

## K:Fe Ratio as an Indicator of Cyanobacterial Bloom in a Eutrophic Lake

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**Abstract** The effects of potassium, sodium, calcium, magnesium, and iron on cyanobacterial bloom potentials were investigated in Daechung Reservoir, Korea. Potassium showed the highest correlation with the cyanobacterial cell number ( $r=0.487$ ,  $P<0.05$ ) and phycocyanin concentration ( $r=0.499$ ,  $P<0.05$ ). However, it was not likely that the potassium had directly affected the bloom formation, because the variations of its concentration were not significantly large. In contrast, the Fe concentration fluctuated drastically and exhibited a negative correlation with the cyanobacterial cell number ( $r=-0.388$ ,  $P<0.1$ ) and phycocyanin concentration ( $r=-0.446$ ,  $P<0.05$ ). Accordingly, the K:Fe atomic ratio would appear to reflect the extent of cyanobacterial bloom more precisely than K or Fe alone. The K:Fe ratio specifically correlated with cyanobacterial percentage, the cyanobacterial cell number and phycocyanin concentration ( $r=0.840$ ,  $P<0.001$ ;  $r=0.416$ ,  $P<0.05$ ;  $r=0.522$ ,  $P<0.01$ , respectively). With the K:Fe atomic ratio of over 200, the chlorophyll-*a* concentration, cyanobacterial cell number, and phycocyanin concentration exceeded  $10 \mu\text{g l}^{-1}$ ,  $20,000 \text{ cells ml}^{-1}$ , and  $20 \text{ pM}$ , respectively, the general criteria of eutrophic water.

**Key words:** Bloom, cyanobacteria, Daechung Reservoir, indicator, K:Fe ratio

Freshwater bloom formation requires high concentrations of nutrients, especially phosphorus (P). However, in some cases, no cyanobacterial bloom is observed in eutrophic ponds with optimal environmental conditions. Parker *et al.* [26] discovered that high concentrations of potassium (K) suppressed the growth of *Microcystis* spp. in several Indian eutrophic ponds. Conversely, dinoflagellate blooms co-occur with increased urea concentrations in bass aquaculture ponds [12]. In some regions of the Pacific Ocean, the low availability of iron limits algal growth [6, 7, 8]. All these

facts indicate that some other chemicals may be suppressing the massive growth of phytoplankton or that minor nutrients may be limiting factors for algal growth, even under favorable conditions in eutrophic lakes. Cations have not been paid much attention to principal factors for bloom formation. Therefore, studies on the relationship between cations and algal blooms could provide new insights into mechanisms of bloom formation.

Potassium and sodium are cofactors for several enzymes and involved in protein synthesis and osmotic regulation [5]. Magnesium is a component of chlorophyll [5]. Calcium plays a role in motility, heterocyst differentiation, signal transduction and regulation of enzyme activities [18]. Iron is the fourth most abundant element by weight in the earth's crust and a key nutrient and is essential for the photosynthetic electron transport system. Nevertheless, the acquisition of Fe in aquatic microorganisms remains a difficult problem. Ferrous iron ( $\text{Fe}^{2+}$ ) quickly converts to ferric iron ( $\text{Fe}^{3+}$ ) in water and  $\text{Fe}^{3+}$  forms insoluble hydroxides, thereby making Fe unavailable to algae at neutral and alkaline pHs [31]. Iron usually exists in unavailable particulate and colloidal forms in water. Under Fe stress, algae and bacteria cope with the Fe deficiency by siderophore/flavodoxin production [16, 20], reduction in cell volume [23] and toxin production [17].

Sources of Fe vary significantly with the geographical conditions and climate. Bacteria and protozoan grazers act as sources of Fe in water. Siderophore-producing bacteria may increase the solubility of Fe, thereby enhancing its availability to algae [13]. The digestion of colloidal Fe in the acidic food vacuoles of protozoan grazers may also generate bioavailable Fe from unavailable Fe compounds [4]. The bioavailability of Fe can be improved by chelation to dissolved organic matter, primarily humic acid [15].

The cyanobacterial density, concentrations of phycocyanin, nutrients, and 5 representative cations (K, Na, Mg, Ca and Fe) were measured every week during an algal bloom in Daechung Reservoir in Korea. And the roles of cations and

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associated mechanisms that affected cyanobacterial bloom in the reservoir were investigated.

## MATERIALS AND METHODS

### Study Site and Sample Collection

Daechung Reservoir is located on the upper part of the Geum River in the central region of South Korea (36°50' N; 127°50' E). It is a large branch-type, dimictic, and temperate lake. Precipitation is usually concentrated in summer, particularly in July and August, due to Asian monsoon season. At one fixed site near Daechung Dam, the environmental conditions, nutrient concentrations, and algal biomass were measured at 1-week intervals from 27 April 1999, until 12 October 1999. The sampling depth was within 1 m. The cation concentrations were also measured during the same period except for 27 April. Water samples were transferred to the laboratory in ice for measuring the amounts of N, P, and cations. The water samples for the algal biomass counting and phycocyanin (PC) measurement were concentrated 1,000 times using a plankton net (mesh size 10  $\mu\text{m}$ ).

### Biochemical Analyses

The PC was measured with a spectrophotometer (Shimadzu UV-160A) after extraction using acetone and sodium acetate [24] on the sampling day. The chlorophyll-*a* (Chl-*a*) was extracted with a chloroform-methanol mixture (2:1, vol/vol) and measured with a fluorometer (Turner 450) according to the method of Wood [34].

The orthophosphate was determined by the ascorbic acid method [2]. The total dissolved phosphorus (TDP) was determined after filtration of the water sample through a 0.45  $\mu\text{m}$  cellulose filter (Millipore Type JH) and persulfate oxidation [21]. Total phosphorus (TP) was determined after persulfate oxidation without filtration. Total particulate phosphorus (TPP) was obtained by subtraction of TDP from TP.

### Cations Analyses

The concentrations of K, Na, Mg and Ca were measured using an atomic absorption spectrometer (Smith-Hieftje 4000) and the Fe was measured with an inductively coupled plasma atomic emission spectrophotometer (Shimadzu ICPS 1000III). The samples for the dissolved cationic form were preserved with nitric acid (1 ml l<sup>-1</sup>) after filtration through a 0.45  $\mu\text{m}$  cellulose filter (Millipore Type JH), whereas those for the total form were preserved with nitric acid (5 ml l<sup>-1</sup>) without any further treatment until measurement. The concentrations of the particulate cationic form were calculated by subtracting the dissolved values from the total values.

### Algal Species Composition and Meteorological Data

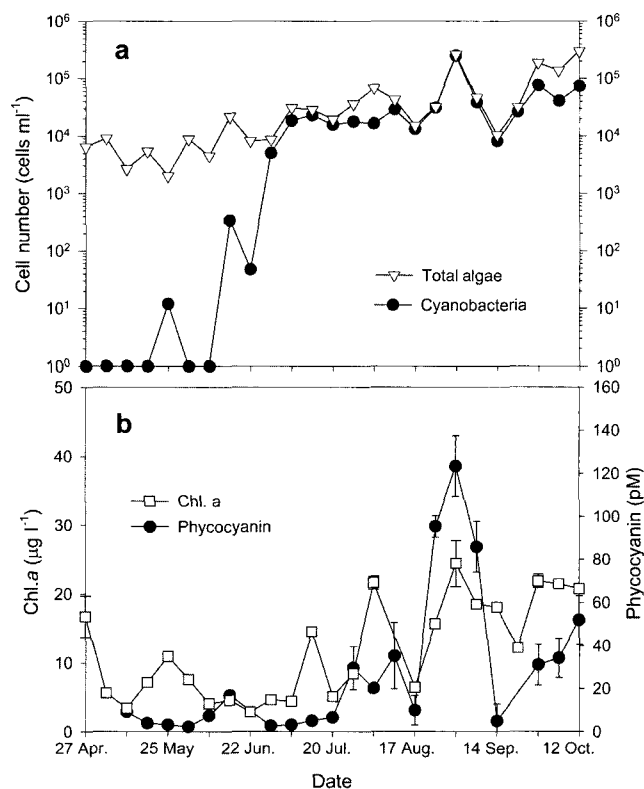
The counting and classification of the algal cells were performed using a hemocytometer (Marienfeld, Fuchs-

Rosenthal) under an optical microscope (Nikon, Microphot-FXA). The cyanobacterial percentage was calculated as total cyanobacterial cell number divided by total algal cell number ( $\times 100\%$ ). The precipitation data were obtained from the Korea Meteorological Administration.

## RESULTS

### Bloom in the Daechung Reservoir

Daechung Reservoir was eutrophic throughout the investigation period, since the average concentrations of TP and TN were about 0.20 mg l<sup>-1</sup> and 1.03 mg l<sup>-1</sup>, respectively. Blooms were attributed to cyanobacteria, accompanying increases in temperature, light intensity, pH, and day length. Concentrations of PC, which is a cyanobacteria-specific pigment protein, followed the same pattern (Fig. 1). Cyanobacteria were dominated by *Microcystis* spp. and *Anabaena* spp., which accounted for over 90% of cyanobacteria during the entire investigation period. *Anabaena* spp. were often the most abundant. Diatoms were dominant in early spring and fall. *Asterionella formosa* and *Aulacoseira* spp. were dominant in spring,



**Fig. 1.** Cell numbers of total algae and cyanobacteria (a) and concentrations of Chl-*a* and phycocyanin (PC) (b) in Daechung Reservoir during the investigation period.

Each value in (b) is the mean of triplicate  $\pm$  SD. Zero cells/ml of cyanobacteria is expressed as 10<sup>0</sup> cells/ml in (a).

**Table 1.** Correlation coefficients of cations with cyanobacterial cell number, phycocyanin, and phosphorus ( $n=24$ ).

Cation <sup>a</sup>	Total cyanobacteria (cells ml <sup>-1</sup> )	Phycocyanin (pM)	<i>Microcystis</i> spp. (cells ml <sup>-1</sup> )	<i>Anabaena</i> spp. (cells ml <sup>-1</sup> )	Total phosphorus (mg l <sup>-1</sup> )	Total dissolved phosphorus (mg l <sup>-1</sup> )
K	0.487*	0.499*	0.503*	0.328	0.757***	0.671***
Na	0.220	0.237	0.419*	0.287	0.498*	0.580**
Ca	0.245	0.402	0.451*	0.279	0.612**	0.707***
Mg	0.269	0.192	0.367	0.220	0.538**	0.547**
Fe	-0.388	-0.446*	-0.538**	-0.352	-0.441*	-0.587**
K:Fe ratio	0.416*	0.522**	0.561**	0.407*	0.454*	0.662***

<sup>a</sup>Cations of total form were used.

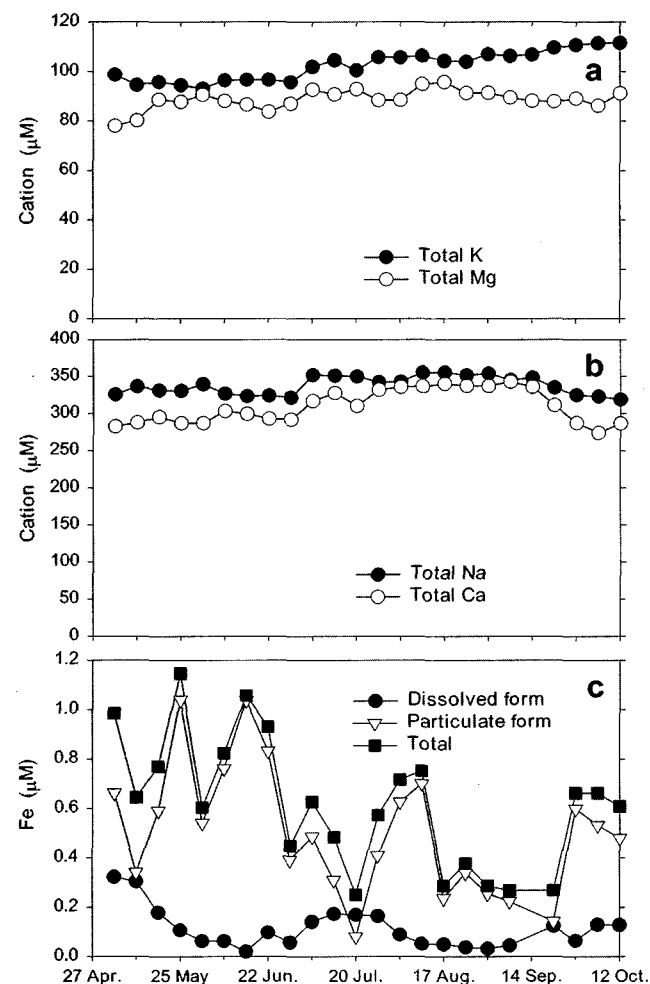
\*:  $P<0.05$ . \*\*:  $P<0.01$ . \*\*\*:  $P<0.001$ .

whereas *Aulacoseira* spp. and *Fragilaria crotonensis* dominated in fall.

### Correlations between Cations and Cyanobacterial Bloom

Among the five cations measured, only total forms of K (hereafter cations refer to total forms without special

comments) showed a clear correlation with the cyanobacterial cell number (Table 1). Although K exhibited a strong correlation with cyanobacterial cell density ( $r=0.487$ ,  $P<0.05$ ), it did not appear to have a direct influence on the bloom formation, because its concentrations stayed within a narrow range without a significant change for about 6 months (Fig. 2a). The Na, Mg, and Ca concentrations also did not show large variations (Fig. 2a and 2b). Accordingly, nearly constant concentrations of the 4 cations would not appear to be significantly related with cyanobacterial bloom. In contrast, the Fe concentration fluctuated dramatically (Fig. 2c) and exhibited a strong negative correlation with *Microcystis* spp. ( $r=-0.538$ ,  $P<0.01$ ) and a comparable negative correlation with the PC ( $r=-0.446$ ,  $P<0.05$ ) (Table 1). Therefore, these results indicate that K and Fe had a stronger effect on the bloom formation than Na, Mg, and Ca.



**Fig. 2.** Concentrations of (a) K, Mg, (b) Na, Ca, and (c) Fe in Daechung Reservoir.

Error bars of standard deviations were omitted because they were too small to be shown in the graphs.

### K:Fe Atomic Ratio and Cyanobacteria

Potassium exhibited a positive correlation with the cyanobacterial cell number and PC, while Fe showed a negative one. By combining their inverse relations, the resulting K:Fe atomic ratio could be used as a new parameter that simultaneously expresses the two cationic characteristics. The K:Fe atomic ratio showed a better correlation with the *Microcystis* spp. and *Anabaena* spp. numbers and the PC than K or Fe alone (Table 1). The effects of K and Fe were more prominent on *Microcystis* spp. than on *Anabaena* spp. (Table 1). However, correlations using ratios or percentages can be problematic, because their distribution may be rather unusual and not normally distributed. This difficulty can be overcome by log transformation of the variable [30]. The log transformation of K:Fe ratio and cyanobacterial percentage produced almost similar correlation results (data not shown), confirming validity of the previous correlation analysis.

Over 90% of the cations were in dissolved form during the entire period of the investigation except K and Fe (Fig. 3). A high proportion of dissolved form usually indicates that these cations are not cellular constituents. The dissolved portion of K was slightly lower than that of other cations, whereas that of Fe was much lower.

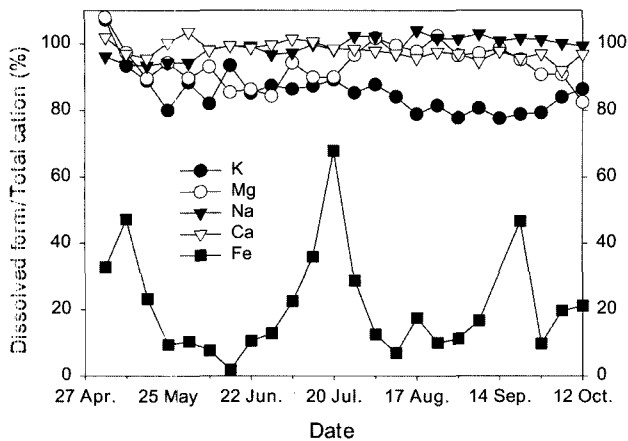


Fig. 3. Percentages of dissolved cationic forms.

The high correlation between the particulate K and the cyanobacterial percentage ( $r=0.599$ ,  $P<0.002$ ) implies that the particulate K was principally constituted in cellular form. Conversely, the negative correlation between the particulate Fe and the cyanobacterial percentage ( $r=-0.756$ ,  $P<0.001$ ) indicates that, even though it existed in particulate form, most of the particulate Fe was not a cellular component.

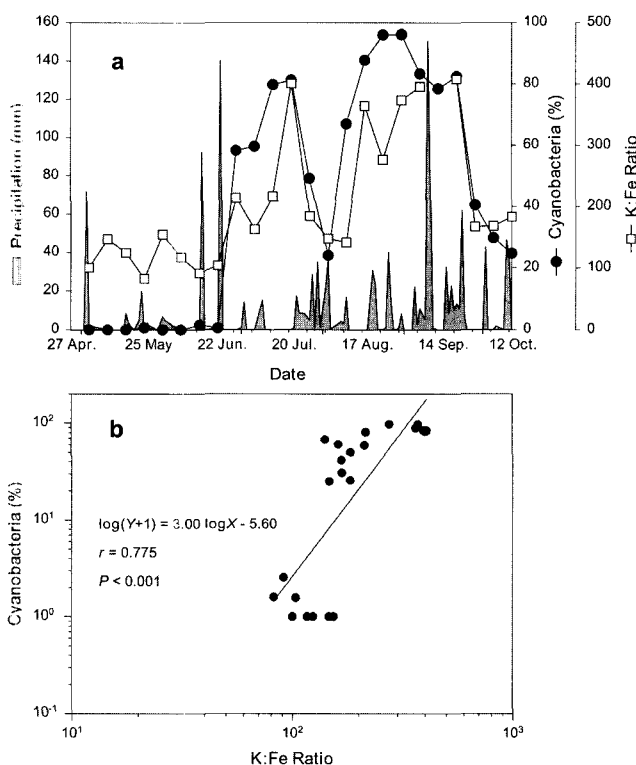


Fig. 4. (a) Precipitation and changes in cyanobacterial percentage and K:Fe atomic ratio during the period of algal bloom in Daechung Reservoir, (b) Correlation between K:Fe ratio and cyanobacterial percentage.

By plotting the K:Fe atomic ratio along with the cyanobacterial percentage, it was found that the curve of the K:Fe ratio was very similar to that of the cyanobacterial percentage with a strong correlation (Fig. 4). Both the cyanobacterial percentage and K:Fe ratio increased after rain, whereas they decreased during rain. The concentrated precipitation in late July and late September induced a rapid decline in both the cyanobacterial percentage and the K:Fe atomic ratio.

#### Change of P Concentration with Bloom

Orthophosphate, TPP and Fe concentration exhibited the same pattern of rise and fall particularly in blooming season (August-September). On the other hand, nitrogen did not exhibit any meaningful relationship with cyanobacterial bloom (data not shown). Although it is not shown critically in Fig. 5, orthophosphate concentration on 31 August, when bloom was at peak, was  $0.011\pm 0.001$  (mean $\pm$ SD,  $n=3$ )  $\text{mg l}^{-1}$ , the minimum value during the entire period of investigation. Orthophosphate concentrations earlier and after 2 weeks of the bloom peak, were about two times higher,  $0.018\pm 0.002$  (mean $\pm$ SD,  $n=3$ )  $\text{mg l}^{-1}$ . This indicates that severe bloom formation induced a substantial uptake of phosphate or sedimentation of Fe with orthophosphate. Sedimentation of P is more definitely shown by the decreased TPP with bloom (Fig. 5).

#### Validity of K:Fe Ratio as a Bloom Indicator

The criteria of a bloom include a cyanobacterial cell concentration of  $20,000 \text{ cells ml}^{-1}$  [11] and PC of  $20 \text{ pM}$  [1]. The linear regression of the cyanobacterial cell concentration relative to the K:Fe ratio showed that 197.1 was the critical K:Fe ratio value for determining a bloom ( $r=0.416$ ,  $P<0.05$ ). The linear regression of the PC relative to the K:Fe ratio produced a critical value of 192.1 ( $r=0.522$ ,  $P<0.01$ ).

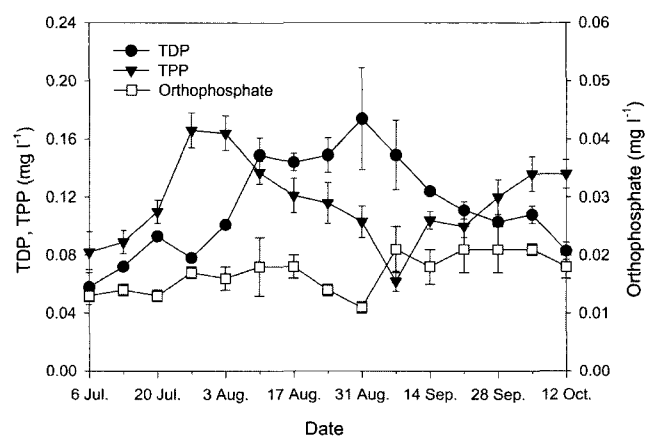


Fig. 5. Changes in phosphorus (P) concentrations in Daechung Reservoir. Mean of triplicate $\pm$ SD.

## DISCUSSION

### Effect of K on Cyanobacteria

Among the various cations, K and Fe were more highly correlated with cyanobacterial density and percentage than Na, Ca and Mg. While a high concentration of K inhibited the growth of *Microcystis* spp. in eutrophic ponds in India [26], the K concentration increased with cyanobacterial bloom in Daechung Reservoir. The discrepancy between these two results was caused by a significant difference in the respective concentrations. The inhibitory concentration of K in the Indian ponds was above 2.8 mM, while the K concentration in Daechung Reservoir was only about 0.1 mM. Therefore, the K concentration was too low in Daechung Reservoir to inhibit the growth of *Microcystis* spp. and instead promoted cyanobacterial growth with an increased concentration at that level. Daechung Reservoir can be classified as an argillaceous type according to Stumm and Baccini [32], because of low concentrations of Ca, Mg and Na. On the other hand, Indian ponds that Parker *et al.* [26] studied were rather salty due to high concentrations of Na (ca. 200 mg l<sup>-1</sup>) and K (ca. 100 mg l<sup>-1</sup>). Potassium inhibition of *Microcystis* spp. at high concentrations is a very specific phenomenon in saline ponds and lakes. Therefore, it could not be applied generally to Daechung Reservoir. K:Fe ratio as an indicating parameter for cyanobacterial bloom cannot be applied to all kinds of lakes for the same reason. Nevertheless, K:Fe ratio could be a good criterion for cyanobacterial bloom in lakes similar to Daechung Reservoir, i.e. an argillaceous-type, temperate lake influenced by Asian monsoons.

### The Role of Fe in Daechung Reservoir

Cyanobacteria require less Fe than P for growth and maintenance. However, Fe is essential for photosynthesis, nitrogen fixation, and nitrate reduction. Usually, higher concentrations of nutrients promote faster algal growth. But, the results shown in Table 1 and Fig. 2 are contrary to this expectation, as Fe is negatively correlated with cyanobacteria. However, the amount of Fe available for algal growth is generally insufficient because Fe usually exists in unavailable forms in water [4, 7, 10]. Even worse problem is that the half-life of soluble Fe<sup>2+</sup> is too short, e.g. only 7–60 sec in Lake Greifen, a temperate and eutrophic lake in Switzerland [9]. The actual amount of Fe accessible in bioavailable forms is still unknown because a high concentration of Fe does not necessarily ensure the large amount of bioavailable forms. In contrast, as shown in Table 1 and Fig. 2 the results suggest that the bioavailability of Fe could be inversely related with its concentration. Particulate forms of Fe constituted about 80% of the total Fe, and the concentration changes of particulate Fe were found to be quite similar to those of the total Fe. Algae seemed to have utilized Fe in small amounts because the

concentrations of particulate Fe changed in a converse pattern with the cyanobacteria. This indicates that the majority of Fe was not a cellular constituent but rather in inorganic and organic detritus forms. Additionally, it was also clear that Fe, at least, did not limit the growth of algae in Daechung Reservoir. A molar ratio of dissolved ionic Fe (Fe<sub>i</sub>) to orthophosphate (PO<sub>4</sub>) lower than 10<sup>-2</sup> to 10<sup>-4</sup> has been suggested as indicating Fe limitation of algal growth [22]. The Fe<sub>i</sub>: PO<sub>4</sub> atomic ratio in Daechung Reservoir was 0.21±0.12 (mean±SD, n=24), higher than the above criterion value for Fe limitation. This fact further supports the belief that algal growth was not limited by Fe in Daechung Reservoir.

The bioavailability of Fe can be increased through several processes, e.g. thermal dissolution, photochemical reduction, and ligand complexation [4]. The latter two processes may have occurred in Daechung Reservoir. Although combination of ultraviolet and heavy metal decreases the primary production of cyanobacteria [3], sunlight is known to be effective in the dissolution of colloidal Fe oxides particularly in the presence of naturally occurring organic materials that increase the bioavailability [33]. Irrespective of the Fe concentration, the availability of Fe to the algae in Daechung Reservoir could have been enhanced by photochemical reduction as a result of the high intensity of sunlight after rainfall, thereby promoting cyanobacterial bloom. The Fe in Daechung Reservoir is believed to be mainly supplied from the upstream inflow after rain, since Fe in freshwater is known to be supplied by particulate inputs [29]. Figure 2c and 4a show the increase of Fe concentration after much rain.

Ferric sulfate is commonly used to remove algae and P in lakes through the formation of ferric hydroxide flocs [19]. Therefore, the decline of the Fe concentration relative to the cyanobacterial bloom could be attributed to the sedimentation of particulate Fe through the formation of flocs with the algae or P. The simultaneous decrease in the Fe concentration, TPP, and orthophosphate also supports the sedimentation of Fe with P at the time of bloom (Fig. 2c and 5). *Microcystis* spp. appeared to have been sedimented much more than *Anabaena* spp. with Fe (Table 1). Cyanobacteria in general produce a polysaccharide capsule with a high negative charge, thereby facilitating metal accumulation [14, 27]. In freshwater lakes, manganese nodules are known to precipitate through adsorption within these *Microcystis* spp. capsules [28]. An increase in the viscosity of this extracellular polysaccharide by cations could make it much easier for *Microcystis* spp. flocs to sediment [25]. The relatively high concentration of Fe in early August would then seem to be facilitated by the temporarily lower cyanobacterial percentage at that time, even with bloom (Fig. 2c and 4a). These results indicate that a cyanobacterial bloom, particularly *Microcystis* spp., promotes the sedimentation of Fe with P. This is also

supported by the fact that the cell number of *Anabaena* spp. culminated on 31 August, up to  $1.84 \times 10^5$  cells  $\text{ml}^{-1}$  at the time of the most severe bloom, whereas the cell number of *Microcystis* spp. was one-third ( $6.12 \times 10^4$  cells  $\text{ml}^{-1}$ ) of that of *Anabaena* spp. In this study, Fe is not regarded as one of the limiting nutrients, because algae are not limited by Fe in Daechung Reservoir. Instead, Fe is regarded as a cyanobacteria-sedimenting agent. In other words, high concentrations of Fe reduce cyanobacterial bloom. Concentrated rainfalls in late July and late August brought about increased Fe and simultaneous decrease of cyanobacterial percentage (Fig. 2c and 4). *Microcystis* spp. followed such pattern of cyanobacterial percentage more similarly than *Anabaena* spp., indicating that the sedimentation of *Microcystis* spp. with Fe occurred much more than that of *Anabaena* spp. (data not shown).

### K:Fe Ratio as a New Indicator

Potassium acted as a growth-limiting factor, where Fe did not. Yet, Fe sedimented with P during bloom formation. By combining these two cation characteristics, the K:Fe atomic ratio can be used as a new parameter for indicating cyanobacterial bloom. Linear regression showed that a K:Fe ratio of about 200 agreed with the known criteria of bloom formation, e.g. a cyanobacterial concentration of 20,000 cell  $\text{ml}^{-1}$  [11] ( $r=0.416$ ,  $P<0.05$ ) or PC concentration of 20  $\mu\text{M}$  [1] ( $r=0.522$ ,  $P<0.01$ ). Another criterion of bloom, a Chl-*a* concentration of 10  $\text{mg l}^{-1}$  [19], also confirmed that K:Fe ratio of 200 is a good indicator. Accordingly, it would appear that the K:Fe atomic ratio is an effective parameter for estimating cyanobacterial bloom.

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