TheScientificWorldJOURNAL
www.thescientificworld.com

Prediction of *Microcystis* Blooms Based on TN:TP Ratio and Lake Origin

Yoshimasa Amano[1,*], Motoi Machida[1], Hideki Tatsumoto[1], Dennis George[2], Sharon Berk[2], and Kazuo Taki[3]

[1]*Graduate School of Engineering, Chiba University, Chiba, Japan;* [2]*Center for the Management, Utilization and Protection of Water Resources, Tennessee Technological University, Cookeville, Tennessee, U.S.A.;* [3]*Faculty of Engineering, Chiba Institute of Technology, Chiba, Japan*

E-mail: amano.yoshimasa@nies.go.jp

We evaluated the relationship between TN:TP ratio and *Microcystis* growth via a database that includes worldwide lakes based on four types of lake origin (dammed, tectonic, coastal, and volcanic lakes). We used microcosm and mesocosm for the nutrient elution tests with lake water and four kinds of sediment (nontreated, MgO sprinkling treated, dissolved air flotation [DAF] treated, and combined treated sediment) in order to control TN:TP ratio and to suppress *Microcystis* growth. *Microcystis* growth was related to TN:TP ratio, with the maximum value at an optimum TN:TP ratio and the minimum values when the TN:TP ratios reached to 0 or ∞. The kurtosis of the distribution curve varied with the type of lake origin; the lowest kurtosis was found in dammed lakes, while the highest was found in volcanic lakes. The lake trophic state could affect the change in the kurtosis, providing much lower kurtosis at eutrophic lakes (dammed lakes) than that at oligotrophic lakes (volcanic lakes). The relationship between TN:TP ratio and *Microcystis* growth could be explained by the nutrient elution tests under controlled TN:TP ratios through the various sediment treatments. A significant suppression of *Microcystis* growth of 70% could be achieved when the TN:TP ratios exceeded 21. Lake origin could be regarded as an index including morphological and geographical factors, and controlling the trophic state in lakes. The origin rather than trophic state for lakes could be considered as an important factor of TN:TP influences on *Microcystis* growth.

KEYWORDS: *Microcystis* growth, prediction, TN:TP ratio, lake origin, sediment treatment

INTRODUCTION

The outbreak of algae blooms is a worldwide problem[1,2]. Negative effects of algae blooms include odors, fish mortality, and toxin production[3,4,5]. One of the most common nuisance species that forms water blooms is blue-green alga, mainly *Microcystis* species[6,7]. *Microcystis* blooms are related to factors such as light intensity, temperature, and nutrient concentrations, such as nitrogen and phosphorus. These factors have been studied in detail for *Microcystis* growth[8,9,10,11].

The ratio of total nitrogen (TN) to total phosphorus (TP) concentration by weight (TN:TP ratio) influences the occurrence of *Microcystis* blooms. Several studies have reported that *Microcystis* blooms favor the low TN:TP ratio[12,13,14] because *Microcystis* grows well at high phosphorus concentrations[15,16], it is not nitrogen-fixing cyanobacteria, and is thus not a good nitrogen competitor[17,18].

The TN:TP ratio impacts on *Microcystis* imply a balance of nitrogen and phosphorus concentrations, and *Microcystis* could achieve a significant growth at the balanced TN:TP ratio. Contrarily, unbalanced TN:TP ratios could result in the limited growth of *Microcystis*. Neither laboratory nor on-site experiments have shown the quantitative model between TN:TP ratio and the growth of *Microcystis*.

The objective of this study was to reveal the relationship between TN:TP ratio and the growth of *Microcystis* (Chlorophyll a [*Chl.a*] concentration) via a database that includes worldwide lakes based on four types of lake origin (i.e., dammed, tectonic, coastal, and volcanic). A reliability of the relationship was verified through nutrient elution tests by microcosm and mesocosm with lake water and four kinds of sediment: nontreated, MgO sprinkling treated, dissolved air flotation (DAF) treated, and the combination of the MgO and the DAF treatments for the control of TN:TP ratio. The relationship between lake origin and trophic state was also investigated. The importance of lake origin for prediction of *Microcystis* growth is discussed.

QUANTITATIVE RELATIONSHIP BETWEEN TN:TP RATIO AND *MICROCYSTIS* GROWTH

The growth of *Microcystis* is generally limited by nitrogen concentration at low TN:TP ratios, and also limited by phosphorus concentration at high TN:TP ratios. Alternately, maximum growth is achieved at the balanced TN:TP ratio or the optimum TN:TP ratio[19]. *Microcystis* growth, therefore, would be represented as a type of Gaussian distribution curve as a function of TN:TP ratio, which generally reveals a similar relationship between the gross photosynthetic rate (P_g) and light intensity (I)[20] as:

$$\frac{P_g}{P_{max}} = \frac{I}{I_{opt}} \exp\left(1 - \frac{I}{I_{opt}}\right) \tag{1}$$

where P_{max} and I_{opt} are the maximum gross photosynthetic rate and the optimum light intensity, respectively. The value of P_g/P_{max} in Eq. (1) increases with increase in I and goes up to the maximum value at $I = I_{opt}$. Afterward, the P_g/P_{max} decreases with increase in I because of the photoinhibition effect.

Under enough solar energy, *Microcystis* growth would be supposed to be represented as follows:

$$\frac{C}{C_{max}} = \left\{\frac{R}{R_{opt}} \exp\left(1 - \frac{R}{R_{opt}}\right)\right\}^{\alpha} \tag{2}$$

where C and R are the *Chl.a* concentration and the TN:TP ratio, C_{max} and R_{opt} are the maximum *Chl.a* concentration and the TN:TP ratio at C_{max}, and α is defined as the coefficient expected to differ among the types of lake origins. The physical characteristic of α is described below.

Eq. (2) could be characterized by a grouping of lakes as long as the trophic state is similar, where the maximum *Chl.a* concentration can be used as C_{max} and its TN:TP ratio as R_{opt}. In Eq. (2), the distribution curve for C/C_{max} in grouped oligotrophic lakes could entirely show the lower values for the corresponding R/R_{opt}, representing that the kurtosis of the distribution would be higher or sharper than that in grouped eutrophic lakes. Hence, the shape of the distribution curve would be expected to differ according to lake trophic state, i.e., TN and TP concentrations. Because the C/C_{max} values for each group of lakes show 1 at $R = R_{opt}$, the kurtosis of the distribution could be represented by α. The kurtosis could increase with the

increase in the value of α. Thus, the variation of trophic state on the grouped lakes could be expressed by α in Eq. (2).

It has been suggested that the lake trophic state would be controlled by the depth, the area of watershed, and the ratio between the area of watershed and surface area[21]. A study for characteristics of water quality on geographical factors reported by Taki and Tanaka[22] indicated that chemical oxygen demand (COD) was in inverse proportion to Secchi disc transparency (SD), and the relationship between COD and SD was featured by lake origin. Based on these reports, the lake trophic state would eventually be controlled by the geographical and physical factor, the so-called lake origin, and accordingly, α in Eq. (2) could be regarded as the coefficient related to the lake origin. Lakes with shallow depth, a large area of watershed, and subsequent large amounts of sedimentary phosphate generally cause a eutrophic condition and, consequently, a high *Chl.a* concentration. For those lakes, α would be given as a low value. On the other hand, oligotrophic lakes and a low *Chl.a* concentration result from a small area of watershed, deep depth, and small amount of sedimentary phosphate. For such lakes, α would show a high value.

Microcystis growth, therefore, could be expressed by TN:TP ratio and the coefficient α. The finding of α values for lake origins would enable us to predict *Microcystis* growth. In the latter section, the values of α for four types of lake origins are revealed through data analysis of worldwide lakes. The laboratory experiment for the control of TN:TP ratio by means of lake sediment treatment was also conducted to examine the relationship between TN:TP ratio and the growth of *Microcystis*.

MATERIALS AND METHODS

Data Set of the Lakes

The relationship between TN:TP ratio and *Microcystis* growth was examined based on a database of 27 worldwide natural lakes[23,24,25,26,27,28,29,30], which provides algal species including *Microcystis* species, TN and TP concentrations, TN:TP ratio, *Chl.a* concentration, and lake origin. All lakes are listed in Table 1 and include the name, origin, location, trophic state, surface area, mean water depth, water temperature, and pH values. The trophic state for these lakes shows that 22 lakes were eutrophic and five were mesotrophic. The information on origin for these lakes is also represented as 12 lakes that are categorized as dammed lakes, three lakes as tectonic lakes, and six lakes as coastal and volcanic lakes, respectively.

Control of TN:TP Ratio by Removing Organic Matter in Sediment

The TN:TP ratio was experimentally controlled by removing organic matter from sediment through the DAF method. The sediment was obtained from Lake Tega, located at 35°50′N, 140°03′E. The lake has a small surface area of 6.5 km^2, shallow water depth of 0.9 m, and is well known as one of the most eutrophic lakes in Japan. The sediment was collected using Ekman-Birge–type bottom sampler at the middle part of the lake. The collected sediment was carried to the laboratory and stored at room temperature under the direct rays-free condition.

Fig. 1 shows an apparatus for the DAF treatment. As a procedure of the DAF process, the sediment treatment tank (a) and the circulation tank (b) were filled with 130 l of tap water and microbubbles were generated by the microbubble generator (c). Afterward, 7 kg of the sediment (wet volume) was charged from the top of the sediment treatment tank. At the same time, the inside of the sediment treatment tank was stirred using mixer equipment (d). The beginning of the DAF process was set as 0 min (t = 0) when the sediment was charged, and the flocked and accumulated organic matter (froth) at the upside of sediment treatment tank was removed by aspirating at t = 10, 20, 30, and 40 min. As a coagulant, the polyferric sulfate solution (200 mg-Fe l^{-1}) was injected with the dosage of 0.5, 1.0, and 2.5 l at t = 0, 20, and 35 min

TABLE 1
Classifications of Worldwide Natural Lakes where *Microcystis* has Appeared Based on Lake Origin and Trophic State
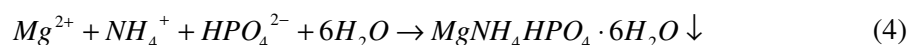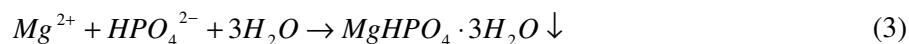
Name	Location	Origin	Trophic state	Surface Area (km²)	Mean water depth (m)	Water temperature (°C)	pH	Reference
L. Karasu	39°44'N, 140°05'E	Dammed	mesotrophic	< 1	5*	28	8.7	[23]
L. Sohara	37°41'N, 140°04'E	"	"	< 1	5	28	7.3	[24]
L. Onogawa	37°40'N, 140°06'E	"	"	1	21*	25	7.3	"
L. Oike	38°12'N, 138°23'E	"	eutrophic	< 1	2	26	9.2	"
L. Asahi	37°14'N, 138°22'E	"	"	< 1	1	33	7.8	"
L. Unoike	37°13'N, 138°21'E	"	"	< 1	2*	32	8.5	"
L. Amagaike	37°13'N, 138°20'E	"	"	< 1	< 1	33	9.2	"
L. Hasuike	36°43'N, 138°30'E	"	"	< 1	< 1	25	9.4	[26]
L. Wakuike	36°35'N, 138°04'E	"	"	< 1	7	30	8.9	"
L. Jonuma	36°14'N, 139°33'E	"	"	1	1	31	8.4	"
L. Teganuma	35°51'N, 140°05'E	"	"	7	1	30	10.4	[25]
L. Inbanuma	35°46'N, 140°13'E	"	"	12	2	30	9.4	"
L. Buhi	13°05'N, 123°05'E	Tectonic	eutrophic	17	7	28	8.2	[29]
L. Kinneret	32°50'N, 35°35'E	"	"	170	26	28	8.7	"
L. Suwa	36°03'N, 138°05'E	"	"	1330	5	26	9.6	[30]
L. Kasumigaura	36°02'N, 140°24'E	Coastal	eutrophic	168	3	27	9.3	[25]
L. Kitaura	36°01'N, 140°34'E	"	"	34	5	27	8.7	"
L. Sotonakaura	35°55'N, 140°36'E	"	"	6	9	28	8.8	"
L. Sanaru	34°43'N, 137°42'E	"	"	1	1	30	9.7	[27]
L. Hanareko	35°41'N, 135°03'E	"	"	< 1	3	28	8.4	"
L. Oike	32°37'N, 129°50'E	"	"	< 1	5	29	8.7	[28]
L. Rotorua	38°05'S, 176°15'E	Volcanic	eutrophic	80	11	20	6.7	[29]
L. Trasimeno	43°09'N, 12°06'E	"	"	124	5	21	9.3	"
L. Imutaike	31°49'N, 130°28'E	"	"	1	1	26	6.9	[25]
L. Sumiyoshiike	31°46'N, 130°36'E	"	"	< 1	23	27	8.2	"
L. Ikedako	31°14'N, 130°34'E	"	mesotrophic	11	126	28	8.0	"
L. Unagiike	31°13'N, 130°36'E	"	"	1	35	30	8.4	"

*Maxmum water depth (m)

from the coagulation injection point (e) on the side of the sediment treatment tank. The timing and dosage of the injection provided the highest efficiency to remove the organic matter in the DAF treatment process[31]. After the separation of the organic matter from the sediment, the treated sediment left at the bottom of the sediment treatment tank was collected and used for the nutrient elution tests as described below.

Control of TN:TP Ratio by Restraining the Nutrient Concentration and by Reducing Organic Matter and Nutrient Concentrations

The restraint of nutrient salts eluted from the sediment was demonstrated through a chemical sprinkling method. The powdered magnesium oxide (MgO) was used as a chemical because MgO would restrain both nitrogen and phosphorus as a form of magnesium ammonium phosphate or magnesium phosphate[32,33], as shown in the following equations:
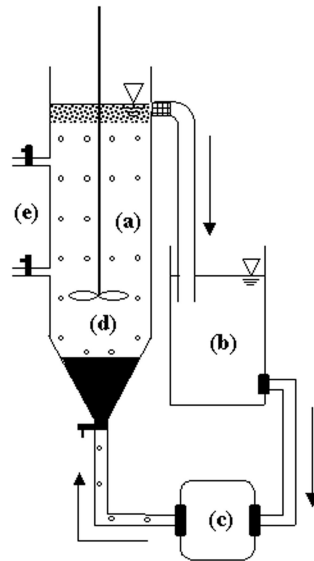
$$Mg^{2+} + HPO_4^{2-} + 3H_2O \rightarrow MgHPO_4 \cdot 3H_2O \downarrow \qquad (3)$$

$$Mg^{2+} + NH_4^+ + HPO_4^{2-} + 6H_2O \rightarrow MgNH_4HPO_4 \cdot 6H_2O \downarrow \qquad (4)$$

FIGURE 1. Set-up for the DAF treatment. (a) Sediment treatment tank, (b) circulation tank, (c) microbubble generator, (d) mixer equipment, (e) coagulation injection point. Arrows represent the direction of water flow.

The reactions shown in Eqs. (3) and (4) proceed under high pH condition. Accordingly, this method would be advantageous for the restraint of nutrient salts in eutrophic lakes because pH value always rises when water blooms occur.

The dosage of MgO on the sediment was 100, 400, and 2000 g m^{-2}.

The hybrid method (combining the DAF method and the MgO sprinkling method) was also demonstrated to control TN:TP ratio. The dosage of MgO sprinkled was the same as the MgO sprinkling method.

Influence of Change in TN:TP Ratio on the Growth of *Microcystis*

Nutrient Elution Test by Microcosm

There were 100 g of nontreated or DAF-treated sediment separately charged into transparent glass vessels of 470-ml volume. Afterward, lake water containing blue-green algae, mainly *Microcystis* species, obtained at Akebono Bridge, Lake Tega, was added by siphoning, using a rubber tube with a diameter of 1 mm. Since the nondimensional ratio between water depth and sediment thickness in Lake Tega was approximately 4 according to the article reported by Kobayashi and Kusuda[34], the ratio for the nutrient elution test was determined with the same condition for Lake Tega. Each glass vessel was covered using a transparent plastic sheet, and incubated under light (2×10^4 lux) and dark (0 lux) conditions with an incubation temperature of 20°C.

The experimental runs were set as shown in Table 2. Run 1 was incubated three times. The results of analyses for each water quality were represented as a mean value for $n = 3$.

Nutrient Elution Test by Mesocosm

There were 7 kg of nontreated or DAF-treated sediment separately charged into transparent acrylic reactors (70-l volume). Then the raw water was added by siphoning, using a rubber tube with a diameter of 1 cm. The reactors were set outside without any covers.

TABLE 2
Experimental Runs for the Nutrient Elution Test*

Run	Non-treated	DAF treated	MgO treated		
			MD: 100	MD: 400	MD: 2000
Run1	○				
Run2	○		○		
Run3	○			○	
Run4	○				○
Run5		○			
Run6		○	○		
Run7		○		○	
Run8		○			○
RunI	○				
RunII	○			○	
RunIII		○			
RunIV		○		○	

*   Runs 1–8: elution test by microcosm; Runs I–IV: elution test by mesocosm. MD, MgO dosage (g m^{-2}).

The experimental runs (Runs I–IV) for the nutrient elution test using mesocosm were the same as conditions with Runs 1, 3, 5, and 7 as shown in Table 2. Run I was incubated two times. The results of analyses for each water quality were represented as a mean value for $n = 2$.

Water Analyses

To examine the effect of the removal of organic matter, the ignition loss (I.L., %) and the grain distribution (μm) were measured by ignition loss method and grain size distribution method, respectively. TN (mg l^{-1}), TP (mg l^{-1}), and *Chl.a* concentrations (μg l^{-1}) were analyzed on the first and last day in the nutrient elution experiment with spectrophotometric method, ascorbic acid method, and spectrophotometric method, respectively.

RESULTS

Characteristic of Water Quality Based on Lake Origin

The values of TN, TP, *Chl.a* concentrations, and TN:TP ratio in accordance to lake origin are summarized in Table 3. The mean TN, TP, and *Chl.a* concentrations (TN$_{mean}$, TP$_{mean}$, and C_{mean}) were one order of magnitude higher for dammed, tectonic, and coastal lakes than for volcanic lakes. The TN' and TP', defined as the concentrations at C_{max}, clearly varied with lake origin, representing that the concentrations were higher in dammed lakes and tectonic lakes, and then decreased in the order of coastal lakes and volcanic lakes, which was proportional to the corresponding C_{max} values. The relationship between *Microcystis* growth and TN:TP ratio is illustrated in Fig. 2. Data plots of *Chl.a* concentration were entirely scattered for

TABLE 3
Characteristics of Water Qualities for the Lakes Used
in this Study*

	Dammed	Tectonic	Coastal	Volcanic
TN_{mean} (mg L^{-1})	1.43	0.98	1.38	0.26
TN' (mg L^{-1})	2.40	2.54	1.05	0.31
TP_{mean} (mg L^{-1})	0.103	0.124	0.137	0.027
TP' (mg L^{-1})	0.333	0.360	0.100	0.023
R_{mean}	22	17	16	18
R_{opt}	7	7	11	14
C_{mean} (μg L^{-1})	73	64	60	7
C_{max} (μg L^{-1})	316	217	122	23

*   Sample size: n = 21 for dammed lake, n = 14 for tectonic lake,
      n = 13 for coastal lake, n = 20 for volcanic lake. TN_{mean}, TP_{mean},
      R_{mean}, and C_{mean}; mean TN, TP, TN:TP ratio, and *Chl.a*
      concentration, TN' and TP'; TN and TP concentration at C_{max},
      R_{opt} and C_{max}; TN:TP ratio at C_{max} and maximum *Chl.a*
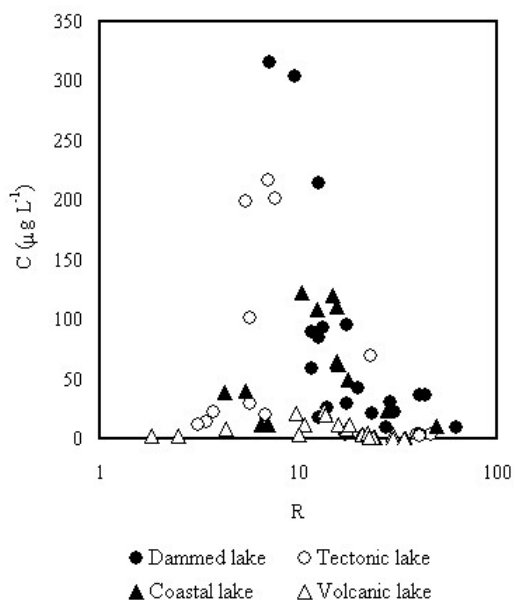   concentration.

FIGURE 2. Relationship between *Chl.a* concentration and
TN:TP ratio in the lakes used in this study.

TN:TP ratios. The mean TN:TP ratios (R_{mean}) and the TN:TP ratios at C_{max} (R_{opt}) showed similar values, but the R_{opt} values for each lake origin were somewhat lower than the R_{mean} values.

Removal Efficiency of Organic Matter by DAF Treatment

The efficiency of the removal of organic matter from the sediment by the DAF treatment was evaluated by the I.L. and D_{50} as tabulated in Table 4. The I.L. value in the raw sediment for Lake Tega was 15.9% ($n = 12$), whereas the value for the DAF-treated sediment was 15.1% ($n = 12$), representing a significant decrease ($p < 0.05$) in the organic matter than that obtained from the raw sediment. The D_{50} in the raw sediment was 6.8 μm, while that in the DAF-treated sediment was 40 μm. Thus, DAF treatment could remove approximately 5% of the organic matter from the raw sediment that would be small particles.

TABLE 4
Influence of the DAF Treatment on Removal of
Organic Matter in Sediment

Treatment	Ignition loss (%)	Mean diameter (μm)
Non-treated	15.9	6.8
DAF treated	15.1	40

Influence of Sediment Treatments on the Change in Nutrient Salts and TN:TP Ratio

Change in nutrient salts eluted from the sediment and the corresponding TN:TP ratio through the sediment treatments are illustrated in Fig. 3, based on the microcosm test. The restraint efficiencies of TN and TP could be seen in all of the sediment-treated runs compared with the nontreated run. Here, the restraint efficiency of nutrient salts was calculated as [100–{(concentration for sediment treated run ∕ concentration for nonsediment treated run)} × 100]. In the MgO sprinkling treatment (Runs 2–4), the restraint efficiencies of TN concentrations were in the range between 29 and 73%, except Run 3 (–10%), while significant restraint efficiencies of TP concentrations were found for all runs except Run 6 (17%), and the efficiency for TP concentration increases with the increase in MgO dosage. Consequently, TN:TP ratios for the sediment-treated runs showed higher values (21 < TN:TP ratio < 107), although Runs 5 and 6 remained at low values (TN:TP ratio = 9 for Run 5, TN:TP ratio = 7 for Run 6).

Although less restraint efficiencies of TN concentrations were observed in the nutrient elution test using mesocosm for all treated runs (below 30 %) as displayed in Fig. 4, significant restraint efficiencies of TP concentrations were represented for all runs. Accordingly, TN:TP ratios for all runs were raised and ranged between 20 and 94%, representing the same trends as with the nutrient elution test by microcosm.

Influence of Change in TN:TP Ratio on the Growth of *Microcystis*

The relationship between C/C_{max} and R/R_{opt} by lake origin, based on Eq. (2) and using the values listed in Table 3, is illustrated in Fig. 5. The plots by lake origin were well fitted to the model expressed in Eq. (2) with the correlation coefficients of 0.88, 0.69, 0.80, and 0.85 for dammed, tectonic, coastal, and volcanic lakes, respectively. The α values can be determined by the best-fitted curve to the resultant values for lake origin. The α values for dammed, tectonic, coastal, and volcanic lakes were calculated to be 8.5, 11.0, 11.5, and 18.5, respectively, which showed negative correlation with the nutrient concentrations for each lake origin. When our data for the nutrient elution tests were applied to the model, the relationship between
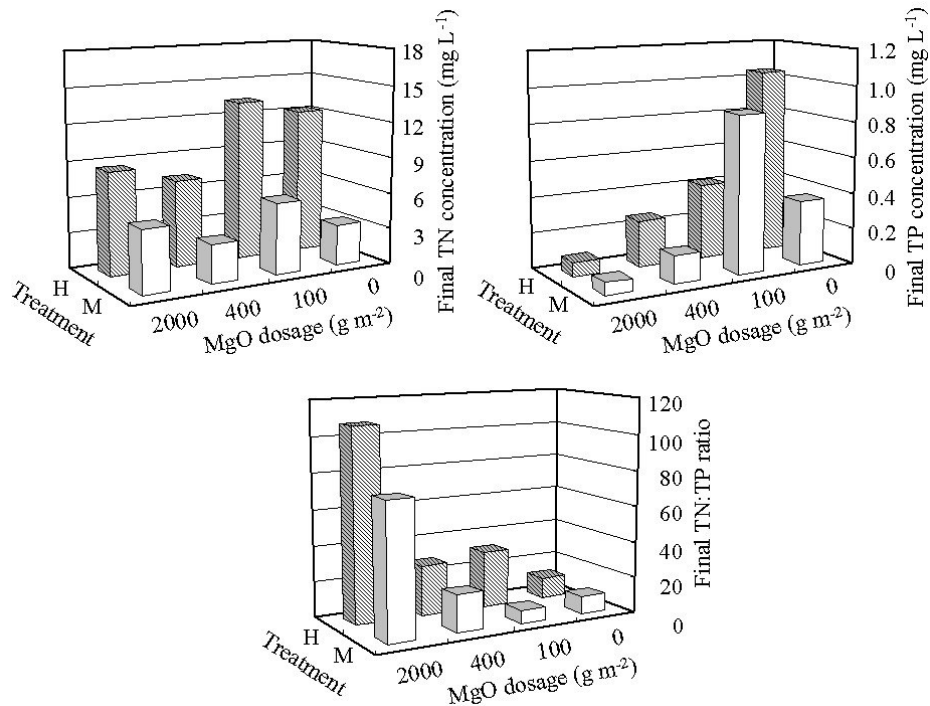
FIGURE 3. Changes in the final TN and TP concentrations, and the TN:TP ratios through the nutrient elusion test (microcosm). N, nontreated sediment; M, MgO-treated sediment; D, DAF-treated sediment; H, hybrid-treated sediment.
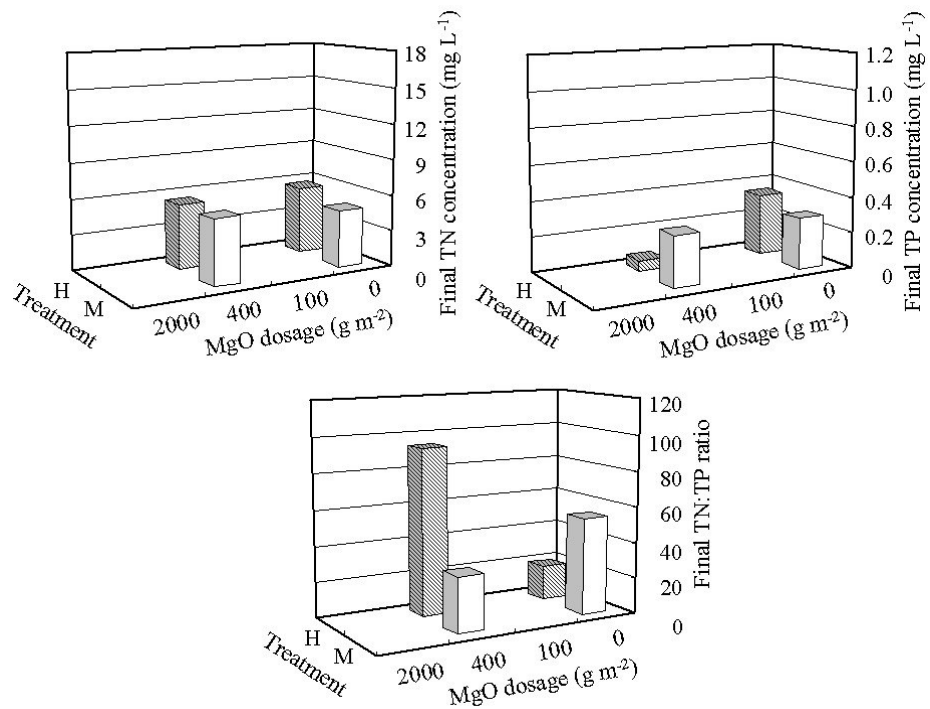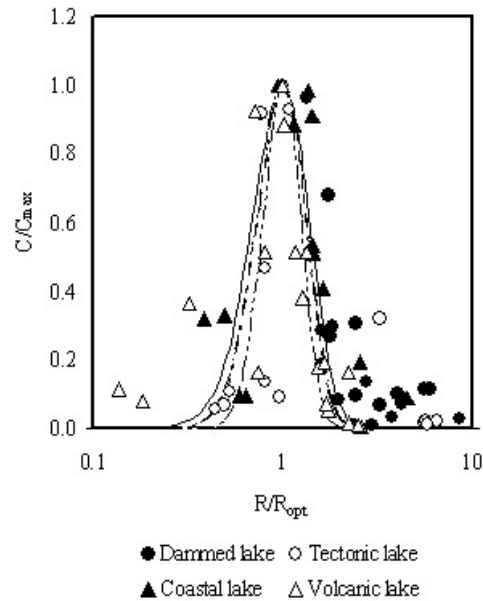

FIGURE 4. Changes in the final TN and TP concentrations, and the TN:TP ratios through the nutrient elusion test (mesocosm). N, nontreated sediment; M, MgO-treated sediment; D, DAF-treated sediment; H, hybrid-treated sediment.

FIGURE 5. Data plots of *Chl.a* concentrations and TN:TP ratios by Eq. (2) for each lake origin. Each approximation curve is represented by: solid curve for dammed lake ($\alpha = 8.5$, $r = 0.88$), dashed curve for tectonic lake ($\alpha = 11.0$, $r = 0.69$), dash-dotted curve for coastal lake ($\alpha = 11.5$, $r = 0.80$), and dash-double dotted curve for volcanic lake ($\alpha = 18.5$, $r = 0.85$).

TN:TP ratios and *Microcystis* growth could be represented as in Fig. 6, where the C_{max} and R_{opt} for the nutrient elution tests were based on the values of nontreated runs. In the figure, the data for dammed lakes were also added because the sediment and lake water for the nutrient elution test was obtained from Lake Tega, of which origin was specified as a dammed lake.

In Fig. 6, the distribution of C/C_{max} based on the sediment treatments was in correspondence with the model ($r = 0.73$), representing that *Chl.a* concentrations decreased with increase or decrease in the TN:TP ratios. Also, the data clearly indicated that approximately 90% of *Chl.a* concentrations in Runs 7 and 8 were suppressed compared with the nonsediment-treated run (Run 1).

DISCUSSION

Relationship Between TN:TP Ratio and *Microcystis* Growth

Considerable studies have dealt with blue-green algae and/or *M. aeruginosa* growth based on the TN:TP ratio. Our previous study[13] discovered, through the culture experiment with lake sediment and lake water, that blue-green algae were the dominant algal species when the TN:TP ratios were between 5 and 18 for the summer season. Fujimoto et al.[10] compared the growth abilities for *M. aeruginosa* and *Phormidium tenue* through the semi-continuous culture under various N:P supply ratios, and found that *M. aeruginosa* dominated when N:P ratios were less than 20. Furthermore, in a study of 17 lakes by Smith[12], blue-green algae tended to appear at TN:TP < 29. In contrast to those findings, Xie et al.[14] concluded that *Microcystis* blooms appeared not to be a function of the TN:TP ratios. However, their data showed that the reproduction of *Microcystis* blooms was optimal when the TN:TP ratios were approximately 8 to 9, which agrees with the findings (<10) of Takamura et al.[35].
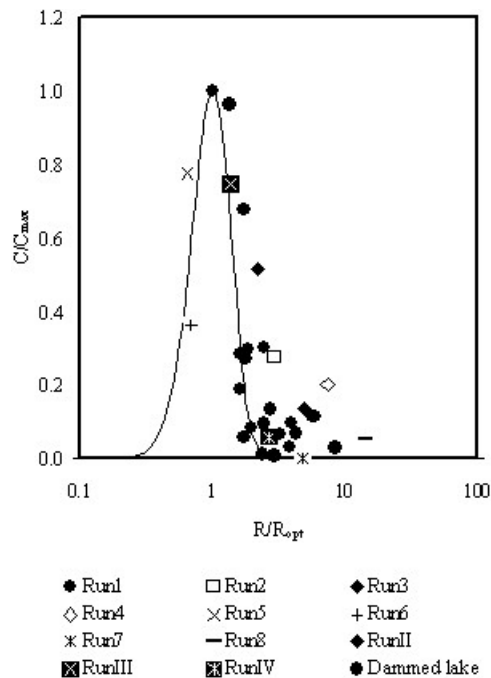
FIGURE 6. Influence of changes in TN:TP ratios by each sediment treatment on *Chl.a* concentrations. Solid curve represents the estimated distribution of C/C_{max} for damped lake ($\alpha = 8.5$).

In our data analysis for lake origins, approximately 70% of the data used were in the range of 5 < TN:TP < 29. Moreover, the R_{opt} values were in correspondence with the field observations that *Microcystis* blooms experience a significant growth as mentioned above, which agrees with the results obtained from the nutrient elusion tests (Run 1: $R_{opt} = 12$, $n = 3$, for microcosm; Run I: $R_{opt} = 19$, $n = 2$, for mesocosm). Our results could follow the conventional TN:TP considerations that *Microcystis* could achieve a significant growth where the TN:TP ratio was under low condition, and could support the hypothesis that *Microcystis* growth could be expressed as a function of TN:TP ratio, i.e., *Microcystis* could significantly grow at the optimum TN:TP ratio and decrease with decreasing or increasing the TN:TP ratio.

Influence of the Factors Related to *Microcystis* Growth

There are possibilities of factors other than the TN:TP ratio that may influence *Microcystis* growth. A trend of increase in algal growth with increasing nutrient concentration has widely been found in many studies[13,16]. This fact indicates that the same TN:TP ratio with different nutrient concentrations considerably causes the variance of *Microcystis* biomass, which is reflected as the relationship between *Chl.a* concentration and TN:TP ratio. The scattering of *Chl.a* concentrations at the same TN:TP ratio could be converged via standardization that the maximum values of C/C_{max} and the corresponding values of R/R_{opt} for each group of lakes by the origin were set to be 1. Then, the influence of nutrient concentrations would be reflected as the kurtosis for C/C_{max} distribution or α value. For example, the C/C_{max} values at the same R/R_{opt} show higher in damped lakes than in volcanic lakes, implying the difference in trophic state. Thus, the nutrient concentrations would affect the C/C_{max} distribution and/or α value.

Although *Chl.a* data were used to represent *Microcystis* growth, the data included algal species other than *Microcystis*, implying that algal species could influence the relationship between C/C_{max} and R/R_{opt}. In the competition experiment carried out by Fujimoto et al.[10] and Holm and Armstrong[16], the biomass of *M. aeruginosa* was one or two orders higher than that of the competitor when the dominant species was *M. aeruginosa*. Similarly, a significant abundance of *Microcystis* biomass was found in a field observation in a eutrophic lake[35]. The *Chl.a* data used in this study included a dominant species of *Microcystis*, so that a high rate of *Microcystis* dominance for the *Chl.a* data used in this study could be expected.

Fujimoto et al.[10] examined the difference of the growth rate of *M. aeruginosa* under different temperatures and found the significant influence of temperature on the growth rate in both phosphorus-limited medium (0.46 day^{-1} at 20°C and 0.88 day^{-1} at 30°C) and nitrogen-limited medium (0.84 day^{-1} at 20°C and 1.45 day^{-1} at 30°C). Jin et al.[36] discussed the influence of pH on the release of phosphorus from sediment. Their experimental results clearly show that pH prompted the phosphorus release, and a remarkable release of phosphorus occurred under pH < 5 and under pH > 7. Thus, *Microcystis* growth, i.e., the distribution of C/C_{max}, could remarkably be influenced by both temperature and pH. The mean water temperature and pH for the lakes used in this study were 28°C and 8.6, respectively, which follows the widely accepted facts that *Microcystis* tends to be abundant at high temperature conditions and, thereby, tends to raise the pH value in lake water. The C/C_{max} distribution represented in this study could be regarded as the result of the substantially constant high water temperature and pH, and these conditions would, therefore, cause little influence on the C/C_{max} distribution.

Lake Origin Controlling Lake Trophic State

Several studies have discussed the factors concerning the trophic state in lakes. Taki and Tanaka[22,37] reported the water qualities for 62 Japanese lakes from the viewpoint of lake origin. A part of their studies can be illustrated in Fig. 7 with some arrangements, i.e., where data other than lakes they used were added. Also, the index represented as the ratio between COD and SD was adopted in this figure to indicate lake trophic state more clearly.

It is obvious that the difference in the COD/SD values could be recognized among lake origins, being much higher values in dammed and coastal lakes than in tectonic and volcanic lakes. This trend would effectively agree with the nutrient concentrations for the lakes used in this study. Fig. 7 also represents the difference in population that followed in order of coastal, dammed, tectonic, and volcanic lakes. Volcanic lakes would significantly be influenced by population in watershed per surface area compared with other types of origin, implying a high sensitivity to even slight increases in the population. Conversely, the COD/SD values in dammed, coastal, and tectonic lakes show the scattered plots against the population. This would indicate the influences of factors other than the population, e.g., surface area, depth, area of watershed, and probably the amount of phosphate in sediment. These factors could be attributed to lake origin, of which consideration would be supported by the reports from Goldman and Horne[21].

The difference of trophic states in lakes would result from an interaction between size and area of watershed for lakes, and these factors would be characterized by lake origin. Lake origin could be regarded as an index including morphological and geographical factors, and controlling the trophic state in lakes, so that the origin rather than the trophic state for lakes could be considered as an important factor of TN:TP influences on *Microcystis* growth.

CONCLUSIONS

This study reveals the relationship between TN:TP ratio and the growth of *Microcystis* via a database that includes worldwide lakes based on four types of lake origin. The nutrient elution tests using microcosm and mesocosm with lake water and four kinds of sediment were also conducted in order to control TN:TP ratio and to suppress *Microcystis* growth. The main conclusions could be summarized as follows:
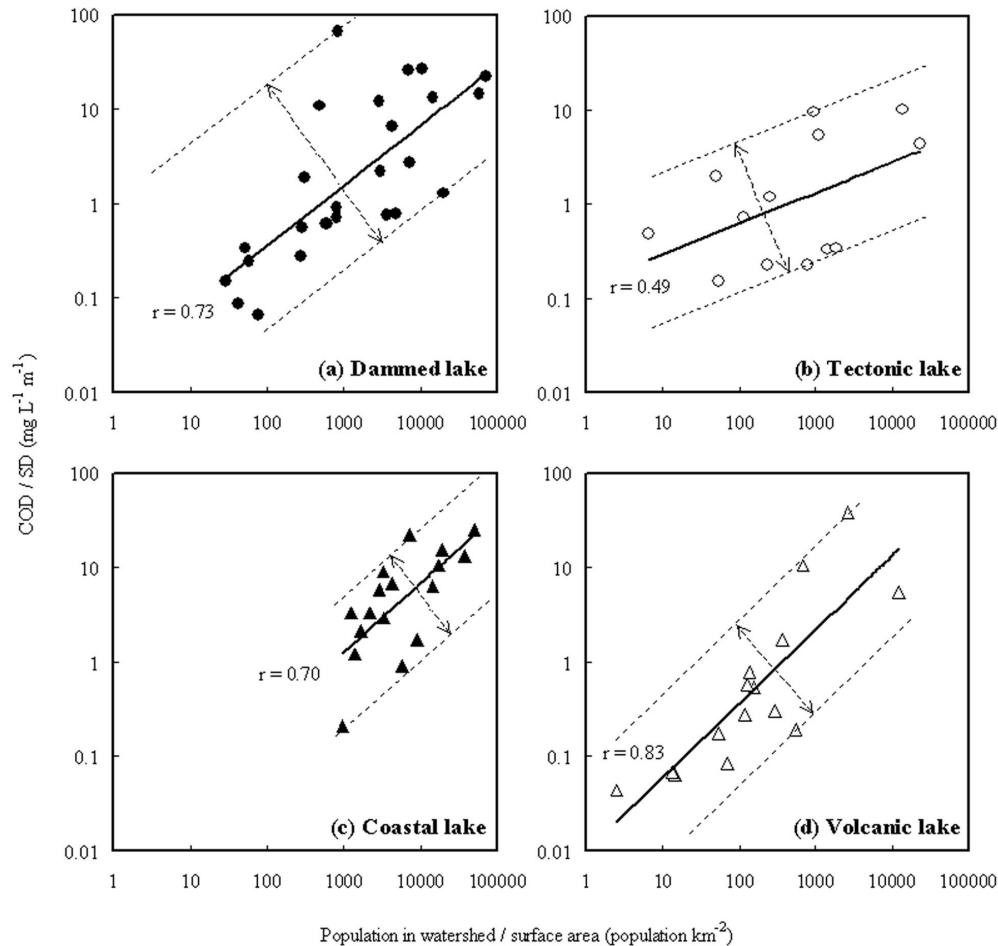
FIGURE 7. Difference in trophic state represented from the viewpoint of lake origin. Data plots were cited from the articles reported by Taki and Tanaka[22,37] with some arrangements. Sample size: $n = 26$ for dammed lake, $n = 13$ for tectonic lake, $n = 17$ for coastal lake, $n = 15$ for volcanic lake.

1. *Microcystis* growth could be expressed by TN:TP ratio and described as a distribution curve showing the maximum value at an optimum TN:TP ratio and the minimum values at the TN:TP ratio nearly equal to 0 or ∞. The kurtosis of the distribution curve varied with the type of lake origin and could be expressed by the α value.

2. The α values for dammed, tectonic, coastal, and volcanic lakes were calculated to be 8.5, 11.0, 11.5, and 18.5, respectively. The lake trophic state affected the change in α value, providing the much lower α value at the eutrophic state (dammed lakes) than that at the oligotrophic state (volcanic lakes).

3. The nutrient elution tests controlling the TN:TP ratios through the various sediment treatments showed that a significant suppression of *Microcystis* growth (70%) could be achieved when the TN:TP ratios exceeded 21.

4. Lake origin could be regarded as an index including morphological and geographical factors, and controlling the trophic state in lakes. Therefore, the origin rather than the trophic state for lakes could be considered as an important factor of TN:TP influences on *Microcystis* growth.
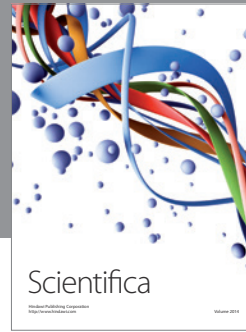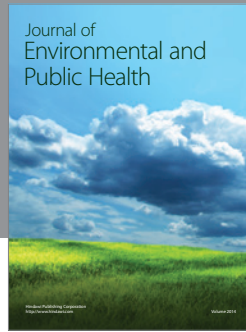
ACKNOWLEDGMENTS

REFERENCES

1. Pearl, H.W. (1998) Nuisance phytoplankton blooms in coastal estuarine inland waters. *Limnol. Oceanogr.* **33**, 823–847.
2. Rittmann, B.E. and McCarty, P.L. (2001) *Environmental Biotechnology: Principles and Applications.* McGraw-Hill, New York.
3. Clark, J.W., Viessman, W., Jr., and Hammer, M.J. (1971) *Water Supply and Pollution Control.* 2nd ed. International Textbook, Scranton, PA.
4. Meißner, K., Dittmann, E., and Börner, T. (1996) Toxic and non-toxic strains of the cyanobacterium *Microcystis aeruginosa* contain sequences homologous to peptide synthetase genes. *FEMS Microbiol. Lett.* **135**, 295–303.
5. Orr, P.T. and Jones, G.J. (1998) Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanogr.* **43**, 1604–1614.
6. Amano, Y., Murakami, K., Ishii, T., Taki, K., and Matsushima, H. (2001) Elution of nutrient salts from sediment layer in eutrophicated lake. *Proceedings of the 1st IWA Asia-Pacific Regional Conference, ASIAN WATERQUAL 2001*, Fukuoka, Japan. pp. 193–198.
7. Xie, L. and Xie, P. (2002) Long-term (1956-1999) dynamics of phosphorus in a shallow, subtropical Chinese lake with the possible effects of cyanobacterial blooms. *Water Res.* **36**, 343–349.
8. Søndergaard, M., Jeppesen, E., and Jensen, J. P. (2000) Hypolimnetic nitrate treatment to reduce internal phosphorus loading in a stratified lake. *Lake Reserv. Manage.* **16**, 195–204.
9. Yagi, O., Okubo, N., Tomioka, N., and Okada, M. (1989) Limiting nutrients of algal growth in Ushiku Marsh. *Jpn. J. Limnol.* **50**, 139–148.
10. Fujimoto, N., Sudo, R., Sugiura, N., and Inamori, Y. (1997) Nutrient-limited growth of *Microcystis aeruginosa* and *Phormidium tenue* and competition under various N:P supply ratios and temperatures. *Limnol. Oceanogr.* **42**, 250–256.
11. Jüttner, F. (1977) Thirty liter tower-type pilot plant for the mass cultivation of light- and motion-sensitive planktonic algae. *Biotechnol. Bioeng.* **19**, 1679–1687.
12. Smith, V.H. (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in Lake phytoplankton. *Science* **221**, 669–671.
13. Amano, Y., Taki, K., Murakami, K., Ishii, T., and Matsushima, H. (2002) Sediment remediation for ecosystem in eutrophic lakes. *TheScientificWorldJOURNAL* **2**, 885–891.
14. Xie, L., Xie, P., Li, S., Tang, H., and Liu, H. (2003) The low TN:TP ratio, a cause or result of *Microcystis* blooms?. *Water Res.* **37**, 2073–2080.
15. Olsen, Y. (1989) Evaluation of competitive ability of *Staurastrum luetkemuellerii* (Chlorophyceae) and *Microcystis aeruginosa* (Cyanophyceae) under P limitation. *J. Phycol.* **25**, 486–499.
16. Holm, N.P. and Armstrong, D.E. (1981) Role of nutrient limitation and competition in controlling the populations of *Asterionella formosa* and *Microcystis aeruginosa* in semicontinuous culture. *Limnol. Oceanogr.* **26**, 622–634.
17. Havens, K.E., James, R.T., East, T.L., and Smith, V.H. (2003) N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution, *Environ. Pollut.* **122,** 379–390.
18. Howarth, R.W., Marino, R., and Lane, J. (1988) Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnol. Oceanogr.* **33,** 669–687.
19. Rhee, G.-Y., and Gotham, I.J. (1980) Optimum N:P ratios and coexistence of planktonic algae. *J. Phycol.* **16**, 486–489.
20. Steele, J.H. (1962) Environmental control of photosynthesis in the sea. *Limnol. Oceanogr.* **7**, 137–150.
21. Goldman, C.R. and Horne, A.J. (1983) *Limnology*. McGraw-Hill, New York.
22. Taki, K. and Tanaka, T. (2004) Characteristics on water environment involving geophysical factors in Japanese Lakes. *Papers Environ. Inform. Sci.* **18**, 483–488. [Japanese with English abstract]
23. Environment Agency (1987) Report of the National Survey on the Lake and Marsh: Tohoku version I. 4th survey. Environment Agency, Tokyo. [Japanese]
24. Environment Agency (1987) Report of the National Survey on the Lake and Marsh: Tohoku version II. 4th survey. Environment Agency, Tokyo. [Japanese]
25. Environment Agency (1993) Report of the National Survey on the Lake and Marsh: Kanto version. 4th survey. Environment Agency, Tokyo. [Japanese]

26. Environment Agency (1987) Report of the National Survey on the Lake and Marsh: Hokuriku and Koshinetsu version. 4th survey. Environment Agency, Tokyo. [Japanese]

27. Environment Agency (1987) Report of the National Survey on the Lake and Marsh: Tokai and Kinki version. 4th survey. Environment Agency, Tokyo. [Japanese]

28. Environment Agency (1993) Report of the National Survey on the Lake and Marsh: Chugoku, Shikoku, Kyushu and Okinawa version. 4th survey. Environment Agency, Tokyo. [Japanese]

29. International Lake Environmental Committee. Index of World Lakes: http://www.ilec.or.jp/database/index/idx-lakes.html (accessed 24 February 2008).

30. Nagano Research Institute for Health and Pollution (1982) Study on Eutrophication of Lake Suwa. Nagano Prefecture, Nagano [Japanese]

31. Suwa, T., Amano, Y., Murakami, K., Ishii, T., Taki, K., and Matsushima, H. (2001) Fueiyouka kosyouni okeru cyokusetsu zyouka (1) gyoshuzai tenka taiminguniyoru yuukibutsu zyokyo tokusei. *Proceedings of the Annual Conference of the Japan Society of Civil Engineering* (Kanto Shibu). pp. 832–833. [Japanese]

32. Tsuno, H., Somiya, I., and Yoshida, M. (1991) Syokaso Datsurieki karano sutorabaitono kaisyuni kansuru kenkyu. *Gesuido kyokai ronbunsyu* **28**, 68–77. [Japanese]

33. Schulze-Rettmer, R. (1991) The simultaneous chemical precipitation of ammonium and phosphate in the form of magnesium-ammonium-phosphate. *Water Sci. Technol.* **23**, 659–667.

34. Kobayashi, S. and Kusuda, T. (1985) Teganuma no teishitsu –Osendei no taisekito seizyo–, *Yosuito Haisui*, **27**, 150–158. [Japanese]

35. Takamura, N., Otsuki, A., Aizaki, M., and Nojiri, Y. (1992) Phytoplankton species shifts accompanied by transition from nitrogen dependence to phosphorus dependence of primary production in lake Kasumigaura, Japan. *Arch. Hydrobiol.* **124**, 129–148.

36. Jin, X., Wang, S., Pang, Y., and Wu, F.C. (2006) Phosphorus fractions and the effect of pH on the phosphorus release of the sediments from different trophic area in Taifu Lake, China. *Environ. Pollut.* **139,** 288–295.

37. Taki, K. and Tanaka, T. (2004) Characteristics on water environment for typical lakes due to the origin factors in Japan. *Proceedings of the Second International Symposium on Southeast Asian Water Environment*, Hanoi, Vietnam. pp. 302–309.

Journal of
Waste Management

The Scientific
World Journal

Journal of
Environmental and
Public Health

Scientifica

International Journal of
Ecology

Journal of
Ecosystems

International Journal of
Oceanography

International Journal of
Atmospheric Sciences

Journal of
Marine Biology

Submit your manuscripts at
http://www.hindawi.com

Hindawi

International Journal of
Biodiversity

Journal of
Geological Research

International Journal of
Forestry Research

Applied &
Environmental
Soil Science

Journal of
Climatology

Journal of
Earthquakes

Journal of
Computational
Environmental Sciences

International Journal of
Geophysics

Advances in
Meteorology

Advances in
Environmental
Chemistry