Polish Journal of Environmental Studies Vol. 9, No. 3 (2000), 171-178

The First Report about the Presence of Cyanobacterial Toxins in Polish Lakes

A.K.M.Kabziński[1]*, R. Juszczak[1], E. Miękoś[1], M. Tarczyńska[2], K. Sivonen[3], J. Rapala[3]

[1] University of Lodz, Department of General and Inorganic Chemistry, Chromatography, Biochromatography and Environmental Analysis Laboratory, Narutowicza 68, 90-136, Lodz, Poland [2] University of Lodz, Department of Applied Ecology, Banacha 12/16, 90-237, Lodz, Poland [3] University of Helsinki, Department of Applied Chemistry and Microbiology, Helsinki; Finland

Received 25 May, 1999
Accepted 18 January, 2000

Abstract

The occurence of toxic freshwater blooms of cyanobacteria (blue-green algae) has been reported in many countries. These toxic water blooms have caused the deaths of domestic animals and wildlife. Cyanobacteria may produce acute toxins such as hepatotoxic peptides (microcystins, nodularins and cylindrospermopsin) and neuro-toxic alkaloids (anatoxin-a, anatoxin-a(s), homoanatoxin and aphanotoxins). Very important toxins are microcystins (MCYST) with high biological activities and wide distribution all over the world. They have been produced by *Microcystis, Oscillatoria, Anabaena* and *Nostoc.* All MCYST can play the role of tumor-promoting activators.

This paper for the first time presents results showing the presence of microcystin hepatotoxin in Polish lakes and drinking water.

Keywords: Cyanobacterial toxins, microcystine, environmental analysis, high performance liquid chromatography

Introduction

The occurence of toxic freshwater blooms of cyanobacteria (blue-green algae) has been reported in many countries. These toxic water blooms have caused deaths of domestic animals and wildlife [1, 2]. Cyanobacterial toxins are produced by terrestrial-, fresh-, brackish- and sea-water cyanobacteria at cosmopolitan occurence. These toxins present acute and chronic hazards to human and animal health and are responsible for isolated, sporadic animal fatalities (mammals, fishes, birds, etc.) each year. Human health problems are associated with the ingestion of and contact with cyanobacterial blooms and their toxins. Cyanobacteria may produce acute toxins such as hepatotoxic peptides (microcystins, nodularins and cylindrospermopsin) and neurotoxic alkaloids (anatoxin-a, anatoxin-a(s), homoanatoxin and aphanotoxins) [3] (Table 1). There are approximately about 1,500 known species of cyanobacteria, but only about 100 can synthesize different toxins [4]. The main producers are: *Oscillatoria, Anabaena, Aphanizomenon, Cylindrospermum, Synechocystis, Cylindrospermopsis, Scytonema, Hapalosiphon, Schizotrix, Gleotrichia, Nodularia* and *Nostoc.* Within an individual water sample the toxin concentration per unit of cyanobacterial biomass may vary widely from week to week at the same sampling station. The concentration of toxins varies not only for different strains of cyanobacteria, but also for different clones of the same isolate. Workers found a mixed pattern of high and low toxicity per unit of cyanobacterial biomass when sampling a lake on a single occasion [4-6]. An investigation over 3 years showed that each year cyanobacterial

* Corresponding author.

blooms were nontoxic at the begining of the growing season but developed high toxicity during the first strong biomass increase in the summer [7].

Table 1. The characterization of diffrent toxic substances and comparision with toxicity of cyanobacterial toxins.

No.	Toxic substances	Toxicity LD_{50} (μg/kg)	Molecular weight (Da)
1.	Sodium cyanide NaCN	15,000	51
2.	Potasium cyanide KCN	10,000	67
3.	Strychnine	500	334
4.	Microcystine-RR	600	1037
5.	Microcystine-YR	100-200	1044
6.	Microcystine-YA	100	959
7.	Microcystine-LR	50	994
8.	Microcystine-LA	<100	909
9.	Microcystine-AR	200-400	952
10.	Microcystine-YM	56	1018
11.	Microcystine-WR	100-200	1067
12.	Microcystine-LY	<100	1001
13.	Nodularine	30-50	824
14.	Anatoxin-a	200	165
15.	Anatoxin-a(s)	20	252
16.	Homoanatoxin	200-300	179
17.	Neosaxitoxin	10	315
18.	Saxitoxin	10	299
19.	Scytophycin A	x	821
20.	Scytophycin B	650	819

Much attention has been paid to microcystes (MCYST), because of their biological activities and wide distribution all over the world. They have been produced by *Microcystis, Oscillatoria, Anabaena* and *Nostoc* [1]. MCYST consist of a common moiety composed of seven amino acids (3-amino-9-methoxy-10-phenyl-2,6,8--trimethyldeca-4,6-dienoic acid (Adda), N-methyldehydroalanine (Mdha), D-alanine, β-linked *D-erythro- β*-methylaspartic acid, γ-linked D-glutamic acid and two L-amino acids as variants), and approximately 50 MCYSTs have been isolated so far [8-11]. These toxins also inhibit phosphatases 1 and 2A in a similar manner to ocadoic acid and have tumor-promoting activity not only in rat liver [12-16]. Yu and coworkers [17] reported that there are about 100,000 deaths annually from primary liver cancer (PLC) in China. In several areas of China, the rates of PLC do not corelate with PLC-causing agents such as aflatoxin and hepatitis B virus, and people who drink pond and ditch water have a higher risk of PLC than people who drink well water. This may suggest that incidences of PLC are related to MCYST in drinking water. Falconer [18] achieved an oral experiment of chronic ingestion of crude extract of *Microcystis* and the result suggested the possibility of MCYST promoting tumors after long-term exposure.

The conventional method for purification of cyanobacterial peptide toxins involves freezing and liophilization of cells, followed by solvent extraction and preconcentration of the toxins by adsorption on C18 solid-phase extraction (SPE) cartridges [19], than low-pressure gel permeation chromatography (LPGPC) on Sephadex in some cases and invariable purified by reverse-phase high performance liquid chromatography (RP-HPLC) [20-25]. The use of the internal surface reversed-phase (ISPR) column for peptide toxins purification was introduced by Meriluoto and Eriksson [26]. The more popular detection method is HPLC-UV or HPLC-DAD with detection limit about 0.1-1.0 μg MCYST-LR, but also fluorescence (FL), poexyolate chemiluminescence (POCL), mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA), or linked protein phosphatese bioassay (PPB) detections were used.

The paper presents results showing the presence of microcystin hepatotoxin in Polish lakes and drinking water.

Experimental

Samples and Materials

Field samples, water blooms of cyanobacteria and lake water were collected from lakes Sulejow, Jeziorsko and Wtodawek in 1993-1998 from June to November. All of them are artificial lakes used as reservoirs of drinking

Table 2. The retention times for different microcystines standards hile separation by RP-HPLC technique on column CI 8.

No.	Microcystine	Retention tim (t_R) (min.)		
		column 1 100x4.0 mm ID	column 2 250x4.0 mm ID	column 3 300x4.9 mm ID
1.	MCYST-LA	1.396	3.386	3.277
2.	MCYST-LR	1.652	4.074	3.109
3.	MCYST-[Dha^7]LR	1.630	3.989	4.068
4.	MCYST-[D-Asp^3]LR	1.633	3.953	3.705
5.	MCYST-RR	2.110	5.246	5.844
6.	MCYST-[Dha^7]RR	1.938	4.846	6.155
7.	MCYST-[D-Asp^3]RR	1.969	4.898	5.737
8.	MCYST-YR	2.543	6.142	3.515
9.	MCYST-YA	1.405	3.627	4.251
10.	MCYST-AR	2.053	5.232	6.256
11.	MCYST-LY	2.317	5.764	7.918

(1) Column Spherisorb 5S ODS2, 100x4.0 mm ID (Hewlett--Packard)
(2) Column Spherisorb 5S ODS2, 250x4.0 mm ID (Hewlett-Packard)
(3) Column Resolve CI8, 300x3.9 mm ID (Waters)

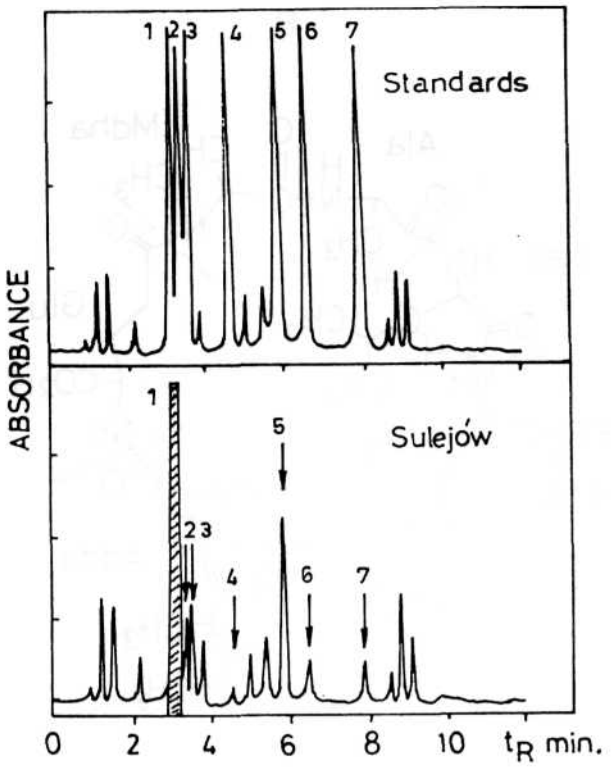

Fig. 1. The separation of MCYSTs standards mixture and extracts of blooms samples received from Sulejow Lake, where: (1) MCYST-LR; (2) MCYST-LA; (3) MCYST-YR; (4) MCYST-YA; (5) MCYST-RR; (6) MCYST-AR and (7) MCYST-LY, (column Resolve CI 8 from Waters, 300x3.9 mm ID, the flow rate 1.0 ml/min, absorbance at 240 nm was measured).

water. The cyanobacterial bloom samples were colected by skimming across the water surface with 25 mm plankton net. The larger particles and zooplankton were separated by using different sieves. Later the bloom samples were liophilized. The water samples were collected from a depth of 0.3-0.6 m, in dark glass bottles with the addition of sodium azide NaN_3 (0.05% w/v), and were stored at +4°C. The standards of microcystins: MCYST-YR, MCYST-LR and MCYST-RR and were received from Calbiochem (USA) and Sigma (USA). The other standards were received from Dr. Karina Sivonen laboratory (University of Helsinki, Finland). The following SPE cartridges were used: Bakerbond C18, SAX, DEA, NH_2, CN (1000 mg, J.T.Baker, USA). All chemicals used in the experiments were of analytical grade (J.T.Baker, USA and Merck, Germany). All experiments utilized single use equipment [27-36].

Extraction and Clean-up Methods

200 or 500 mg of freeze-dried (liophilized) algal cells were extracted in 100ml of mixture (5% n-Bu-OH, 20% MeOH, 75% H_2O) for 30 min. using a magnetic stirrer at low speed. Later the mixture was ultrasonificated in ultrasonificator type XL 2020 (Misonix Inc., USA) with a full power 500W by 10 min. The extract was centrifuged by 30 min. (2,000xg, ultracentrifuge WE-6 from Unipan, Poland and microcentrifuge XX-42 from Milipore, Japan). The supernatant was separated by ultrafiltration (0.47 µm GF52 filter from Schleider and Schnell, Germany) and in the inert gas flow to volume 15-20 ml. Later samples were preconcentrated and partially prepurified by SPE technique (SPE-12G from J.T.Baker, USA) and finally filtered on 0.45 µm filter (Amersham, USA) to 0.5 ml volume [37-41].

The water samples (1000 ml) were filtered for removal of the mechanical inorganic and organic pollutants (0.47 µm GF52 filter from Schleider and Schnell, Germany) and later were separated and partially prepurified by SPE method (SPE-12G from J. T.Baker, USA) by the same method as extracts of bloom samples [37-41].

Chromatographic Analysis

The water and bloom samples after preconcentration by SPE technique were separated by RP-HPLC method (HP 1050 with UV/VIS detection from Hewlett-Packard, USA and HPLC 600E with 990 DAD detector from Waters, USA) on columns types C18 (columns Spherisorb 5S ODS2 100x4.0 mm ID and 250x4.0 mm ID, equipped with precolumns C18 4x 4.0 mm ID from Hewlett-Packard, USA; column Resolve C18 300x3.9 mm ID from Waters, USA and Econosil C18 250x4.6 mm ID from Alltech , USA) with detection at 240 nm, volume of injection loops were 20.0 and 250.0 µl. The samples were separated by gradient and isocratic methods with application of acetonitryl, methanol, ammonium-acetate buffer, phosphate buffer and addition of sodium sulfate, with a flow-rate of 1.0 ml/min. [41].

Results and Discussion

The retention times for different microcystine toxins are given in Table 2. The chromatograms of different MCYST standards and extract of biological samples (blooms) from Sulejow Lake is presented in Figure 1 and the main toxic components in Polish lake bloom samples are given in Table 3. The chemical structure of main

Table 3. The main microcystine compounds in blooms collected from Polish Lakes.

No.	Microcystine	Content (%)	MW (Da)	LD_{50} (µg/g)
1.	MCYST-LR	85 – 95	994	50
2.	MCYST-RR	2 – 8	1037	600
3.	MCYST-LA	1 – 4	909	50
4.	MCYST-YR	1 – 2	1044	70
5.	MCYST-YA	(*)	959	100
6.	MCYST-AR	(*)	952	250
7.	MCYST-LY	(*)	1001	90

(*) MCYSTs probably present in blooms but not quantitative determined in collected samples.

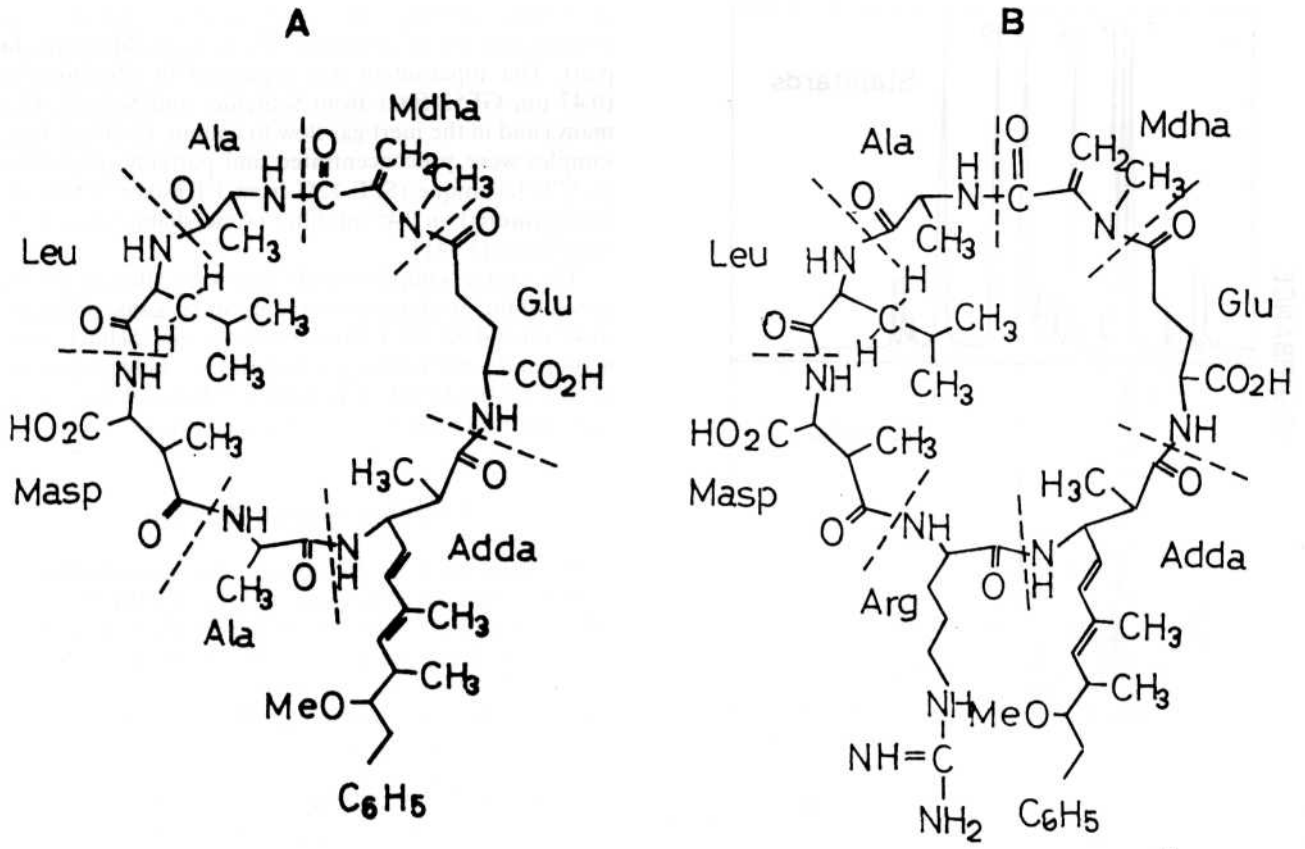

Fig. 2. The chemical structures of main microcystines present in blooms samples from Polish lakes: (a) MCYST-LR and (b) MCYST-RR.

microcystines present in blooms in Poland are present in Figure 2. The chromatograms of MCYST-LR standard in different concentrations and calibration line are presented in Figures 3 and 4. The UV/VIS spectrum of MCYST-LR standard are presented in Figure 5. The chromatograms of cyanobacterial bloom extracts are presented in Figure 6 and the chromatograms of lake water and tap water from Lodz town are presented in Figures 7 and 8. The mean concentration of MCYST-LR in blooms samples, Sulejow lake water and MCYST-LR concentrations in drinking water from Lodz town area are given in Tables 4 and 5. The exemplar relation between MCYST-LR concentrations in bloom samples, Sulejow lake water and drinking water in Lodz at 1995 year is presented in Figure 9. The mean concentration of MCYST-LR in blooms from Jeziorsko and Włocławek are given in Table 6.

The main component of microcystines present in tested Polish lakes (Sulejow, Włocławek and Jeziorsko) was MCYST-LR (Figures 1 and 2), which make about 85-95% of all microcystines contained in bloom (Table 3). It is a similar situation to that in other countries, where the main components of toxin are: MCYST-LR, MCYST-RR, MCYST-YR and their isoforms [Dha^7], [D-Asp^3] and [Dha^7,D-Asp^3] [42-53]. In the tested materials the content of MCYST-LR were higher than was observed for Finish lakes, where the content of MCYST-LR ranged between 14-57% and were 12-35% for MCYST-RR [44] or 31-81% of MCYST-LR in blooms from Suwa Lake in Japan [52]. Only in Brazilian

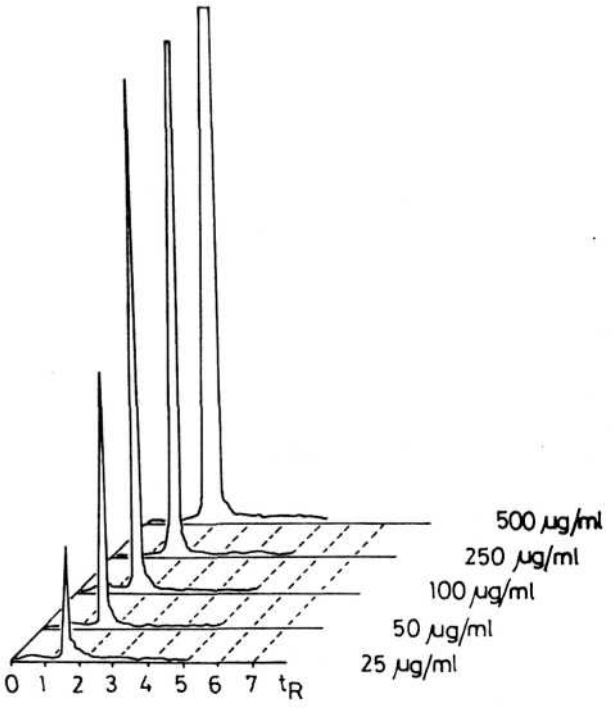

Fig. 3. The chromatograms of MCYST-LR standard separated in different concentrations by RP-HPLC method on column CI 8 (column Spherisorb 5S ODS2 from Hewlett-Packard, 100x4.0 mm ID, the flow rate 1.0 ml/min, absorbance at 240 nm was measured).

Table 4. The concentration of microcystine-LR in blooms and water from Sulejow Lake in period 1993-1998.

No.	Year (*)	Mean content of microcystine-LR			
		Blooms sample (µg/g)		Lake water (µg/L)	
		range	mean	range	mean
1.	1993	16 – 149	74+/– 56	x	x
2.	1994	5 – 191	81+/– 71	x	x
3.	1995	8 – 427	120+/– 111	0.0 – 6.8	2.3+/– 1.8
4.	1996	6 – 227	99+/– 67	0.0 – 5.3	1.9+/– 1.2
5.	1997	7 – 286	103+/– 86	0.0 – 4.6	1.5+/– 1.4
6.	1998	4 – 168	76+/– 53	0.0 – 2.7	0.8+/– 0.7

(*) Samples were collected from June to November.

Table 5. The concentration of microcystine-LR in drinking water from Lodz town area in 1995-1998.

No.	Year (*)	Content of microcystine-LR in drinking water from Łódź (µg/L)	
		range	mean
1.	1995	0.0 – 3.1	0.7 +/– 0.9
2.	1996	0.0 – 1.9	0.4 +/– 0.5
3.	1997	0.0 – 2.0	0.5 +/– 0.6
4.	1998	0.0 – 1.7	0.3 +/– 0.4

(*) Samples were collected from June to November.

Table 6. The concentration of microcystine-LR in bloom samples from Jeziorsko and Włocławek Lakes in 1996-1998.

No.	Year (*)	Content of MCYST-LR in bloom samples (µg/g)			
		Jeziorsko Lake		Włocławek Lake	
		range	mean	range	mean
1.	1996	15 – 390	187+/–115	8 – 460	210+/–135
2.	1997	18 – 450	212+/–101	12 – 438	206+/–112
3.	1998	10 – 275	93+/–102	6 – 283	84+/–100

(*) Samples were collected from June to November.

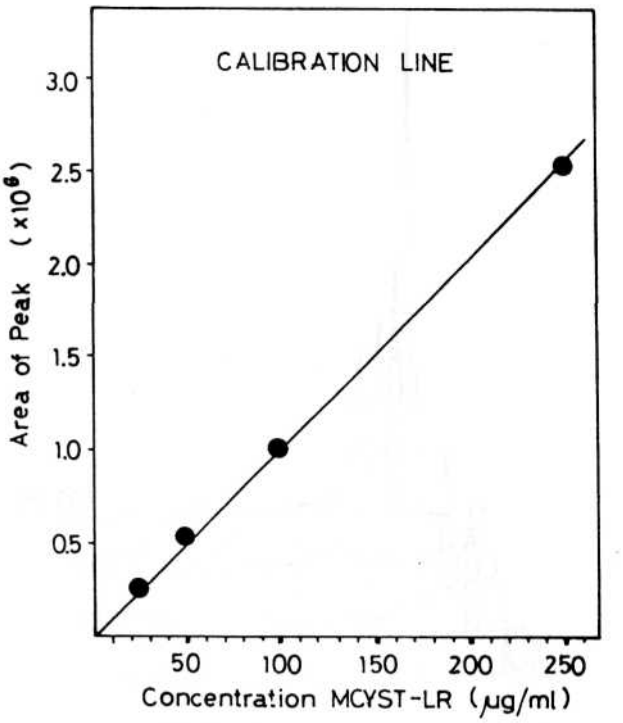

Fig. 4. The calibration line between MCYST-LR concentration and area of peaks (chromatographic conditions the same as in Figure 3).

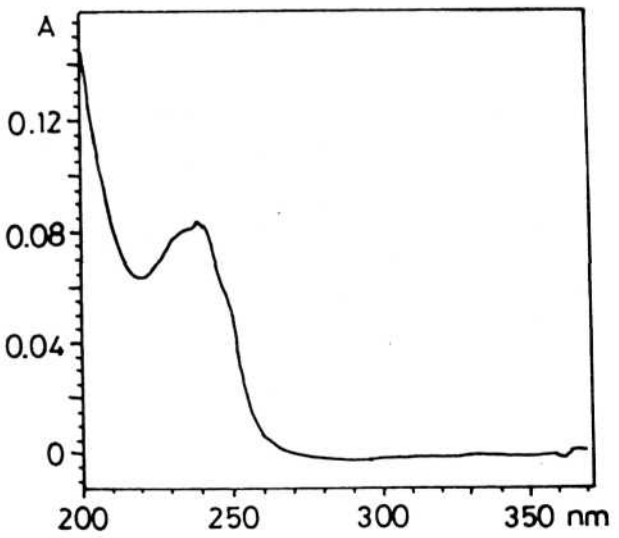

Fig. 5. The UV/VIS spectrum of MCYST-LR standard (Sigma, USA).

lakes [51] was MCYST-LR in general the main fraction, as in Poland. In difference to the observations of Watanabe [52], this composition of microcystine in bloom was very constant and didn't change during the summer of 1995. Very similar results were obtained for other summer seasons in 1993-1998, where the main compound was MCYST-LR in concentrations ranging between 85-95% of mass of microcystine toxins.

Concentrations of MCYST-LR were determined from calibration line with a very good linearity at range 25-500 µ^ml (Figures 3 and 4). Concentrations of MCYST-LR were determined in bloom samples (Figure 6), lake water (Figure 7) and tap water from Lodz (Figure 8). In addition, to all samples was added MCYST-LR standard for control of quality of separation and stability of retention time. The level of MCYST-LR concentration in bloom samples ranged between a few micrograms at the beginning of summer season to a few hundred micrograms in

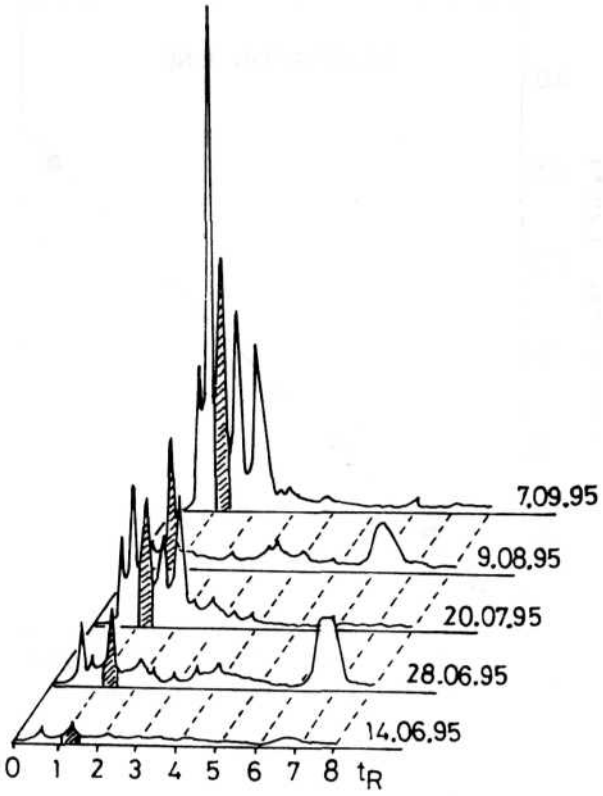

Fig. 6. The chromatograms of bloom samples extracts separated by RP-HPLC method on column C18 type (column Spherisorb 5S ODS2 from Hewlett-Packard, 100x4.0 mm ID, the flow rate 1.0 ml/min, absorbance at 240 nm was measured, samples were collected between 14.06.1995-7.09.1995).

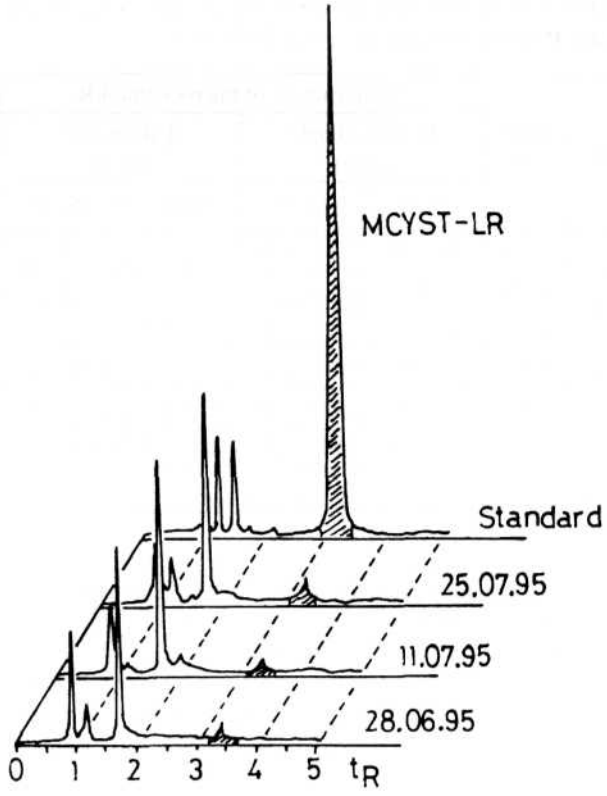

Fig. 7. The chromatograms of water samples from Sulejow Lake separated by RP-HPLC method on column C18 type (column Spherisorb 5S ODS2 from Hewlett-Packard, 250x4.0 mm ID, the flow rate 1.0 ml/min, absorbance at 240 nm was measured, water samples wre collected between 28.06.1995-25.07.1995).

the autumn (Tables 4 and 6, Figure 9). The similar results were obtained for all three lakes tested in experiment (0.010-0.450 mg/g Jeziorsko, 0.006-0.460 mg/g Wloclawek and 0.004-0.427 mg/g Sulejow). The amounts of MCYST-LR in particular material (bloom samples) from lakes: Sulejow, Wloclawek and Jeziorsko were lower than reported in: Lake Tuusulanjarvi in Finland (0.01-0.79 mg/g) [50], Alberta lakes in Canada (0.01-0.74 mg/g) [54], Suwa Lake in Japan (0.05-0.82 mg/g) [55] or in Slovenian lakes (0.01-0.85 mg/g) [53].

The concentrations of microcystine-LR in Sulejow lake water ranged between 0.0-6.8 µg/L (Table 4, Figure 9) and were similar to those reported by Lahti and coworkers [50] for Tuusulanjarvi Lake in Finland (0.01-7.40 µg/L) and were lower than reported by: Lindholm and Meriluoto [56] for other few Finish lakes (20-30 µg/L), and Park and coworkers [55] for Suwa Lake in Japan (0.1-30.1 µg/L for MCYST-RR and about 0.1-20.4 µg/L for MCYST-LR). Only Watanabe and coworkers [57] (1.1 µg/L), and Jones and Orr [58] (4.7 µg/L) reported lower concentrations of MCYST-LR in lake water. The maximums of MCYST-LR concentrations in lake and drinking water were removed in relation to maximums of MCYST-LR concentrations in bloom for about 7 -14 days (Figure 9).

There are not so much publications about present and degradation of cyanobacterial toxins in natural water.

Microcysins are normally present only inside cyanobacterial cells and enter the surrounding water after cell lysis [59,60]. In laboratory studies the decimal reduction time (biodegradation time) for the dissolved microcystins in water varied from 3 to 40 days [58, 60-63]. In natural system the full biodegradation of microcystins disolved in lake water were about 45 days (Figure 9). Based on literature data, the level of MCYST concentrations in lake water used as reservoirs of drinking water ought to be lower than 2.0 µg/L.

We also observed the presence of MCYST-LR in drinking water from Lodz, well correlated with the level of MCYST-LR concentrations in Sulejow reservoir (Figure 9). The concentrations in drinking water ranged between 0.0-3.1 µg/L (mean 0.7 +/- 0.9 µg/L in 1995) (Table 5). The time for full dissappearance was about 35-40 days.

Conclusions

1. The dissolved microcystins were detected in all three Polish lakes water during decomposition of cy anobacterial bloom, and were observed about 60 days after maximum concentration of microcystines in bloom.
2. The main component was MCYST-LR (85-95%) and in addition also were observed: MCYST-RR,

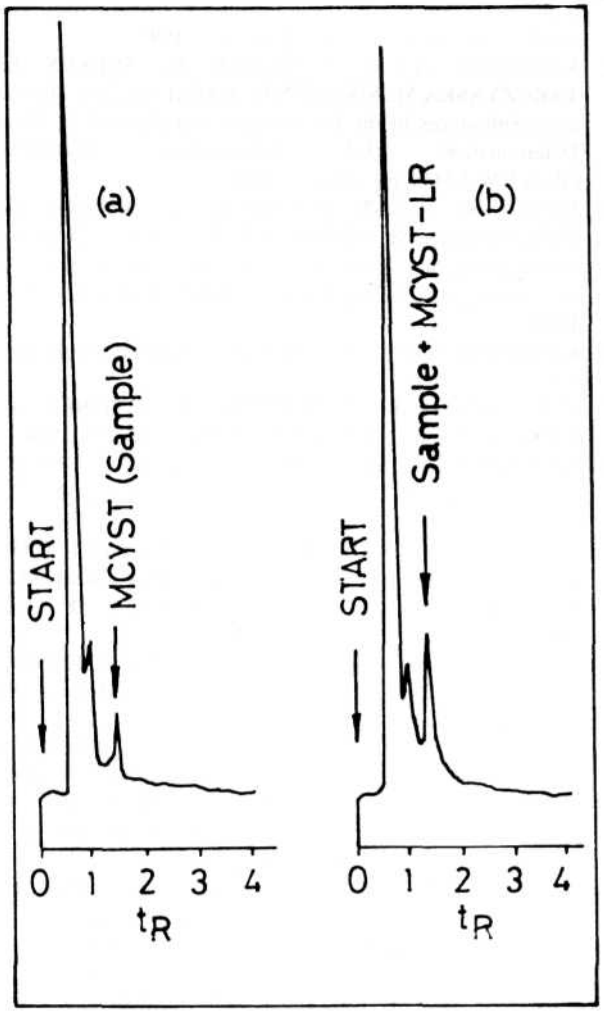

Fig. 8. The chromatograms of drinking water from Lodz town area collected at 3.08.1995, (a) chromatogram of extract od drinking water prepared by SPE method, (b) chromatogram of extract of drinking water with addition of MCYST-LR standard (column Spherisorb 5S ODS2 from Hewlett-Packard, 100x4.0 mm ID, the flow rate 1.0 ml/min, absorbance at 240 nm was measured).

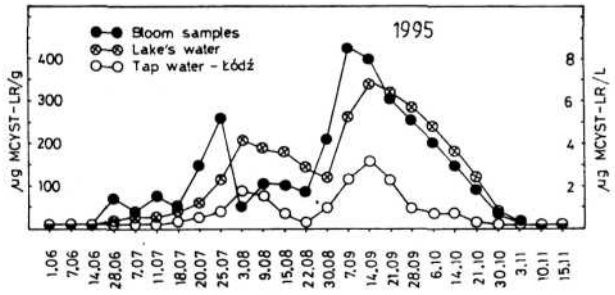

Fig. 9. The exemplar relation between MCYST-LR concentrations in blooms sam-ples and MCYST-LR concentration in Sulejow Lake water (June-November 1995).

MCYST-LA and MCYST-YA (together about 4-14%). Probably also were detected: MCYST-YA, MCYST-AR and MCYST-LY.

3. The content of MCYST-LR in bloom samples were about: 0.004-0.427 mg/g for Sulejow Lake, 0.006-0.460 mg/g for Wloclawek Lake and 0.010-0.450 mg/g for Jeziorsko Lake.

4. The concentration of MCYST-LR in water from Sulejow Lake ranged between 0.0-6.8 µg/L and reduction time for disappearance of toxin was about 45 days from the maximum concentration.

5. Also were observed the varied contents of MCYST-LR in drinking water in Lodz, ranging between 0.0-3.1 ug/L.

6. Microcystins were detected in Polish lakes, similar for the other Europen countries. Water quality ought to be controlled and there must be a described method for drinking water pretreatment.

Aknowledgement

The authors are very grateful to Dr. Karina Sivonen, Dr. Iarrko Rapalla and Dr. Leo Rouhiainen from the Department of Applied Chemistry and Microbiology of Helsinki University for microcystine standards preparation and very effective practice in their laboratory.

References

1. CARMICHAEL W.W. [in:] A Status Report on Planctonic Cyanobacteria (Blue-Green Algae) and their Toxins, US Envi ronmental Protection Agency, pp. 15-26, **1992.**
2. CARMICHAEL W.W., Sci.American, **270,** 78, **1994.**
3. CARMICHAEL W.W., J.Appl.Bacteriol., **72,** 445, **1992.**
4. CODD C.A., BEL S.G., Water PolIut.Control, **84,** 225, **1985.**
5. GORHAM P.R., MAC LACHLAN J., HAMNER V.T., KIM W.K., Verh.Int.Ver.Limnol. **15,** 796, **1964.**
6. CARMICHAEL W.W., GORHAM P.R., BIGGS D.F., Can.Vet.J-, **18,** 71, **1977.**
7. BENFORD J., HENNING M., Int.Rev.Gesamten Hydrobil., **74,** 233, **1989.**
8. NAMIKOSHI K., RINEHART K.L., SAKAI R., STOTTS R., DAHLEM A., BAYSLEY V., CARMICHAEL W.W., EVANS W.R., J.Org.Chem., **57,** 866, **1992.**
9. KIVIRANTA J., NAMIKOSHI M., SIVONEN K., EVANS W.R., CARMICHAEL W.W., RINEHART K.L., Toxicon, **30,** 1093, **1992.**
10. SIVONEN K., SKULBERG O.M., NAMIKOSHI M., EVANS W.R., CARMICHAEL W.W., RINEHART K.L., Toxicon, **30,** 1465, **1992.**
11. LUUKKAINEN R., NAMIKOSHI M., SIVONEN K., RINEHART K.L., NIEMELA S.I., Toxicon, **32,** 133, **1994.**
12. NISHIWAKI-MATSUSHIMA R., OHTA T., NISHIWAKI S., SUGANUMA M., KOHYAMA K., ISHIKAWA T., CAR MICHAEL W.W., FUJIKI M., J.Cancer Res.Clin.Oncol., **118,** 420, **1992.**
13. YOSHIZAWA S., MATSUSHIMA R, WATANABE M.F., HARADA K.I., ICHIHARA A., CARMICHAEL W.W., FUJIKI J., J.Cancer.Oncol., **116,** 609, **1990.**
14. MATSUSHIMA R., YOSHIZAWA S., WATANABE M.F., HARADA K.I., FURUSAWA M., CARMICHAEL W.W., FUJIKI H., Biochem.Biophys.Res. Commun., **173,** 867, **1990.**
15. MAC KINTOSH C, BEATTIE K.A., KLUMPP S., COHEN P., CODD G.A., Febs Lett., **264,** 197, **1990.**
16. ERIKSSON J.E., TOIVOLA D., MERILUOTO J.A.O., KARAKI H., HAN Y.G., HARTSHORNE D., Biochem.Biophys.Res.Commun., **173,** 1347, **1990.**

17. YU S.Z., [in:] Primary Liver Cancer, (Eds., Z.Y.Tang, M.C.Wu, S.S.Xia), Beijing, Springer Verlag, pp. 30-37, **1989.**
18. FALCONER I.R., Environ.Toxicol.Water Quality, **6,** 177, **1991.**
19. MARTIN C, KAARINA S., MATERN U., DIERSTEIN R., WECKESSER J., Ferns Microbiol.Lett., **68,** 1, **1990.**
20. BROCKS W.P., CODD G.A., Lett.Appl.Microbiol., **2,** 1,**1986.**
21. DIERSTEIN R, KAISER I, WECKESSER J., Ferns Microbiol.Lett., **49,** 143, **1988.**
22. KRISHNAMURTHY T., CARMICHAEL W.W., SARVER E.W., Toxicon, **24,** 865, **1986.**
23. GOTHERCOLE P.S., THILE P.G., J.Chromatogr., **408,** 435, **1987.**
24. BIRK I.M., MATERN U., KAISER I., MARTIN C, J.Chromatogr., **449,** 423, **1988.**
25. HARADA K.I., MATSUURA K., SUZUKI M., OKA H., WATANABE M.F., OISHI S., DAHLEN A.M., BAYSLEY V.R., CARMICHAEL W.W., J.Chromatogr., **448,** 275, **1988.**
26. MERILUOTO J.A.O., ERIKSSON J.E., J.Chromatogr., **438,** 93, **1988.**
27. KABZINSKI A.K.M., SCHOLL H., JUSZCZAK R., DOMAGALA S., TARCZYNSKA M., International Symposium on Environmental Analytical Chemistry, 9-12.04. Vienna - Austria, Book of Abstracts, p. 44, **1996.**
28. KABZINSKI A.K.M., SCHOLL H., DOMAGALA S., JUSZCZAK R., TARCZYNSKA M., 10th Anniversary Meeting of the Europiean Society for Bio-Chromatography, 13-15.05. Paris - France, Book of Abstracts, p.55, **1996.**
29. KABZINSKI A.K.M., SCHOLL H., DOMAGALA S., JUSZCZAK R., 20th International Symposium on HPLC and Related Techniques, 16-21.06.1996, San Francisco - USA, Book of Abstracts, p.231, **1996.**
30. KABZINSKI A.K.M., SCHOLL H., DOMAGALA S., JUSZCZAK R., TARCZYNSKA M., 7th International Symposium on Pharmaceutical and Biomedical Analysis, 20-23.08.1996, Osaka - Japan, Abstract Book, p.46, **1996.**
31. KABZINSKI A.K.M., SCHOLL H., JUSZCZAK R., 4th Soil and Sediment Contaminant Analysis Workshop, 22-25.09.1996, Basel - Switzerland, Abstracts, p.86, **1996.**
32. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., TARCZYNSKA M., 21st International Symposium on HPLC and Relation Techniques, 22-27.06.1997, Birmin-gham - England, Abstracts of Papers, p.43, **1997.**
33. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., 12th International Conference on Chromatographic Methods and Human Health, 10-13.11.1997, Piestany - Slovak Republik, Conference Materials, p.114, **1997.**
34. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., TARCZYNSKA M., 28th Annual Intrnational Symposium on Environmental Analytical Chemistry, 1-5.03.1998, Geneva - Switzerland, Conference Materials, p.163, **1998.**
35. KABZINSKI A.K.M., International Conference - Forum Chemistry, 27-29.04.1997, Warsaw - Poland, Conference Materials, p.85, **1997.**
36. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., 1st International Odra Research Conference, 16-19.06.1998, Krakow - Poland, Conference Materials, p.93, **1998.**
37. KABZINSKI A.K.M., SCHOLL H., [in:] Analytical Problems with Determination of Cancerogenic Substances in Water, (Ed. J.R.Dojlido), Warszawa, PAN-PZH-IMIGW, pp. 121-141, **1997.**
38. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., SCHOLL H., TARCZYNSKA M., SIVONEN K., [in:] The Chromatography and other Separation Techniques in Eco-Analysis, (Ed. B.Buszewski), Torun, UMK, pp. 407-419, **1997.**
39. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., TARCZYNSKA M., SIVONEN K., RAPALA I., [in:] The Organic Substances in the Environment and Methods of Their Determination, (Ed. J.Bartulewicz), Warszawa, GIOS-UW-NFOS, pp. 241-243, **1998.**
40. KABZINSKI A.K.M., JUSZCZAK R., MIEKOS E., TARCZYNSKA M., SIVONEN K., RAPALA I., [in:] The Chromatographic Methods in Foods Analysis and in Ecotoxicology, (Ed. Z.Suprynowicz), Lublin, UMCS, pp. 47-58, **1998.**
41. KABZINSKI A.K.M., J.Chromatogr., submitted for publication.
42. LUUKKAINEN R., NAMIKOSHI M., SIVONEN K., RINEHART K.L., NIEMELA S.I., Toxicon, **32,** 133, **1994.**
43. NAMIKOSHI M., SIVONEN K., EVANS W.R., SUN F., CARMICHAEL W.W., RINEHART K.L., Toxicon, **30,** 1473, **1992.**
44. SIVONEN K., NAMIKOSHI M., EVANS W.R., CARMICHAEL W.W., SUN F., ROUHIAINEN L, LUKKAINEN R., RINEHART K.L., Appl.Environ.Microbiol., **58,** 2495, **1992.**
45. SIVONEN K., NAMIKOSHI M., EVANS W.R., GROMOV B.V., CARMICHAEL W.W., RINEHART K.L., Toxicon, **30,** 1481, **1992.**
46. VASCONEELOS V.M., EVANS W.R., CARMICHAEL W.W., NAMIKOSHI M., J.Environ.Sci.Health, **28,** 2081,**1993.**
47. KONDO F., YKAI Y., OKA H., ISHIKAWA N., WATANABE M.F., WATANABE M., HARADA K.I., SUZUKI M., Toxicon, **30,** 227, **1992.**
48. WATANABE M.F., OISHI S., HARADA K.I., MATSUURA K., KAWAI H., SUZUKI M., Toxicon, **26,** 1017, **1988.**
49. SHIRAI M., OHTAKE A., SANO T., MATSUMOTO S., SAKAMOTO T., SATO A., AIDA T., HARADA K.I., SHIMADA T., SUZUKI M., NAKANO M., Appl.Environ.Microbiol., **57,** 1241, **1991.**
50. LAHTI K., RAPALA J., FORDIG M., NIEMELA M., SIVONEN K., War.Res., **31,** 1005, **1997.**
51. AZEVEDO S.M.F.O., EVANS W.R., CARMICHAEL W.W., NAMIKOSHI M., J.Appl.Phycol., **6,** 261, **1994.**
52. WATANABE M.F., HARADA K.I., MATSURA K., WATANABE M., SUZUKI M., J.Appl.Phycol., **1,** 161, **1989.**
53. SEDMAK B., KOSI G., Nat.Toxins, **5,** 64, **1997.**
54. KOTAK B.G., LAM A.K.Y., PREPAS E.E., KENEFICK S.L., HRUDEY S.E., J.Physiol., **31,** 248, **1995.**
55. PARK H.D., WATANABE M.F., HARADA K.I., SUZUKI M., HAYASHI H., OK1NO T., Environ.Toxicol.Wat.Qual., 8, 425, **1993.**
56. LINDHOLM T., MERILUOTO J.O.A., Can. J. Fish. Aquat. Sci., **48,** 1629, **1991.**
57. WATANABE M.F., KAYA K., TAKAMURA W., J.Phycol., **28,** 761, **1992.**
58. JONES C.F., ORR P.T., Wat.Res., **28,** 871, **1994.**
59. WATANABE M.F., TSUJI K., WATANABE M., HARADA K.I., SUZUKI M., Nat.Toxins, **1,** 48, **1992.**
60. BERG K., SKULBERG O.M., SKULBERG R., Arch.Hydrobiol., **108,** 549, **1987.**
61. KENEFICK S.L., HRUDEY S.E., PETRSON H.G., PREPAS E.E., Wat.Sci.Technol., **27,** 433, **1993.**
62. LAM A.K.Y., FEDERAK P.M., PREPAS E.E., Environ.Sci.technol., **29,** 242, **1995.**
63. LAM A.K.Y., PREPAS E.E., SPINK D., HRUDEY S.E., Wat.Res., **29,** 1845, **1995.**