Hepatotoxic Microcystins and Neurotoxic Anatoxin-a in Cyanobacterial Blooms from Korean Lakes

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ABSTRACT: Cyanobacterial bloom samples were collected in the warm season during 1992–1995 from the 12 lakes in Korea. Six species each of *Microcystis* and *Anabaena*, and two of *Oscillatoria* were identified in these lakes. The cyanotoxins of 47 samples collected from the lakes were identified as microcystins-RR, -YR, -LR; desmethyl-7-microcystin-LR (7-DMLR), plus anatoxin-a. Microcystins were the main components of these cyanotoxins, while anatoxin-a was detected in samples from a few lakes. Thirty-four of the 47 samples, included microcystins and the total amounts of microcystin ranged between 20–1500 μg/g freeze-dried bloom material. In four of the 26 samples, the samples contained anatoxin-a, though the amounts varied. The total microcystin concentration in 30 samples from the lakes was equal to the cellular microcystin in these lakes because no extracellular microcystin was detected. All the lakes except for Lakes Younglang and Mijae are a source of drinking water, so the presence of cyanotoxin can be a potential threat and requires more attention to water treatment. Environ Toxicol Water Qual 13: 225–234, 1998

Keywords: microcystin; hepatotoxin; anatoxin-a; neurotoxin; cyanotoxin; cyanobacteria bloom; Korean lakes

INTRODUCTION

Heavy blooms of cyanobacteria are one of the consequences of the worldwide trend for increasing eutrophication in many waters. This phenomenon is thought to result from increased exogenous nutrient loadings. Toxic cyanobacterial blooms in eutrophic lakes and reservoirs have been reported in many countries (Skulberg et al., 1984; Gorham and Carmichael, 1988; Carmichael, 1992). These toxic blooms have caused death to livestock and wildlife, in addition to cases of illness and death in humans (Billings, 1981; Falconer, 1989; Carmichael et al., 1996).

Toxins from freshwater cyanobacteria are classified functionally into two groups, hepatotoxins and neurotoxins. *Microcystis aeruginosa* is the most common toxic cyanobacterium worldwide, and it produces potent cyclic peptide hepatotoxins, termed microcystins (Carmichael, 1988, 1992; Carmichael et al., 1988) of which more than 50 variants have been isolated. Microcystins are also found in *Microcystis viridis* (Watanabe et al., 1986, 1989; Kusumi et al., 1987), *Anabaena flos-aquae* (Krishnamurthy et al., 1986), *Oscillatoria agardhii* (Meriluoto et al., 1989), and *Nostoc* sp. (Sivonen et al., 1990). The chemical structures of the

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hepatotoxins in *M. aeruginosa* have been elucidated by Botes et al. (1984, 1985). These cyclic heptapeptides are composed of five common amino acids with variations combining a pair of *L*-amino acids. The structural differences among the toxins are related to the two *L*-amino acids (Fig. 1).

Recently, desmethyl derivatives have been reported (Harada et al., 1991a), in which methyl groups of *N*-methyldehydroalanine and *N*-methyl aspartic acid are replaced by hydrogen atoms. Microcystins and nodularin inhibit protein phosphatase activity, especially type 1 and 2A, in a manner similar to that of okadaic acid (Matsushima et al., 1990; Yoshizawa et al., 1990; Mackintosh et al., 1990).

Anatoxin-a is produced by *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Oscillatoria* sp., and is an alkaloidlike compound that has been already synthesized (Carmichael, 1992). It is a secondary amine (Fig. 1), 2-acetyl-9-azabicyclo[4.2.1]non-2-ene (Huber, 1972; Devlin et al., 1977), with a molecular weight of 165. Anatoxin-a is a potent postsynaptic cholinergic nicotinic agonist, which acts as a depolarizing neuromuscular blocking agent (Carmichael et al., 1975, 1979; Spivak et al., 1980, 1983; Aronstam and Witkop, 1981). According to Stevens and Krieger (1991), there are several nontoxic degradation products of anatoxin-a, and these degradation products are considered to play an important role in clarifying the detoxification route of anatoxin-a.

Progressive eutrophication in Korean waters has led cyanobacteria to become dominant in several man-made dams, estuary dams, and reservoirs. Even though potentially toxic cyanobacterial species occur in Korea, there have been no reports of mortality in wild or domestic animals. Aqueous extracts from cyanobacteria have been found to be lethal, however, upon intraperitoneal injection into mice (Kim et al., 1995). The aim of this study was to investigate the distribution of the dominant species of cyanobacteria in Korean lakes and the amount of hepatotoxic microcystins and neurotoxic anatoxin-a in cyanobacterial blooms.

MATERIALS AND METHODS

Bloom Samples

Cyanobacterial bloom samples were collected (Fig. 2) in the warm season from seven onstream man-made dams (Soyang, Choongju, Daechong, Jangsong, Hapchon, Andong, Imha), two estuary dams (Nakdong, Youngsan), two small reservoirs (Gunsan, Mijae), and one coastal lagoon (Younglang). All provide drinking water sources except for Lakes Younglang and Mijae which are used only for recreation. Cyanobacteria cells from natural waters were concentrated by plankton net (NXXX 25, 40 μm aperture) for use in the analysis of cyanotoxins and species composition. Formaldehyde (1.4%) was added to a part of the samples for species identification. The remaining bloom samples were lyophilized and stored at -30°C until the chemical analysis was performed.

Simultaneous Analysis of Microcystin and Anatoxin-a

A simultaneous analysis of anatoxin-a and microcystin was devised based on methods by Harada et al. (1988, 1989, 1993). This method is applied to natural bloom samples dominated by several species of cyanobacteria which are able to produce both anatoxin-a and microcystins (Park et al., 1993). The method was as follows (Fig. 3): lyophilized cells (about 100 mg) were extracted three times with 10 mL of 0.05 M acetic acid for 30 min

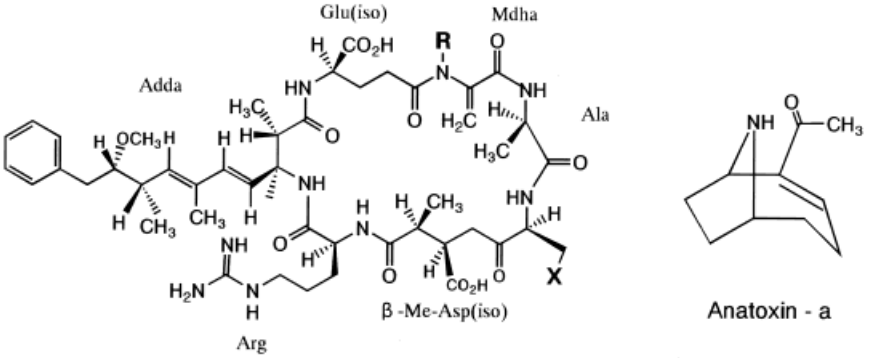

Fig. 1. Structures of four microcystins, and anatoxin-a.

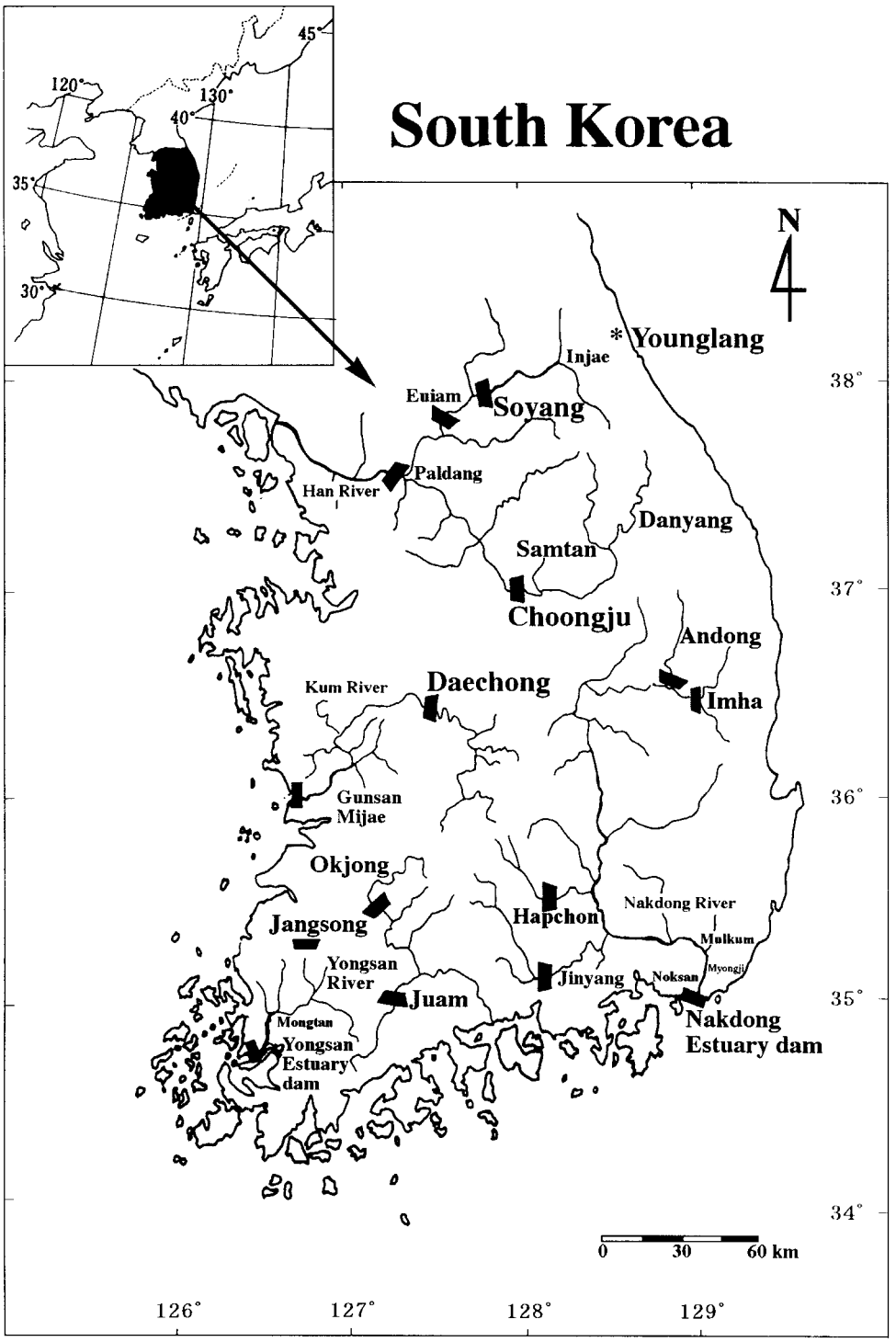

Fig. 2. Map of sampling sites of cyanobacterial blooms in South Korea.

while stirring. The extract was centrifuged at 4000 or 10,000 rpm and the supernatant was adjusted to pH 10 with 7% ammonium hydroxide. This pH 10 extract was directly applied to 0.2 g of a reversed phase ODS-disposable extraction column (Bakerbond SPE 7020-03, Octadecyisilane(C18), ODS (Octadecylsilance) type, 3-mL; J. T. Baker, Phillipsburgh, NJ, USA), which had been preconditioned by washing with 10 mL of methanol and 10 mL of water. The column containing toxins was washed with water (10 mL), followed by water–methanol (9 : 1, 10 mL). Elution from the column with methanol (20 mL) gave the toxins-containing

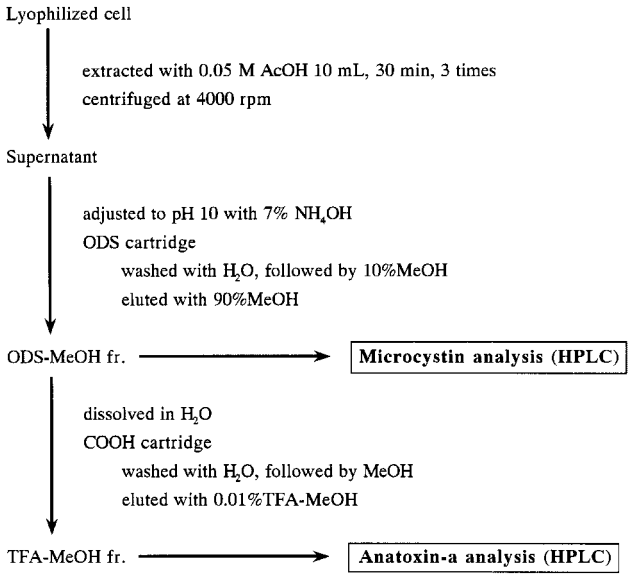

Fig. 3. Scheme for simultaneous analysis of microcystin and anatoxin-a.

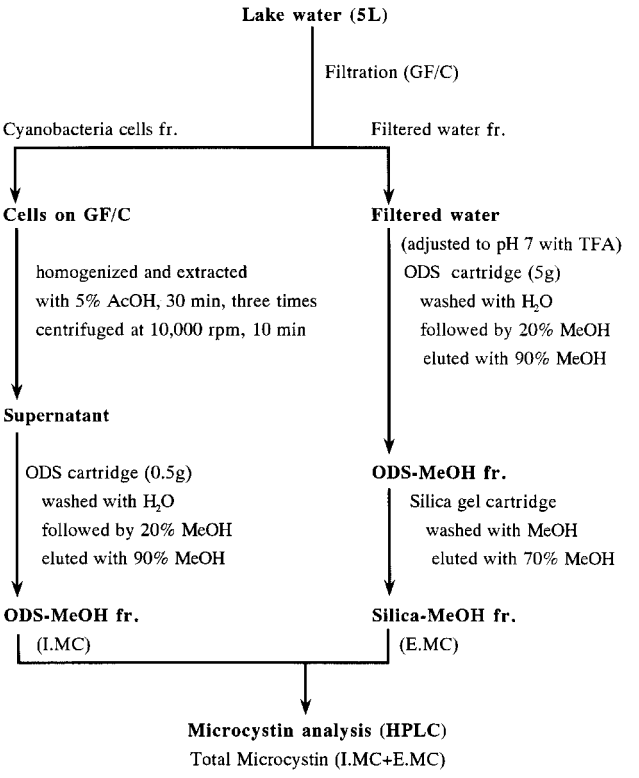

Fig. 4. System for analysis of total microcystin in lake water. I.MC: Intracellular microcystin, E.MC: Extracellular microcystin.

fraction. A portion (5 μL) of the fraction was applied to a high performance liquid chromatography (HPLC) system for the analysis of microcystins. The remaining fraction, dissolved in 20 mL water, was absorbed onto a disposable solid phase column (0.5 g) of a cation exchanger organosilan bonded to silica gel (Bond Elut 615303, CBA 1210-2038, COOH type, 3-mL; Analytichem International, Varian Associates Inc., Harbor, CA, USA) which had been preconditioned by washing with 0.1% trifluoroacetic acid–methanol (10 mL), followed by methanol (10 mL) and water (10 mL). The column was rinsed with 10 mL water and then 10 mL methanol. The toxins-containing fraction was eluted with 20 mL of 0.01– trifluoroacetic acid–methanol to give the crude toxin fraction. This fraction was dissolved with methanol and injected to a HPLC. The HPLC was carried out under the following conditions: LC: pump, Shimadzu (Kyoto, Japan) LC-9A; detector, Shimadzu SPD-M10A photodiodearray detector; Column, Cosmosil 5PH (150 × 4.6 mm; Nacalai Tesque Inc., Kyoto, Japan); mobile phase, methanol : 0.1 M ammonium acetate (adjusted pH 5 with TFA) = 14 : 86; flow rate, 1.2 mL/min; detection, UV (227 nm). Anatoxin-a and microcystin standards were a gift from Dr. K.-I. Harada. All chemicals used were of analytical grade.

Microcystin Analysis in Lake Water

Measurements of microcystin concentrations and cleanup methods for analysis of trace amounts of microcystins in lake water were done according to Harada et al. (1988) and Tsuji et al. (1994), respectively, (Fig. 4). A lake water sample (about 5 L) was filtered through a glass microfiber filter (Whatman GF/C) and adjusted to pH 7. The filter and its content were homogenized and extracted with 5% aqueous acetic acid and the supernatant was applied to an ODS cartridge (0.5 g, Bakerbond SPE 7020-03) after centrifugation. The 90% methanol-extracted eluate from the cartridge was applied to an HPLC system equipped with an ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan). The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-9A pump coupled to a SPD-10A set at 238 nm and SPD-M10A photodiodearray detector, and C-R6A integrator. Microcystins were quantified by comparing the absorbance peak area of test samples, at 238 nm, after separation with a methanol : 0.05 M phosphate buffer (pH 3.0, 58 : 42) with those of standards. The flow rate was 1 mL/min. The filtrated water was applied directly to an ODS silica gel cartridge (5g, Chromatorex ODS, 100–200 mesh, which was packed into a polypropylene cartridge). The cartridge was rinsed with water and 20% methanol–water. The eluate from the cartridge with 90% methanol-water was evaporated to dryness, and the residue was dissolved in methanol. The methanol solution was applied to a silica gel cartridge (2 g,

Sep-Pak), which was preconditioned with methanol, and the cartridge was rinsed with methanol. The eluate from the cartridge with 50% methanol–water was evaporated to dryness; the residue was then dissolved in methanol. The methanol solution was subjected to HPLC analysis for the resolution of microcystins.

RESULTS

Dominant Species in Cyanobacteria Blooms

Twenty samples of cyanobacterial blooms were collected in the warm season during 1992–1995 from the 12 lakes. Cyanobacteria were the dominant phytoplankton in all these lakes. Three genera of cyanobacteria, representing 14 species, were identified (Table I). Six species of *Microcystis* and six *Anabaena*, and two *Oscillatoria* appeared in the lakes during the season. Lakes Soyang, Choongju, Daechong, and Jangsong were dominated by *Anabaena* or *Oscillatoria*, while *Microcystis* was predominant in the other eight lakes. The species that showed the highest appearance frequency among the 14 species was *M. aeruginosa*. Half the cyanobacterial bloom samples in Korean lakes were dominated by *M. aeruginosa and/or M. ichthyoblabe*. Lakes Soyang, Daechong, and Jangsong were always dominated by *Anabaena*. The order of dominance in the blooms was *Microcystis*, *Anabaena*, and *Oscillatoria*, the percent values being 60, 30, 10%, respectively.

Microcystin and Anatoxin-a in Cyanobacterial Blooms

Table II shows the amounts of microcystin and anatoxin-a in the 47 samples of cyanobacterial bloom collected from the 12 lakes in the warm season during 1992–1995. The cyanotoxins were identified as microcystins-RR, -YR, -LR; desmethyl-7-microcystin-LR

TABLE I. Dominant species in cyanobacterial blooms, Korean waters

Sampling Sites	Date	Dominant Cyanobacteria Species
Lake Soyang	09/20/92	*Anabaena mucosa*, *Microcystis aeruginosa*
	10/26/92	*A. mucosa*, *M. aeruginosa*
	08/24/95	*A. mucosa*, *M. aeruginosa*
Lake Choongju	10/05/92	*Oscillatoria agardhii*, *M. wesenbergii*, *M. ichthyoblabe*, *M. aeruginosa*
Lake Daechong	10/06/92	*A. spiroides*, *M. ichthyoblabe*, *M. aeruginosa*
	08/29/95	*A. spiroides*, *A. circinalis*, *M. ichthyoblabe*, *A. planctonica*
Lake Jangsong	10/07/92	*A. citrispora*, *M. ichthyoblabe*, *M. aeruginosa*, *A. spiroides*
	08/29/95	*O. tenuis*, *M. aeruginosa*, *M. ichthyoblabe*, *M. wesenbergii*
Lake Hapchon	10/09/92	*M. aeruginosa*, *M. ichthyoblabe*, *M. novacekii*, *M. flos-aquae*
	08/26/95	*M. ichthyoblabe*
Lake Yongsan[a]	10/17/92	*M. ichthyoblabe*, *M. aeruginosa*
	08/29/95	*M. aeruginosa*, *M. ichthyoblabe*
Mongtan[b]	08/28/95	*M. ichthyoblabe*, *M. aeruginosa*
Lake Yonglang[c]	08/17/93	*M. ichthyoblabe*, *M. aeruginosa*, *A. flos-aquae*, *M. wesenbergii*
Lake Andong	08/25/95	*M. aeruginosa*
Lake Imha	08/26/95	*M. aeruginosa*, *M. nobacekii*, *M. wesenbergii*
Lake Nakdong[a]	08/27/95	*M. aeruginosa*, *M. wesenbergii*
Noksan[a]	08/27/95	*M. viridis*, *M. wesenbergii*, *M. ichthyoblabe*, *M. aeruginosa*
Gunsan Reservoir	08/29/95	*M. viridis*, *M. aeruginosa*
Mijae Reservoir	08/29/95	*M. ichthyoblabe*, *M. aeruginosa*, *M. wesenbergii*

[a] Estuary dam.
[b] downstream region of the river.
[c] Lagoon.

TABLE II. Amounts of microcystins and anatoxin-a in cyanobacterial blooms from Korean lakes

Sites	Date	MC (μg/g) RR	MC (μg/g) YR	MC (μg/g) LR	Total MC (μg/L)	Total MC (μg/g)	ANTX-a (μg/g)
Soyang	09/20/92	37	N.D.[a]	2.4	—[b]	39	—
	10/26/92	133	N.D.	N.D.	—	133	—
	09/08/93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10/06/93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Injae[c]	10/20/93	19	N.D.	N.D	1.9	19	N.D.
	08/23/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
	10/08/95	N.D.	N.D.	29	N.D.	29	—
Choongju	09/27/92	29	N.D.	5.7	—	35	1190
	10/05/92	24	N.D.	N.D.	—	24	—
	10/11/93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	10/18/93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Samtan[c]	10/18/93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Danyang[c]	10/18/93	—	—	—	81	—	N.D.
Daechong	09/27/92	57	N.D.	39	—	96	—
	10/06/92	76	N.D.	N.D.	—	76	N.D.
	10/05/94	96	N.D.	45	—	141	N.D.
	10/15/94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	08/30/95	140	N.D.	78	0.7	218	—
Jangsong	10/07/92	167	1062	N.D.	—	1229	1444
	10/15/94	84	N.D.	31	12	115	N.D.
	08/29/95	164	N.D.	70	1.9	234	—
	09/30/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
Hapchon	10/06/92	441	N.D.	126	—	567	N.D.
	10/06/93	514	N.D.	104	—	618	N.D.
	10/14/94	10	N.D.	244	—	254	N.D.
	08/26/95	N.D.	N.D.	207	0.9	207	—
	09/29/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
Youngsan[d]	10/17/92	N.D.[e]	N.D.	181	—[f]	181	570
	09/26/94	303	N.D.	N.D.	1.6	303	N.D.
	07/21/95	N.D.	177	74	—	251	—
	08/29/95	788	N.D.	204	—	992	—
Mongtan[g]	08/28/95	595	N.D.	354	17	949	—
Younglang[h]	08/17/93	793	N.D.	410	—	1203	N.D.
	10/16/93	317	N.D.	91	—	408	417
	09/15/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
Andong	08/25/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
	09/28/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
Imha	08/26/95	N.D.	N.D.	N.D.	N.D.	N.D.	—
Nakdong[d]	09/24/94	60	N.D.	9.0	1.0	69	N.D.
Mulkum[g]	07/26/94	30	N.D.	N.D.	0.6	30	N.D.
	07/28/94	561	N.D.	519	171	1080	N.D.
	08/18/94	56	N.D.	N.D	0.9	56	N.D.
Myongji[d]	09/24/94	107	N.D.	N.D.	2.4	107	N.D.
Noksan[d]	08/27/95	433	N.D.	202	30	635	—
Gunsan R.	08/29/95	424	169	263	856	—	—
	09/30/95	146	N.D.	188	344	—	—
Mijae R.	08/29/95	565	384	540	—	1489	—

[a] N.D.: toxins not detected.
[b] —:not determined.
[c] stream inlet site.
[d] Estuary dam.
[e] N.D.: toxins not detected.
[f] —: not determined.
[g] downstream region of the river.
[h] Lagoon.

(7-DMLR), plus anatoxin-a. Microcystins were the main components of the cyanotoxin of these blooms, anatoxin-a being detected in only a few lakes. Thirty-four of the 47 cyanobacteria samples, included microcystins and the total amounts of microcystin ranged between 20–1500 μg g freeze-dried bloom material. Microcystin-RR and -LR were detected in 66 and 55– samples, respectively; microcystin -YR was detected in only four lakes (Jangsong, Youngsan, Gunsan, Mijae). 7-DMLR was detected in two lakes, Lake Jangsong (10/07/92) and Lake Youngsan (10/17/92), the amounts being 80 and 39 μg/g freeze-dried material (no data in Table II), respectively. High amounts of total microcystin (> 1 mg/g freeze-dried material) were detected in Lakes Jangsong, Mijae, Younglang, and Nakdong.

Four of the 26 samples contained anatoxin-a, though with varying amounts. Anatoxin-a was high in Lakes Choongju and Jangsong (1.190 and 1.144 mg g freeze-dried material, respectively).

Microcystin Concentration in Lake Water

The estimates of total microcystin concentrations (μg/L) included intracellular and extracellular microcystin. The former consisted of the cyanobacterial cell fraction and the latter the filtered lake water fraction. The total microcystin concentration in the 30 samples equaled the intracellular microcystin, because no extracellular microcystin was detected in any sample. These concentrations ranged between 0.6–171 μg/L (Table II). High concentrations of microcystin were observed on 28 July 1994 (Mulkum) and 17 October 1993 (Danyang). At Mulkum, microcystin-RR and -LR were 89 and 82 μg/L, respectively, at Danyang 68 and 13 μg/L, respectively.

DISCUSSION

Relationship of Dominant Cyanobacteria Species with Cyanotoxin

Natural blooms in eutrophic lakes of Korea are frequently dominated by several species of cyanobacteria (Kim et al., 1995). Twenty samples of cyanobacteria blooms were collected in the warm season during 1992–1995 from 12 lakes. Cyanobacterial cells from natural water sources were concentrated in plankton nets for use in the analysis of toxins and species composition. The samples used in the present study were composed mainly of *Anabaena citrispora*, *A. mucosa*, *A. spiroides*, *Oscillatoria agardhii*, and *O. tenuis*, but most of these blooms were also composed of *Microcystis* and other cyanobacteria. *M. aeruginosa* and *M. ichthyoblabe* were the most abundant cyanobacteria species in natural bloom samples in Korean lakes. Cyanobacteria were dominant phytoplankton in all the investigated lakes, with three genera and, 14 species (Table I). This suggests the possibility of cyanotoxin production there. According to the current morphology-based taxonomy, the present known toxic cyanobacteria constitute about 40 species (Skulberg et al., 1993). In freshwater sources in Japan, the most common bloom-forming species, *M. aeruginosa*, is also the most widely distributed (Watanabe et al., 1986; Park et al., 1993; Park and Watanabe, 1996) and thus, it is commonly associated with hepatotoxicity (Watanabe et al., 1989). Sivonen et al. (1989) reported *Anabaena* to be the most frequent genus in freshwater blooms in Finland, and is thus most frequently responsible for neurotoxic blooms. Natural blooms are occasionally dominated by several genera of cyanobacteria, such as *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Microcystis* and *Oscillatoria*. According to Sivonen et al. (1989), a simultaneous occurrence of neurotoxin and hepatotoxin, as well as atypical toxic responses, was found in some Finnish samples using the mouse bioassay. Anatoxin-a content in blooms from these freshwater sources ranged from 12 to 4360 μg/g freeze-dried material (Sivonen et al., 1989). The cyanotoxins of the 47 samples from Korean lakes were identified as microcystins-RR, -YR, -LR; desmethyl-7-microcystin-LR (7-DMLR), and anatoxin-a. The total amounts of microcystins and anatoxin-a in Korean water samples ranged from 20 to 1500 μg/g and 417 to 1190 μg/g, respectively. Anatoxin-a has also been reported in North America and elsewhere in northern Europe, but to our knowledge, this is the first report of detection of anatoxin-a in freshwater sources in Korea. Although natural blooms in bodies of water in Korea are frequently dominated by several species, microcystin is the main toxin in the blooms, with anatoxin-a detected in only a few lakes.

Al-Lay et al. (1988) and Harada et al. (1991b) reported that *Anabaena flos-aquae* (NRC-44-1) originating from Canada produces both anatoxin-a and microcystin, and another *Anabaena flos-aquae* (NRC 525-17) from Canada produces simultaneously anatoxin-a(s) and microcystins, respectively. These were the first studies suggesting that cyanobacteria may be able to produce simultaneously neurotoxins and hepatotoxins. In the present study four samples of cyanobacteria blooms contained both anatoxin-a and microcystin. A method for the simultaneous determination of anatoxin-a and microcystins will contribute to the monitoring of natu-

ral blooms and strains in which these toxins apparently often coexist.

Microcystin Concentration in Lake Water

Lake water microcystin was present in the *Microcystis* cells (intracellular microcystin) and free in filtered lake water (extracellular microcystin). The total microcystin concentration in lake water was calculated by adding intracellular microcystin and extracellular microcystin. The amounts of toxin have usually been expressed in units of mass per unit mass (e.g., $\mu g/g$). Volumetric units (e.g., $\mu g/L$) are more appropriate for the estimation of risk levels for aquatic biota. Only a few researchers, however, have reported microcystin concentrations in volumetric units (Lindholm et al., 1989; Lindholm and Meriluoto 1991; Kotak et al., 1995). Lindholm and Meriluoto (1991) reported that the highest toxin (desmethyl-microcystin-RR) levels in the metalimnion were 20–40 $\mu g/L$ in the summers of 1988–1990, in a Finish lake. The total microcystin concentrations in 30 samples in Korean lakes was equal to the intracellular microcystin because extracellular microcystin was not detected in all samples. These concentrations ranged between 0.6–171 $\mu g/L$. In the Japanese hypertrophic lake, Lake Suwa, high concentrations of microcystin were found during the exponential growth phase of the bloom; the highest concentration of microcystin was 184 $\mu g/L$ on 10 October 1994. However, the amount of microcystin in the filtered lake water was highest at the end of the bloom; this amount was very low ($< 4\ \mu g/L$) during the period of the study. The high percentage of extracellular microcystin in filtered lake water ($> 20\%$) at the end of blooms suggests that release of microcystin from cells occurs during the senescence and the decomposition periods of *Microcystis* cells (Park et al., 1996).

All the studied lakes except Lakes Younglang and Mijae are sources for drinking water, so the presence of cyanobacterial toxin is a potential threat and requires more attention to water treatment. In Korea the rapid sand filter system is employed in most water supply treatments. Slow sand filter or charcoal treatment that are more effective in removing cyanobacterial cells and toxins are not common, so this may lead to a public health problem. There has been no debate about the hazards of cyanobacterial toxins in Korea. People are also suffering from odor problems in many reservoirs, mainly caused by cyanobacteria.

A complete understanding of toxins in lakes cannot be obtained from only a few water samples. Temporal and spatial measurements of cyanotoxin and toxic species composition in lakes and reservoirs are necessary to assess the risks for the health of humans, aquatic animals, livestock, and wildlife.

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