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The distribution of cyanobacterial toxins in Korean lakes

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INTRODUCTION

Toxic cyanobacterial blooms have been reported in many countries [1][2][3][4]. Toxic waterblooms cause death of domestic animals and wildlife, and human illness. Cyanobacterial toxins were also demonstrated to be toxic to zooplankton and fish[5][6][7][8].

Structures and function of toxin are classified into three groups, neurotoxin, hepatotoxin and lipopolysaccharide. *Microcystis aeruginosa* that is the most common toxin-producing cyanobacteria found worldwide produces cyclic heptapeptide toxins named microcystins[9][10]. Microcystins are also found in *Microcystis viridis*, *Anabaena flos-aquae*, *Oscillatoria agardhii*, and *Nostoc* sp.[4].

Recent studies showed that these microcystins and nodularin inhibit in vitro activity of protein phosphatase in a cytosolic fraction of mouse liver[11][12]. Liver is reported as the target organ that shows the greatest degree of histopathological change when animals are poisoned by these cyclic peptide. The cause of death in mice is at least partially known and is concluded to be hypovolemic shock caused by interstitial hemorrhage[13].

The summer of 1989 was particularly notable in Britain and several other European countries for the outbreak of toxic algal bloom in eutrophic lakes and reservoirs. If not serious in terms of magnitude of the effects, the incidents were thought to have generated a great deal of publicity and public concern. Although cyanobacterial blooms are ubiquitous in Korea, there was not any report on lethal effects on livestock or wild animals, possibly due to lack of examination. In this study, we investigated the distribution of dominant species of cyanobacteria in Korean lakes. The occurrence of toxic bloom was examined with mouse bioassay and the content of two cyanobacterial hepatotoxin, microcystin-RR and microcystin-LR, which are known to be the most common toxin in Japan, were analyzed with HPLC.

MATERIALS AND METHODS

Cyanobacterial cells were collected in late summer from 6 middlestream reservoirs(Soyang, Chungju, Daechong, Jangsung, Hapchon, Jinyang, Okjong), one estuarine reservoir(Yongsan), and one coastal lagoon(Yonglang). Phytoplankton cells were collected with plankton net or surface scums were collected by a dip net when dense surface scum was floating.

A representative aliquot of sample was examined microscopically to identify cyanobacterial species and their relative concentration. Algal cells were freeze-dried for mouse bioassay and chemical analysis. Dried cells(about 20-300mg d.w.) were extracted in saline solution(about 2-3ml) by soaking and centrifugation followed by filtration with a 0.45mm membrane filter. Extracts were injected intraperitoneally to ICR mice of 20-25g body weight. The injected volume of extract was 0.5 to 1.0 ml according to available amount of extract (Table. 1). After injection, the signs of toxicity was observed for mice and all mice were examined by necropsy (surviving mice were sacrificed at 24hr) so that liver engorgement or intestinal petechia was observed.

Table 1. The concentration of dried algal cell in extracts, the volume of extract injected to a mouse, and the dose of dried cell per mouse.

Lakes	Concentration of algal extract (mg dried cell/ml)	Injection volume (ml)	Dose per mouse (mg)
Control	0	0.5	0
Daechung	100	0.5	50
Choongju	150	0.5	75
Soyang	83	1.0	83
Hapchun	150	0.8	120
Jangsung	182	0.9	164
Youngsan	200	0.9	180
Younglang	300	1.0	150
	200	1.0	100
	100	0.5	75
	50	0.5	25

Lyophilized cells(about 100mg) for chemical analysis of toxin were extracted with 5% aqueous acetic acid and the supernatant was applied to a CN cartridge after centrifugation. The methanol extracted eluate from the cartridge was applied to an HPLC and a UV monitor. Amount of toxins were estimated by comparison of the peak area of a test sample, at 238nm, after separation with methanol: 0.05M phosphate buffer with those of standard samples. [14]

RESULTS AND DISCUSSION

Cyanobacteria were dominant phytoplankton in all the studied lakes. This implys possibility of toxin production there. In Lake Soyang and Lake Chungju, dominant species were *Anabaena* and *Oscillatoria* respectively, whereas *Microcystis* was dominant in L. Daechong, L. Hapchon, L. Yongsan, L. Yonglang (Table. 2).

Table 2. Dominant phytoplankton species in study lakes.

Lakes	Date	Dominant phytoplankton
Daechong	1992 Sep.27	*Anabaena spiroides*, *Microcystis ichthyoblabe*, *M.aeruginosa*
Chungju	1992 Oct.5	*Oscillatoria agardhii*, *Microcystis wesenbergii*, *M.ichthyoblabe*, *M.aeruginosa*
Soyang	1992 Sep.20	*Anabaena macrospora*, *Microcystis aeruginosa*
Jangsong	1992 Oct.7	*Anabaena citrispora*, *M.ichthyoblabe*, *M.aeruginosa*, *Anabaena spiroides*
Hapchon	1992 Oct.9	*M.aeruginosa*, *M.ichthyoblabe*, *M.novacekii*, *M.flos-aquae*
Yongsan	1992 Oct.17	*Microcystis ichthyoblabe*, *M.aeruginosa*
Yonglang	1993 Aug.17	*Microcystis ichthyoblabe*, *M.aeruginosa*, *M.flos-aquae*, *M.wesenbergii*

Lake Soyang and Lake Chungju are located in less populated northern part of South Korea and less eutrophicated than those located in southern coastal regions. Because these two reservoirs are located at higher altitude and latitude, water temperature is lower than others. Lower temperature might explain partially the difference of phytoplankton community, especially lack of *Microcystis* dominancy. In Lake Soyang *Anabaena* has been dominant species every summer since 1986[15]. Several species of *Anabaena* alternates as the major component were mainly *A. spiroides* and *A. macrospora*.

Test of toxicity by mouse bioassay showed lethal effect for L. Yonglang, L. Hapchon, L. Okjong, L. Jangsung, and L. Jinyang(Table 3). Respiratory distress and muscle fasciculation symptom was obvious in case of L. Hapchon, L. Yonglang and L. Yongsan. Livers of the mice became darker, lager and increased weight by more than 10%. In cases when liver weight increase over 20%, lethal effect of mice were observed within 1-2hrs.

All the studied lakes except Lake Yonglang provide for the drinking water sources, the presence of cyanobacterial toxin can be a potential threat and requires more attention to water treatment. In Korea rapid sand filter system is employed in most of water supply treatment plants. Slow sand filter or charcoal treatment that are more effective in removing cyanobacterial cells and toxins are not common, which might provoke public health problem. We did not have debate about the hazard of cyanobacterial blooms in water supply resources because we did not have any data about cyanobacterial toxins in Korea. People are also suffering from odor problem in many reservoirs mainly caused by cyanobacteria.

Table 3. The result of mouse bioassay and the contents of microcystin-RR and -LR measured by HPLC. (-);not observed, (+);observed, (n.d.);not detected.

Lakes	Mouse bioassay			Content of microcystin (mg/g d.w.)	
	Toxic symptom	Weight increase of liver	Lethal effect (dead/total)	RR	LR
Deachong	-	-	-	0.074	n.d.
Chungju	-	-	-	0.024	n.d.
Soyang	-	-	-	0.133	n.d.
Hapchon	+	+	+(5/6)	0.291	0.172
Jangsong	+	+	+(1/5)	0.087	0.119
Yongsan	+	+	+(3/5)	n.d.	0.234
Yonglang	+	+	+(20/20)	0.183	0.181

Lake Yonglang showed the highest density of phytoplankton and the highest toxicity. Dense 'pea soup' was observed at the leeward shore of this circular lake. Cyanobacterial cells could be collected from the surface scum just by taking surface water with a vat. Though LD_{50} was not measured, it is inferred to be much smaller than 25mg dried algae per mouse. The smaller dose of 25mg dried algae per mouse showed 100% mortality in mouse bioassay. Compared with the high mortality in mouse bioassay, the contents of microcystin-RR and -LR were not so much higher than other lakes. It can be inferred that several other toxins which are not measured in this study may have exerted cooperatively. Because this lake is used only for recreational resort and not for drinking water source, there is no threat to human health. Originally Lake Yonglang was a brackish lagoon having water exchange with sea. But after a watergate is constructed to raise water level and enlarge water surface in the course of recreational resort town development, the lakewater became eutrophic freshwater. Sewage coming from resort town flow into the lake without any treatment is the major cause of eutrophication.

SUMMARY

Cyanobacterial blooms in eutrophic lakes often produce algal toxins causing death of livestock, wild animals, and fish. In this work, phytoplankton communities were examined for the appearance of toxic species. The occurrence of toxic bloom was examined with mouse bioassay. Phytoplankton were collected in late summer at the surface of 6 middlestream reservoirs(Soyang, Chungju, Daechong, Jangsung, Hapchon, Jinyang, Okjung), an estuarine reservoir(Yongsan), and a coastal lagoon(Yonglang). Algal cells were freeze-dried for the use in mouse bioassay and chemical analysis. The occurrence of toxic bloom was examined with mouse bioassay and the content of two cyanobacterial hepatotoxins, microcystin-RR and -LR, that are known to be most common in freshwater algae were analyzed with HPLC.

In all the study lakes cyanobacteria were dominant phytoplankton. The dominant species implied the possibility of toxin production(*Microcystis*, *Anabaena* etc.). In L. Soyang and Chungju, dominant species were *Anabaena* and *Oscillatoria*, respectively. In L. Jangsung and Daechong, *Anabaena* and *Microcystis* appeared together.

Test of toxicity by mouse bioassay showed lethal effect for L. Yonglang, L. Hapchon, L. Yongsan, L. Jangsung. L. Yonglang showed strong lethal effect. Microcystin-LR and -RR both were detected in L. Hapchon, L. Jangsung and L. Yonglang, and microcystin-LR was detected in L. Yongsan, -RR was detected in L. Daechong, L. Chungju, and L. Soyang.

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