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Luxurious Phosphorus and Phosphorus Limitation for Epiphytic and Planktonic Algal Growth in Reed Zones of Lake Biwa

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To evaluate the limitation for epiphytic and planktonic algal growth, acid extractable inorganic phosphorus (AP), implying the luxury uptake phosphorus, was measured in five reed zones of Lake Biwa. The AP in epiphytic substances was 0.7 to 1.4 mg P surface stem m^{-2} in summer and 1.2 to 2.8 mg P m^{-2} in winter. On the other hand, the amount in planktonic substances was 1.4 to 5.7 mg P m^{-3} and 0.8 to 5.4 mg P m^{-3} in both seasons. Contribution of AP in the epiphytic and planktonic phosphorus was 23 to 31% and 8 to 27% in summer, and 17 to 22% and 9 to 17% in winter. It suggests that in summer both epiphytic and planktonic algae had been luxuriously taken up phosphate into cells. The weight ratios of C : N : P were averaged 79 : 20 : 1 for the epiphytic substances and 81 : 12 : 1 for the particulate substances. On the other hand, the ratios without the luxurious phosphorus were 93 : 24 : 1 and 103 : 15 : 1, showing much higher values than the Redfield ratio. High ratio in the epiphytic substances indicates that the phosphorus is the limiting parameter, rather than nitrogen, regulating the growth of epiphytic algal populations.

Key words : luxurious phosphorus, phosphorus limitation, epiphytic and planktonic algae, reed zone, Lake Biwa

INTRODUCTION

In freshwater lakes, phosphorus often functions as the limiting parameter rather than nitrogen, regulating the growth of phytoplankton populations (e.g., Goldman, 1960; Satoh *et al.*, 2006). To estimate the limitation levels of phosphorus in natural waters, several procedures, such as the nitrogenous and phosphorus concentration in dissolved or particulate forms and its ratio (Forsberg and Ryding, 1980; Healey and Hendzel, 1980; Nakanishi *et al.*, 1990; Hecky *et al.*, 1993), the alkaline phosphatase activity (Fitzgerald and Nelson, 1966; Reichardt *et al.*, 1967; Jansson *et al.*, 1988), and/or the examination of algal and nutrient addition bioassays for biologically available phosphorus (Holmboe *et al.*, 1999; Satoh *et al.*, 2006), have been attempted. However, it has been well known that in times of an appreciable concentration of phosphate in water, the phytoplankton sometimes luxuriously take up phosphorus nutrient in their environment and stored as available phosphorus like polyphosphate until the assimilation into phosphoric organic compound in cell structure. The luxurious phosphorus has sometimes been observed (Fitzgerald and Nelson, 1966; Stewart and Alexander, 1971; Senft, 1978; Eixler *et al.*, 2005). It indicates that the previous procedures without the considera-

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tion of luxurious phosphorus make no accurate assessment of limitation for algal growth. Furthermore, the evaluation of the limiting parameter for the growth of epiphytic algae on reed stems has been quite limited. The luxurious phosphorus in algal cells practically remains in existence as the extractable fraction by diluted acid. In the present investigations, the acid extractable inorganic phosphorus, implying the luxury uptake phosphorus, was measured to evaluate the limitation for the epiphytic and planktonic algal growth in the reed zones of Lake Biwa.

STUDY AREA AND METHODS

1. Study site and investigation procedure

Lake Biwa is the largest lake in Japan, covering an area of 674 km^2 with a maximum depth of 104 m. The present investigations were carried out at five typically large-scale reed zones, each showing a different trophic character (Fig. 1). Field investigations were conducted on calm days during summer (July) and winter (December) at the boundary between the littoral and pelagic zones, where the *Phragmites* zone extended from land to water. In the present investigations, a mesotrophic St. A (35° 24.3′N, 136° 13.2′E), located in the north basin of Lake Biwa covers an area of 300 m^2 with an average *Phragmites* stem density of 60 m^{-2}. Eutrophic Sts. B (35° 02.7′N, 135° 52.5′ E), C (35° 02.2′N, 135° 55.0′E) and D (35° 03.5′N, 135° 56.0′E) in the south basin cover 300, 400 and 250 m^2 with 80, 20 and 40 m^{-2}, respectively. A eutrophic St. E (35° 09.9′N, 136° 06.9′E), a lagoon connected with Lake Biwa, covers an area of 600 m^2 with stem density of 200 m^{-2}. At center station in each reed zone, the water depth of submerged portion of the stems was 0.5 to 0.9 m in summer and 0.2 to 0.6 m in winter. The grain size of bottom sediments was composed of sand at Sts. A, B and C, sand-mud at St. D and mud at St. E, respectively. In advance of the present investigations, no vertical changes in the chemical parameters were observed in respective reed zones. Water samples, therefore, were drawn gently with a plastic tube from a 0.2 m in summer and 0.1 m in winter seasons depth at five inside sampling points in respective reed zones.

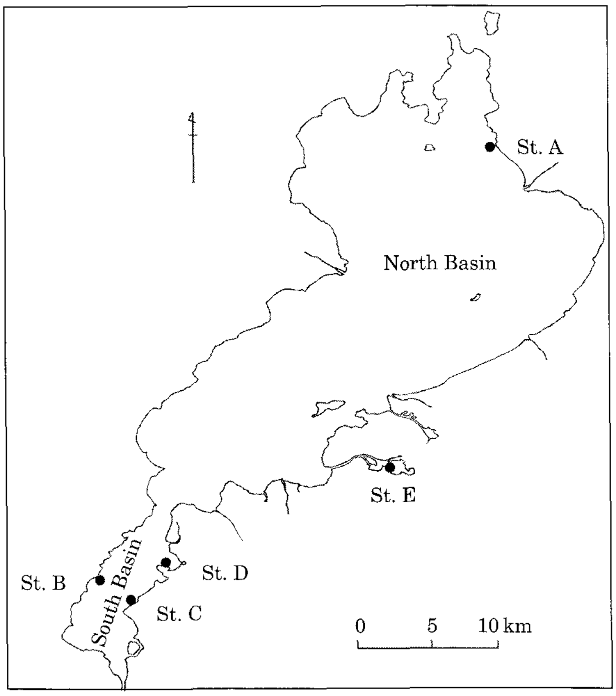


Fig. 1. Map showing investigation reed zones at the boundary between the littoral and pelagic areas of Lake Biwa.

Old submerged stems of *Phragmites* accounted for approximately 60% of the total reed stems in summer and 30% in winter. The standing crop of epiphytic algae on old reed stems was generally greater than that on new reed stems. Then, the field investigations were carried out in both seasons of small (July) and large amounts of epiphytic substances (December). The reed stems employed in this study, therefore, were chosen proportionally in the ratio of new to old numbers. Both new and old stems of *Phragmites* taken from five inside sampling points in respective reed zones were cut above the rhizomes and returned to the laboratory. The substrate section used was a length of approximately 5 cm. Low chlorophyll *a* (Chl. *a*) concentrations on the reed stems were observed near the water surface and just above the sediment. Muller (1995) reported the vertical fluctuations in the standing crop of epiphytic algae on *Phragmites*, showing a similar tendency to the present results. Therefore, random sections of the stem were used for the experiments. Periphytic substances were gently removed from the surface of reed stems using a paintbrush with filtered water through a glass fiber filter (Whatman GF/F). The stripped periphytic substances were subdivided for chemical analyses.

2. Analytical procedure of dissolved and particulate biogeochemical constituents

Epiphytic or particulate phosphorus (EP or PP) was analyzed using the method described by Menzel and Corwin (1965) after digestion with perchloric acid and nitric acid (Saijo and Mitamura, 1995). Acid extractable inorganic phosphorus (AP) in EP or PP was measured as the fraction of the extracted phosphorus by acidification treatment. After adding appropriate volume of 0.1 M HCl solution to the epiphytic or particulate substance which was chopped into small pieces, the sample was incubated for 24 hours at 20°C. On the other hand, the hot water soluble inorganic phosphorus (HP) in EP or PP was measured as the fraction of the released phosphorus with boiling treatment. After adding appropriate volume of pure water to the epiphytic or particulate substance, the sample was boiled up for 90 minutes at 100°C. After centrifugal separation of both the cloudy AP and HP fluids, the concentration of phosphate in supernatant solutions was determined by the method described by Murphy and Riley (1962). The AP and HP indicate the intracellular accumulation phosphorus, although both fractions might exclude the imperceptible polyphosphate amount. Therefore, it is just conceivable that the AP and HP fractions represent the available phosphorus for algal growth, such as inorganic phosphate and polyphosphate in algal cells, as suggested by Fitzgerald and Nelson (1966) and Eixler *et al.* (2005). The AP fraction also might be consisted of inorganic phosphorus absorbed to inorganic and organic particles (Sanudo-Wilhelmy *et al.*, 2004; Fu, *et al.*, 2005). The standing crop of epiphytic algae on the reed stems was estimated in terms of Chl. *a*, and measured using 90% acetone as the extraction solvent according to the method of SCOR/Unesco (1966). Epiphytic or particulate carbon (EC or PC) and nitrogen (EN or PN) were determined with a CHN Corder (Yanaco MT-5 type).

To determine the concentration of particulate matter and nutrients, the collected water samples were immediately filtered through glass fiber filters (Whatman GF/F) treated by ignition at 420°C. The filters and filtrates were then stored at −20°C in a deep freezer until chemical analyses in the laboratory. Phosphate (DIP) was determined by the method described Murphy and Riley (1962). Ammonia was determined by Sagi (1966), nitrite after Bendschneider and Robinson (1952), nitrate after Wood *et al.* (1967), urea after Newell *et al.* (1967), silicate after Mullin and Riley (1955). Dissolved organic phosphorus (DOP) was determined by the method of Menzel and Corwin (1965). Dissolved organic carbon (DOC) was determined by Menzel and Vaccaro (1964) using an infra-red analyzer (Beckman, Model 864), dissolved organic nitrogen (DON) by the Kjeldahl technique with selenium dioxide as a catalyst described by Mitamura (1994).

RESULTS AND DISCUSSION

1. Distributions of inorganic and organic forms of phosphorus and other bioelements

Concentrations of DIP were generally limited in summer and winter seasons, with the exception of high concentrations at Sts. B and E in summer (Table 1). Predominant component of nitrogenous nutrients was nitrate. High values of ammonia and nitrite concentrations were observed in the reed zones in the south basin of Lake Biwa. Urea nitrogen was generally low, much lower than those of ammonia and nitrate. An appreciable concentration of silicate was observed in both seasons. Considerable variations in these nutrients concentrations were locally found among the reed zones, indicating that the trophic levels differed widely among five reed zone.

Concentrations of DOP were comparable or lower than those of DIP concentrations. The DOP accounted for 30% of dissolved total phosphorus (DTP: sum of DIP and DOP). The DOC concentrations at St. A showed lower values than those in the other four reed zones. At St. E, much high DOC concentrations were obtained in summer. These concentrations showed no regular horizontal variations in any reed zone. In general, the DOC concentrations were higher in summer than in winter. Distributions of DON concentrations showed a similar pattern to the DOC distribution. An approximate 40% was occupied by the DON in dissolved total nitrogen (DTN: sum of DIN and DON) in the five reed zones. The water at a lagoon St. E had high concentrations of dissolved organic matter. The DOC and DON distributions had

Table 1. Concentrations of nutrients (phosphate, ammonia, nitrite, nitrate, urea and silicate) and dissolved organic phosphorus, nitrogen and carbon (DOP, DON and DOC) at inside area in reed zones of Lake Biwa. Data show an average value with standard deviation at five inside sampling points in respective reed zones of Lake Biwa.

	St. A		St. B		St. C		St. D		St. E	
	July	December	July	December	July	December	July	December	July	December
Phosphate (μg P L^{-1})	7±1	8±2	85±16	18±5	6±2	7±1	13±1	25±4	41±3	8±1
Ammonia (μg N L^{-1})	31±12	29±6	190±30	18±5	42±10	85±34	77±15	17±12	160±10	20±4
Nitrite (μg N L^{-1})	3±0	8±1	39±6	9±2	21±2	19±2	22±1	12±1	13±1	8±1
Nitrate (μg N L^{-1})	43±9	240±30	710±90	290±60	160±30	380±90	300±30	400±80	120±10	410±70
Urea (μg N L^{-1})	13±2	9±2	20±4	11±2	15±4	15±2	22±3	15±2	52±6	13±1
Silicate (mg Si L^{-1})	1.5±0.1	2.3±0.1	2.8±0.2	2.6±0.2	0.9±0.2	2.3±0.2	3.1±0.1	5.0±0.2	4.8±0.0	5.0±0.2
DOP (μg P L^{-1})	6±1	4±1	5±1	7±2	11±1	7±1	14±2	6±1	13±3	5±1
DON (μg N L^{-1})	240±30	160±10	330±40	200±20	250±10	210±20	340±30	230±10	450±30	300±10
DOC (mg C L^{-1})	1.8±0.1	1.4±0.1	2.2±0.1	1.7±0.1	2.2±0.1	1.8±0.1	2.5±0.1	1.7±0.1	4.0±0.3	2.5±0.1

a different distribution compared with those of the DOP.

Thus, the concentrations of dissolved inorganic and organic phosphorus and other biogeochemical parameters varied among the five inside sampling points in each reed zone (Table 1). The present results indicate that the reed zone in the littoral area of Lake Biwa has a heterogeneous environment, contrary to a relatively homogeneous environment in the open water areas of the littoral and pelagic zones.

2. Luxurious phosphorus of epiphyton and plankton

In the present investigations, the concentration of particulate (planktonic) phosphorus (PP) ranged from 5.3 to 21.6 mg P m^{-3}, as an average value at five inside sampling points in each reed zone, in summer (July) in summer (July) and 4.5 to 24.9 mg P m^{-3} in winter (December) investigations (Table 2). The particulate carbon (PC) and nitrogen (PN) levels showed similar patterns noted in the PP distribution. The amount of epiphytic phosphorus (EP), on the other hand, ranged from 3.9 to 10.4 mg P surface stem m^{-2} in summer, and 9.9 to 28.6 mg P m^{-2} in winter seasons, showing similar distribution of epiphytic carbon (EC) and nitrogen (EN), respectively. The amounts of epiphytic substances on reed stems at inside sampling points in respective reed zones fluctuated widely (Table 2). The EP concentration in winter showed much higher than in summer season. High concentrations of EP were observed at Sts. C and E.

Many algae take up luxuriously phosphate from their environments and store in their cell as available phosphorus for the algal growth, such as inorganic phosphate and polyphosphate granules (Stewart and Alexander, 1971; Eixler *et al.*, 2005). Fitzgerald and Nelson (1966) measured the polyphosphate amount, as an indicator of cellular phosphorus depletion, from the released phosphorus with boiling treatment. Acid extractable (AP) and hot water soluble (HP) inorganic phosphorus implies the available luxurious phosphorus in algal cell or the absorbed inorganic phosphorus on surface of particle. The absorbed phosphorus is an available form for algal utilization. The determination of AP and HP including this fraction is preferable to evaluate the nutrients limitation. In the present investigations, the HP amounts of the epiphytic and planktonic substances in respective reed zones were similar or slightly higher than those of the AP, namely 1.04 to 1.28 times higher. Therefore, it

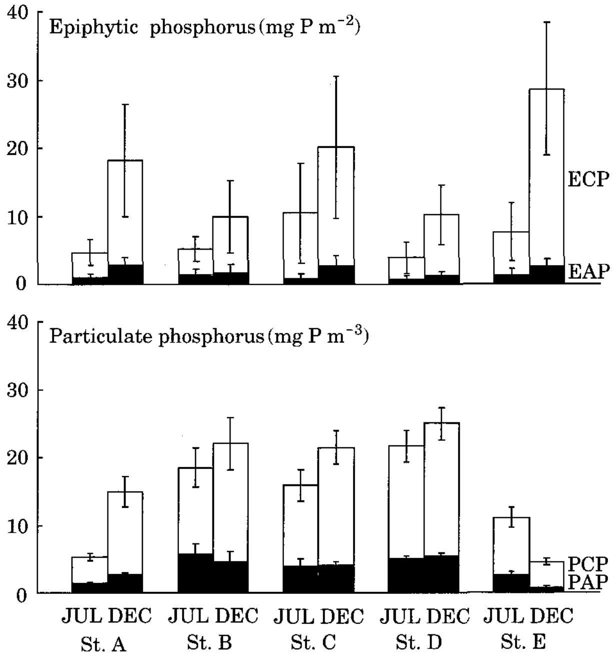
Table 2. Amounts of epiphytic (unit surface area of *Phragmites* stem) and particulate (unit volume of water) phosphorus, nitrogen and carbon at inside area in reed zones. Data show an average value with standard deviation at five inside sampling points in respective reed zones.

Reed zone		Phosphorus		Nitrogen		Carbon	
		Epiphytic (mg P m^{-2})	Particulate (mg P m^{-3})	Epiphytic (mg N m^{-2})	Particulate (mg N m^{-3})	Epiphytic (mg C m^{-2})	Particulate (mg C m^{-3})
St. A	July	4.6±2.4	5.3±0.7	115±65	53±5	0.35±0.21	0.40±0.05
	December	18.2±9.5	15.0±2.2	383±210	169±22	1.65±1.08	1.39±0.02
St. B	July	5.2±2.6	18.5±4.1	94±46	100±16	0.25±0.15	0.72±0.11
	December	9.9±6.5	22.0±5.0	124±92	199±28	1.03±0.65	0.96±0.16
St. C	July	10.4±8.0	16.0±2.9	119±99	182±24	0.34±0.31	1.30±0.17
	December	20.1±11.9	21.4±2.9	319±201	330±33	1.60±0.96	1.77± 0.21
St. D	July	3.9±2.8	21.6±2.4	103±79	212±35	0.34±0.32	1.71±0.20
	December	10.2±5.0	24.9±2.7	164±79	269±28	0.75±0.44	1.67±0.20
St. E	July	7.6±5.2	11.0±1.2	193±156	93±9	0.62±0.55	0.76±0.07
	December	28.6±10.7	4.5±0.6	869±358	107±8	3.18±1.48	0.80±0.06

was considered the AP fraction as the luxurious phosphate fraction.

Acid extractable inorganic phosphorus of planktonic substances (PAP) was ranged from 1.4 to 5.7 mg P m^{-3}, as an average value at respective stations, in summer and 0.8 to 5.4 mg P m^{-3} in winter (Fig. 2). The amount of acid extractable inorganic phosphorus (AP) of epiphytic substances (EAP) was 0.7 to 1.4 mg P surface stem m^{-2} in summer and 1.2 to 2.8 mg P m^{-2} in winter. Furthermore, the cellular phosphorus of planktonic substances (PCP) calculated as the difference between PP and PAP, although imperceptible amount of polyphosphate might be consisted of this fraction. The PCP amount was within the range of 3.9 to 16.6 mg P m^{-3} in summer and 3.7 to 19.5 mg P m^{-3} in winter. In the case of epiphytic substances, the cellular phosphorus (ECP) was 3.2 to 9.6 mg P surface stem m^{-2} in summer and 8.3 to 26.0 mg P m^{-2} in winter seasons, respectively.

The contribution of EAP in EP was 8 to 27% in summer and 9 to 17% in winter (Table 3). The percentage of PAP in PP, on the other hand, ranged from 23 to 31% and 17 to 22% in both seasons. The contributions were higher in summer than in winter season and also in epiphytic substance than in planktonic one. It suggested that the epiphytic algae had a disadvantage in terms of luxury uptake than the planktonic algae, although the ability of the luxury phosphorus uptake were widely among algal species (e.g., Tilman and Kilham, 1976; Brown and Button, 1979), and both algae had been luxuriously taken up phosphate into cells from the water in summer than in winter season.


Epiphytic phosphorus (mg P m^{-2})

Particulate phosphorus (mg P m^{-3})

Fig. 2. Distributions of acid extractable inorganic phosphorus (EAP or PAP) and cellular phosphorus (ECP or PCP) in the epiphytic or particulate substances in reed zones of Lake Biwa in summer (July) and winter (December) seasons. Data show as an average value at five inside sampling points in respective reed zones. Error bars indicate the standard deviations (n=5). The amounts of epiphytic and particulate phosphorus are in unit surface area of *Phragmites* stem and unit volume of water, respectively.

Table 3. Contributions of luxurious phosphorus (acid extractable phosphorus) in total phosphorus for epiphytic (EAP/EP) and particulate (PAP/PP) substances at inside area in reed zones. Data in parentheses show an average value at five inside sampling points in respective reed zones.

Reed zone	EAP/EP (%)				PAP/PP (%)			
	July		December		July		December	
	Range	Average	Range	Average	Range	Average	Range	Average
St. A	16~27	(21)	13~18	(15)	21~30	(26)	14~21	(18)
St. B	22~30	(27)	14~19	(17)	26~37	(31)	17~25	(21)
St. C	6~9	(8)	11~15	(13)	20~31	(25)	16~21	(19)
St. D	15~20	(18)	9~14	(12)	21~28	(23)	15~19	(22)
St. E	11~18	(16)	8~10	(9)	16~33	(23)	13~21	(17)

3. Limitation for epiphytic and planktonic algal growth

The Redfield stoichiometric ratio with C : N : P of 41 : 7 : 1 (by weight) has become a widely cited standard reference value for assessing the nutrient limitation in natural water (Redfield, 1958). Several workers examined the limiting parameter as the growth limiting nutrients of phytoplankton. Forsberg and Ryding (1980) reported that DIN : DIP ratio of 5 or less (by weight) indicated nitrogen limitation, and a ratio of 12 or greater meant phosphorus limitation. The total nitrogen to total phosphorus ratio of 10 and 17 was also used by them as a boundary value for the limitation. Healey and Hendzel (1980) discussed the nutrient deficiency for phytoplankton using the ratio of PN : PC (<0.13; nitrogen limitation), PP : PC (<0.02; phosphorus limitation) and PN : PP (>10; phosphorus limitation). The limiting parameter in each reed zone was estimated from these ratios in dissolved and particulate forms.

As shown in Table 4, the weight ratio of TNN to DIP was calculated as 9 to 37, as an average value at five inside sampling points in respective reed zones, in summer (July) and 18 to 68 in winter (December). High ratios were obtained at Sts. C and D in summer. The ratios in winter showed higher values than in summer on the whole. These results indicate that each of the five reed zones in the present study had a different trophic character, and that phosphorus was generally the limiting parameter. The average weight ratio of DON to DOP was 23 to 66 in summer and 29 to 62 in winter at five reed zones. The DON : DOP ratios at St. B in summer and at St. E in winter were much higher than those in other observations. It has been investigated that some dissolved organic nitrogenous and phosphorus compounds are utilized as a nitrogen or phosphorus source for phytoplankton (e.g., Berman and Bronk, 2003). The ratio of total dissolved nitrogen (DTN) to phosphors (DTP) was 14 to 28 and 21 to 59 by weight ratio in both summer and winter seasons. High ratios were obtained in winter season. The present results in dissolved forms suggest that the phosphorus is also a limiting parameters rather than the nitrogen. Mitamura *et al.* (1999) indicated that the phosphorus was generally the limiting parameter for the growth of pico- and larger phytoplankton in the pelagic areas of Lake Biwa, showing a similar tendency to the present investigations in the reed zones.

The EC : Chl. *a* or PC : Chl. *a* ratios was measured relatively low values, namely averaged 53 and 42 mg C mg chl. a^{-1}. In the present investigations, therefore, a large number of the epiphytic and planktonic substances seemed to be composed of algae. The weight ratios of EC : EN : EP, based on phosphorus, in epiphytic algae were 32 : 11 : 1 to 89 : 27 : 1, as an average value at five inside sampling points in respective reed zones, in summer and 74 : 16 : 1 to 111 : 30 : 1 in winter (Table 4). The planktonic PC : PN : PP, on the other hand, were 39 : 5 : 1 to 82 : 11 : 1 in summer and 44 : 9 : 1 to 178 : 24 : 1 in winter, respectively. The present epiphytic EC : EN : EP and planktonic PC : PN : PP ratios showed somewhat high compared with the Redfield stoichiometric ratio. Sanudo-Wilhelmy *et al.* (2004) noted that the stoichiometric ratio of phytoplankton was strictly influenced by the surface adsorbed and intracellular phosphorus pools. Then, the EC : EN : ECP and PC : PN : PCP ratios without luxurious

Table 4. Ratios of phosphorus to other parameters in respective reed zones. Values are expressed by weight ratios of an average value at five inside sampling points in respective reed zones. TNN: sum of ammonia, nitrite, nitrate and urea nitrogen; DIP: phosphate phosphorus; DON or DOP: dissolve organic nitrogen or phosphorus; DTN or DTP: dissolved total nitrogen or phosphorus (sum of inorganic and organic forms of nitrogen or phosphorus); TN or TP: total nitrogen or phosphorus in water (sum of dissolved and particulate forms of nitrogen or phosphorus); PC, PN or PP: particulate carbon, nitrogen or phosphorus; EC, EN or EP: epiphytic carbon, nitrogen or phosphorus; PCP or ECP: cellular phosphorus without luxurious phosphorus (difference between PP or EP and acid extractable phosphorus (PAP or EAP) in particulate or epiphytic substances); ATN or ATP: areal total nitrogen or phosphorus (sum of dissolved, particulate and epiphytic forms of nitrogen or phosphorus per unit area of reed zone), respectively.

	St. A		St. B		St. C		St. D		St. E	
	July	December	July	December	July	December	July	December	July	December
TNN:DIP	13.0	35.3	11.4	18.4	36.7	68.3	32.8	18.0	8.6	57.2
DON:DOP	38.2	40.5	66.1	29.2	23.0	32.2	23.7	36.6	35.2	62.3
DTN:DTP	25.0	37.0	14.4	21.4	28.1	51.3	28.0	21.8	15.0	59.2
TN:TP	20.7	22.7	12.9	15.6	20.1	29.5	19.9	16.9	13.9	49.9
PC:PN:PP	76:10:1	93:11:1	39:5:1	44:9:1	82:11:1	83:15:1	79:10:1	67:11:1	69:8:1	178:24:1
EC:EN:EP	76:25:1	91:21:1	48:18:1	104:13:1	32:11:1	80:16:1	89:27:1	74:16:1	82:25:1	111:30:1
PC:PN:PCP	102:13:1	113:14:1	56:8:1	55:11:1	108:15:1	102:19:1	103:13:1	85:14:1	90:11:1	215:29:1
EC:EN:ECP	97:31:1	107:25:1	66:25:1	125:15:1	35:12:1	92:18:1	108:32:1	84:18:1	98:30:1	122:33:1
ATN:ATP	21.8	21.9	13.3	14.7	18.9	26.5	20.4	16.8	18.1	32.5

and adsorbed phosphorus were calculated to evaluate the originated cellular ratio and to assess the accurate limitation. The weight ratios of EC : EN : ECP ranged from 35 : 12 : 1 to 108 : 32 : 1 and 84 : 18 : 1 to 125 : 15 : 1 in both summer and winter seasons, The PC : PN : PCP ratios, on the other hand, were 56 : 8 : 1 to 108 : 15 : 1 and 55 : 11 : 1 to 215 : 29 : 1 in both seasons, respectively. These epiphytic and planktonic ratios were much higher than the Redfield stoichiometric ratio. In the pelagic areas of Lake Biwa, Tezuka (1985), Nakanishi *et al.* (1990) and Seike *et al.* (1996) reported that the phytoplankton were exposed frequently to moderate or serve phosphorus deficiency, whereas the phytoplankton were not nitrogen deficient at any time, from the particulate C : N : P ratios. The present results were similar to those by them. High ratios without luxurious phosphorus in the epiphytic algae in the present investigations indicated that the phosphorus is the limiting parameter regulating the growth of epiphytic algal populations, especially in winter season.

Areal amounts of the epiphytic and planktonic nitrogen and phosphorus in respective reed zones were estimated from both the stem density of *Phragmites* and the water depth at sampling points. To evaluate the potential limitation in respective reed zones, the ratio of the areal total nitrogen (ATN: sum of TNN, DON, PN and EN amounts per unit area of reed zone) to phosphorus (ATP: sum of DIP, DOP, PP and EP amounts per unit area of reed zone) was calculated. The ATN : ATP ratio was 13.3 to 21.9, as an average value at five inside sampling points in respective reed zones, in summer and 14.7 to 32.5 in winter investigations (Table 4). High ratios in entire investigations in both seasons were obtained. The present assessments from the measurement of the luxury phosphorus fraction and the stoichiometric analyses of carbon, nitrogen and phosphorus indicated that the phosphorus was the limiting parameter for the epiphytic and planktonic algal growth in the reed zones at the littoral areas of Lake Biwa, and agreed with the results by previous workers obtained in the pelagic open areas of Lake Biwa.

The present study could provide only approximations under the environmental conditions in respective investigations. The stoichiometric C : N : P ratio of epiphytic and planktonic algae might vary widely among the algal growth under different nutrient status (Guildford and Hecky, 2000) and taxonomic group even in the saturated levels of nutrients concentrations for algal growth (Sakushaug *et al.*, 1983; Quigg *et al.*, 2003). To elucidate the luxury uptake and limitation of phosphorus for epiphytic algal growth in the reed zone ecosystem, further investigations of several fluxes, such as the uptake activity, the algal species and the biofilm structure, were required.

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