

# Major Nutrients and Their Stoichiometry over 45 Years (May 1963-May 2008) at the Northern and Southern Basin of Lake Biwa, Japan

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**Abstract** On the basis of the chemical components found in Lake Biwa, Japan from May 1963 to May 2008, the vertical variation (0~70 m depth) of total phosphorus (TP) and total nitrogen (TN) concentrations in the Northern Basin (Ie-1) were evaluated. Similarly, TP and TN concentrations were also discussed at the surface of the Southern basin (Nb-5). It was concluded that the second stage nutrient concentrations had decreased compared to Northern basin due to increased macrophyte growth, improvement in inflow river waters and implementation of laws. The long term variations in the TN/TP ratios were also discussed in relation to propagation of plankton. The lower TN/TP ratios observed in the Northern and Southern Basins waters were well coincident with the more frequent and denser propagation of N<sub>2</sub>-fixing cyanobacteria. Recently, the TN/TP ratios have shown a tendency of increase in Southern Basin suggesting a first sign for the restoration of water.

**Keywords:** lake biwa, total phosphorus, total nitrogen, vertical distribution, long term trends

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## 1. Introduction

A period of 45 years is relatively short when compared with the time needed for geological and meteorological changes in the natural environment. However, in the 45 years from May 1963 to May 2008, human activity increased rapidly due to industrialization and upset the material balance in the freshwater ecosystem of Lake Biwa. The perturbation has affected the air, soil and water environments. The water environment has been especially afflicted with the setting in of eutrophication in various water reservoirs.

In August, 1962 and January, 1963, as a part of a project for an integrated assessment of animated resources in Lake Biwa, the water research group headed by Prof. Fujinaga carried out a survey of the chemical component of Lake Biwa and the entire data was denoted as "BST". Following the BST-observations, Prof. Fujinaga and his coworkers made another series of observations from May 1963 to June 1981 and called them hydrosphere research Group (HRG). After "HRG", Prof. Hori and his coworkers conducted a new series of observations termed "PHOS" during the period of 1980 to 1982. The next series of observations termed "SIAL" was carried out monthly from April 1992 to March 1995, during which period usual [1992-93] and unusually wet [1993-94], as well as unusually drought [1994-96] conditions were included [1].

After that, phosphorus and nitrogen levels from May 1996 to May 2008 were deduced and referred as "NUTR". From the HRG to the recent NUTR, the whole data set thus covers 45 years.

Lake Biwa is the largest freshwater source in Japan. It is a source of drinking water for 14 million residents in Kyoto, Osaka and Kobe cities. There are more than 400 rivers and streams flowing into the lake, whereas the single natural outlet with an annual flow of 90 m<sup>3</sup>.s<sup>-1</sup> is the Sita River [2].

In May 1969, a musty odor was first noticed in the tap water that supplied water from Lake Biwa to Kyoto city. Several eutrophication-related phenomena were observed, such as excessive growth of *Egeria densa*, and an outbreak of freshwater red tide caused by the blooming of *Uroglena americana*. The alarmed residents started a movement to promote the replacement of phosphorus-containing synthetic detergents with soap, and the Shiga assembly passed the Eutrophication Control Ordinance in 1980. As a result of an increase in nutrient loading due to urbanization, bloom of *Uroglena americana* occurred since 1977 and cyanobacteria since 1983 at several coastal areas of Lake Biwa [3]. In 1979, the government enacted the ordinance for prevention of eutrophication of Lake Biwa including the legal regulation of nitrogen and phosphorus discharge from domestic and industrial sources [4]. *Anabaena* and *Microcystis*, which are nuisance blue green algae became dominant in the 1980s and tended to increase in summer in the Southern Basin of

Lake Biwa [5]. In 1992, the Shiga Prefecture government enacted the ordinance concerning the conservation of reed colonies around Lake Biwa without consideration of the comprehensive conservation of Lake Biwa and its watershed; in 2002, the ordinance was therefore revised and a new project was started with the cooperation of scientists, local citizens, NGOs and governmental organizations [6].

The effects of inflows to Lake Biwa are very complicated because widely different quality of water can be observed, viz., rainwater, groundwater, agriculture or industrial drain water, or surface water due to heavy rainfall or flood containing various chemicals. These are non-point sources, and most of them were not measured well for analysis [7,8]. A long term (45 years) data set from Lake Biwa provides an opportunity to correlate nutrient (total-nitrogen, total-phosphorus) concentrations with the impact of anthropogenic influences on Lake Biwa, such as implementation of the Shiga Prefecture Ordinance on Prevention of Eutrophication in Lake Biwa in 1979, promotion of septic tanks in 1981 and Reed Belt Conservation Ordinance in 1992 etc. The sewage system has been constructed in the watershed since the 1970s and coverage of the systems reached 82.2% in 2006 [9].

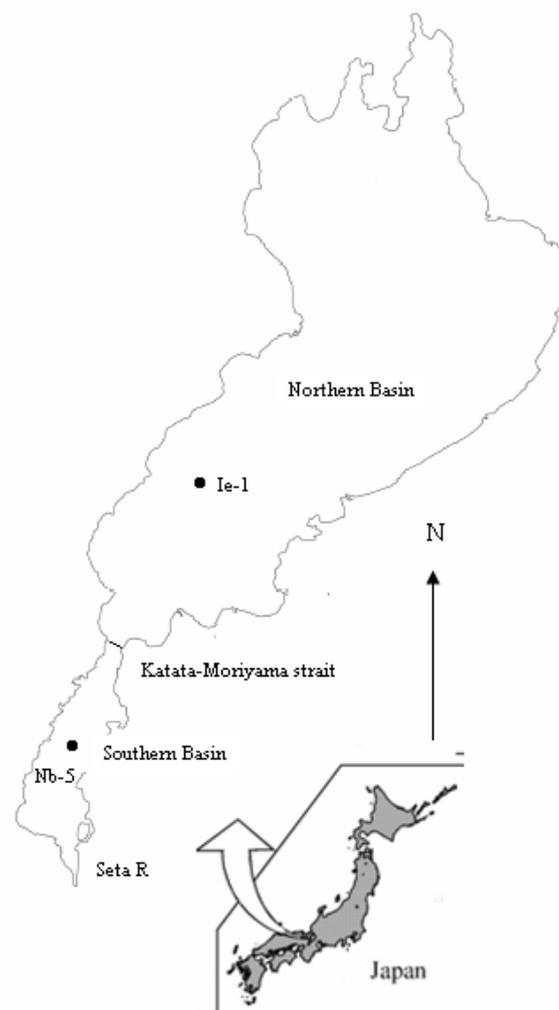
Lake Biwa is geometrically and limnologically divided into two parts, Northern Basin and Southern Basin. The Northern Basin is the main basin and has a surface area of 616 km<sup>2</sup> and a capacity of 27.3 km<sup>3</sup>. Its average and maximum depths are 44 m and 104 m, respectively. The residence time of lake water is estimated to be 5.5 years. The Southern Basin of the lake has a surface area of 58 km<sup>2</sup> and a capacity of 0.2 km<sup>3</sup>, with average and maximum depth of 3.5 m and 8 m, respectively. The residence time of the water is calculated to be 0.04 years [10].

It is scientifically significant that the 45 year span of the present study includes two important periods for Lake Biwa: The first stage covers the years of 1963 - 1982 when human activities were initially so subtle that the lake retained its original state. Subsequently, eutrophication of the lake proceeded rapidly due to industrialization, and later local laws were enacted for the restoration of the lake. The second stage covers 1992-2008 when self-cleaning effects may have appeared. In this research, our purposes were to compile and analyze historical (1963-2008) TN, TP and TN/TP ratio data and how it is affected by industrialization and natural cleaning process in Lake Biwa. As a subject of study by environmental chemists, however, Lake Biwa provides a unique opportunity because many chemical components, including phosphorus and nitrogen, have been monitored continuously through periods before and after its eutrophication. In the present study, 45 years (1963 - 2008) data of Ie-1 (depth wise 0 ~ 70 meters) and Nb-5 (0 meter) were analyzed against time to deduce long term variations of chemical components such as phosphorus and nitrogen.

## 2. Experimental Details

Water samples were collected from the surface at Ie-1 (35° 12' 58'' N 135° 59' 55'' E) and Nb-5 (35° 04' 02'' N 135° 54' 27'' E). The location of Ie-1 and Nb-5 is shown

in Figure 1. At Ie-1, samples were also taken at depths of 1, 5, 10, 15, 20, 30, 40, 50, 60, 70 and 73 m (bottom) using a Van Dorn sampler.



**Figure 1.** Map of Lake Biwa and the location of sampling stations Ie-1 and Nb-5

### 2.1. Total Phosphorus, TP

A fixed amount of unfiltered sample (200 mL) was taken into a beaker and dried on a hotplate to dryness, followed by both incineration with 2 mL of 60% HClO<sub>4</sub> and quantization of phosphorus content according either to heteropoly blue spectrophotometric method at 700 nm [11] from 1963 to 1980 observation and to the indirect spectrophotometry at 545 nm [12] from 1981 to 2008 observations.

### 2.2. Total Nitrogen, TN

A fixed amount (100 mL) of unfiltered sample was subjected to total Kjeldahl digestion and the resulting ammonia-N was analyzed spectrophotometrically at 660 nm according to the indophenol method [13]. For NO<sub>3</sub>-N 100 mL of unfiltered sample was dried in a beaker and nitrate in the residue was quantized spectrophotometrically at 410 nm by using phenoldisulphonic acid [14]. TN defined as total Kjeldahl nitrogen plus NO<sub>3</sub>-N.

### 3. Results and Discussion

#### 3.1. Vertical Distribution of Total Phosphorus at Ie-1

The long term variation of monitored total phosphorus concentrations are shown in Figure 2. It is indicated from the available data that, in the early stage the total phosphorus concentrations were low at the bottom, but increased after some time due to rapid industrial growth. The loading of total phosphorus into Lake Biwa increased from 1960 to 1975 and then decreased, reflecting economic growth and regulation of total phosphorus [15]. The phosphorus concentrations reached a maximum in 1976 and then declined until 1982, and fluctuated around a stable value thereafter. A water treatment regulation was enforced in 1982, and nutrient loading was progressively reduced and then stabilized after 1985 in Lake Biwa [16]. In Japan, there was an unusually cool and rainy summer in 1993 while an unusually hot and dry summer was recorded in 1994. In 1993, abundant rain and weak thermal stratification supported high nutrient concentrations in the epilimnion. In 1994, nutrients were depleted and a high transparency and low concentration of chlorophyll a were observed in the epilimnion of the Northern Basin. This is because the supply of nutrients was limited owing to little rain and a well-developed thermocline [17]. Over the whole stage, the TP concentration at Ie-1 has been increasing at a rate of  $3.0 \times 10^4 \mu\text{M}$  per month; in other words, the amount of phosphorus contained in the water phase of the entire lake ( $2.75 \times 10^{13} \text{ dm}^3$ ), has increased by  $3.0 \text{ ton yr}^{-1}$ . The total phosphorus concentrations were high in 2000, 2003 and 2006 because of high dissolved organic phosphorus and particulate phosphorus concentrations, respectively [18]. In the surface waters, phosphorus was essentially present in particulate phosphorus form, while bottom waters contained an important fraction of the element in dissolved organic phosphorus and orthophosphate form. The recent increase of total phosphorus at Ie-1 can better be attributed to supply from coastal regions where phosphorus compounds accumulated, and also from the Southern Basin [10].

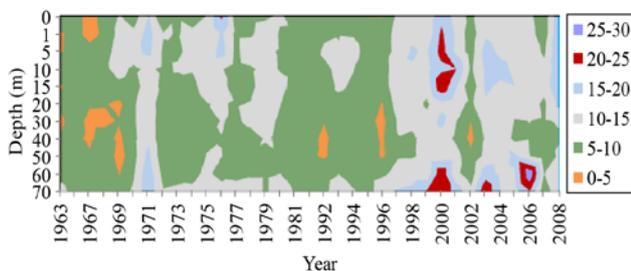


Figure 2. Mean annual vertical distribution of Total phosphorus (ppb) at Ie-1 (0~70 m depths)

#### 3.2. TP Concentration at Nb-5

Since the water depth at Nb-5 is around 3.5 m and stratification hardly occurs throughout the year, the concentration profile of TP along the depth is mostly uniform and, in consequence, the concentration at the surface may well represent the average concentration of TP to the full length of Nb-5. The high TP concentration

in 1963-1982 can be attributed firstly to higher anthropogenic activity in the surrounding urbanized area, secondly to the geological nature of the Southern Basin having a shallower and more gradually shaped bottom [3,19], and, thirdly to high population level of seston [20]. Recently, waters of the Southern Basin showed a greater and more rapid reduction in total phosphorus concentration as compared to the Northern Basin due to improvement in the water quality of tributary streams entering the Southern Basin. The quality was much better at the beginning of the 1990s, compared with the 1980s. In fact, the mean concentration of total phosphorus contained in the river water flowing into the Southern Basin during 1978-1999 was decrease from 455 to 93 mg/L [21], whereas, total phosphorus concentration in the river water flowing to the Northern Basin during 1996-2007 was increased or stable [4]. According to monitored phosphorus records over the whole stage, the mean value was 19.6 (min. 8; max. 37), whereas, the average total phosphorus concentration from 1963 to 1985 and 1996 to 2008 were 21.3 (min. 8; max. 43) and 20.5 (min. 10; max. 37), respectively (see Figure 3).

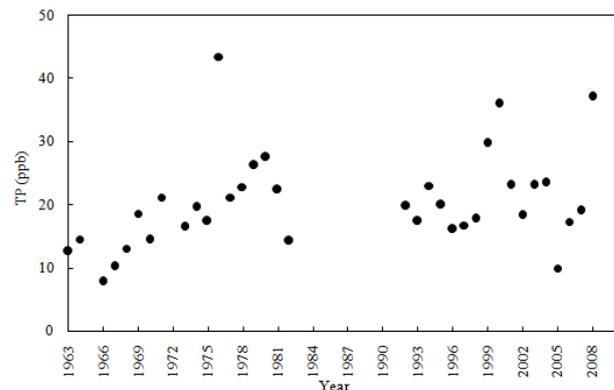
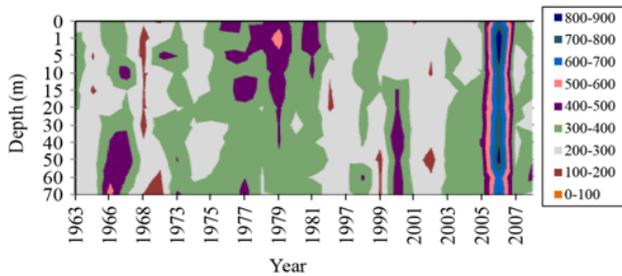


Figure 3. Mean annual variations in Total Phosphorus concentrations at Nb-5

#### 3.3. Vertical Distribution of Total Nitrogen at Ie-1

Since Lake Biwa had an apparently oligotrophic nature in 1963 and exhibited a truly eutrophic nature in 1973, the variation depicted in Figure 4 represents the eutrophication processes in terms of total nitrogen. It is indicated from the available data that, at the bottom (60~70 m depths) in the early stage the total nitrogen concentrations were generally lower mainly due to low nitrate nitrogen. The total nitrogen concentrations were high during 1975 to 1980 due to rapid industrial growth, whereas, after 1981 decreased rapidly due to regulation of law. Most of the nitrogen load during 1976 to 1985 decreased from the non-point sources as the Japanese government tried many ways to control the non-point sources pollution [22]. The high TN concentrations were observed in 2000 and 2006 due to  $\text{NO}_3\text{-N}$  and particulate organic nitrogen, respectively [18]. The Basic Law for Environmental Pollution Control (1967), Water Pollution Control Law (1970), Pollution Prevention Ordinance (1973) and Shiga Prefecture Basic Environment Ordinance (1996) were implemented to control the increased TN concentrations in the Northern Basin. The

submerged macrophyte growth also increased by 15% in the Northern Basin in 2002, compared to 1997 [23]. However, in spite of these various countermeasures, the TN concentrations in the Northern Basin have not improved due to loads from nonpoint sources are unexpectedly greater than those from point sources such as domestic wastewater. Total nitrogen concentration has increased in the Northern Basin due to increased  $\text{NO}_3\text{-N}$  concentration during the past 40 years [24].



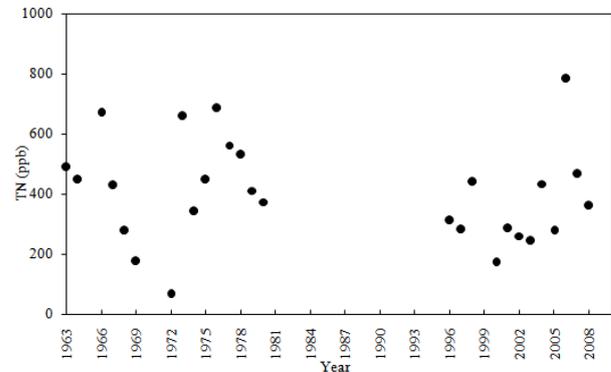
**Figure 4.** Mean annual vertical distribution of Total Nitrogen (ppb) at Ie-1 (0~70 m depths)

### 3.4. TN Concentration at Nb-5

The total nitrogen concentrations were high from 1963 to 1982, likely due to increased anthropogenic activities in the surrounding area. In other words, the Southern Basin was strongly influenced by the input of nitrogen compound over the period. Whereas, in the recent stage low TN concentration at Nb-5 suggests that the TN concentration decreases much faster due to development of macrophyte and improvement of the water quality of tributary streams entering the Southern Basin. The water quality was much more improved in the Southern Basin after 1994 due to development of macrophytes [25]. The area covered by submerged macrophytes reached 80% of the entire area of the south basin in 2006 [26]. Moss (2001) argued that macrophytes are particularly sensitive to TN loading and will show low species diversity at high TN and high diversity at low TN [27]. The dominance of submerged macrophytes usually influences cyanobacteria negatively, and release of allelochemicals by macrophytes is regarded as a major inhibitor of their growth [28]. Faafeng and Mjelde (1998) postulated the following mechanism by which macrophytes may maintain a clear-water state; competition with phytoplankton for nutrient, competition with epiphyte algae for light, and the release of allelopathic substances [29]. The aquatic plants that form dense vegetation, e.g. reed colonies in Lake Biwa, make a great contribution to nutrient binding in the shore zone. In view of these relationships, the recovery of macrophytes presumably played an important role in recent water quality improvement [30].

The TN concentration from nonpoint sources from forest, paddy and farm land is decreasing from 1971 to 2006 due to increasing urbanization of Lake Biwa basin from 12.5% to 21.9%, while forest and agricultural land area decreased from 51.2% to 45.2% and 27.6% to 21.8%, respectively [31]. Another important factor was the improvement in the water quality of the inflow river water at the beginning of the 1990s compared with the 1980s. At Southern Basin, TN concentration from inflow rivers decreased from 2.80 mg/L in 1980 to 1.17 mg/L in 2007 [4]. According to monitored nitrogen records over the

whole stage mean value is 364 (min.66; max.784), whereas, the average total nitrogen concentration from 1963 to 1985 and 1996 to 2008 were 402 (min.66; max. 684) and 359 (min.172; max. 784), respectively (Figure 5). Recently, the decline in total nitrogen concentration is principally the result of a marked decline in total organic nitrogen, but not of  $\text{NO}_3\text{-N}$  at the Southern Basin [8].



**Figure 5.** Mean annual variations in Total Nitrogen concentrations at Nb-5

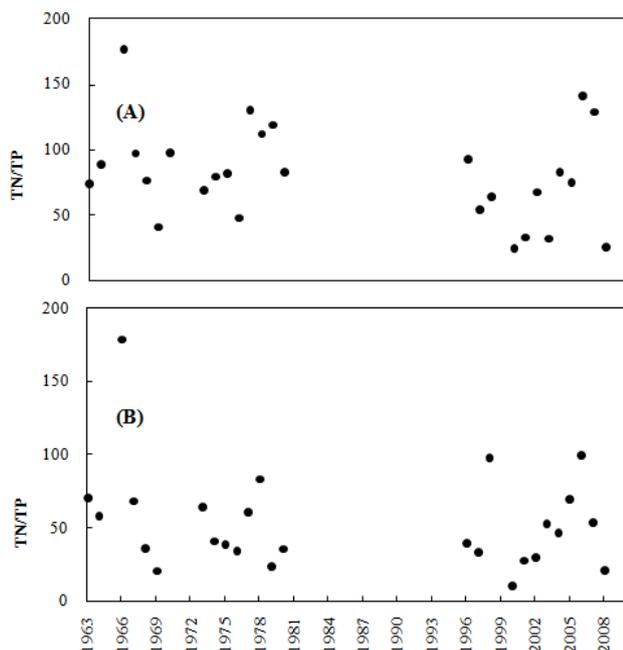
In the first stage, TN concentration at Nb-5 is 2.6 times higher than that of Ie-1, in other words, the Southern Basin was strongly influenced by the input of nitrogen compounds over the period. In the second stage, however, decrease in the nitrogen concentration at Nb-5 than at Ie-1 suggests that the water quality regarding TN is improving faster than in the Northern Basin [18].

### 3.5. Variations in TN/TP Ratio at Ie-1 (0 m) and Nb-5

Although the absolute value of TN may not be a strong predictor of N limitation, the TN/TP ratio may be useful in determining the potentially limiting nutrient. Changes of TN/TP stoichiometry indicate altered patterns of phytoplankton nutrient limitation [32]. The most widely accepted hypothesis about cyanobacterial dominance in low TN/TP ratio was first proposed by Pearsall [33], and later elaborated by Schindler [34] and Smith [35]. This hypothesis maintains that cyanobacteria are masters of survival in environments poor in N and that their dominance in eutrophic systems is related to the low TN/TP ratios of major anthropogenic pollutants.

Tilman (1982) suggested that cyanobacteria should typically be dominant in the lake with a low TN/TP ratio and strong competitors under condition of nitrogen limitation because they can fix new nitrogen from  $\text{N}_2$ , a gaseous source of inorganic nitrogen that is not available for other phytoplankton [36]. Smith compiled extensive data sets for several lakes in which cyanobacteria dominance and TN/TP ratios varied, and showed that cyanobacteria dominance became low when the TN/TP ratios were greater than 65 (by atoms). However, when the ratio was less than 65, the potential for cyanobacteria dominance increased dramatically, and in the special case where lakes showed smaller ratios than 25 the dominance of cyanobacteria became mostly 100% [35]. The phytoplankton growth is limited by phosphorus but not inorganic nitrogen in the south basin of Lake Biwa [37]. Freshwater red tide caused by *Uroglena americana*

propagated suddenly in 1977 and changed the surface water to dark brown color and it has occurred every year from 1977 to 1986 (especially frequently and widespread from 1977 to 1979) in Lake Biwa [38].



**Figure 6.** (A) Variations of annual mean TN/TP ratio at Ie-1(0 m) and (B) that of Nb-5

The TN/TP ratios were discussed at Ie-1 (0 m) and Nb-5 in detail. At Ie-1(0 m), the mean TN/TP ratio over the whole stage was 76 (min. 25; max. 141), whereas, in the first and second stages the mean values were 87 (min. 25; max. 141) and 62 (min. 25; max. 141), respectively. At Nb-5, the mean TN/TP ratio over the whole stage was 50 (min. 11; max. 179), whereas, in the first and second stages the mean values were 53 (min. 41; max. 119) and 46 (min. 25; max. 141), respectively (Figure 6). The low TN/TP ratios in the Southern Basin have the capacity to support large cyanobacterial blooms [39]. From 1997 onwards, bloom forming cyanobacteria have been observed more frequently in the offshore water of the Northern Basin [40]. Tsugeki et al. (2010) showed that phytoplankton biomass had increased during 1960s-1970s and indicated that the Northern Basin had become eutrophied. Since the 1980s, however, algal remains have decreased or stabilized [41]. The TP showed a significant positive correlation with phytoplankton biomass and biomass in the 2000s was almost half of that in the 1980s [16]. At the Northern and Southern Basins of Lake Biwa, TN/TP ratios have been reported by several researchers; Ichise et al. [42] reported that during the period of 1979 to 1999, the mean of TN/TP ratios in the Northern and Southern Basins increased, respectively, from 67 to 104 and from 29 to 48, with the decrease of population of phytoplankton. According to Environmental White paper Shiga Prefecture [43], the mean of TN/TP ratios at the Northern and Southern Basins during 1993-2000 were found to be 91 and 43, respectively. Nakanishi et al. (2001) reported that the mean in the respective Basins were 77 and 41 during 1980-1992 and 134 and 99 during 1993-2000 and pointed that the higher TN/TP ratios observed at the Northern and Southern Basins and such high TN/TP ratios compared to the other lakes seemed to be one of the

limnological characteristics of Lake Biwa. Recently, more high TN/TP ratios were observed at the Southern Basin due to decrease of the total phosphorus concentration in inflow river waters and increase of the macrophyte growth in the basin [44].

## 4. Conclusion

Over 45 years, trend of total phosphorus and total nitrogen in the Northern and Southern Basins were reported. As a result, it was found that TP was still increasing in the Northern and Southern Basins. On the other hand, TN increased slightly in the Northern Basin, but decreased in the Southern Basin. Particularly, in the recent second stage, the decreases in TP and TN and the slight increase in the TN/TP ratio at Nb-5 were noted as the first signs of the restoration of water quality in the Southern Basin. However, enough information is not available to draw sturdy conclusions about the Southern Basin due to a significant lack of data on bioactivities in the water mass. However, based on a 45 year study (1963-2008) of limnological records for Lake Biwa it is suggested that the water quality of the Southern Basin is improving much faster than in the Northern Basin. Recently, the TN/TP ratio has shown a tendency to increase, suggesting a certain reduction in algal blooms to be expected in the Southern Basin. It is also concluded that any reduction of external nutrients has a quicker response in the Southern Basin of Lake Biwa.

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