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Water Quality and Cyanobacterial Anatoxin-a Concentration in Daechung Reservoir

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The current study was performed to elucidate the relationship between the anatoxin-a produced by cyanobacteria and aquatic environmental factors. Algal and water samples were collected from the Daechung Reservoir from June to November 2001. The physical factors of the water quality were measured *in situ*, while the biological and chemical factors were examined in the laboratory. The concentrations of anatoxin-a in the algal and water samples were analyzed by HPLC using a fluorescence detector and ranged from 0.61-8.68 μg/g dw in the algal samples and 0.01-0.08 μg/L in the water samples. The suggested maximum concentration of anatoxin-a for safe drinking water is 1 μg/L. The concentrations of anatoxin-a in the algal and water samples were highest in July. The relationships between the aquatic environmental factors and the anatoxin-a concentration were also analyzed to identify the crucial elements for toxin production. The anatoxin-a concentrations in the algal samples exhibited a high correlation with nitrate, the TN/TP ratio, TDN ($P < 0.05$), and TPN/TPP ratio ($P < 0.01$), whereas the anatoxin-a concentrations in the water samples were highly related to the water temperature, conductivity ($P < 0.01$), pH, phycocyanin, and phycocyanin/chlorophyll *a* ratio ($P < 0.05$).

Key words : Anatoxin-a, cyanobacteria, environmental factors, water quality

INTRODUCTION

In many countries of the world, lakes and reservoirs achieve eutrophication based on the nutrient loading of nitrogen (N) and phosphorus (P). However, the blooms of cyanobacteria are causing environmental problems (Rapala and Sivonen, 1998; Andrea, 1999; Andrea *et al.*, 2000; Xu *et al.*, 2000). The occurrence of cyanobacterial blooms deteriorates water quality due to scum formation on the water surface and the production of malodorous compounds, such as geosmin (*trans*-1, 10-dimethyl-*trans*-decalol, GSM) and 2-methylisoborneol (MIB) (Park *et al.*, 2001; Tarczynska *et al.*, 2001). About 50% of water blooms produce both hepatotoxins and neurotoxins (Takino *et al.*, 1999; Gajdek *et al.*, 2001; Villatte *et al.*, 2002), which are known to have adverse effects on animals and humans (Rapala *et al.*, 1993; Onodera *et al.*, 1997; Takino *et al.*, 1999; Kaas and Henriksen, 2000). These harmful toxins are produced by cyanobacteria of the genera *Microcystis*, *Anabaena*, *Oscillatoria*, and *Aphanizomenon* (WHO, 1999).

Anatoxin-a, 2-acetyl-9-azabicyclo[4, 2, 1]-non-2-ene, is an alkaloid-type neurotoxin that binds to the acetylcholine receptor and acts as a postsynaptic neuromuscular blocking agent. Anatoxin-a has a LD_{50} of 200 μg/kg, and the maxi-

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mum concentration for safe drinking water is 1 μg/L (Takino *et al.*, 1999).

Many studies have already revealed that the toxicity of cyanobacterial blooms is connected to water quality, and several studies have investigated the relationship between toxin production and environmental factors. For example, light, temperature, nitrate, and orthophosphate have all been found to affect hepatotoxin production by *Oscillatoria agardhii* strains (Sivonen, 1990). While other studies have examined anatoxin-a concentrations in *Anabaena* and *Aphanizomenon* under different environmental conditions (Rapala *et al.*, 1993), microcystin production in *Anabaena* spp. as a function of growth stimuli (Rapala *et al.*, 1997), toxin production in *Anabaena* spp. at different temperatures (Rapala and Sivonen, 1998), microcystin production in *Microcystis viridis* under various culture conditions (Song *et al.*, 1998), and toxin production and the growth characteristics of *Anabaena flos-aquae* in different culture media (Gupta *et al.*, 2002).

Most domestic studies about cyanobacteria can be divided into two groups, new methods for the assay of toxins and analyses of the relationship between cyanobacterial blooms and environmental factors. Methods for assaying cyanobacterial toxins include the development of new analysis methods (Pyo *et al.*, 1994), separation/purification from toxin materials (Yoon *et al.*, 1998), and toxin analysis using HPLC (Yu *et al.*, 1999), while the analyses of the relationship between cyanobacterial blooms and environmental factors have investigated variations in the N/P ratio and algal blooms (Heo *et al.*, 1991), changes in the biological community due to cyanobacterial blooms (Kim *et al.*, 1995), short-term prediction of algal blooms (Oh and Kim, 1995), and diel variations in environmental factors and the carbon cycle in cyanobacterial blooms (Kim and Kim, 1997). Despite such studies, further investigation of the relationship between toxin production and environmental factors is still needed.

Until now, studies on the Daechung Reservoir have been focused on water quality, variations in the algal community, and microcystin production. As such, researchers have examined the water quality relative to algal bloom (Kim, 1996; Oh *et al.*, 1997; Chung, 1998; Seo, 1998; Lee *et al.*, 2000), changes in the biological community due to cyanobacterial blooms (Kim *et al.*, 1995), short-term prediction of algal blooms (Oh and Kim, 1995), the diurnal vertical migration of phytoplankton (Oh *et al.*, 1995), variations in the environmental factors and phytoplankton (Shin *et al.*, 1999), and seasonal variations and the monitoring of microcystin concentrations (Oh *et al.*, 2001). However, no previous study has focused on the relationship between anatoxin-a production and environmental factors.

Accordingly, the current study investigated the environmental factors responsible for cyanobacterial toxin production in the Daechung Reservoir. In addition, anatoxin-a concentrations were quantitatively measured and the correlation between environmental factors and anatoxin-a concentrations analyzed. Therefore, this data will be quite useful for future water quality management.

MATERIALS AND METHODS

Sampling

The sampling site was located in the vicinity of the Daechung Dam, about 20 m from the bank, at a depth of about 10 m.

The sampling period was from 18 June 2001 to 5 November 2001, which included the rainy season in early summer and the time of intense algal blooms. The sampling was carried out at intervals of 1-2 weeks during five months. Samples were collected from the surface water for analysis of the environmental factors, transported on ice, and stored in a refrigerator until analyzed.

Analysis of physicochemical factors

The physical factors were measured *in situ*. The water temperature, pH, and conductivity were measured using a conductivity meter (YSI 63). The dissolved oxygen (DO) was measured using a DO meter (YSI 95) and the turbidity using a turbidimeter (HF Scientific DRT-15CE).

The total nitrogen (TN) and total phosphorus (TP) were analyzed after persulfate oxidation into nitrate (D'Elia *et al.*, 1977) and orthophosphate (Menzel and Corwin, 1965), respectively. The nitrate was determined using the method of Crumpton *et al.* (1992) and the orthophosphate using the phosphomolybdate method (APHA, 1995). The total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were analyzed after filtration of the water samples using filter paper

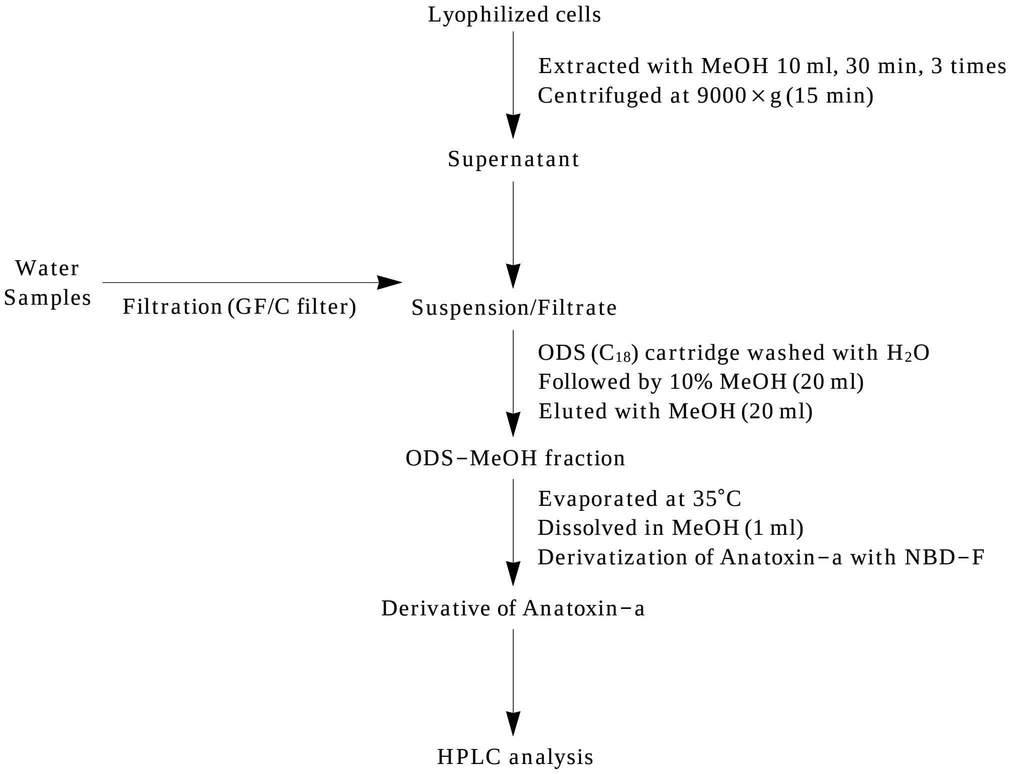

Fig. 1. Procedure used to extract anatoxin-a from algal and water samples.

(Whatman GF/C) and persulfate oxidation. The total particulate nitrogen (TPN) and phosphorus (TPP) were obtained by subtracting the dissolved form values from the total form values.

Analysis of biological factors

The water samples (30 mL) were filtered using filter paper (Whatman GF/C) for the chlorophyll *a* fraction. Chlorophyll *a* was extracted with a chloroform-methanol mixture (2 : 1, v/v) and measured using a fluorometer (Turner 450) (Wood, 1985). The phycocyanin was measured using a spectrophotometer (Shimadzu UV-160A) after extraction with 80% acetone and resuspension in a sodium acetate buffer (Myers *et al*., 1978).

Anatoxin-a analysis

The water samples were collected within 1 m from the water surface, transported to the laboratory, and stored at 4°C after filtration through filter paper (Whatman GF/C). The samples for the algal biomass and cellular anatoxin-a analysis were concentrated about 1,000 times using a plankton net (mesh size 25 μm) and lyophilized.

Table 1. Optimum conditions for HPLC analysis.

Model	LC-10AD (Shimadzu), Fluorescence detector (Waters 470)
Column	Nucleosil C_{18}(4.6 × 250 mm)
Mobile phase	acetonitrile : water = 75 : 25
Temperature	30°C
Flow rate	0.5 ml/min
Injection volume	20 μl

The anatoxin-a in the lyophilized algal samples was extracted three times with 10 mL of methanol per 100 mg of sample for 30 min. The extract was then centrifuged at 9,000 × g and the supernatant evaporated using an evaporator (Büchi 461) after being adjusted to pH 10 with 7% ammonium hydroxide. Thereafter, the residue was dissolved in 70% methanol (10 mL). The following procedure was identical to the anatoxin-a extraction from the water samples (Fig. 1). The supernatant was passed through an ODS cartridge (C_{18} Supelco Park Supelco), rinsed with water (20 mL) and 10% methanol (20 mL), and finally eluted with methanol (20 mL). The eluate was then evaporated using an evaporator at 35°C and the residue dissolved in methanol (1 mL). Next, the purified anatoxin−a was reacted with 7−fluoro−4−nitro−2, 1, 3−benzoxadiazole (NBD−F) to produce a measurable derivative for a HPLC analysis (Harada *et al.*, 1993). The optimum conditions for the HPLC anatoxin−a analysis are shown in Table 1.

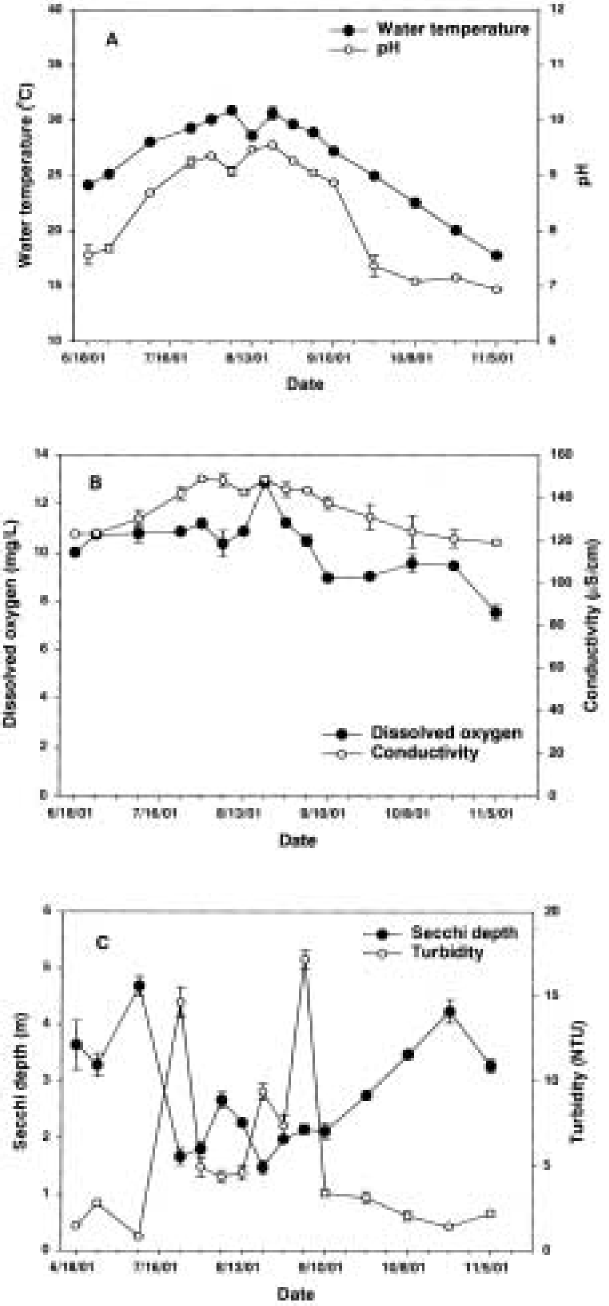

Fig. 2. Variation of aquatic environmental factors in Daechung Reservoir.

RESULTS

Environmental factors

The water temperature was highest at 30.9°C on August 6 and then gradually decreased to 17.8°C by November 5 (Fig. 2A). The pH, DO, and conductivity exhibited the same tendency as the water temperature (Fig. 2A and 2B). The water temperature was highly correlated with the pH, DO, and conductivity ($r = 0.780$, $P < 0.001$)

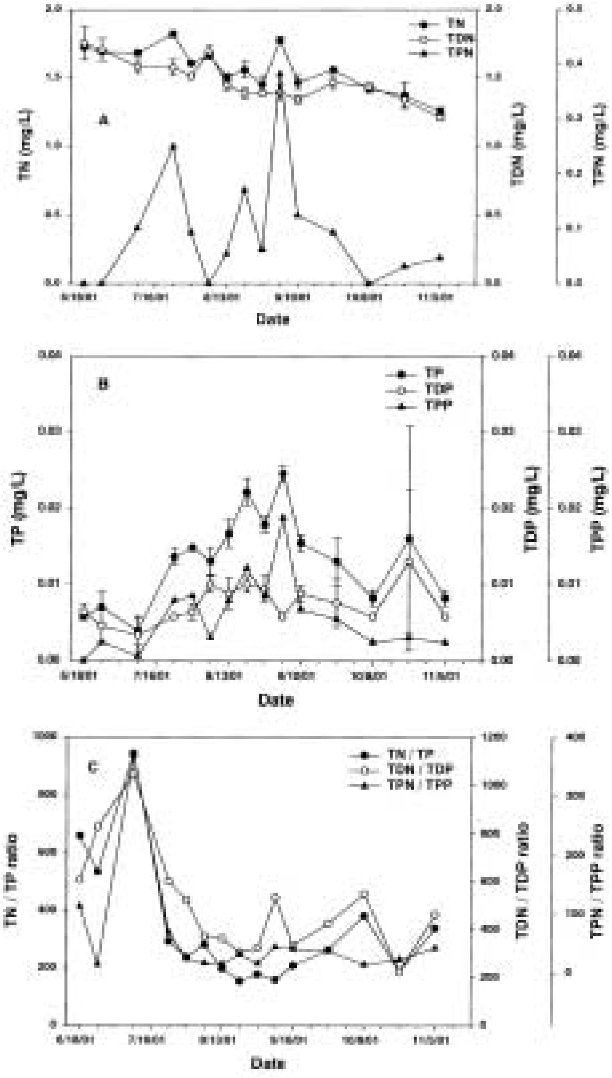

Fig. 3. Variation of chemical factors in Daechung Reservoir.

Table 2. Correlation coefficients between environmental factors ($n = 15$).

Factors	Correlation coefficient													
	Secchi depth	Turbidity	Chlorophyll *a*	Phycocyanin	TN	TDN	TPN	TP	TDP	TPP	TN /TP	TDN /TDP	TPN /TPP	Cyanophyceae
Secchi depth	1													
Turbidity	-0.682**	1												
Chlorophyll *a*	-0.749**	0.924***	1											
Phycocyanin	-0.710**	0.591*	0.722**	1										
TN	-0.176	0.505	0.373	0.330	1									
TDN	0.179	-0.106	-0.244	-0.109	0.747**	1								
TPN	-0.499	0.869***	0.870***	0.621*	0.406	-0.304	1							
TP	-0.710**	0.702**	0.765***	0.522*	0.016	-0.455	0.647**	1						
TDP	-0.199	-0.048	0.030	0.000	-0.413	-0.323	-0.147	0.545*	1					
TPP	-0.737**	0.855***	0.889***	0.617*	0.229	-0.373	0.840***	0.904***	0.134	1				
TN/TP	0.714**	-0.463	-0.575*	-0.458	0.308	0.557*	-0.325	-0.835***	-0.653**	-0.654**	1			
TDN/TDP	0.464	-0.119	-0.262	-0.189	0.515*	0.538*	0.000	-0.656**	-0.876***	-0.329	0.871***	1		
TPN/TPP	0.498	-0.163	-0.213	-0.163	0.283	0.192	0.142	-0.425	-0.469	-0.263	0.774**	0.678**	1	
Cyanophyceae	-0.768***	0.946***	0.908***	0.699**	0.423	-0.165	0.833***	0.717**	0.069	0.813***	-0.511	-0.224	-0.150	1

*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

(Table 2), while the Secchi depth and turbidity displayed the opposite pattern of change (Fig. 2C).

The TN concentration changed from 1.26 to 1.82 mg/L with an average of 1.57 mg/L. The monthly average was highest at 1.70 mg/L in July (Fig. 3A). The TN concentration was mainly composed of TDN ($r = 0.747$, $P<0.01$) and the major constituent of TDN was nitrate ($r = 0.852$, $P<0.001$). The TP concentration was highest at 0.025 mg/L on September 3 (Fig. 3B). The TPP concentration showed a high correlation with the TP concentration ($r = 0.904$, $P<0.001$). The much higher TDN/TDP ratio compared to the TPN/TPP ratio also supported the relatively high proportion of TDN (Fig. 3C).

Cyanobacterial bloom

The chlorophyll *a* concentration fluctuated during the period of investigation and showed a high peak in September (Fig. 4). The phycocyanin concentration (cyanobacterial-specific pigment protein) also exhibited the same pattern as chlorophyll *a* and was high related with the chlorophyll *a* concentration ($r = 0.722$, $P<0.01$). In addition, the algal peaks coincided with those of chlorophyll *a* and phycocyanin. Cyanobacteria occupied 95% of the total algae on average. The turbidity and Secchi depth were easily measurable indicators, representing the degree of algal bloom in the Daechung Reservoir. The chlorophyll *a* concentration revealed a highly negative correlation with the Secchi depth ($r = -0.749$, $P<0.01$), yet highly positive correlation with the turbidity ($r = 0.924$, $P<0.001$). Furthermore, despite seasonal variations in turbidity, the chlorophyll *a* and phycocyanin concentrations remained very similar (Figs. 2 and 4). Therefore, using chlorophyll *a*, the Secchi depth, and TP as water quality parameters for evaluating the trophic state during the investigation period, the trophic level in the Daechung Reservoir was examined based on the boundary values of the Organization for Economic Cooperation and Development (OECD) (Table 3). Early in the investigation, on June 18, the

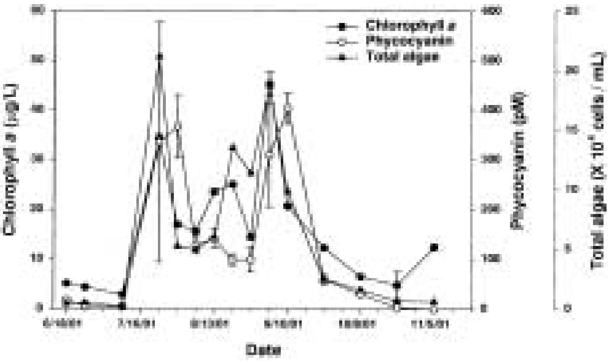

Fig. 4. Variation of chlorophyll *a*, phycocyanin, and total algal cell number in Daechung Reservoir.

Table 3. Trophic state based on OECD boundary values in Daechung Reservoir, 2001.

Date	Trophic categories		
	Chlorophyll *a*	TP	Secchi depth
06/18	M	O	M
06/25	M	O	M
07/09	M	U	M
07/23	H	M	H
07/30	E	M	H
08/06	E	M	E
08/13	E	M	E
08/20	E	M	H
08/27	E	M	H
09/03	H	M	E
09/10	E	M	E
09/24	E	M	E
10/08	M	O	M
10/22	M	M	M
11/05	E	O	M

U: Ultra−oligotrophic, O: Oligotrophic, M: Mesotrophic
E: Eutrophic, H: Hypertrophic

chlorophyll *a* concentration and Secchi depth indicated mesotrophy, while the TP indicated oligotrophy. Throughout the investigation period, the highest trophic level was measured on July 23 when the chlorophyll *a* and Secchi depth indicated hypertrophy, while the TP indicated eutrophy. As such, the average trophic level for the Daechung Reservoir was determined to be eutrophic based on the OECD criteria.

Analysis of anatoxin−a production

The concentration of anatoxin−a in the water samples ranged from 0.01−0.08 μg/L, while the concentration in the algal samples ranged from 0.61−8.68 μg/g dw (Fig. 5). The highest anatoxin−a concentration in the water samples was 0.08 μg/L on July 30, and that in the algal samples was 8.68 μg/g dw on July 9. A monthly average of the anatoxin−a concentrations in the water and algal samples revealed peaks in July. Generally, the intracellular and extracellular anatoxin−a exhibited the same trend except for initial three points.

The relationship between the anatoxin−a concentration and environmental factors was examined (Table 4). The anatoxin−a concentration in the algal samples demonstrated a correlation with the TPN/TPP ratio ($r = 0.646$, $P < 0.01$), TN/TP ratio, nitrate, and TDN ($P < 0.05$), whereas the anatoxin−a concentration in the water samples was highly related to the water temperature ($r = 0.643$, $P < 0.01$), conductivity ($r = 0.658$, $P < 0.01$), pH ($r = 0.616$, $P < 0.05$), phycocyanin ($r = 0.554$, $P < 0.05$), and phycocyanin/chlorophyll *a* ($r = 0.640$, $P < 0.05$).

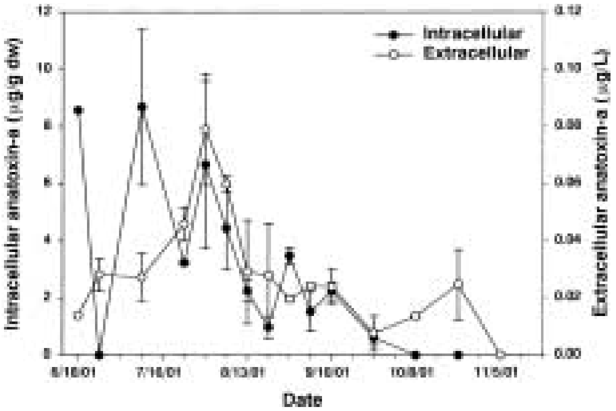

Fig. 5. Concentrations of intracellular and extracellular anatoxin−a in Daechung Reservoir.

Table 4. Correlation coefficients between environmental factors and anatoxin−a concentrations (n = 15).

Factors	Correlation coefficient	
	Intracellular anatoxin−a	Extracellular anatoxin−a
Water temperature	0.404	0.643**
pH	0.343	0.616*
Conductivity	0.219	0.658**
Phycocyanin	0.124	0.554*
Phycocyanin /Chlorophyll *a*	0.278	0.640*
Nitrate	0.567*	0.154
TDN	0.565*	0.389
TN/TP	0.583*	−0.142
TPN/TPP	0.646**	0.093

*: $P < 0.05$, **: $P < 0.01$

DISCUSSION

Water temperature is known to be a basic environmental factor affecting the biological community, such as cyanobacteria (Kim and Kim, 1997), along with the pH, DO, and conductivity ($P < 0.001$). In general, the pH increases with algal blooms, as photosynthesis by algae occurs according to the following reaction at a pH of over 6.3 (Lampert *et al.*, 1997).

$6HCO_3^- + 6H^+ = C_6H_{12}O_6 + 6O_2$

In the current study, TPN and TPP showed a high correlation with chlorophyll *a*, indicating that nitrate and phosphate changed into TPN and TPP during the algal blooms, which was connected with algal growth.

Meanwhile, chlorophyll *a* was found to be related to TP, TPN, and TPP ($r = 0.765$, $P < 0.001$). Oh and Kim (1995) and Shin *et al.* (1999) previously reported that TP was related with chlorophyll *a*. As such, the present results confirmed that P was a major limiting factor on algal growth in the Daechung Reservoir.

During the period of investigation, the algal bloom in the Daechung Reservoir occurred from July to September. This bloom period was seemingly caused by the increased nutrient loadings after the rainy spell in early summer and subsequent rise in the water temperature due to the higher intensity of radiation after the rainy spell. Oh and Kim's study (1995) of the Daechung Reservoir also found that algal growth was affected by the nutrient concentration, water temperature, radiation intensity, and rainfall. In the current study, the chlorophyll *a* concentration exhibited a high correlation with the phycocyanin concentration, and Oh and Kim (1995) previously reported that algal bloom in the Daechung Reservoir mainly occurred due to cyanobacteria, such as *Anabaena* sp., *Microcystis aeruginosa*, and *Oscillatoria* sp. In addition, *Anabaena* sp. and *Oscillatoria* sp. have been reported to produce anatoxin-a (WHO, 1999). During the investigation period, the highest anatoxin-a concentrations in the water and algal samples were 0.08 μg/L on July 30 and 8.68 μg/g dw on July 9, respectively. The highest content of anatoxin-a detected in Korea was 1,444 μg/g dw in Lake Jangsong (Park *et al.*, 1998). The monthly average of anatoxin-a concentrations in the water and algal samples was highest in July. Anatoxin-a was detected from early July until early September, indicating that the eutrophication of the water body was highly related to the algal bloom, and during this period anatoxin-a was produced by *Anabaena* sp. and *Oscillatoria* sp. Anatoxin-a has a LD_{50} of 200 μg/kg and the suggested maximum level of anatoxin-a for safe drinking water is 1 μg/L (Takino *et al.*, 1999). Therefore, the anatoxin-a concentrations in the Daechung Reservoir were substantially below the suggested guideline.

As regards the relationship between the anatoxin-a concentration and environmental factors, the concentration of anatoxin-a in the algal samples was highly related to the TN/TP ratio, nitrate, TDN ($P < 0.05$), and TPN/TPP ratio ($P < 0.01$), suggesting that the production of anatoxin-a was affected by N rather than P. In addition, anatoxin-a includes N in its molecular structure. The highest TN/TP ratio co-occurred with the highest concentration of anatoxin-a on July 9. Rapala *et al.* (1993) also reported that P did not influence the anatoxin-a concentration.

However, the concentration of anatoxin-a in the water samples revealed a positive correlation with the phycocyanin concentration ($P < 0.05$). Therefore, the anatoxin-a in the water samples may have been released into the water due to the death of cyanobacteria during the algal bloom (Rapala *et al.*, 1993).

In conclusion, the concentration of anatoxin-a in the algal samples was found to be highly related to the N/P ratio, while a low TP content effected the anatoxin-a concentration by causing stress to the algae. Oh *et al.* (2000) reported that the microcystin-producing rate of *M. aeruginosa* was dependent on the concentration of P. That is, P-limited condition increased the microcystin content. Moreover, Lee *et al.* (2000) stated that the microcystin of *M. aeruginosa* was closely connected with the concentration of chlorophyll *a*.

Therefore, although the current study examined the relationship between aquatic environmental factors and anatoxin-a production, further research is still required to control the algal bloom and toxin production in the Daechung Reservoir.

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<국문적요>

대청호의 수질과 남조류 독소 Anatoxin-a 농도의 관계

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남조류에 의해 생성되는 anatoxin-a의 양과 환경요인과의 관계를 알아보기 위해 대청호에서 2001년 6월부터 2001년 11월까지 조류 및 물 시료를 채취하였다. 환경요인 중 물리적 요인은 현장에서 측정하였고, 생물 · 화학적 요인은 실험실에서 측정하였다. 조류 및 물 시료에 존재하는 anatoxin-a의 양은 fluorescence detector를 이용하여 HPLC로 측정하였고, 조류 시료와 물 시료의 경우 각각 0.61–8.68 μg/g dry wt, 0.01–0.08 μg/L로 측정되었다. 음용수의 안전을 고려한 anatoxin-a의 권고 기준 농도는 1 μg/L로 제안되고 있다. 조류세포와 물 시료에서 anatoxin-a 농도가 가장 높게 검출된 시기는 7월이었다. 독소 생성에 중요한 요인을 확인하기 위해 환경요인과 anatoxin-a 농도와의 상호관계를 살펴보았다. 조류 시료내 anatoxin-a 농도는 nitrate, 총질소와 총인 비율, 총용존질소 ($P<0.05$) 및 총입자성질소와 총입자성인 비율($P<0.05$)과 높은 상관관계를 나타내었고, 물 시료내 anatoxin-a 농도는 수온, 전기전도도($P<0.01$), 수소이온농도, phycocyanin, phycocyanin과 엽록소 *a* 비율($P<0.01$)과 높은 상관관계를 나타내었다.