and sanitary sewer
Construction sites runoff (>2 ha)	Construction sites runoff (2 ha)
Range and pasture runoff	Mines, oil fields, unsewered industrial sites
Atmospheric deposition	Animal feedlots
Land use activities; logging, wetland conversion	Storm sewer outfalls from cities

2.1.1 Eutrophication and trophic status in lakes and reservoirs

Eutrophication is known as a worldwide problem causing excessive algal growth in water catchments. The process occurs either naturally over centuries as the lake starts to age and fill in with sediments (Carpenter et al., 1998) that known as lake succession process or due to cultural eutrophication. The cultural eutrophication regarding to the anthropogenic activities by human that heightened the rate and extent of eutrophication process through both points and non-point sources. This factor includes nitrogen and phosphorus loading that are known as limiting nutrient into aquatic ecosystems (Carpenter et al., 1998). Eutrophication also affects the ecological functioning, aquatic life, biodiversity of the aquatic ecosystem, water quality and the domestic water supply (Salameh & Harasheh, 2011). Globally, the increase of nitrate loads into both ground and surface water rise the safeguard to human and livestock health (Ji et al., 2017) increasing the NO_3^--N contamination that cause eutrophication problem and impairment of water quality through oxygen deficiency condition (Nestler et al., 2011; Paerl et al., 2010).

Asian countries like China and Japan faced similar problem of eutrophication, which potentially worsens without proper management of conservation (Inamori et al., 2001). In Malaysia, a desk study by NAHRIM (2005) regarding the trophic status of lakes had classified 90 of them into mesotrophic and eutrophic condition respectively. Out of those 90 lakes, 34 lakes or 38% were evaluated to be in mesotrophic condition while another 56 lakes or 62% were in eutrophic condition including BMR. Some studies had been conducted in Malaysian lakes (Table 2.2) within 2011 to 2017 focusing on nutrient level, water quality and the trophic status. However, till present there is no existing study conducted on tracing the nitrate sources which is one of the major factors that may lead into eutrophication problem in all freshwater catchments.

Table 2.2 Previous study on water quality, nutrients, fish and phytoplankton distribution in lakes in Malaysia.

Author(s), Year	Publication
(Ismail & Najib, 2011)	Sediments and nutrients balance of BMR, Perak, Malaysia.
(Gasim et al., 2006)	Hydrological assessment and water quality characteristics of Chini Lake, Pahang, Malaysia.
(Gharibreza et al., 2013)	Effects of agricultural projects on nutrient levels in Lake Bera (Tasek Bera), Peninsular Malaysia.
(Akinbile et al., 2013)	Qualitative analysis and classification of surface water in BMR in Malaysia.
(Ain Nihla et al., 2011)	Study of Point and Non Point Sources Pollution – A Case Study of Timah Tasoh Lake in Perlis, Malaysia.
(Jamal et al., 2014)	Littoral and Limnetic Phytoplankton Distribution and Biodiversity in a Tropical Man- Made Lake.
(Sharip et al., 2014)	Lakes of Malaysia : Water quality, eutrophication and management.
(Shafiq et al., 2014)	An annotated checklist of fish fauna of BMR and its catchment area, Perak, Malaysia.
(Shafiq et al., 2014)	The effects of seasonal changes on freshwater fish assemblages and environmental factors in BMR (Malaysia).
(Wan Maznah & Makhlough,2015)	Water quality of tropical reservoir based on spatio-temporal variation in phytoplankton composition and physico-chemical analysis.
(Suratman et al., 2015)	A preliminary study of the distribution of phosphorus and silicon compounds in Tasik Kenyir, Hulu Terengganu, Malaysia.
(Zakeyuddin et al., 2016)	Spatial and Temporal Variations of Water Quality and Trophic Status in BMR, Perak.
(Baharim et al., 2016)	The relationship between heavy metals and trophic properties in Sembrong Lake, Johor.
(Talib et al., 2016)	Nutrient concentration in sediment and overlying water at BMR, Perak.
(Rahaman et al., 2016)	Rivers and lakes as natural heritage: Water quality status in Northern States of Peninsular State.

(Najib et al., 2017)	Suspended sediment and phosphorus budget and trophic status of Bukit Merah Reservoir, Perak, Malaysia.
(Sharip & Md Yusoff, 2017)	Plankton community characteristics of natural and man-made tropical lakes.

Among all, there were two studies at BMR by Zakeyuddin et al. (2016) and Sharip et al. (2014) which showed the adaptation on Carlson's Trophic State Index (TSI) in classifying reservoir into their productivity status. Level of primary productivity or growth of algae and plant community as a response of the biological properties of the lake as it aged, reaching different biological growth states are known as their trophic state (Murthy & Hosmani., 2008). This Carlson's index is used to categorise the lake into its trophic status without tracing the source of nutrient that contributed to the eutrophication problem.

The increment of eutrophication problem and nitrate contamination in water catchment results in a wide application of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ especially in Asian countries like China, Vietnam and Japan. However, there is no application of nitrate stable isotopes in Malaysia on freshwater management. Table 2.3 presents studies that had been conducted by researchers from different geographical area.

Table 2.3 Application of nitrogen and oxygen stable isotopes of nitrate in water catchment.

Watersheds	Title	Author
Liao River	Using dual isotopes to evaluate sources and transformation of nitrogen in the Liao River, northeast China.	(Yue et al., 2013)
Songhua River	Analysis of $\delta^{15}N$ and $\delta^{18}O$ to identify nitrate sources and transformations in Songhua River, Northeast China.	(Yue et al., 2014)
Shijiazhuang, China	Evaluating the sources and fate of nitrate in the alluvial aquifers in the Shijiazhuang Rural and Suburban Area, China: Hydro-chemical and Multi-Isotopic Approaches	(Zhang et al., 2015)
Taihu Lake	Tracing the nitrate sources of the Yili River in the Taihu Lake watershed: A dual isotope approach	(Zeng & Wu, 2015)
Mt. Yulong Region	Variability of stable isotope in lake water and its hydrological processes identification in Mt. Yulong Region	(Shi et al., 2017)
Xijiang River	Identification of sources and transformations of nitrate in the Xijiang River using nitrate isotopes and Bayesian model	(Li et al., 2019)
Japan & China	Microbial denitrification dominates nitrate losses from forest ecosystems	(Fang et al., 2015)
Tatara River Basin, Japan	Differences in stream water nitrate concentrations between a nitrogen-saturated upland forest and a downstream mixed land use river basin	(Shinozuka et al., 2017)
Hai Duong	Interpretation of anthropogenic impacts (agriculture and urbanization) on tropical deltaic river network through the spatio-temporal variation of stable (N, O) isotopes of NO_3^-	(Ta et al., 2016)

From the table, we can see that the pattern of stable isotopes studies started with tracing and identifying the sources of nitrate and the processes that affected the transformation of nitrate in water catchment, which later discovered the application of Bayesian mixing model to calculate the contribution of identified sources in water catchment. Hence, in order to narrow the gap of knowledge regarding the application of nitrate dual-stable isotopes tools, our study managed to start with tracing the nitrate sources using nitrate dual-stable isotopes technique, identifying the processes that took place in BMR in order to get the insight of local N cycle and lastly to come out with new ranges of isotopic composition of nitrogen and oxygen of nitrate from potential local nitrate sources at BMR.

2.2 Nitrogen sources and processes involved in the nitrogen cycle

Nitrogen cycle describes the motion and transformation of nitrogen in the ecosystem on land, ground and surface water. About 78% of the atmosphere is being covered by atmospheric nitrogen gas in the form of N_2 which is not in the reactive form (Berner & Berner, 1987; Ramakrishnan et al., 2015). In the atmosphere, the most abundant nitrogen gases are available in the forms of elemental nitrogen (N_2), nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2) and ammonia (NH_3).

Some nitrogen in ecosystem is not in the active form that can be used directly by plant or organisms but instead need to be fixed through several processes. In aquatic ecosystem, dissolved inorganic nitrogen (DIN) including the ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) are known as their reactive forms (Camargo & Alonso, 2006). These DIN can be found naturally either through atmospheric deposition, runoff from surface and groundwater, geological factors and biological organic matter decomposition or N_2 fixation by certain types of cyanobacteria (Kinne, 1984; Camargo & Alonso, 2006). Generally, the processes involved in nitrogen cycling reactions in the ecosystem are nitrogen fixation, nitrification, denitrification, assimilation and mineralization (Camargo & Alonso, 2006). The nitrogen cycle and end concentration value of nitrogen in ecosystem is affected by those processes.

2.2.1 Nitrogen fixation

Nitrogen fixation takes place when there is the presence of organisms such as phytoplankton and bacteria. During the nitrogen fixing, N_2 will be converted into one of the usable form of nitrate, NH_3 either by bacteria in soil (legumes) or algae in water, or atmospheric phenomena or lightning (Kendall et al., 2003; Ramakrishnan et al., 2015) or human activities through energy production, fertilizer production and crop cultivation (Kendall et al., 2003). The biological fixation is the dominant fixation processes, compare to lightning and industrial fixation. However, human activities had been reported to add the sum of additional N load per year, which cause critical changes in ecosystem worldwide. In soil, fixation of nitrogen occurs in legumes plant with the presence of heterotrophic bacteria. While in aquatic ecosystem, cyanobacteria with nitrogenase enzyme allows conversion of N_2 to NH_3 (Durand et al., 2011). The biological nitrogen fixation requires energy supply hence, this process needs bacteria that live in symbiosis with plant as in equation 2.1:

$$N_2 + 3H_{2\ \text{(pyruvic acid donor)}} \rightarrow 2NH_3 \qquad (2.1)$$

The stoichiometry of nitrogen fixation requires 8 electrons and 16 molecules of ATP to reduce one molecule of N_2, as shown below:

$$N_2 + 8H^+ + 8e^- + 16MgATP \qquad\qquad 2NH_3 + H_2 + 16MgADP + 16P_i \quad (2.2)$$

Fixation due to lightning will occur under a very high pressure and temperature, where N_2 will combine with O_2 to produce NO which is then oxidised to form NO_2 or HNO_3 and will be eventually remove through wet and dry deposition (Galloway et al., 2004). Deposition introduce the reactive nitrogen in both wet and dry forms into the ecosystems especially in tropical regions. Generally, nitrate from the atmospheric deposition is known as one of the important nitrogen sources on earth. The industrial nitrogen fixation started the evolution of the N fertilizer to cope with increasing food demands results from escalating number of population and rapid urbanization. The industrial fixation including N fertilizer production, fossil-fuel combustion cause significant alteration in the nitrogen cycle pathway as more reactive N is readily available to the ecosystem (Galloway et al., 2014; Li et al., 2015). Kendall (1998) predicted that the increasing fossil fuel combustion and fertilizer use will cause 60% increment in anthropogenic fixation rate by 2020 especially in Asia, while Chindler et al. (1997) reported on the transfer of N from the atmosphere to biologically available pool on land had doubled as a consequence from increasing human activities.

2.2.2 Ammonification and mineralization

Ammonification or also known as mineralization of nitrogen is mediated by heterotrophic organisms that are engaged in decomposition of organic matter that produce ammonium (Foth, 1990; Kendall et al., 2007).

$$N_2 + 3H_2 \rightarrow 2NH_3 + H_2O \rightarrow NH_4^+ + OH^- \qquad (2.3)$$

Through ammonification process, the microbial decomposition will convert the organic nitrogen to the ammoniacal form either in water column of lakes and rivers or in sediments (Heathwaite, 1993). In the organic matter decomposition, different kind of bacteria are engaged to the mineralization of nitrogen to ammonia (Foth, 1990; Stein & Klotz, 2016). The first product of NH_3 produced will either volatilize and escape into the atmosphere or reacts with water to combine with H^+ to form ammonium, NH_4^+ (Foth, 1990). At certain condition, under anammox (anaerobic ammonia oxidation), NH_4^+ will be directly transferred into N_2 gas and release to atmosphere. The equation evolved as;

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \tag{2.4}$$

2.2.3 Nitrification

The ammonia produced from ammonification or mineralization is then oxidized and transformed into nitrate with availability of oxygen. This conversion process of the usable form of N for organisms, NO_3^- is known as nitrification process. The nitrification process converts ammonia to NO_3^- via NO_2^-, by different autotrophic organisms; nitrifying bacteria of *Nitrosomonas* and *Nitrobacter* (Nestler et al., 2011). Nitrification is described by two parts of oxidation reaction that can be summarised as below;

$$NH_4^+ + O_2 \rightarrow NO_2^- + O_2 + 2H_2 \rightarrow NO_3^- + H_2O \tag{2.5}$$

Equation (2.5) shows the formation of NO_3^- with NO_2^- as the by-product of the reaction. The nitrification process requires minimum oxygen concentration of at least 2 mg/L, in which in some aquatic systems the minimal amount of oxygen may create hypoxic conditions (Heathwaite, 1993; Kendall et al., 2007; Yang & Toor, 2016). This process is important when retention times are longer and under high temperature, where the temperature limitation of bacteria should be basically above 15ºC (Heathwaite, 1993). This oxidation process causes the NO_2^- to convert into NO_3^- that results in usually lower and decreasing concentration of NO_2^- in aquatic system.

2.2.4 Denitrification

The balance nitrogen cycle is achieved through balance input and output of nitrogen into earth system in which, the denitrification process plays important role in the cycle. Denitrification process occurs through the microbial reduction of converting NO_3^- into gaseous products, N_2 in the absence of or low saturation of oxygen content in ecosystem. In nitrogen cycle, denitrification is one of the key process where, nitrate is reduce to nitrogen gases as their end-product and will be release into atmosphere for balancing the nitrogen cycle (Zhao et al., 2015). It occurs when O_2 concentration are less than 20µM in soils, aquifers, riparian zones, benthic and river sediment (Kendall et al., 2007). Denitrifying bacteria will eventually transforms the NO_3^- to nitrogen gases via NO_2^-, NO and N_2O by the enzymatic activity (Nestler et al., 2011). Generally, the denitrification process helps in reduction of nitrate contain in water body. The denitrification process is important for the pools of reactive N to significantly reduce the excess amount of NO_3^-, NO and N_2O that will cause diverse problem of earth pollution (Davidson et al., 2012). However, since it requires an anaerobic condition and a fixed bacterial for carbon supply, this factor may cause some limitation in the process. Once the conversion of nitrate to inert gas, N_2 in the biosphere is limited, it will results in excess production of various form of N reactive that lead to accumulation of reactive N in the earth's atmosphere, soil and water (Galloway et al., 2014). Thus, due to the addition of N reactive (NO_3^-, NO, N_2O, NH_3) in the N pools, the NO_3^- concentration cannot be solely reduced by denitrification process unless the inputs to aquatic ecosystem is control and monitor.

2.3 Stable Isotopes

Throughout the past few decades, the application of various stable isotope techniques has emerged as a complementary tool to the traditional method of determining concentrations alone (Kendall et al., 2010). Nutrients in the water system are influence by different physical, chemical or biological conditions in which eventually affect the fates, pathways and transformation of these nutrients. The application of stable isotope is applicable for identification and trace of the nutrient sources and as one of the way to heighten the knowledge of biological processes in aquatic ecosystem (Kendall et al., 2010). According to Kendall & Doctor (2003), environmental isotopes are typically used to identify water and solutes sources, the flow paths of water and assessment of nutrient biological cycle within the ecosystem. The stable isotope technique is widely applied by researchers due to the ability of the isotopes to act as a tracer through the isotopic signature (the distinct isotopic ratios) that helps in tracing the source of nutrients or pollutants in waterbodies. One example is where the solutes in water that are derived from atmospheric sources can be differentiated from the other biological sources in the waterbodies by using isotopic ratios (Kendall & Doctor, 2003).

The use of isotopic tools in environmental studies as hydrologic tracers for freshwater systems are divided into two, which act as water tracers itself (water isotope hydrology) and solutes tracers in the water (solute isotope biogeochemistry) as mentioned by Kendall & Doctor (2003). This study, focuses on the use of solute isotopes, which is the nitrogen isotopes in the form of nitrate in water by using nitrate dual-stable isotope application, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$. Chang et al., (2003) discussed on the application of the solute isotopes that are apparently involved in the reaction or fractionation process in water system that will subsequently alter the ratio of the solute isotope.

2.3.1 Properties of stable isotopes

The term environmental isotopes refers to both stable and radioactive isotopes that can be found present in the natural environment, either from natural processes or introduced by humans to nature (Miljevic & Golobacanin, 2007). The environmental isotopes can be used to assist in finding the solution to different hydrogeochemical problem due to its wide distribution in the hydrosphere (Kendall & Caldwell, 1998). Isotopes are known as any element that shares similar protons and electrons number, with distinct in neutrons number. The element shows different masses from each other based on their different neutron numbers (Figure 2.1). The variations of this similar element are called isotopes (Kendall & Caldwell, 1998). Through the geologic time scale, the stable isotopes would not decay to other form of isotopes (Kendall & Caldwell, 1998), which differentiates it from the radioactive isotopes that usually unstable.

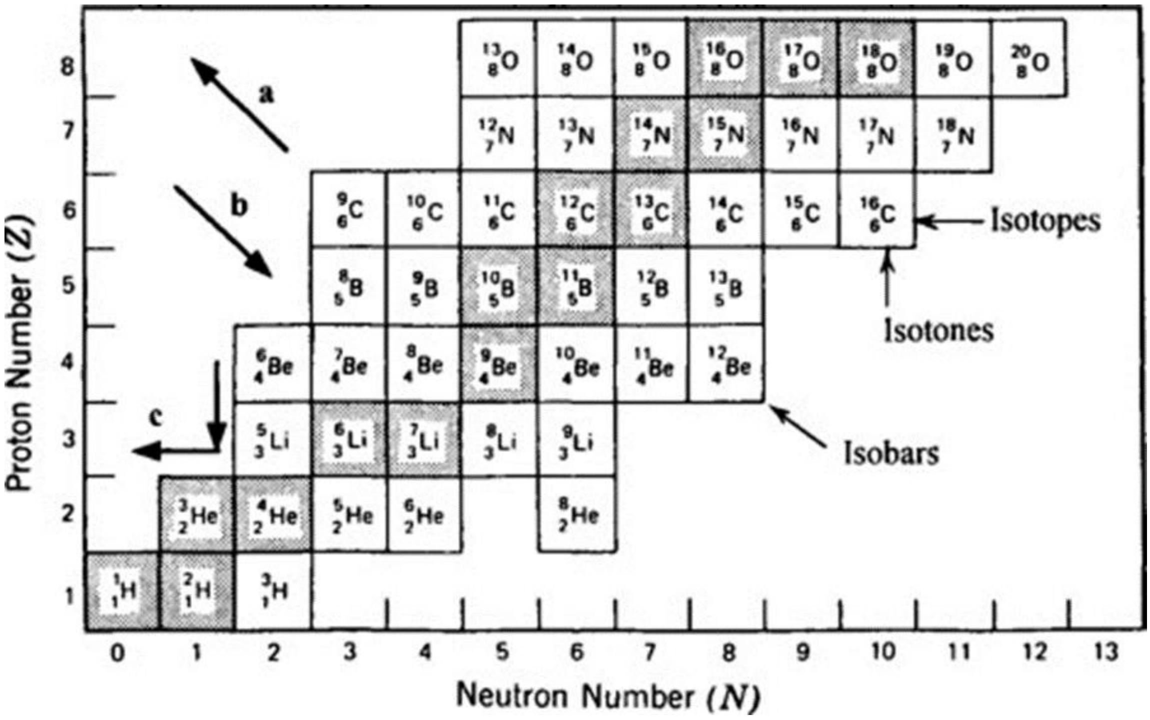

Figure 2.1. Partial chart of the elements that show the stable atoms (shaded square) with same number of protons but different number of neutrons. The superscript on the left show the mass number of same element from the sum of proton and neutron number (Faure, 1986; Kendall & Caldwell, 1998).

Stable isotope composition in sample are usually reported as δ values or known as delta in units of part per thousands (‰) denoted as per mil. The δ values of the stable isotope elements are calculated by:

$$(\text{‰}) = ((R_{sample}/R_{standard}) - 1) \quad (2.6)$$

Where R is the heavy to light isotopes ratio, R_x and R_s are the sample and standard ratio, respectively. The delta value can be both positive and negative value. δ with positive value means that the sample's isotopic ratio is higher than in the standard, reported as enrichments or more positive value/ heavier relative to the known composition of the standard. The reported negative value of δ means that the isotopic ratio of the sample is lower than the standard, reported as depletions or more negative/ lighter relative to known composition of the standard (Kendall & Caldwell, 1998; Kendall et al., 2003). For instance, in our study, +0.8 ‰ $\delta^{15}N\text{-}NO_3^-$ means an enrichment or more positive values than the known standard composition of atmospheric N (AIR).

The tracing process of sources can be complicated by the existence of dynamic processes that alter the original isotopic fingerprint of the source material. Isotope fractionation changes the isotopic composition of an element by conversion of the compound into a different form (Gat, 2001). However, the isotope fractionation that took place gives an important details (Wexler et al., 2011; Xu et al., 2016) in tracing the transformation processes. Isotopic fractionation can occur either due to the driven energy of kinetic condition or at equilibrium. The fractionation is known as the reversible equilibrium reaction or irreversible unidirectional kinetic reaction from the chemical, physical and biological processes (Kendall, 1998).

Equilibrium isotopic reactions involved the identical or same reaction rates either forwards or backwards for the specific isotopes. This condition results in the constant ratio of different isotopes in each compound. This is demonstrated by the equation;

$$AX^0 + BX^1 \leftrightarrow AX^1 + BX^0 \tag{2.7}$$

Where X^0 and X^1 indicate the common element with different weight of heavy or light isotopes. This condition will only occur in different isotopic ratios between phases of the same elements and when there is reversible reaction in either closed or well-mixed systems. In different phase of the similar compound or dissimilar species of the similar elements, the heavier isotope tends to be enriched in much denser material such as, ^{18}O that when in liquid state will be more enriched, which differs than the lighter isotopes, ^{16}O that stay in the vapour state (Kendall, 1998).

On the other hand, the kinetic fractionation that are generally unidirectional, they possesses different rates both forward and backward reactions. The vibrational energies of the isotopes and their ratio masses will affect the kinetic fractionation reactions. Since the bond between the lighter isotopes are easily broken, it will have faster reaction than heavier\ isotopes and it will be more concentrated in the end product (Kendall, 1998; Miljevic & Golobacanin, 2007). In general, it will results in products that are lighter than the reactants whilst in reversible equilibrium reaction, the products produced can be both lighter and heavier than the reactants (Kendall, 1998). For example, in nitrification process where ammonium is converted into nitrate, nitrate products is always lighter with lower $\delta^{15}N$ value than the ammonium.

2.3.2 Fundamentals of nitrogen stable isotopes of nitrate, $\delta^{15}N$-NO_3^-

0.03% of earth comprises of N in which, about 97.8% of the total N is located in rocks and about 2% in the atmosphere while the remainder in the hydrosphere and biosphere (Heaton, 1986; Kendall, 1998). The nitrogen dual-stable isotopes technique allow the detection of NO_3^- sources in water through the characteristic or distinctive composition of nitrogen (Kendall et al., 2007; Wexler et al., 2012; Yue et al., 2014). ^{14}N and ^{15}N are the two stable isotopes with isotopic composition ranging from +5‰ to -3‰. The nitrogen isotopic ratio generally reported as relative to N_2 in atmospheric air as their standard reference as below;

$$\delta^{15}N\ (‰) = \{[(^{15}N/\ ^{14}N)_{X}\ /\ (^{15}N/^{14}N)_{AIR}] - 1\} \qquad (2.8)$$

Where X = sample and AIR= the reference standard gas (atmospheric N)

To report the $\delta^{15}N$ values, air (AIR) is used as the reference since the average abundance of ^{15}N (0.366%) in air is constant (Junk & Svec, 1958). The $\delta^{15}N$ values of terrestrial materials ranged between -20‰ and +30‰. Theoretically, in most natural ecosystem the atmosphere is known as the major source of nitrogen ($\delta^{15}N$ = 0‰). Therefore, the biological fixation by plants were reported to have $\delta^{15}N$ values of 0‰ to +2‰ which is near with the $\delta^{15}N$ value of atmospheric N_2, due to the fixation of N_2 from the atmosphere, while for most plants the values are between -5‰ to +2‰ for the $\delta^{15}N$ (Fry et al., 1991). This is supported by (Kendall & Doctor, 2003) that showed the other potential nitrogen sources that produced from the nitrogen in atmosphere include fertilizers will have value of the $\delta^{15}N$ from 0 ± 3‰ and animal and manure are generally in the range of +10 to +25‰.

However, the fractionation processes in the N cycle will eventually cause changes in the characteristic of the original source and shows an overlapping of some sources based on the values of $\delta^{15}N\text{-}NO_3^-$ alone (Kendall et al., 2007) may constraint its application in NO_3^- source identification. Therefore, the employment of ^{18}O is suggested based on the wide variation of oxygen isotopic composition among different sources that are suitable to be used as an additional tools (Kendall et al., 2007; Zeng & Wu, 2015).