Aquatic Services

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RE: Analysis Report –Twenty-nine freeze dried AFA samples representing the 2007 harvest season were received for Microcystin analysis by enzyme linked immunosorbent assay (ELISA). Fifteen samples were Alpha product and 14 were Omega. To determine recovery of microcystin in the AFA algae matrix, microcystin-LR was spiked into a negative control sample and the amount present was determined by ELISA. This value was used to determine % recovery.

Extraction. The samples were stored at 4°C prior to analysis. An aliquot (100 mg)*of the dried material was extracted in 0.1 M acetic acid in 100% methanol at a ratio of 1 g/50 mL and sonicated for 5 minutes. The extract was centrifuged. The supernatant was dried, and fractionated by solid phase extraction (C18) using water; 20% methanol and then 100% methanol. The 100 % methanol elution was used for analysis of Microcystins. This fraction was dried and taken up in 1 mL of PBS buffer. The filtrate was stored at -20 C and used as needed for ELISA.
** 100 mg of dried cells were weighed out. If the weight was not 100 mg the extraction volume was adjusted to give 100 mg/5 ml methanol*.

A. ELISA assay for the cyclic peptides microcystin and nodularin. The ELISA method is based upon the original polyclonal antibody method described by Chu *et al.* (1989, 1990) and adapted by An and Carmichael (1994) and Carmichael and An (1999). These methods have been adapted to a commercial ELISA kit (Microcystin Plate Kit, EP-022-kit batch#040307) that is produced by Envirologix, Inc. (Portland Maine). This microcystin (MCYST) Plate Kit is calibrated to measure MCYST concentrations between 1.6 and 0.16 μg/L. Samples containing MCYST in greater or lesser amounts of this range are diluted or concentrated, respectively, to bring them into this range for measurement. This range covers the WHO guideline level for MCYST in finished drinking water supplies (Chorus and Bartram 1999). Twenty microliters (20 μl) of sample, (replicated 2x) were used for the assay, providing a minimum detection level of 5 pg**. Full strength and 1:5 dilution were used to run the assay in triplicate.

Microcystin Results.

∝Sample #	. Mcyst conc. (μg/g)
477	BLD**
478	BLD
479	BLD

480	BLD
482	BDL
483	BLD
484	BDL
485	BDL
486	BDL
487	BDL
488	BDL
489	BDL
490	BDL
491	BDL
Ω Samples	
735	BDL
736	BDL
737	BDL
738	BDL
739	BDL
740	BDL
741	BDL
742	BDL
743	BDL
744	BDL
745	BDL
746	BDL
747	BDL
748	BDL

**BLD=below level of detection (0.005 µg/g dry weight)

MATRIX EFFECT ON MICROCYSTIN DETECTION

Detection of microcystin in the *Aphanizomenon flos-aquae* cell extract matrix was tested by spiking extracted ∝491 batch. Microcystin-LR reference standard was diluted with phosphate buffered saline (PBS) to working concentrations of 100 µg/ml. Dilutions were

made at .001, .01, 10 and 100 μg/ml into PBS. All dilutions were run in duplicate on the same ELISA plate as the batch samples.

Results:

Concentration (MCYST-LR)	amount determined ug/g	% recovery
.001	BDL	not determined
.01	.0095	95%
10	10.05	105%
100	95.0	95%

References:

An, J-S. and Carmichael, W.W. (1994). Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon **32**: 1495-1507.

Carmichael, W.W. and An, J-S. (1999) Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. Natural Toxins. **7**: 377-385.

Chu, F.S., Huang, X., and Wei, R.O. (1990). Enzyme-linked immunosorbent assay for microcystins in blue-green algal blooms. J. Assoc. Off. Analyt. Chem. **73**: 451-456.

Chu, F.S., Huang, X., Wei, R.O., and Carmichael, W.W. (1989). Production and characterization of antibodies against microcystins. Appl. Environ. Microbiol. **55**: 1928-1933.

Summary: Twenty-nine samples were received from Simplexity Inc. representing their 2007 harvest season. Samples were tested for Microcystins by an immunoassay (ELISA). All samples were below the level of detection: < 0.005 ug/g. All samples were well below the Oregon Department of Agriculture regulatory value of 1.0 µg/g.

A recovery experiment to determine algae matrix effects was done. All samples had good % recovery-ranging from 95-105%.

Further details on these guideline determinations and other questions of treatment and management of toxic cyanobacteria can be found in:
Chorus, I. and Bartram, J. (eds.) Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. World Health Organization, E&FN Spon, Routledge, London, 1999.

Signed

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