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Phosphatase Activity in Cheonho Reservoir

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Phosphatase activity was measured with other environmental factors in Cheonho reservoir in 1994. It ranged from 95 to 1,685 nM/l/h and was correlated significantly with chlorophyll-*a*. Such a close relation well matched the fact that over 90% of phosphatase activity was detected in >3 μm fraction. The phosphatase activity also correlated negatively with dissolved inorganic phosphate concentration, which implies derepression of phosphatase production by phosphate limitation. Significant correlation was analyzed between phosphatase activity and BOD, which also appeared to be closely correlated with chlorophyll-*a*. A great percentage of organic materials seems to be generated autochthonously by algae and extracellular enzyme even though allochthonous influence was thought to be stronger in Cheonho reservoir.

Key words: phosphatase activity, dissolved inorganic phosphate, chlorophyll-*a*, BOD

Generally, dissolved inorganic phosphate (DIP) is found to be a limiting nutrient for algal growth and eutrophication, especially in freshwater lakes (13). The phosphorous cycle is significantly dependent on rapid regeneration of DIP from particulate and dissolved organic phosphate by phosphatase. Thus phosphatase activity is important for estimating the portion of phosphate available to algae in addition to DIP, with the aim of improving understanding of phosphorus cycling in lakes (3, 5, 12).

A large portion of phosphatase activity was thought to originate from algae. This conclusion was mainly based on indirect evidence that phosphatase activity could be used as an indicator of algal phosphorus limiting and that algal biomass and phosphatase activity were closely related (16). In field and laboratory studies, the quantitatively important contributors to the overall phosphatase activity appear to be bacteria and algae, though some experts have emphasized excretion of phosphatase from zooplankton (6, 15). In recent reports, many scientists have been emphasized the algal portion in total phosphatase activity though some have reported reverse results (11).

Phosphatase is repressed under conditions of excess phosphorus and derepressed (induced) when phosphorus concentrations become limiting. Moreover the activity of these enzymes is competitively inhibited by DIP (1, 3, 5, 10, 11, 12, 16). It was reported that the algal phosphatase was directly inhibited by DIP, but inhibition of bacterial phosphatase activity was only slight (10). Induction, where phosphatase activity is enhanced by additions of organic phosphate substrate, seems rather uncommon (or less investigated than inhibition by DIP) (5, 16).

Phosphatase activity was investigated in a shallow and small reservoir, and the relations between phosphatase activity and other environmental factors such as water temperature, pH and DIP concentration were analyzed.

Materials and Methods

Sampling and station description

From March to December 1994, phosphatase activity, water temperature, pH, DIP, total phosphorus, chlorophyll-*a*, and heterotrophic bacterial numbers were analyzed 10 times monthly.

Station 1 is the inlet of a small stream from Munam reservoir and the Shincheon area, which has an agricultural region. Station 2 is affected by a more polluted stream from the Anseo area that has a much higher population than the Shincheon area. Station 3 is located near the outlet of a reservoir in front of the dam. Cheonho reservoir is a small eutrophic lake with a total water volume of 1,250,000 m^3 (area 0.32 km^2, mean depth 4 m) that shows a high chlorophyll-*a* and DIP concentra-

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tion, with algal blooms two or three times annually.

Physico-chemical environmental factors

Water temperature and pH were measured by thermometer and portable pH meter at *in situ*. Dissolved inorganic phosphate (DIP) was analyzed by the stannous chloride method (4). Total phosphorus was analyzed in the same method as DIP measurement after the hydrolysis of the particulate and organic phosphorus to DIP by autoclave at 121℃ for 30 minutes. BOD was analyzed by the Standard method (4). Precipitation data for Ahsan city (near Cheonan city) made use of the "Annual Weather Report (1994)" by the Korean Meteorological Administration.

Chlorophyll-*a* and heterotrophic bacteria

Chlorophyll-*a* was measured by spectrophotometer at 664, 647 and 630 nm after acetone extraction (4). Samples were incubated in nutrient agar medium for heterotrophic bacteria (4). The inoculated medium was incubated at 30℃ for 48 hours.

Phosphatase activity

Phosphatase activity was determined by a modified method (3, 10, 14). Two hundred μl of fluorescent substrate (4-methylumbelliferyl-phosphate, 5 μM) was added to a 1.8 ml sample and incubated in a cuvette at its *in situ* temperature for 30~60 minutes. Then glycin-sodium hydroxide buffer (pH 10.5) was added to the incubated sample and fluorescence was measured by fluorometer (Hoefer, TKO 100). Both non-filtered and filtered ($\not<$ 3 μm) samples were used for fractionated phosphatase activity.

Glucose and glycollate uptake rate

[U-^{14}C] glucose (271 mCi/nmol, ICN Biomedical, Inc., USA) and glycollate (30 mCi/nmol, ICN Biomedical, Inc., USA) were used as a substrate. The radio active tracer [U-^{14}C] was added to the sample for a final concentration of 1.5 μg-C/l and then it was incubated *in situ* temperature for 1 hour (2). After glucose and glycollate incorporation were quantified by liquid scintillation counter (Beckman LS9800, Beckman Instrument, Inc., USA), the uptake rate (%/h) was calculated.

Statistical analysis

A statistical analysis software (MS-Excel 5.0) was used to calculate the mean values and correlations.

Results and Discussion

Environmental factors

The range and mean values of investigated environmental factors are listed in Table 1. Water temperature ranged from 3℃ to 36℃. In summer, the water temperature was high (35℃) in all stations. pH ranged from 6.9 to 9.6 and appeared high in spring and summer when algae bloomed. Dissolved oxygen (DO) was also high in the algal blooming season and correlated closely with chlorophyll-*a*, which also indicated strong oxygen generation by algal photosynthesis. The range of biological oxygen demand (BOD) was 0.2~16.6 mg/l. BOD correlated positively with chlorophyll-*a*.

Dissolved inorganic phosphate (DIP) and total phosphorus

The DIP concentration ranged from 1~72 μg/l and showed low values in April and July, when the DIP was assumed to be rapidly used by overgrown algae at station 1 and 2 (Table 2). The DIP at station 3 had the lowest value in April and had relatively high values of 30~70 μg/l thereafter, which coincides inversely with

Table 1. Range and mean values of environmental factors in Cheonho reservoir.

Factors	Station 1			Station 2			Station 3		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Temperature (℃)	3.0	35.0	19.0	4.0	36.0	19.1	4.0	35.0	19.0
pH	6.9	9.3	8.1	7.3	9.6	8.6	7.2	9.6	8.5
DO (mg/l)	4.4	13.4	8.4	6.2	14.6	9.2	6.9	12.4	8.8
BOD (mg/l)	0.5	16.6	8.1	0.7	16.2	8.0	0.2	16.1	7.0
NH_4 (μg/l)	14	275	106	12	262	113	12	392	114
NO_2 (μg/l)	4	178	75	2	272	87	4	299	92
NO_3 (μg/l)	53	1180	511	138	1006	490	66	719	443
PO_4 (μg/l)	1	47	27	1	52	25	1	72	34
Total-P (μg/l)	34	917	371	34	369	313	34	1270	452
Chlorophyll-*a* (μg/l)	17	330	74	24	148	80	32	109	70
Heterotrophic Bacteria (CFU/ml)	1.0	64.0	23.9	1.5	76.0	1.0	1.0	36.0	10.6
Glucose uptake rate (%/h)	1.8	44.0	18.7	7.0	75.8	36.1	0.1	84.8	39.6
Glycollate uptake rate (%/h)	0.9	27.4	7.9	2.4	88.1	23.1	0.5	79.9	21.7

Table 2. Variations in DIP and total phosphorous (μg/l) in Cheonho reservoir.

Month	Station 1		Station 2		Station 3	
	DIP[a]	TP[b]	DIP	TP	DIP	TP
March	17	599	13	510	15	510
April	1	34	1	34	1	34
May	47	563	34	578	70	1139
June	43	917	52	369	34	387
July	17	207	1	171	34	138
August	38	309	31	316	72	256
September	34	246	33	246	33	246
October	27	246	17	263	22	263
November	26	352	34	281	34	281
December	17	242	34	359	20	1270

[a] Dissolved inorganic phosphate (μg/l).
[b] Total phosphorous (μg/l).

Table 3. Variations in Chlorophyll-*a* (μg/l) in Cheonho reservoir.

Month	Station 1	Station 2	Station 3	Mean
March	47	59	59	55
April	330	148	108	195
May	29	145	109	94
June	61	97	101	86
July	104	147	95	115
August	47	43	49	46
September	46	46	49	47
October	30	46	36	37
November	31	24	32	29
December	17	41	63	41

chlorophyll-*a* concentration.

Total phosphorus ranged approximately from 200 to 600 μg/l (Table 2), except in April when it showed minimal values (34 μg/l). However, it increased to about 1, 200 μg/l) at station 3 in May and December. It seemed that a high level phosphorus was loaded from outside and induced the phosphatase activity (16).

Chlorophyll-*a*

The chlorophyll-*a* concentration ranged from 17 μg/l to 330 μg/l with an annual average of 75 μg/l, which could indicate a eutrophic lake (Table 3). Massive algal blooms occurred twice in April and July. At station 1, the value of chlorophyll-*a* was recorded as the highest at 330 μg/l in April, decreased dramatically to 29 μg/l in May, and increased to the second highest value in July. It decreased to 47 μg/l in August and leveled off at low values till the end of the year. At station 2, two peaks of chlorophyll-*a* appeared in April and July, but values did not show significant fluctuation during that season. At station 3, chlorophyll-*a* showed high values from April to July and low values in other months. Thus algal blooms continued for several months at station 3,

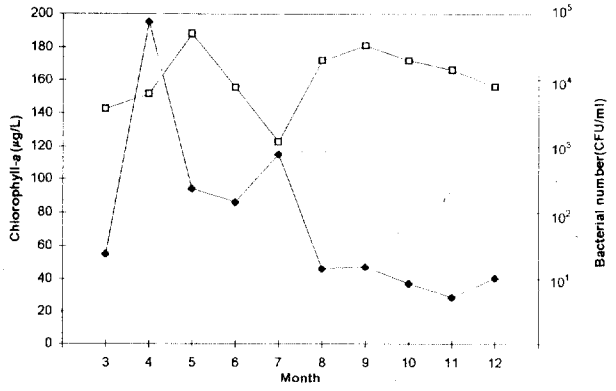

Fig. 1. Variation of mean values in chlorophyll-*a* and heterotrophic bacterial number in Cheonho reservoir, 1994 (heterotrophic bacterial number: □—□, chlorophyll-*a*: ◆—◆).

whereas they occurred twice at station 1.

Bacteria

The number of heterotrophic bacteria ranged 1,000~76,000 CFU/ml. At station 1, the number of heterotrophic bacteria was highest in May and September and was almost as high in April and August. Station 2 was similar to station 1, but the second highest bacterial number in August was lower (17,000 CFU/ml) than in station 1. There were also two peaks of heterotrophic bacteria in May and August. The heterotrophic bacteria showed two peaks at station 3 in April and September, but the numbers were lower than at other stations. The relationship between algae and bacteria is shown distinctly in Fig. 1. Following algal blooms, bacterial number increased after one or two months.

The variation in heterotrophic bacterial numbers differed from other studies (2, 7) that showed high numbers in summer and low numbers in winter. Especially low numbers (10^3 CFU/ml) of heterotrophic bacteria and coliform bacteria were detected in July. It was reported by Reinheimer (21) that higher bacterial numbers obtained during the cold season are due to favorable conditions of low temperatures for the nutrition and life of the bacteria, which come mainly from sewage.

Phosphatase activity

The total phosphatase activity was 95~1,685 nM/l/h (Table 4). Maximum total phosphatase activity was determined at all stations in April when during the highest chlorophyll-*a* concentration, and minimum activities were detected in November (station 1 and 2) and December (station 3) when water temperature and chlorophyll-*a* concentration were low. The seasonal fluctuation in total phosphatase activity was similar to that of chlorophyll-*a*, but it showed the 3rd small peak in December.

At station 1, total phosphatase activity showed the hi-

Table 4. Variation in phosphatase activity (nM/l/h) in Cheonho reservoir.

Month	Station 1		Station 2		Station 3	
	Total	3 μm-filtrate	Total	3 μm-filtrate	Total	3 μm-filtrate
March	ND*	ND	ND	ND	ND	ND
April	1685	880	517	94	588	412
May	148	6	455	20	542	30
June	449	20	447	32	190	8
July	349	17	610	58	508	32
August	192	5	521	11	134	21
September	458	9	453	57	104	33
October	484	47	140	7	140	6
November	103	0	136	12	95	4
December	204	10	106	14	355	15

* Not determined.

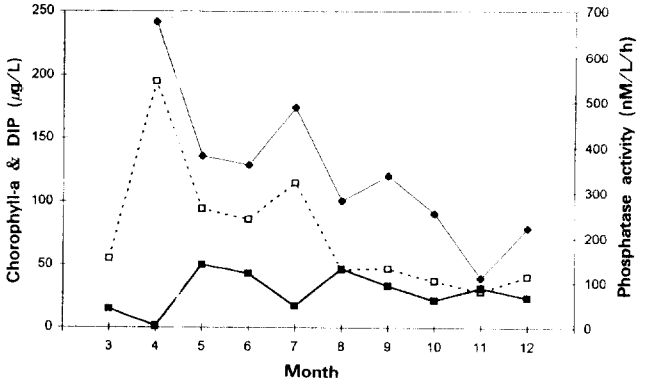

Fig. 2. Variation of mean values in phosphatase activity, chlorophyll-*a* and DIP in Cheonho reservoir, 1994 (chlorophyll-*a*: □—□, phosphatase activity: ◆—◆, DIP: ■—■).

ghest peak in April and 2nd highest peak (449 nM/l/h) in June. The third highest peak of 204 nM/l/h occurred in December following the decrease in July and August and the second increase in October. In June, the high concentration of organic phosphorus was assumed to induce the repressed total phosphatase activity, as shown by the highest total phosphorus value (917 μg/l) even though chlorophyll-*a* was decreased. At station 2, total phosphatase activity increased twice in April and July. The variation in the total phosphatase activity at station 2 was smaller than at station 1. The fluctuation in total phosphatase activity at station 3 was similar to that at station 2.

Phosphatase could be quickly derepressed by deficiency of DIP (12, 22). This phosphatase activity is thought to be switched on or off (induced or inhibited) by over or under a critical DIP concentration adequate for an aquatic system rather than changed by the variations in DIP level. The critical value of DIP concentration for switching on phosphatase activity in the aquatic systems is reported to be 15 μg/l (3, 12). In this study, it was not clear whether phosphatase activity was proportionally changed according to DIP level or sharply switched on or off by a critical DIP value. According to the annual mean values of 3 stations, higher phosphatase activity was estimated when DIP was low (Fig. 2). Interestingly, activity higher than 517 nM/l/h was shown whenever DIP values were 1 μg/l (Table 2 and 4). The extremely high total phosphorus concentration over 1,000 μg/l coincided with high phosphatase activity (June at station 1; May and December at station 3). It was likely that this high substrate load might induce phosphatase activity.

The relationship between phosphatase activity and bacterial number was obscure. This can be explained by the fact that most of the activity originates from algae and peaks of bacterial number did not coincide with algal blooms. Filtered phosphatase activity was estimated to be below 10% of non-filtered activity, and most of that of filtrate was assumed to be dissolved free enzyme originating from algae. It seemed that filtered phosphatase activity also did not show a direct relation with bacterial number and was similiar to non-filtered activity in correlation analysis.

Both non-filtered and filtered (< 3 μm) samples were used for fractionated phosphatase activity. Non-filtered samples were used to study total enzyme activity. Activity in filtered samples could be regarded as bacterial phosphatase activity, which is lower than algal phosphatase activity. Algal phosphatase activity was calculated by substracting the bacterial phosphatase activity from total phophatase activity.

In studies of the size-fraction, the variation in algal phosphatase activity (< 3 μm fraction) and bacterial phosphatase activity (> 3 μm fraction) was almost similar to total phosphatase activity (non-filtered fraction). Algal phosphatase activity made up over 90% of total phosphatase activity. Furthermore bacterial phosphatase activity (< 3 μm fraction) was assumed to be free dissolved phosphatase which mainly orginated from algae. Thus bacterial phosphatase activity (< 3 μm fraction) showed the same fluctuations as total phosphatase activity and did not have any relation with heterotrophic bacterial number.

Statistical analysis

Correlations among the investigated data were analyzed (Table 5). Dissolved oxygen correlated positively with pH. Chlorophyll-*a* correlated positively with pH ($r=0.76$, $p<0.05$) and DO ($r=0.63$, $p<0.05$) at station 1. Such a fact could represent an increase in pH and DO as a result of algal photosynthesis. Chlorophyll-*a* correlated positivly with BOD ($r=0.62$, $p<0.05$), which could suggest that BOD would be increased by autochthonous

Table 5. Correlation coefficiency among various parameters based on data from all stations in Cheonho reservoir.

Station	Factor	Temp.[a]	Prec.[b]	pH	DO	BOD	DIP	Chl-*a*[c]	Glu.[d]
1	DO			0.66					
	Chl-*a*			0.76	0.63	0.65	−0.65		
	Glu.		*0.80						
	Gly.[e]		0.76						
	T-AP[f]						−0.65	*0.95	
2	DO			0.69					
	Chl-*a*			0.7		0.69			
	Gly.							*0.91	
	T-AP	0.68				0.75		0.69	
3	DO	−0.71							
	Chl-*a*					0.75			
	Gly.					0.72			
	T-AP				0.72			*0.83	−0.78
Total	DO	*−0.51		*0.62					
	Chl-*a*			*0.57	*0.56	*0.62			
	Glu.	*0.53							
	Gly.			*0.48	0.38	*0.50			
	T-AP			0.45	0.43	*0.55	−0.37	*0.87	

[a] Water temperature (℃), [b] Sum of 3 days precipitation, [c] Chlorophyll-*a* (μg/l), [d] Glucose uptake rate (%/h), [e] Glycollate uptake rate (%/h), [f] Total phosphatase activity (nM/l/h), *: $P<0.01$, others: $P<0.05$.

algal exudates. The correlation between BOD and glycollate could support such a assumption because glycollate is thought to be a typical algal exudate (19). Phosphatase activity correlated significantly with chlorophyll-*a*. This fact matched the reports that most phosphatase originated from algae (16). Both phosphatase activity and chlorophyll-*a* correlated negatively with DIP and this suggested that DIP could have a role as a trigger to rapid increase of algal growth and phosphatase production. Glucose and glycollate uptake rates correlated with the total precipitation for 3 days. It seemed that large amounts of bacteria flowing in from streams during rainy periods affected uptake rates for glucose and glycollate.

The relations among pH, DO, chlorophyll-*a*, and BOD at station 2 were almost similar to those at station 1. Phosphatase activity at station 2 was more loosely coupled with chlorophyll-*a* than at station 1 and showed additional positive correlations with water temperature and BOD. It could be assumed that organic material might increase autochthonously by phosphatase.

At station 3, pH was not correlated with DO and chlorophyll-*a*. A typical negative correlation between DO and water temperature appeared at station 3. Chlorophyll-*a* was more closely correlated with BOD than at station 2. Phosphatase activity showed a positive correlation with DO and chlorophyll-*a*, and a negative correlation with glucose uptake rate. If products of phosphatase from algae were able to decrease glucose uptake rate, the negative correlation between phosphatase activity and glucose uptake rate might be explained by this fact.

In all stations, DO correlated negatively with water temperature ($r=-0.51$, $p<0.01$) and positively with pH ($r=0.62$, $p<0.01$). These are thought to be a result of algal photosynthesis. Chlorophyll-*a* correlated significantly with pH, DO, and BOD that was due to algal photosynthesis. Glucose uptake rate correlated with precipitation. Glycollate uptake rate also correlated with pH, DO and BOD. Phosphatase activity correlated positively with pH and chlorophyll-a. As shown in the correlation constant of 0.45 ($P<0.05$) between phosphatase activity and pH, the higher pH coincided with higher activity. The activity was not low in pH 9.55 whereas Münster (18) reported it rarely ocurred at a pH over 9.0. It was assumed that phosphatase might be adapted to the pH of the aquatic system (9, 17, 18, 20). Relations between water temperature and activity was not shown clearly. However, the activity was very low during the cold season. Annual variations in total phosphatase activity were almost same as that of chlorophyll-*a*. Their close relations were reported in many papers (8, 16). In this study of size-fraction, over 90% of total phosphatase activity was found to originate from algae. Such a close relation has been observed between chlorophyll-*a* and 5′-nucleotidase activity in the Hudson river in USA (3). For reference, the correlation constants between chlorophyll-*a* and phosphatase activity were reported to be 0.69 (8) and 0.44 (1) in Lake Soyang. The relation between phosphatase activity and DIP ($r=-0.37$, $p<0.05$) implied a possibility of con-

trolling phosphatase production by DIP concentration in aquatic sytems.

Since glucose uptake rate seemed to be inhibited and underestimated by a lot of low-molecular exudates and hydrolyzed products from high densities of algae and extracellular enzymes, it hardly appeared to be correlated with the concerned environmental factors. Glycollate uptake rate seemed to be less influenced by extracellular enzyme products as shown by the clearer correlation with other factors.

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