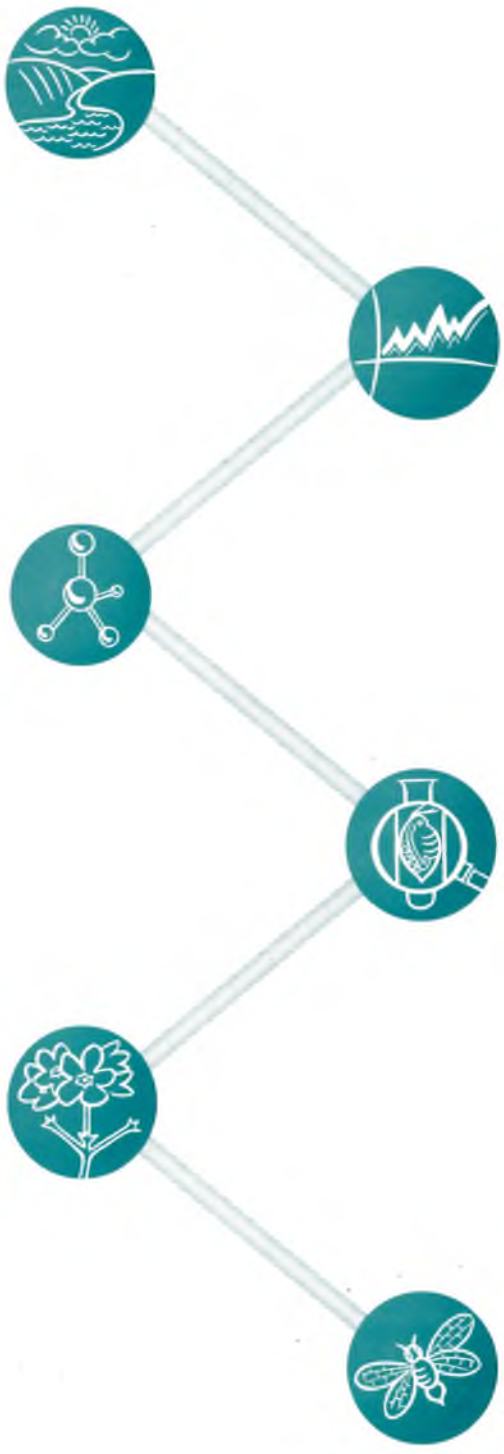
SUMMARY REPORT ON BLUE-GREEN ALGAE TO ASSESSMENT AND REPORTING TO THE ENVI



ENVIRONMENT AGENCY

SUMMARY REPORT ON BLUE-GREEN ALGAE TOXIN ANALYSIS, TOXICITY ASSESSMENT AND REPORTING TO THE ENVIRONMENT AGENCY, 1998

G.A. CODD

UNIVERSITY OF DUNDEE

MAY 1999

CONTENTS

1. INTRODUCTION

A Technical Service has been provided by the Department of Biological Sciences, University of Dundee for the Environment Agency since 1995, to examine the toxicity of environmental samples containing blue-green algae (cyanobacteria). The establishment of this service followed an annual series of investigations performed by the University of Dundee, from September 1989, until 1995 for the National Rivers Authority into blue-green algal bloom toxicity and associated problems. The 1998 data therefore complete a decade of such investigations by this laboratory, with NRA, and now Environment Agency colleagues throughout England and Wales. The activities continue to include:

- the application, as appropriate, of toxin analysis and toxicity assessment methods for the characterisation of environmental samples of water, cyanobacterial blooms, scums and mats, and of associated material

- reporting of results to the originators of the samples throughout the Regions, initially by telephone or fax, followed by a written report, plus copy to the National Centre for Ecotoxicology and Hazardous Substances, Evenlode House, Wallingford

- provision of comments and recommendations arising from the findings, where appropriate, with the telephone/fax and written reports

- recording of novel developments and findings in the recognition, characteristics and significance of cyanobacterial ecotoxicology

- preservation and storage of environmental samples of cyanobacterial blooms, scums and mats for future reference and research.

This report is structured in the same style as that of the preceding 3-year Summary Report (1995-1997; Codd, 1998) to enable comparisons to be made since the beginning of the present Technical Service agreement.

2. SUMMARY OF ACTIVITIES AND FINDINGS

Samples from cyanobacterial blooms, scums and mats have again been received from a wide range of waterbodies throughout the Regions including freshwater lakes, reservoirs, ponds and canals. The samples have continued to be received on a largely reactive "one-off" basis. One sample was received from one of the previously designated Blue-Green Algal Long-Term Monitoring Sites (Weirwood Reservoir). Some of the samples were from sites at which fish- and bird-kills had occurred.

The number of samples received in 1998 for which cyanobacterial toxin analysis/toxicity assessment was carried out was 32. Three samples were received which did not merit toxin analysis. Table 1 lists the results obtained for the samples examined. Samples included cyanobacterial blooms, scums and mats plus sediments containing cyanobacteria, with bloom and scum samples being the most common (Table 2). This pattern was similar to that which emerged in 1995-1997.

Table 2 also summarises the cyanobacterial genera present in the samples. *Microcystis* species were the most frequently encountered (in 34% of the samples). An *Arthrospira* bloom sample was received for the first time.

The results of toxin analysis and toxicity assessment are summarised in Table 3. Sixty two per cent of the samples examined by bioassay were toxic, the highest incidence according to this criterion since the start of the current service in 1995. The percentage of the toxic samples which were hepatotoxic in 1998 was 52%, also the highest value since 1995. Fifty per cent of the samples analysed for microcystins by high-performance liquid chromatography were positive for the toxins.

As in previous years, we did not detect the neurotoxin anatoxin-a in 1998. However, as in 1995, neurotoxic saxitoxin(s) was/were present in 1998. (It is not known whether the saxitoxin-containing samples contained one or more of this family over 20 neurotoxins, since the new neo-saxitoxin antibodies which we are using do not permit the individual identification of toxin variants.)

As in previous years, positive findings of microcystins in the samples were shown to be accounted for by a range of microcystin variants, up to 4 being found in 1998 (Table 4).

The concentrations of microcystins detected in positive samples are also listed in Table 4. In the case of microcystin-positive bloom samples, these included samples above (numbers 5, 18, 19) and below (numbers. 3, 30) the provisional World Health Organization Guideline Value for microcystins in drinking water of 1 microgram microcystin-LR equivalents per litre.

Concentrations of microcystins in positive scum samples include some very high values in 1998. In particular, sample 8 contained 3,310 micrograms, and sample 16 contained 33,000 micrograms microcystin-LR equivalents per gram dry weight of scum, respectively. The microcystin concentration in sample 16 (Farmoor) was higher than the highest microcystin concentrations previously published in the world, namely in Japan (19,500 micrograms; Nagata *et al.*, 1997) and in Germany (25,000 micrograms; Chorus *et al.*, 1998). Whether the high toxin concentration in sample 18 was typical of the scum present at Farmoor and the persistence of this microcystin concentration are not known. However, this highly toxic sample should bring into operation management plans and preventative measures in a structured, alert levels framework system for the control of toxic cyanobacterial blooms problems. Consistent with the high variability in cyanobacterial toxin concentrations per unit of scum or bloom, it is noted that in contrast to the highly toxic Farmoor samples of 1998 (numbers 18, 23) the Farmoor scum sample received in 1997 (number 11) was non-toxic (Codd, 1998).

We have not sought to identify novel microcystins in the 1998 samples so far, although large stocks of samples 18 and 23 have been lyophilized for future investigation (see later). The two new dehydrobutyrine-containing microcystins from an *Oscillatoria agardhii* bloom associated with cattle deaths in Scotland have been identified as [D-Asp^3, (Z)-Dhb^7]microcystin-HtyR and [D-Asp^3, (Z)-Dhb^7]microcystin-LR, where Asp is aspartate, Hty is homotyrosine, R is arginine and L is leucine (Sano *et al.*, 1998).

3. SCHEDULES OF SAMPLE COLLECTION, RECEIPT, ANALYSIS AND REPORTING, 1998

Our understanding from the beginning of the Technical Service agreement remains that the provision of the results of analysis is not required urgently. However, in practice, the findings in 1998, as in 1995-1997, were often sought/required as soon as possible after receipt. We have continued to endeavour to provide initial information by telephone or fax with minimal delay. Table 5 lists the dates of sample collection by EA laboratories, arrival dates in Dundee, dates of initial telephoned/faxed information on results and the dates of the final written reports. The high level of rapid response with initial results being supplied on the day of sample receipt or on the following day is apparent in Table 6.

4. PURIFIED CYANOBACTERIAL TOXINS

Cyanobacterial hepatotoxins and neurotoxins, as listed in Table 13 of Codd (1998) continue to be used and replaced by in-house purification from laboratory cultures of cyanobacteria.

5. ARCHIVE OF STORED ENVIRONMENTAL SAMPLES

The material remaining after the analysis of 1998 samples was lyophilized before storage at -20°C. Stored samples, which are being used for research purposes are listed in Table 7.

6. COMMENTS AND RECOMMENDATIONS

Procedures for the supply, packaging and despatch of samples in 1998 were generally satisfactory. Only 3 samples received did not merit toxicity assessment or toxin analysis in our opinion, indicating a continuing development in the Regions of the ability to recognise cyanobacterial materials which merit hazard characterization. Samples arriving in Dundee *via* the various courier services were generally received in good order as "next morning" deliveries.

No additional recommendations, or changes to the detailed recommendations for the collection and processing of samples from waterbodies, as given in the previous report (Codd, 1998), are deemed necessary from the 1998 programme.

The findings at Long Hall Nurseries, Longton, Preston (samples 8, 9) were novel in the work performed by this laboratory for the EA. Sample 8, which included *Microcystis* scum and microcystins was from water used for the spray irrigation of a crop of salad lettuce which had been intended for sale. Sample 9, in which *Microcystis* colonies and microcystin toxins were present was a lettuce plant from the exposed crop. The possibility of the accumulation of cyanobacterial toxins on or in plant material, *via* spray irrigation from sources containing cyanobacterial blooms and toxins was raised earlier by this laboratory, based on laboratory and greenhouse studies (Abe *et al.*, 1996). This scenario has been discussed with EA colleagues and presents a route for the potential exposure of humans and animals to the toxins *via* the consumption of spray-irrigated plant food. The Long Hall Nurseries samples in 1998 represent the first case in our experience in the UK of the transfer of cyanobacterial cells and toxins to plant material *via* spray irrigation in the commercial sector. It is recommended that this experience is considered by relevant EA colleagues to determine whether current policies on the management of water abstraction for spray irrigation are sufficient.

7. REFERENCES

Abe, T., Lawson, T., Weyers, J.D.B. and Codd, G.A. Microcystin-LR inhibits photosynthesis of *Phaseolus vulgaris* primary leaves: implications for current spray irrigation practice. *New Phytol.* **133**, 651-658 (1996).

Chorus, I. Die Blaualgen "Cyanobakterien" – eine Berliner Besonderheit welcher Zusammenhang besteht zwischen dem Massenhaften Auftreten von Blaualgen und der Nutzung von Badegewässern? In: Nachhaltigeit im Wasserwesen in der Mitte Europas. Symposium 17-19 June, 1998, Berlin (in the press).

Codd, G.A. Summary Report on Blue-Green Algae Toxicity Testing, Assessment and Reporting to the Environment Agency, 1995-1996. University of Dundee, pp. 6 with 14 tables (1998).

Nagata, S., Tsutsumi, T., Hasegawa, A., Yoshida, F., Ueno, Y. and Watanabe, M.F. Enzyme immunoassay for direct determination of microcystins in environmental water. *J. AOAC International* **80**, 408-417 (1997).

Sano, T., Beattie, K.A., Codd, G.A., and Kaya, T. Two (*Z*)-dehydrobutyrine-containing microcystins from a hepatotoxic bloom of *Oscillatoria agardhii* from Soulseat Loch, Scotland. *J. Nat. Products* **61**, 851-853 (1998).

8. ACKNOWLEDGEMENTS

I thank Kenneth A. Beattie and Samantha L. Raggett for their continuing conscientious and thoughtful technical assistance, and Dr Steve G. Bell and James S. Metcalf for their valuable contributions.

Table 1 : Summary of Samples Received and Results of Analysis, 1998

Sample no.	Date of receipt	Sample source	Corresponding lab/office	Sample description	Toxicity assessment/Toxin analysis	
					Bioassay	Analysis
1	7/4/98	Lincoln Pool, Standlake	Isis House, Wallingford	Water from fish-kill	Non-toxic	ND
2	11/3/98	Poole Park Lake	Blandford Forum, Dorset	Sediment	ND	Positive for microcystin (1 toxin variant) (HPLC-DAD); negative for anatoxin-a (HPLC-DAD)
3	30/4/98	Bridge Farm Reservoir	Bodmin	Bloom, *Oscillatoria*	ND	Negative for anatoxin-a (HPLC-DAD); positive for microcystins (HPLC-DAD)
4	20/5/98	Llyn Tegid	Bangor	Bloom, *Oscillatoria*	Hepatotoxic (lethal)	ND
5	22/5/98	Cinnamon Pond – Taswood Lakes	Kelvedon	Bloom, scum, *Aphanizomenon*	ND	Positive for microcystins in filtered cells (HPLC-DAD)

to be continued

Table 1. Summary of Samples Received and Results of Analysis, 1998 (continued)

Sample no.	Date of receipt	Sample source	Corresponding lab/office	Sample description	Toxicity assessment/Toxin analysis	
					Bioassay	Analysis
6	19/6/98	Harrow Lodge Park Lake	Hatfield	Mat, *Oscillatorial/ Phormidium*	Neurotoxic and hepatotoxic (lethal)	ND
7	11/7/98	Combwich Pond	Bridgwater	Bloom, scum, *Aphanizomenon*	Non-toxic	ND
8	22/7/98	Hall Lane Nurseries, Longton, Preston	Preston	Scum, bloom, *Microcystis*	Hepatotoxic (lethal)	Positive for microcystins (HPLC-DAD)
9	24/7/98	Hall Lane Nurseries, Preston	Preston	Lettuce plant, *Microcystis*	ND	Positive for microcystins (HPLC-DAD)
10	28/7/98	Knypersely Reservoir	Lichfield	Scum, *Microcystis*	Hepatotoxic (lethal)	ND
11	28/7/98	Stanley Pool	Lichfield	Scum, *Aphanizomenon*	ND	Positive for saxitoxins (ELISA)
12	28/7/98	Rutland Water	Lincoln	Bloom, scum, *Aphanizomenon*	ND	Negative for saxitoxins (ELISA)
13	30/7/98	Hinstock, Market Drayton	Shrewsbury	Scum, *Aphanizomenon*	Non-toxic	Positive for saxitoxins (ELISA)

to be continued

Table 1. Summary of Samples Received and Results of Analysis, 1998 (continued)

Sample no.	Date of receipt	Sample source	Corresponding lab/office	Sample description	Toxicity assessment/Toxin analysis	
					Bioassay	Analysis
14	30/7/98	Nursery Lane, Hoddesdon	Brampton	Bloom, *Anabaena*, *Aphanizomenon*	ND	Negative for microcystins (HPLC-DAD)
15	13/8/98	Doddington Pool	Sale	Bloom, *Aphanizomenon*	ND	Negative for saxitoxins (ELISA)
16	13/8/98	Farmoor 1 Reservoir	Reading	Scum	ND	Positive for microcystins (ELISA)
17	18/8/98	Hilsea Moat, Portsmouth	Waterlooville	Mat, bloom, *Oscillatoria*, *Microcystis*, *Arthrospira*	Hepatotoxic (sub-acute)	Negative for microcystins (HPLC-DAD)
18	20/8/98	Weirwood Reservoir	Addington	Bloom, scum, *Microcystis*, *Oscillatoria*	Hepatotoxic (lethal)	Positive for microcystins (HPLC-DAD)
19	26/8/98	Baffins Pond, Copnor	Waterlooville	Bloom, *Microcystis*, *Anabaena*	ND	Positive for microcystins (HPLC-DAD)
20	26/8/98	Chiltern Trinity Lake	Bridgwater	Bloom, *Anabaena*	Non-toxic	Negative for microcystins (HPLC-DAD); positive for microcystins (ELISA)

to be continued

Table 1. Summary of Samples Received and Results of Analysis, 1998 (continued)

Sample no.	Date of receipt	Sample source	Corresponding lab/office	Sample description	Toxicity assessment/Toxin analysis	
					Bioassay	Analysis
21	28/8/98	Upper Tamar Lake	Bodmin	Scum, *Microcystis*, *Anabaena*, *Oscillatoria*	Non-toxic	Negative for microcystins (HPLC-DAD)
22	28/8/98	Kennet and Avon Canal (All Cannings Bridge0	Blandford Forum	Sediment, *Oscillatoria*, *Microcystis*	ND	Negative for microcystins (HPLC-DAD)
23	2/9/98	Farmoor 1 Reservoir	Reading	Scum, *Microcystis*	Hepatotoxic (lethal)	Positive for microcystins (HPLC-DAD)
24	4/9/98	Bosmere Lake	Kelvedon	Bloom, *Microcystis*	ND	Negative for microcystins (HPLC-DAD)
25	4/9/98	Springfield Lake	Preston	Scum, *Oscillatoria*, *Anabaena*, *Gomphosphaeria*	Hepatotoxic (sub-acute)	Negative for microcystins (HPLC-DAD)
26	8/9/98	Springfield Lake	Preston	Scum, *Gomphosphaeria*, *Anabaena*	Non-toxic	Negative for microcystins (HPLC-DAD
27	22/9/98	Loe Pool	Bodmin	Scum, *Microcystis*	Hepatotoxic (lethal)	ND

to be continued

Table 1. Summary of Samples Received and Results of Analysis, 1998 (continued)

Sample no.	Date of receipt	Sample source	Corresponding lab/office	Sample description	Toxicity assessment/Toxin analysis	
					Bioassay	Analysis
28	22/9/98	Leeds-Liverpool Canal, Barrowford	Preston	Bloom, scum, *Anabaena*, *Gomphosphaeria*	Hepatotoxic (lethal)	ND
29	29/9/98	Longbridge, Lancaster	Preston	Scum *Microcystis*	Hepatotoxic (sub-lethal)	ND
30	30/9/98	Rutland Water	Spalding	Bloom, scum, *Microcystis*, *Aphanizomenon*	Hepatotoxic (sub-lethal)	Positive for microcystins (HPLC-DAD)
31	1/10/98	Tamar Lakes (Upper)	Bodmin	Scum, *Microcystis*, *Aphanizomenon*, *Anabaena*	Non-toxic	Negative for microcystins (HPLC-DAD)
32	16/10/98	Sunnyhill Farm Pond, Monobier	Haverfordwest	Scum, bloom, *Microcystis*	Toxic (sub-lethal)	Negative for microcystins (HPLC-DAD)

ND, not determined; HPLC-DAD, high-performance liquid chromatography with diode array detection; ELISA, enzyme-linked immunosorbent assay; PPASE, protein phosphatase-inhibition assay.

Table 2. Classification of Samples Received in 1998 According to Types of Cyanobacterial Populations and Dominant Genera

Bloom	9
Scum with bloom	7
Semisolid scum	10
Mat/sediment	4
Others	4
Microcystis	15
Anabaena	8
Aphanizomenon	8
Oscillatoria (planktonic)	8
Oscillatoria/Phormidium (benthic)	1
Arthrospira	1
Gomphosphaeria	3

Table 3. Summary of Toxicity Tests and Toxin Analyses Performed and Results, 1998

Procedure	No. samples tested	No. samples positive	% samples positive
Bioassay	21	13	62
		(11, hepatotoxic)	52
		(2, neurotoxic)	10
		(1, hepato- and neurotoxic)	5
		(1 other)	5
Microcystins (HPLC-DAD)	20	10	50
Microcystins (ELISA)	1	1	
Anatoxin-a (HPLC-DAD)	2	0	
Saxitoxins (ELISA)	4	2	

Table 4. Summary of Analytical Findings of Microcystins (MC), 1998

Sample no.	Analytical method	No. of MC variants	Total MC pool (MC-LR equivalents)
2	HPLC	1	14 micrograms per kg sediment
3	ELISA	a	23 nanograms per l itre
5	HPLC	3	5.51 micrograms per litre
8	HPLC	2	3.31 micrograms per mg dry wt of scum
16	ELISA	a	1.27 micrograms per ml scum, and 33 micrograms per mg dry wt of scum
18	HPLC	2	22.85 micrograms per litre
19	HPLC	2	1.12 micrograms per litre
23	HPLC	4	16.34 micrograms per litre
30	HPLC	2	0.45 micrograms per mg dry wt of scum

a, number of toxin variants not indicated by ELISA.

Table 5. Sample Collection, Receipt and Reporting Schedules, 1998

Sample no.	Date of collection of sample by EA	Date of arrival at Dundee	Date of reporting by telephone/fax	Date of written report
1	6/4/98	7/4/98	15/4/98	15/4/98
2	9/2/98	10/2/98	11/3/98	14/4/98
3	29/4/98(?)	30/4/98	6/5/98	17/8/98
4	18/5/98	20/5/98	21/5/98	9/6/98
5	21/5/98	22/5/98	22/5/98, 27/5/98 and 9/6/98	10/6/98
6	18/6/98	19/6/98	22/6/98	18/8/98
7	9/7/98	11/7/98	13/7/98	20/7/98
8	21/7/98(?)	22/7/98	23/7/98, 24/7/98	28/7/98
9	?	24/7/98	24/7/98	28/7/98
10	27/7/98	28/7/98	29/7/98, 30/7/98	30/7/98
11	27/7/98	28/7/98	29/7/98, 30/7/98	30/7/98
12	27/7/98	28/7/98	30/7/98	30/7/98
13	29/7/98	30/7/98	30/7/98	30/7/98
14	28/7/98	30/7/98	4/8/98	4/8/98
15	12/8/98	13/8/98	14/8/98	14/8/98
16	12/8/98	13/8/98	14/8/98	15/8/98
17	17/8/98	18/8/98	18/8/98	21/8/98
18	19/8//98	20/8/98	20/8/98, 21/8/98	21/8/98
19	24/8/98	26/8/98	27/8/98	29/8/98
20	25/8/98	26/8/98	27/8/98	29/8/98
21	26/8/98	28/8/98	1/9/98	1/9/98
22	25/8/98	28/8/98	3/9/98	3/9/98
23	2/9/98	4/9/98	4/9/98	7/9/98
24	2/9//98	4/9/98	7/9/98	7/9/98
25	30/8/98	4/9/98	4/9/98, 7/9/98	7/9/98
26	7/9/98	8/9/98	11/9/98	11/9/98
27	18/9/98	22/9/98	22/9/98	23/9/98
28	21/9/98	22/9/98	22/9/98	23/9/98
29	28/9/98	29/9/98	1/10/98	10/10/98
30	29/9/98	30/9/98	1/10/98	10/10/98
31	29/9/98	1/10/98	2/10/98	12/10/98
32	13/10/09	16/10/98	16/10/98, 19/10/98, 21/10/98, 20/10/98	21/10/98

Table 6. Response Time for Initial Provision of Results by Telephone/Fax, 1998

Days from sample arrival	Number of samples
Same day	11
1	11
2	1
3	4
5	2
6	1
8[a]	1
29[b]	1
TOTAL	32

a, *Prymnesium parvum* bloom (sample 1).
b, Mineral matter sediment (sample 2).

Table 7. List of Archived Lyophilized Environmental Samples Remaining, 1998

Sample no.	Sample source	Grams dry weight
4	Llyn Tegid	11.50
6	Harrow Lodge Park Lake	17.00
9	Hall Lane Nurseries	1.80
10	Knypersley	7.20
11	Stanley Pool	2.40
12	Rutland Water	2.40
16	Farmoor 1 Reservoir	19.00
20	Chiltern Trinity Lake	0.25
21	Upper Tamar Lake	0.65
23	Farmoor 1 Reservoir	52.00
25	Springfield Lake	4.50
26	Springfield Lake	1.50
28	Leeds-Liverpool Canal	0.30
30	Rutland Water	2.30
31	Upper Tamar Lake	0.40

See Table 1 for summary of dominant cyanobacterial genera.