

National Institute for Environmental Studies

Certificate of Analysis

Certified Reference Material No. 26

Water Bloom

This environmental certified reference material (CRM) was developed for use in calibrating and managing the precision of analytical equipment used for the chemical analysis of microcystins contained in blue-green algae, and for the analysis of the elemental composition of the algae. The target chemical substances are the microcystins and the constituent inorganic elements found in *Microcystis aeruginosa*, the main cyanobacterium involved in the formation of blue-green algal blooms in freshwater.

Preparation of the CRM

The starting material for this CRM was a mixture of two strains of the cyanobacterium *Microcystis aeruginosa* that were isolated from natural water blooms. The strains were separately cultured at the algae culture management facility within the National Institute for Environmental Studies (NIES), then freeze-dried. The mixed dry alga (ca. 40 g), powdered by sieving through a 63- μ m screen, was packed into amber glass bottles (636 bottles) with individual sample sizes of 54 mg. After vacuum drying the bottles were filled with an inert gas (argon), capped, and sealed in aluminum packs containing desiccants.

Homogeneity

Homogeneity tests were carried out on 5 sample bottles selected at random. The major elements (Ca, Fe, K, Mg, Mn, and Na) in the samples were determined by plasma emission spectrometry after acid digestion. Microcystins were extracted with solvent and subjected to oxidative decomposition. The MMPB (3-methoxy-2-methyl-4-phenylbutyric acid) thereby formed was measured by HPLC–mass spectrometry¹⁻³⁾ using the method cited in the legend to Table 1-1. Analysis of variance of the analytical results showed the relative standard deviation of all samples was within 4%, and the variation between bottles was within the range of uncertainty for the certified values. It was adjudged that the material was sufficiently homogenous for its intended purpose.

Certified and Reference Values

Of the measured values reported by 14 organizations, those with z-scores of 2 or more, obtained by use of robust statistics, were rejected. Certified values for microcystins, Ca, Fe, K, Mg, Mn, Na, Sr, and Zn were decided in accordance with ISO Guide 35. The uncertainty attached to the certified values is the expanded uncertainty using a coverage factor $k = 2$, corresponding to a confidence interval of approximately 95%. Reference values as additional information were given for S, P, Co, Cu, Ni, and Pb.

Precautions for Storage and Handling for Analysis

1. All certified and reference values refer to the vacuum-dried contents as supplied. Thorough attention must be paid to moisture because the properties of the samples preclude the possibility of drying them with heat.
2. This CRM contains toxic substances. Precautions must therefore be taken to avoid inhalation of, and skin and eye contact with, the sample powder.
3. Precautions must be taken to avoid contamination of the immediate environment when taking a sample.

4. At least 10 mg of sample should be used for analysis.
5. As this CRM absorbs moisture, it is desirable to use up the sample as soon as possible after opening. If storage is necessary, re-seal the bottle containing the remaining sample, and store it in a desiccator at ambient temperature. It is desirable that samples stored for prolonged periods be vacuum-dried before being used for analysis.
6. Do not use for purposes other than research. When disposing of samples, adhere strictly to local laws concerning processing and disposal of waste materials.

Expiration Date of Certification

The expiration date for the certified values of this CRM is August 2022, assuming that above mentioned storage conditions are adhered to. NIES will announce via its website (www.nies.go.jp) if any changes in the contents are noticed within the term of validity.

Collaborating Laboratories in Analysis

The certified values and reference values for this CRM were based on analytical values from the following participating organizations.

National Institute for Environmental Studies; Tohoku University Graduate School; Nara Prefectural Institute for Hygiene and Environment; Institute for Environmental Sciences; Tohoku Nuclear Services Co., Ltd.; Environmental Control Center Co., Ltd.; Environmental Research Center Co., Ltd.; Shimadzu Techno-Research, Inc.; Sumika Chemical Analysis Service, Ltd.; Chikyu Kagaku Kenkyusho Co., Ltd.; Naitoh Environmental Science Co., Ltd.; Japan Chemical Analysis Center; China National Research Center for Environmental Analysis and Measurements; and China Institute of Atomic Energy.

References

- 1) T. Sano, K. Nohara, F. Shiraishi, and K. Kaya, (1992). Intern. J. Environ. Anal. Chem. 49, 163-170.
- 2) K. Kaya and T. Sano, (1999). Anal. Chim. Acta 386, 107-112.
- 3) H. Takagi, M. Shirai, T. Sano, and K. Kaya, (2004). J. Environ. Chem. 14, 587-596 (in Japanese).

Organization Responsible for Preparation, Certification, and Distribution

The Environmental Analytical Chemistry Research Laboratory within the Laboratory of Intellectual Fundamentals for Environmental Studies, NIES, takes entire responsibility for this CRM. Research reports and technical information regarding this sample can be obtained from the following address.

Correspondence should be addressed to:

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Table 1-1. Certified values for NIES CRM No. 26 Water Bloom

	Unit	Certified value	Analysis method
Microcystins	mg/g	4.5 ± 0.4	a, b

The microcystins were determined in accordance with the manual for examination of substances requiring investigation by the Ministry of Environment, Japan (March 2003). This method followed those in reports for the determination of total microcystins¹⁻³⁾. As described in the manual, microcystins were oxidatively decomposed to MMPB, which was determined using HPLC-mass spectrometry (a), or gas chromatography-mass spectrometry after esterification (b) .

Table 1-2. Certified values for NIES CRM No. 26 Water Bloom

	Unit	Certified value	Analysis method
Ca	%	0.56 ± 0.02	1, 2, 6, 7
Fe	%	0.086 ± 0.006	2, 5, 6
K	%	0.90 ± 0.05	1, 2, 6, 7
Mg	%	0.44 ± 0.03	1, 2, 7
Na	%	0.12 ± 0.02	1, 2, 6
Mn	mg/kg	39 ± 3	2, 3, 4, 6
Sr	mg/kg	4.5 ± 0.3	2, 3, 5
Zn	mg/kg	13 ± 2	2, 3, 4, 5, 6

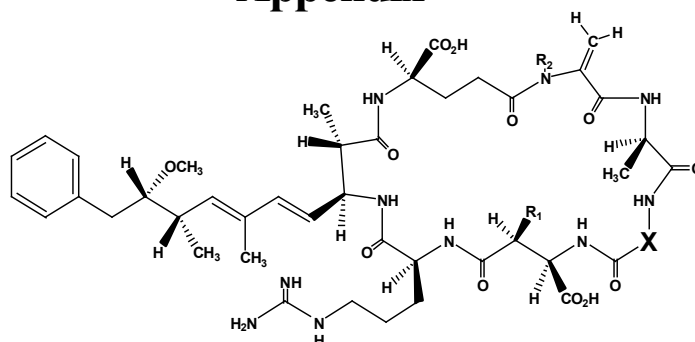
Analysis methods

- 1: Atomic absorption spectroscopy (AAS)
- 2: Inductively coupled plasma atomic emission spectrometry (ICP/AES)
- 3: ICP/mass spectroscopy (ICP/MS)
- 4: High resolution/ICP/MS (HR/ICP/MS)
- 5: Isotope dilution/ICP/MS (ID/ICP/MS)
- 6: Instrumental neutron activation analysis (INAA)
- 7: X-ray fluorescence (XRF)
- 8: Graphite furnace atomic absorption spectrometry (GFAAS)
- 9: Molybdenum blue flow injection analysis (molybdenum blue-FIA)

Table 2. Reference values for NIES CRM No. 26 Water Bloom

	Unit	Reference value	Analysis method
S	%	0.82	2, 7
P	%	0.89	2, 7, 9
Co	mg/kg	0.75	2, 3, 6
Cu	mg/kg	2.3	2, 3, 5, 6, 7, 8
Ni	mg/kg	2.2	2, 3, 5
Pb	mg/kg	4.3	2, 3, 5

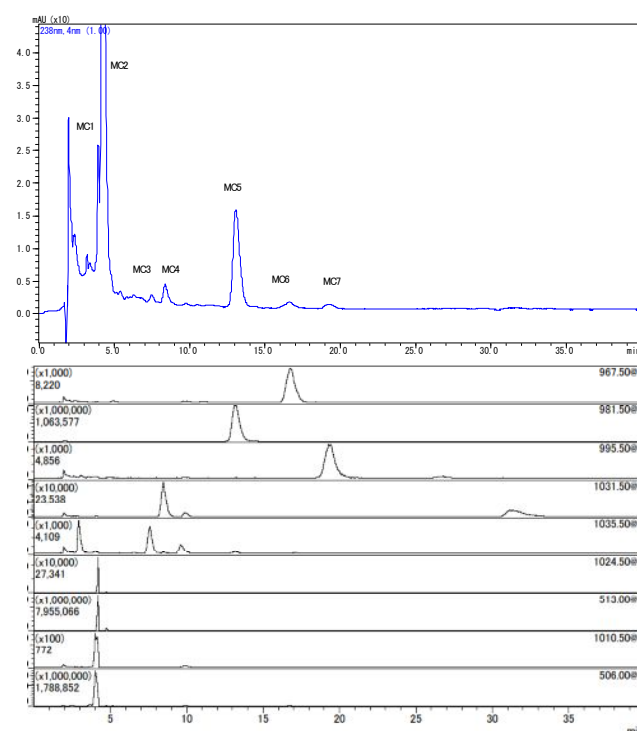
Appendix



MC1: [D-Asp³, Dha⁷]microcystin-RR:
 MC2: [Dha⁷]microcystin-RR:
 MC3: [Dha⁷]microcystin-ThTyr:
 MC4: [Dha⁷]microcystin-YR:
 MC5: [Dha⁷]microcystin-LR:
 MC6: [D-Asp³, Dha⁷]microcystin-LR:
 MC7: [Dha⁷]microcystin-HilR:
 (cf. Microcystin-LR:

R1=R2=H, X=Arg
 R1=Me, R2=H, X=Arg
 R1=Me, R2=H, X=ThTyr
 R1=Me, R2=H, X=Tyr
 R1=Me, R2=H, X=Leu
 R1=R2=H, X=Leu
 R1=Me, R2=H, X=Hil
 R1=R2=Me, X=Leu)

Appendix Fig. 1. Structures of microcystins in NIES CRM No. 26



Appendix Fig. 2. Examples of HPLC/MS chromatograms of microcystins in NIES CRM No. 26

Column: Zorbax XDB Eclipse C-18 2.1 x150 mm, solvent: 55% MeOH in 0.1% formic acid,
 flow rate: 0.2 ml / min, temp.: 40 °C, wave length: 238 nm

Appendix Table 1. Examples of relative retention time ratios of microcystins in NIES CRM No. 26

Column	MC1	MC2	MC3	MC4	MC5	MC6	MC7
Mightysil RP-18	0.164	0.190	0.653	0.751	1.223	1.540	1.803
Super ODS	0.475	0.513	0.720	0.808	1.221	1.591	1.766
Zorbax XDB C18	0.232	0.264	0.605	0.701	1.211	1.573	1.882
SunFire C18	0.022	0.022	0.606	0.710	1.253	1.587	1.933

Relative retention time ratio: retention time of microcystin / retention time of microcystin-LR

Solvent for HPLC was 55% MeOH in 0.1% formic acid.