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Environmental chemistry of rivers and lakes. Part IX†. Variations in the total–nitrogen and –phosphorus concentration and their compositions in the last 12 years in Lake Biwa, Japan.

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Abstract In order to find useful measures to elucidate trophic status, future changes and the degree of natural cleaning processes in Lake Biwa, a series of thirty-three (once every three months) observations of total-nitrogen (TN) and total-phosphorus (TP) were carried out over the last 12 years from September, 1996 to May, 2008, along with fractional analyses of TN into dissolved organic-nitrogen (DON) and particulate-nitrogen (PN) and of TP into dissolved organic-phosphorus (DOP) and particulate-phosphorus (PP). The mean and standard deviation (SD) of the TN/TP ratios and those of the concentrations of DON, PN, DOP, and PP were calculated for the Northern (represented by sampling station Ie–1) and Southern Basins (Nb–5) of the lake. The mean values with SD are expected to be practical measures to elucidate the usual or ordinary trophic status of the lake water and specify water masses with unusual status compared with the usual levels. In the present study, we really noticed unusually high values in TN/TP ratios three times at Ie–1 and once at Nb–5, although no clear explanation for the values was obtained because of a lack of monitoring of bioactivities in the water mass. It is also noteworthy to point out the coincidence between the unusually high ratios in TN/TP and PN/PP.

Keywords : Lake Biwa, Total-nitrogen, Total-phosphorus, TN/TP ratio, Fractional composition, Phytoplankton

Introduction

In 1988, Tezuka[2] noted a coincidence between the appearance of *Anabaena* blooms and nitrogen depletion in the Southern Basin of Lake Biwa. He also reported that during the blooms, the concentrations of TP (total-phosphorus) and PP (particulate-phosphorus) were higher than usual. It was also reported that *Anabaena* blooms could induce phosphorus enrichment in back-streaming water from the Southern Basin of Lake Biwa.[3] In the basin, the so-called freshwater red tides due to the bloom of a species of flagellate algae, *Uroglena Americana*, have been reported in summer since 1977. Moreover, blooms of *Microcystis* sp. have been observed every year since 1985.[4, 5]

The objective of the present study was to elucidate the range of variations in DON (dissolved organic-nitrogen), PN (particulate-nitrogen), and TN (sum of DON and PN) along with the variations in DOP (dissolved organic-phosphorus), PP (particulate-phosphorus), and TP (sum of DOP and PP). In addition, variations in the ratios of TN/TP, DON/DOP, and PN/PP were monitored with time, and coincidence in their variations was analyzed.

†) For part VIII, see reference 1.

Observation Settings

Water samples were collected from sampling stations Ie-1 and Nb–5 (see Fig.1 in reference 1). The geological and limnological characteristics of Lake Biwa and details about sampling stations were described in a previous paper.[1] The present observations of the TN/TP ratios and the fractional composition at sampling stations Ie–1 and Nb–5 cover a period of 12 years from September, 1996 (t=402 months, which is the time elapsed since May, 1963) to May, 2008 (t=540). Whole data termed as "NUTR-observation" are available from http : //w.w.w.oceanochemistry.org/

Methods for chemical analysis

Analytical methods for P(A), Kj-N(A), Am-N and NO_3-N were described previously.[1]

Dissolved-phosphorus, P (FA) : A water sample was filtered by using a Nuclepore@ membrane filter with a pore size of 0.40 μm. The filtered samples were treated in the same manner as for P(A).[1] Here, P(FA) denotes phosphorus contained in filtrable compounds such as orthophosphate, phytates and humic substances.

Orthophosphate, P(FN) : On each filtered sample, PO_4-P was quantified by indirect spectrophotometry at 545 nm.[6] As per the analytical procedure, P(FN) is essentially the same as PO_4-P.

Dissolved organic-phosphorus (DOP) and particulate-phosphorus (PP) : DOP and PP were calculated, respectively, from the differences between P (FA) and P (FN) and between P(A) and P(FA).

The presence of dissolved inorganic phosphorus compared to DOP. Kj-N (FA) : A filtered sample using a Nuclepore@ with a pore size of 0.40 μm was subjected to Kjeldahl digestion[7] and the resulting ammonia-N was analyzed spectrophotometrically at 660 nm according to the indophenol method.[8]

Dissolved organic-nitrogen (DON) and particulate-nitrogen (PN) : DON and PN were calculated, respectively, from the differences between Kj-N(FA) and relatively-trace of Am-N and between Kj-N(A) and Kj-N(FA).

Results and Discussion

Variation in the TN/TP ratio over the last 12 years in Lake Biwa

The nitrogen to phosphorus ratio (N/P) has frequently been used as a key indicator in predicting algal biomass.[9] It can also serve as an index that represents the nutrient limitations for algal growth.[10] The atomic ratio of N/P=16/1, which is known as the Redfield ratio, has generally been used to describe the average elemental composition of phytoplankton.[11–13] Some limnologists believe that cyanobacteria dominance is linked to the concentration of limiting nutrients rather than to the nutrient ratio.[14] Downing *et al*.[15] reported that the respective N/P ratios of phytoplankton and aquatic macrophytes range from 16 to 22 and 13 to 40. In 1983, Smith[10] compiled extensive data sets for several lakes that varied in cyanobacteria dominance.

Figure 1A shows the variation in the TN/TP ratio, observed (three-monthly observation) at the surface of Ie-1 over the 12 years from September, 1996 (t=402) to May, 2008 (t=540 months). Here, the ratio of TN/TP=65/1 is shown by the thicker dotted-line as a reference according to Smith,[10] along with the so-called Redfield ratio[16] of N/P=16/1, which is shown by the thinner dotted-line.

It is seen from Fig. 1A that unusually high ratios were recorded at t=499, 516, and 528 at Ie–1. Although such unusually high ratios are included, the mean (±SD) of the TN/TP ratio is temporally calculated as 62±40.

Figures 1B and 1C show variations in the concentrations of TN (μM) and TP (μM), respectively. As can be seen from the Figures, extremely

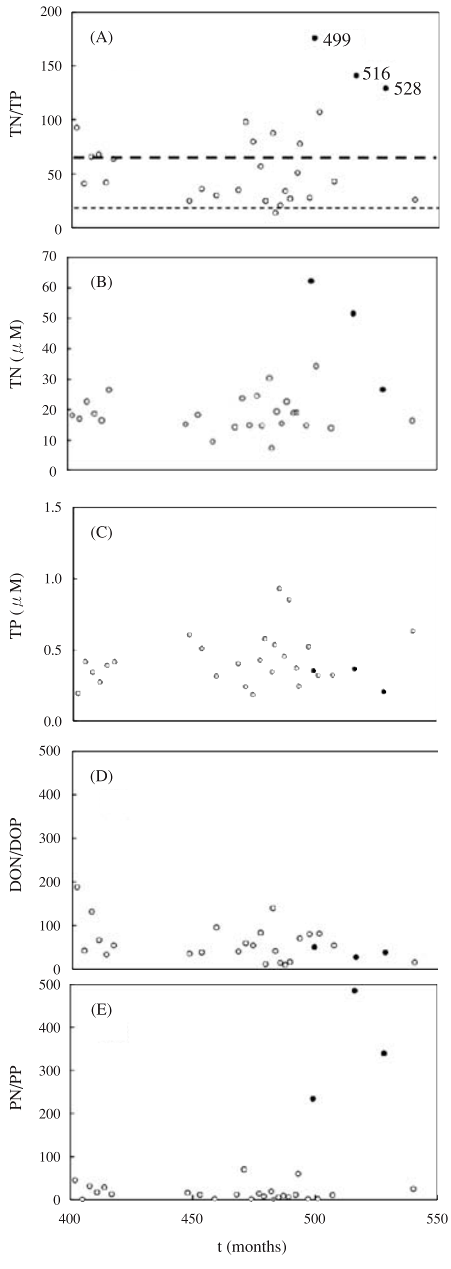

Fig. 1 Variations (every three months) in (A) TN/TP, (B) TN, (C) TP, (D) DON/DOP, and (E) PN/PP observed at Ie-1. Here, t in months shows the time elapsed from May, 1963. In Fig. 1A, TN/TP levels of 16/1 proposed by Redfield and 65/1 by Smith are indicated, respectively, by the thinner and thicker dotted-lines. In the same Figure, unusually high TN/TP values are indicated by closed circles, which occurred at t=499, 516, and 528. These data points are excluded for the calculation of the mean (±SD). For details on the closed circles, see also the text.

high concentrations of TN are observed at t=499 and 516, but the TN concentration at t=528 is normal. Obviously, it can be said that such high TN/TP ratios at t=499 and 516 arise mainly from the unusually high concentrations of TN (Fig. 1B) because the corresponding TP concentrations (Fig. 1C) are normal. In contrast, the high TN/TP at t =528 is due to the low concentration of TP against the normal TN. Thus, extremely high TN/TP ratios above 120 (Fig. 1A) were recorded due to either unusually high TN concentrations at t=499 and 516, or unusually low TP at t=528. Although the reasons for such high ratios of TN/TP are unclear, the mean (±SD) of TN/TP at Ie-1 was evaluated as 51±27 by excluding the data points at t=499, 516, and 528. Incidentally, the TN/TP thus evaluated for the recent period (1996–2008) at Ie-1 is obviously lower than 87±37, which was evaluated for the earlier period (1963–1982).[1)]

Figures 1D and 1E show the variation in the ratios of DON/DOP and PN/PP. In Fig. 1E, extremely high PN/PP ratios are recorded, again at t=499, 516, and 528, while the DON/DOP ratio (Fig. 1D) at each time is fairly normal. Although there is no clear explanation for such a high PN/PP, by temporally excluding these data points from the calculation, means (±SD) of DON/DOP and PN/PP were evaluated as 61±44 and 17±18, respectively. The variations in DON, DOP, PN, and PP will be shown in the next section.

In a similar manner, at Nb-5 the variations in the TN/TP ratio and concentrations of TN(μM), and TP(μM) were observed, and the results are featured in Figs. 2A∼2C.

As shown in Fig. 2A, an extremely high TN/TP ratio above 180 is recorded at t=485. By comparing Figs. 2B and 2C, the high ratio is attributable to the unusually low TP concentration which occurs at t=485 because the corresponding TN remained normal. By excluding the data point

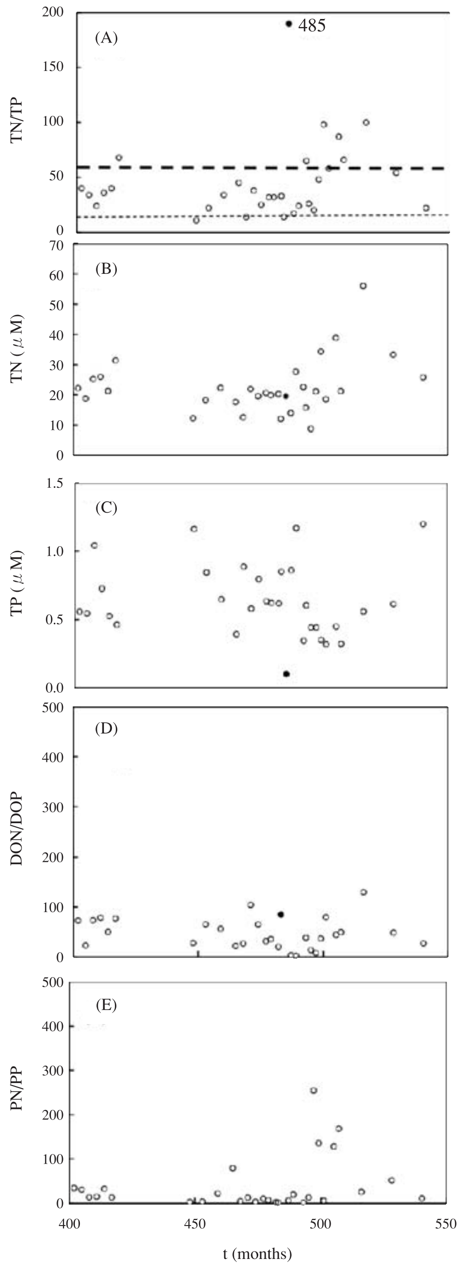

Fig. 2 Variations (every three months) in (A) TN/TP, (B) TN, (C) TP, (D) DON/DOP, and (E) PN/PP at Nb-5. The closed circles in Fig. 2A show that the TN/TP ratio was unusually high at t=485 and this was excluded from the calculation of the mean (±SD). The thicker and thinner dotted–lines are as in Fig. 1A. For details on the closed circles, see also the text.

at t=485 from the calculation, the mean (±SD) of the TN/TP is evaluated as 46±36. The value thus evaluated for the recent period (1996–2008) at Nb–5 is slightly lower than the 53±31 that was evaluated for the early period (1962–1982).[1]

In conclusion, it can generally be said that the mean (±SD) of the TN/TP ratio in the Southern Basin is lower than that in the Northern Basin in both the early and recent periods.

The means (±SD) in the variations of DON/DOP (Fig. 2D) and PN/PP (Fig. 2E) were evaluated as 47±32 and 17±18, respectively. From a comparison of the means (±SD) of the DON/DOP and PN/PP ratios in the recent period (see also Figs. 1D and 1E), it can be said that the DON/DOP ratio in the Southern Basin is lower than that in the Northern Basin, whereas the PN/PP ratio is virtually the same in both basins. These ratios can also be used as a useful tool for evaluating the nutritional status of phytoplankton in lakes.[16–18]

Besides, in the present study we found the unusually high values for TN/TP ratios three times at Ie–1 and once at Nb–5. Although no clear explanation for the values was obtained, it is noteworthy to point out the coincidence between the unusually high TN/TP (Fig. 1A) and PN/PP ratios (Fig. 1E) in the Northern Basin.

In the previous paper,[1] it was reported that a higher rate in the increase of the TN/TP ratio at Nb–5 was observed than that at Ie–1, and it was assumed that water quality in the Southern Basin water improved much faster than the Northern Basin in recent years. In fact, such changes in the water quality were already reported by several researchers in relation to the bioactivities in the lake. For instance, Yoshida[19] carried out yearly observations 1980 to 1993 near the coast in the Southern Basin and reported that the TN and TP concentrations varied, respectively, between 19.6 and 29.6 μM and between 0.38 and 1.00 μM. These results (1989–1994) were basically the same as those obtained in the present observation (1996–2008) ; in other words, it is suggested that the trophic status of the Southern Basin regarding variations in TN and TP has been virtually unchanged for the last 30 years since 1980. As for the status in the early period, Harashima *et al*.[20] reported that loading of TP into Lake Biwa increased from 1960 to 1975 and then decreased, reflecting economic growth and regulation of P loading after that. Loading of TN also increased similarly to that of TP and slightly increased in 1975. In the Northern Basin, Okamura[21] reported that TP concentration varied in a range of 0.004–0.007 mg/l (0.13–0.23 μM) from 1983 to 1990, but the range rose to 0.009–0.017 mg/l (0.29–0.55 μM) after 1991 and maintained the higher level after that. In the same basin, Tsugeki[22] reported that the TN concentration increased from 0.27 mg/l (19.3 μM) to 0.33 mg/l (23.6 μM) from 1980 to 2000, whereas the TP concentration did not change greatly after 1980.

As for the improvement in the lake water, Hamabata and Kobayashi[23] reported two main factors ; one was an increase in macrophyte growth and the other was an improvement in water quality entering the river. Imamoto *et al*.[24] reported that submerged macrophytes increased by 73% in the Southern Basin and 15% in the Northern Basin in 2002, compared with 1997. Taguchi *et al*.[25] reported that aquatic plants that form dense vegetation, *e. g*., reed colonies in Lake Biwa, make a great contribution to nutrient binding in the shore zone. Nakano *et al*.[26] reported that a negligible abundance of nitrogen-fixing cyanobacteria may have a negative effect on cyanobacterial growth by macrophytes in the Southern Basin.

Thus, the present observation revealed usual and unusual levels in the variations of TN/TP, DON/DOP, and PN/PP in both the Northern (Ie–1) and Southern basins (Nb–5) in terms of the mean ($\pm$SD).

Variations in the fractions of DON and PN in TN and those of DOP and PP in TP

Figures 3A〜3D show the concentrations of DON, PN, DOP, and PP observed at Ie–1 during the period t=402〜540 ; here, the data points at t=499, 516, and 528 are shown by closed circles because the TN/TP ratios (Fig. 1A) at these points became extremely high. From the respec-

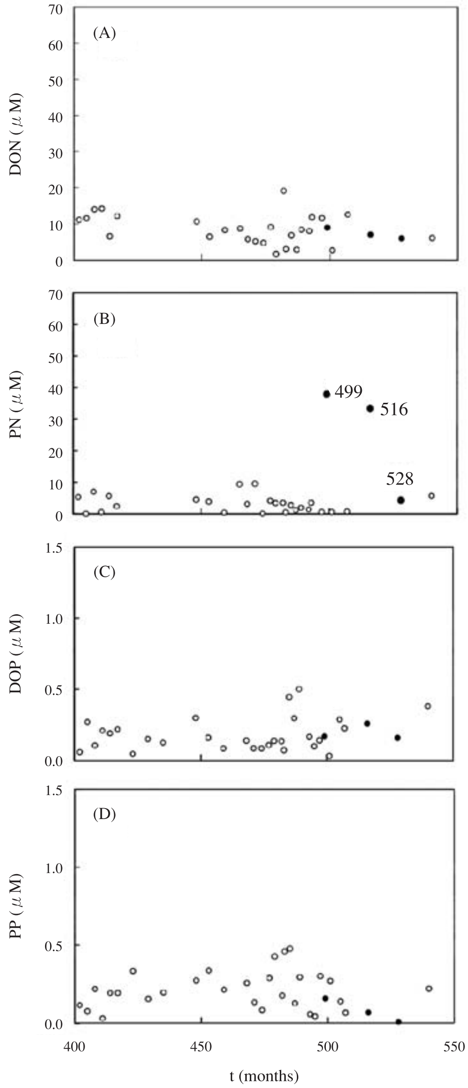

Fig. 3 Variations in (A) DON, (B) PN, (C) DOP, and (D) PP observed at the surface of Ie-1. The data points indicated by closed circles are as in Fig. 1.

tive Figures and by excluding the three data points shown by closed circles, the mean concentrations in μM($\pm$SD) were evaluated as follows: DON$=$ 8.6$\pm$4.2; PN$=$3.2$\pm$2,7; DOP$=$0.18$\pm$0.12; PP $=$0.21$\pm$0.12.

As for Nb–5, the mean concentrations ($\pm$SD) were similarly evaluated from Figs. 4A～4D: DON$=$7.9$\pm$5.3; PN$=$7.1$\pm$6.1; DOP$=$0.20$\pm$ 0.11; PP$=$0.45$\pm$0.26. As described above, in the calculations the data point at t$=$485 was excluded.

Before the present study, the fractions of DON, PN, DOP, PP have been observed by other researchers as measures of the quality of lake water.

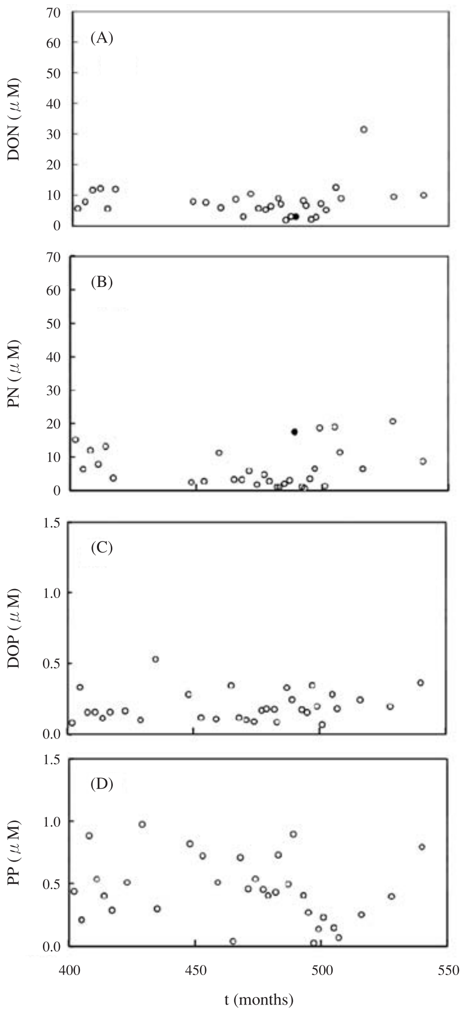

Fig. 4 Variations in (A) DON, (B) PN, (C) DOP, and (D) PP observed at Nb-5. The data points indicated by closed circles are as in Fig. 2.

As for DON, its importance was emphasized as a potential source of available N for phyto-plankton[27–30] and algae.[31] There have been suggestions that increased DON levels may be responsible for the rising incidence of toxic phytoplankton.[32, 33] Takahashi *et al*.[34] reported that DON and Am-N were preferentially utilized by phytoplankton rather than nitrate at higher concentrations. Nakanishi *et al*.[35] reported that in the Southern Basin of Lake Biwa, the DON concentration was stable, varying in a range from 10 to 16 μM, while PN was the most variable and ranged from 6.6 to 24 μM.

As for DOP, there is growing evidence that in freshwater it is a bioavailable nutrient source. In particular, several studies have shown that P from DOP can be taken up by phytoplankton *via* enzymatic hydrolysis depending on competitive strength and storage capacity.[36–38] Inorganic-phosphorus (as orthophosphate) is easily utilized by primary producers, but past and recent studies also indicate their ability to use DOP as a source of P.[16, 40, 41]

Tezuka reported that PP comprises the major part of TP in Lake Biwa and that PP in lake water is potentially bioavailable, that is, most PP can eventually be converted to inorganic-phosphorus.[42] In the Northern Basin of Lake Biwa, phytoplankton largely used DOP to sustain their growth.[43, 44]

As reviewed above, not only TN and TP, but also their fractions DON, PN, DOP, and PP are effective measures, especially for the propagation of phytoplanktons and macrophytes and for changes in dominancy of certain species of plankton. Unfortunately, however, the present study did not observe bioactivities. The means and variances (as $\pm$SD) evaluated in the present study give the usual or normal level for the respective items to estimate the status of lake water both in the Northern and Southern Basin. These values also provide reference levels for the future status of bioactivities and natural cleaning process which may occur in both the Northern and Southern Basins. Limnologically unique characteristics of the water mass will easily or clearly be noticed in the unusually high ratios of TN/TP and PN/PP, which occurred three times at Ie–1 in the last 12 years.

Conclusion

Based on the data known as the "NUTR-observation", variations in TN (together with its components DON and PN) and TP (with its components DOP and PP) in the last 12 years from September, 1996 (t=402 months) to May, 2008 (t=540) in the Northern (Ie–1) and Southern Basins (Nb–5) in Lake Biwa were examined. By comparing the ratios of TN/TP and PN/PP, several unusually high values were noticed. Although such extreme values were temporally excluded from the calculation, the means (±SD) of the variations during the period 1996–2008 for such items as TN/TP, TN, TP, DON/DOP, PN/PP, DON, DOP, PN, and PP were evaluated in both the Northern (at Ie–1) and Southern Basins (Nb–5) as follows : For Ie–1 [and Nb–5 in brackets], TN/TP 51±27 [41±24] ; TN =19±6 [22.7±9.2] μM ; TP=0.43±0.18 [0.65±0.25] μM ; DON/DOP=61±44 [47±32] ; PN/PP=17±18 [17±18] ; DON=8.6±4.2 [7.9±5.3] μM ; DOP=0.18±0.12 [0.20±0.11] μM ; PN=3.2±2.7 [7.1±6.1] μM ; PP=0.21±0.12 [0.45±0.26] μM. The means (±SD) give practical measures to assess the trophic status of Lake Biwa now and in the future. Although unusually high TN/TP ratios were observed three times at Ie–1 and once at Nb–5 over the 12 years, no clear explanation for these ratios was possible because of a lack of data on the bioactivies in the water mass.

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河川と湖沼の環境化学第 IX 報
最近 12 年間に琵琶湖で観測された全リンと全窒素の濃度
並びにそれらの組成の変動について

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概要 1996 年 9 月から 2008 年 5 月に至る 12 年間，びわ湖水中の全窒素（TN）と全リン（TP）濃度をほぼ 3 ヶ月に一回の頻度で連続観測した．またこの観測では，全窒素の画分である溶存態有機窒素（DON）と懸濁態窒素（PN）並びに全リンの画分である溶存態有機リン（DOP）と懸濁態リン（PP）濃度も併せて調査した．びわ湖の北湖（代表点 Ie–1）と南湖（Nb–5）のそれぞれに対して，この 12 年の観測結果を，観測項目毎の平均値（±標準偏差）として表すと次のようになる．すなわち，Ie–1 については [Nb–5 については角括弧内に示す]，TN/TP 51±27[41±24]；TN＝19±6[22.7±9.2]μM；TP＝0.43±0.18[0.65±0.25]μM；DON/DOP＝61±44[47±32]；PN/PP＝17±18[17±18]；DON＝8.6±4.2[7.9±5.3]μM；DOP＝0.18±0.12[0.20±0.11]μM；PN＝3.2±2.7[7.1±6.1]μM；PP＝0.21±0.12[0.45±0.26]μM であった．本研究で得た各項目の平均値と，標準偏差で表現した変動幅は，びわ湖の水質の現状を客観的に評価するための有用な尺度になると考えた．すなわち，観測された栄養状態に関する数値について，それが異常値なのか平常値なのかを判定する基準を得たことになる．これは，将来の水質観測においても同様な働きをすると考えた．なお，今回の観測では，Ie–1 で 3 回，Nb–5 で 1 回，異常に高い TN/TP 比を観測した．しかし，残念ながら生物学的調査を並行して実施していなかったので，異常値出現の理由は不明のままに終わった．加えて特筆できる点は，TN/TP 比に異常値が現れるときには PN/PP 比にも異常値が同期して現れることである．