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Examinations of Toxins That Blue-green Algae Produce -Development of Analysis Methods of Cyanotoxins using LC/MS/MS, Study on Removal Performance and Field Survey-

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Abstract: We developed new rapid analysis methods for typical cyanotoxins, microcystin-LR, YR, RR and anatoxin-a. In addition, we examined the removal performance of these cyanotoxins. As a result, we managed to remove microcystin-LR, YR, RR by treatments using powdered activated carbon and sodium hypochlorite. Although the treatment with sodium hypochlorite did not contribute to the removal of anatoxin-a, it was removed by powdered activated carbon. Furthermore, we conducted a field survey on water resources as well as raw water and purified water in purification plants, which were under the management of Bureau of Waterworks Tokyo Metropolitan Government. Upon this survey, we detected microcystin-LR, YR, RR in blue-green algae blooms in the inflow area of the storage reservoir. However, we did not detect cyanotoxins in the effluent from reservoir and the water resources rivers, as well as in the raw water and purified water in purification plants.

Keywords: Blue-green Algae ; microcystin ; anatoxin

1. Introduction

In August 2014, more than 1μg/L of microcystin, which is one of toxins that is produced by blue-green algae such as microcystis, anabaena and phormidium was detected in a purified water in Ohio. This level exceeded the established limit according to WHO guidelines for the purified water. Therefore, it was banned from drinking. Microcystin is hepatotoxin, and in Japan, microcystin-LR is one of the items for further study (Provisional target value: 0.8 μg/L). Anatoxin-a (hereinafter referred to as “AT-a”), another typical cyanotoxin, along with microcystin, is neurotoxin, and although there is no target value set in Japan for this, in New Zealand the provisional maximum acceptable value is 6 μg/L.

In recent years, blue-green algae blooms have been partially detected in our storage reservoirs and it is necessary for us to take measures to tackle cyanotoxin. With such a situation as a background, as for microcystin-LR, YR, RR, (hereinafter referred to as “MC-LR,” “MC-YR,” “MC-RR,” respectively, and “MCs,” collectively,) along with AT-a, we developed analysis methods, examined the removal performance, and also conducted a field survey on the water resources and the raw water and purified water in purification plants. This report describes the findings obtained by these studies.

2. Methods

2.1 Development of Analysis Methods of MCs and AT-a

We examined rapid analysis methods using LC/MS/MS for four cyanotoxins, MCs and AT-a. Whereas the existing method of analysis of MCs requires 500 mL of water sample for solid-phase extraction as a pretreatment, this new method requires 100 mL for solid-phase extraction using MC-LR$^{-15}N_{10}$, MC-YR$^{-15}N_{10}$ and MC-RR$^{-15}N_{13}$ as surrogates, reducing the analysis time. Furthermore, as for AT-a, we developed a

method by injecting samples directly into LC/MS/MS, without solid-phase extraction. Figure 1 describes the examination procedure, and Table 1 describes the conditions for the analysis of LC/MS/MS.

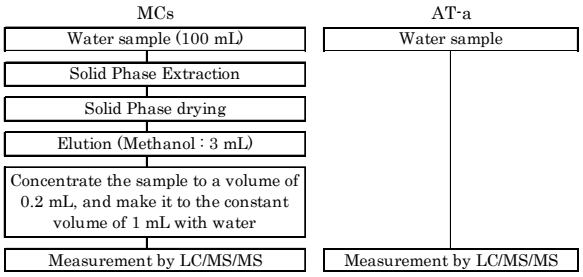

Figure 1 Analysis procedure of MCs and of AT-a

Table 1 Conditions for the analysis by LC/MS/MS

	MCs	AT-a
System	LC/MS/MS	LC/MS/MS
Instrument	AQUITY/TQD (Waters)	AQUITY/TQD (Waters)
Column	Waters Acquity HSS T3 1.8 μm, 100 mm ×2.1 mm (Waters)	Waters Acquity HSS T3 1.8 μm, 100 mm ×2.1 mm (Waters)
Mobile phase	A = Acetonitrile	A = 0.1% formic acid in acetonitrile
	B = 0.1% formic acid in 10 mM ammonium formate aq.	B = 0.1% formic acid aq.
	B : 90%(0min)→90%(0.5min)→10%(5min) →10%(7min)→90%(7min)	B : 95%(0min)→95%(1min)→50%(6min) →30%(8min)→95%(8min)
Flow rate	0.2mL/min	0.2mL/min
Injection volume	50μL	50μL
Ionization	Electro Spray Ionization (+)	Electro Spray Ionization (+)
Monitoring	Multiple Reaction monitoring (MRM)	Multiple Reaction monitoring (MRM)
Analysis time	10 min	12 min

2.2 Removal Performance of MCs and AT-a using Powdered Activated Carbon and Sodium Hypochlorite

We conducted jar tests according to the conditions shown in Table 2 in order to take measures to tackle cyanotoxins in purification plants. We conducted a survey on the removal effectiveness of MCs and AT-a by powdered activated carbon and sodium hypochlorite.

Table 2 Conditions for the jar tests

Experiment No	Water used	The addition amount of MCs and AT-a (μg/L)	Dose rate	Conditions for agitation and leaving to stand
1	Raw water in Ozaku purification plant	MCs: 1 AT-a: 1	Powdered activated carbon (0-100mg/L) Polyaluminum chloride (25mg/L)	Agitation by high-speed mixer (120rpm) : 2minutes Agitation by low-speed mixer (60rpm) : 10minutes Leaving to stand : 10minutes
2	Ultrapure water	MCs: 1、AT-a: 10	Sodium hypochlorite (3mg/L)	Contact time : 60 minutes
3	Raw water in Ozaku purification plant	MCs: 1 (AT-a : Not examined)	Sodium hypochlorite (3mg/L)	Contact time : 60 minutes

2.3 Field Survey on MCs and AT-a

As for Ogouchi Reservoir, our main storage reservoir, we analyzed MCs and AT-a in the blue-green algae blooms around the inflow area at the reservoir (Photo 1). We analyzed the samples that were filtered by a glass filter (dissolved amount) as well as the samples that were disintegrated by ultrasonic waves and then filtered (total amount). We also analyzed the MCs and AT-a in the effluent from reservoirs and the water resources rivers (at 36 points), as well as in the raw water and purified water in our purification plants (9 plants). Figure 2 describes the survey locations.

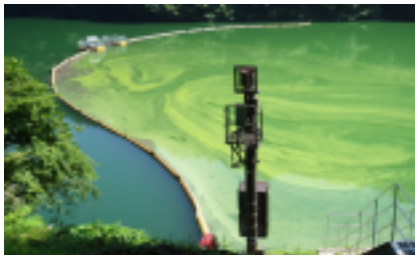

Photo 1 Blue-green algae blooms (The fence prevents blue-green algae blooms from spreading towards the center of the lake)

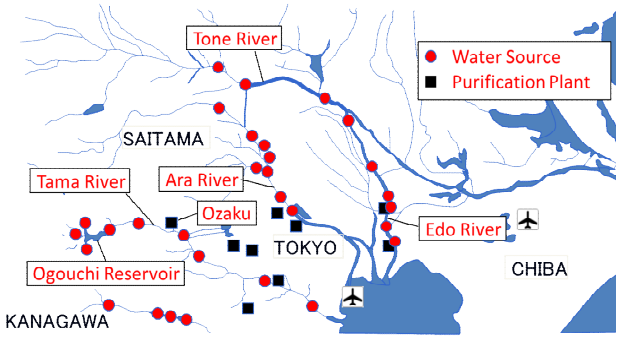

Figure 2 Locations of field survey

3. Results and Discussion

3.1 Development of Analysis Methods of MCs and AT-a

As a result of this study, we developed methods to measure MCs and AT-a using LC/MS/MS. We managed to ensure the values, 0.08μg/L and 0.01μg/L respectively, for the limit of quantitation of MCs and AT-a. Table 3 outlines the developed analysis methods.

Table 3 Outline of the developed analysis methods

Analyte	LOQ (μg/L)	Range of the calibration curve (μg/L)	R^2	RSD (%)	Recovery rate (%)		
					Ultrapure water	River water	Drinking water
MC-LR	0.08	0.08 ～ 1	0.9999	5.2	101	100	107
MC-YR	0.08	0.08 ～ 1	0.9999	3.5	103	109	105
MC-RR	0.08	0.08 ～ 1	0.9997	5.1	91	87	88
At-a (fumarate)	0.01	0.01 ～ 1	1.0000	15.3	100	87	89

3.2 Removal Performance of MCs and AT-a using Powdered Activated Carbon and Sodium Hypochlorite

The results of jar tests show, 40～60% of MCs and AT-a were removed (dose rate : 20mg/L), and 90～100% of MCs and AT-a were removed (dose rate : 100mg/L) by treatments using powdered activated carbon (Figure 3). On the other hand, MCs were removed almost completely by treatments using sodium hypochlorite (dose rate : 3mg/L) in approximately 30 minutes

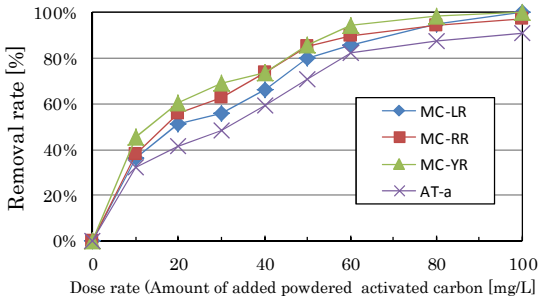

Figure 3 Removal of MCs and AT-a by powdered activated carbon (Experiment No. 1)

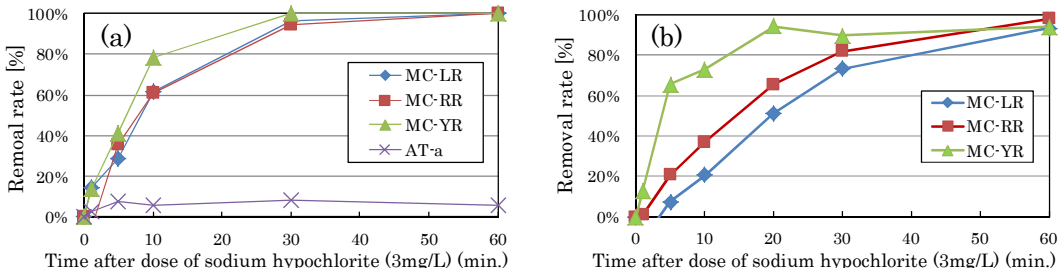

Figure 4 (a) Removal of MCs and AT-a by sodium hypochlorite (Add MCs and AT-a to ultrapure water (Experiment No. 2) ; (b) Add MCs to raw water in Ozaku purification plant (Experiment No. 3))

when using ultrapure water added with MCs (Figure 4 (a)), and in approximately 60 minute when using the raw water from Ozaku Purification Plant added with MCs (Figure 4 (b)). However, approximately 5% of AT-a was removed after 60 minutes even when using ultrapure water added with AT-a (Figure 4 (a)).

These results explain that when cyanotoxins are detected in raw water, MCs can be treated by powdered activated carbon and sodium hypochlorite, whereas AT-a can be treated by powdered activated carbon.

3.3 Field Survey on MCs and AT-a

Upon our field survey, MCs and AT-a were not detected in the effluent from reservoir and the water resources rivers, as well as in the raw water and purified water in purification plants. However, in blue-green algae blooms around the inflow area of reservoir, MC-LR, MC-RR and MC-YR were detected, and the total amount of MC-LR was up to 140 μg/L (Table 4).

Table 4 Field survey results

Items	Provisional target value (μg/L)	Measured value (μg/L)					
		Sampled water in July 2016			Sampled water in May 2015		
		Water from storage reservoir which has blue-green algae blooms		Effluent from reservoirs	Water resources rivers (at 36 points)	Raw Water from purification plants (9 plants)	Purified Water from purification plants (9 plants)
		Dissolved amount	Total amount				
MC-LR	0.8	0.37	140	N.D.	N.D.	N.D.	N.D.
MC-RR	-	0.17	240	N.D.	N.D.	N.D.	N.D.
MC-YR	-	N.D.	16	N.D.	N.D.	N.D.	N.D.
AT-a	-	N.D.	N.D.	-	N.D.	N.D.	N.D.

4 Conclusion

We managed to establish rapid analysis methods for MCs and AT-a, types of cyanotoxin, using LC/MS/MS. As we conducted a survey on the effectiveness of the treatments for MCs and AT-a, we found that MCs could be removed by powdered activated carbon and sodium hypochlorite. AT-a was not removed by a treatment using sodium hypochlorite, however, it could be removed by powdered activated carbon. Furthermore, upon our field survey, we detected microcystin-LR, YR, RR in blue-green algae blooms in the inflow area of the storage reservoir. However, we did not detect cyanotoxins in the effluent from reservoir and the water resources rivers, as well as in the raw water and purified water in purification plants.

We have been conducting regular examinations of MC-LR in raw water and purified water in purification plants, which are positioned as Water Quality Examination Project since FY 2016. In addition , in order to enhance our knowledge on cyanotoxins, we have been conducting field surveys continuously on MCs and AT-a in water resources rivers, and others.

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