AUSTRALIAN WATER QUALITY CENTRE

THE EFFECT OF "CUPRICIDE 110" AND "KUPRAMINE" ON THE CYANOBACTERIUM *Anabaena circinalis*

AUSTRALIAN WATER QUALITY CENTRE

THE EFFECT OF "CUPRICIDE 110®" AND "KUPRAMINE®" ON THE CYANOBACTERIUM *Anabaena circinalis*

Project Officers: Peter Hobson
Renate Velzeboer

Project Manager: **Michael D Burch, Senior Research Biologist**
Tel: (08) 8259 0352
E-mail: mike.burch@sawater.sa.gov.au

Australian Water Quality Centre
Private Mail Bag 3
SALISBURY SA 5108

This report contains an assessment of the effectiveness of CUPRICIDE 110® and KUPRAMINE® against the cyanobacterium *Anabaena circinalis.* The report is in fulfilment for the engagement of the Australian Water Quality Centre by AGMIN CHELATES Pty. Ltd. for this investigation.

THE EFFECT OF "CUPRICIDE 110®" AND "KUPRAMINE®" ON THE CYANOBACTERIUM *Anabaena circinalis*

FEBRUARY 2000

PREPARED BY

AUSTRALIAN WATER QUALITY CENTRE

Peter Hobson, Renate Velzeboer & Michael Burch

AUSTRALIAN WATER QUALITY CENTRE

CONTENTS

EXECUTIVE SUMMARY

This project involved a series of range finding toxicity tests, or bioassays, with CUPRICIDE 110® and KUPRAMINE®. The purpose of the testing was to establish the effective dose rate for a problem cyanobacterium (blue-green alga)–*Anabaena circinalis*, for the two copper chelate algicides in a natural water sample that represents a challenge with respect to pH, alkalinity (TA) and DOC in natural waters.

The test determined the toxicity of the two algicides over 48 hours in natural water that had the following characteristics: pH – 7.45, Dissolved Organic Carbon – 5.4 mg/L, Alkalinity (as $CaCO_3$) – 84 mg/L.

The tests showed that the following dose rates (as mg Cu/L) were 100% effective against *A. circinalis*:

	24 Hours	48 Hours
CUPRICIDE 110®	<0.1	<0.05
KUPRAMINE®	<0.5	<0.2

The key findings from the study were:

- CUPRICIDE 110® was the more effective of the two algicides against *A. circinalis*, with an MLD_{100} of 0.1 mg Cu/L after 24 hours and 0.05 mg Cu/L after 48 hours.
- KUPRAMINE® had an MLD_{100} of 0.5 mg Cu/L after 24 hours and 0.2 mg Cu/L after 48 hours.
- However, under test conditions both CUPRICIDE 110® and KUPRAMINE® performed well, and in accordance with dilutions stipulated in the directions for use.
- The effective or minimum doses are slightly lower than those given in the above table indicating that CUPRICIDE 110® in particular has more effective algicidal action than indicated on the current label.
- CUPRICIDE 110® performed exceptionally well in water that represents a challenge to the toxicity of copper algicides

These tests indicated that CUPRICIDE 110® may be effective at 1/10 the current dose rates for copper sulphate when used to control *Anabaena* in natural water with similar adverse chemistry.

INTRODUCTION

Algicides are used principally by water authorities, reservoir managers and agricultural producers in Australia for the control of the nuisance cyanobacteria *Microcystis aeruginosa* and *Anabaena circinalis.* These species produce odours, and in some cases toxins and represent a threat to water quality, and animal and public health. Copper sulphate has been the algicide of choice for over 50 years. This is because it is generally regarded as effective, economical and safe to use from a water supply operator's viewpoint, although copper can have adverse environmental impacts on the aquatic ecosystem.

In some cases, however, treatment may be unsuccessful or partially successful. This can be due to inadequate dispersal and contact with the target organisms, variable sensitivity of algae and cyanobacteria, and reduced toxicity due to complexation of copper (Burch, 1990). It is widely recognised that the most toxic form of copper to aquatic organisms is the free cupric ion and this can be reduced by complexation with both inorganic ligands (alkalinity and hardness) and organic ligands present in natural waters (McKnight *et al*., 1983).

To overcome these limitations chelated copper algicides have been developed and are recommended by manufacturers for use in hard waters or those with high alkalinity. In these algicides the copper is chelated to a simple organic ligand such as triethanolamine or citric acid and this has been shown to increase algicidal effectiveness in high alkalinity water (Raman, 1985). Although the free cupric ion activity may be lower in natural water with a synthetic chelated algicide it is possible the residual toxic effect will be longer lived than with copper sulphate due to the longer time taken to reach equilibrium complexation with insoluble malachite and tenorite (McKnight *et al*., 1983).

The principal information required by water managers when using algicides is 1) the dose rate, and 2) the time required to achieve a satisfactory kill, under particular operating conditions (eg water quality and temperature). The ambient water quality characteristics which are regarded as the main variables that influence the toxic effect of copper-based algicides are pH, alkalinity, and concentration of dissolved organic carbon (DOC). It is not clear whether these variables have a major influence on the action of chelated copper algicides.

This investigation involved a series of range finding toxicity tests, or bioassays, with CUPRICIDE 110® and KUPRAMINE®. The main purpose of this testing was to establish the effective dose rate for a problem cyanobacterium (blue-green alga), *Anabaena circinalis*, for the two copper chelate algicides in a natural water sample that represents a challenge with respect to pH, alkalinity (TA) and DOC in natural waters.

MATERIALS AND METHODS

The assessment involved three separate laboratory bioassay tests with the two copper chelates, CUPRICIDE 110® and KUPRAMINE®, using cultures of the nuisance cyanobacterium, *Anabaena circinalis*. The algicides were tested in a natural water type selected to represent a potential worst-case for algicides, ie. hard, alkaline water with high DOC (pH ~7.4-7.8, TA >70 mg/L, DOC >5.0 mg/L). The tests were carried out at 20^{o}C under continuous irradiance of 150 µmol photons m^{-2} s^{-1} over a 48-hour growth period. This time period is a relatively standard nominal operational time to define the effectiveness of algicide treatment. Inoculum cultures were grown in WC media and subcultured to maintain them in exponential growth phase. These cultures were then inoculated into natural water to produce a cell concentration of approximately 6 x 10^4 cells/mL. This cell density would represent a relatively heavy algal infestation in surface water, particularly for a drinking water supply. The reservoir water used as the medium for the tests was obtained from Hope Valley Reservoir, South Australia. The water was prepared for the test by filtration (3 µm) to remove wild type algae. Previous experience has indicated that these 'contaminant' natural algae can compete with the test cyanobacteria and interfere with test interpretation. A summary of test conditions is provided in Table 1.

Each test was carried out at range of dose concentrations, plus a control. The nominal dose rates were: **0, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 mg Cu/L** for CUPRICIDE 110® and **0, 0.1, 0.2, 0.5, 1.0 mg Cu/L** for KUPRAMINE®. This range covered the current label recommendations for CUPRICIDE 110®. All dose concentrations were performed in triplicate (ie. x3). A 100 mg Cu/L stock solution was prepared for each algicide according to concentration data on label and was analysed using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) to obtain an accurate measurement of the copper level. This stock was used in subsequent experiments.

Effective toxicity to the *Anabaena circinalis* cells was assessed at 24 and 48 hours after treatment with the algicides. The experimental design involved:

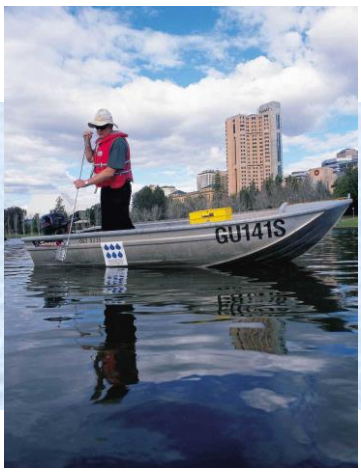
(1) Determination of cell density (Total Cell Number). *A.circinalis* cells were preserved in lugol's iodine and counted in a Sedgewick-Rafter counting chamber using a compound microscope. Cell counts were carried out to a precision of 10%.

(2) Assessment of cell viability (%Viable Cells). The cell viability test procedure is based on determining the integrity of the plasmalemma, which is closely related to viability. *A.circinalis* cells were stained with fluorescein diacetate (FDA) and propidium iodide (PI) and examined with a fluorescence microscope. FDA stains cells with an intact cell membrane (living cells) and they appear bright green under fluorescence microscopy (Rotman and Papermaster, 1966), whereas cells with a damaged cell membrane (dead cells) stain bright orange with PI. A minimum number of cells were counted to achieve a precision of 10%.

This data can be used to further derive the test statistic MLD_{100} (minimum lethal dose to 100% of cells) after 24 hours and 48 hours of Cu treatment. The MLD_{100} is equivalent to the EC_{100} (Effective concentration to 100% of individuals) in this context.

Table 1
Test conditions applied to cultures grown in CUPRICIDE 110® and KUPRAMINE®

Test Conditions	TEST 1	TEST 2	TEST 3
Algicide	**CUPRICIDE 110®**	**CUPRICIDE 110®**	**KUPRAMINE®**
Organism	Anabaena circinalis	Anabaena circinalis	Anabaena circinalis
Temperature	20°C	20°C	20°C
Irradiance	150 µmol m^{-2} s^{-1}	150 µmol m^{-2} s^{-1}	150 µmol m^{-2} s^{-1}
Time	48 hours	48 hours	48 hours
Water Type	Hope Valley Reservoir, 3 µm filtered	Hope Valley Reservoir, 3 µm filtered	Hope Valley Reservoir, 3 µm filtered
Water Characteristics	pH: 7.45 Dissolved Organic Carbon (DOC): 5.4 mg/L Total Organic Carbon (TOC): 5.6 mg/L Dissolved Solids: 334 mg/L Calcium: 23.7 mg/L Magnesium: 18.4 mg/L Potassium: 5.6 mg/L Sodium: 78.1 mg/L Bicarbonate: 103 mg/L Chloride: 125 mg/L Sulphate: 32.4 mg/L Alkalinity as calcium carbonate: 84 mg/L Ion balance: 2.90%		
Replication	3x	3x	3x
Dose Rates -mg Cu/L	0,0.1,0.2,0.5,1.0	0, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0	0,0.1,0.2,0.5,1.0
Test Volume	10 mL	10 mL	10 mL
Initial Cell Density of Anabaena circinalis (cells x 10^4/mL)	6.77 ± 0.23	5.90 ± 0.96	5.89 ± 1.12



Growth inhibition response of *Anabaena circinalis* to algicides at copper concentrations of 0, 0.1, 0.2, 0.5 and 1.0 mg Cu/L are shown in Figure 1 for CUPRICIDE 110® (test 1) and in Figure 3 for KUPRAMINE® (test 3). The data used to construct these figures are presented in Table 2 for CUPRICIDE 110 ® and Table 4 for KUPRAMINE® (see Appendix 1 for raw data). Growth inhibition response of *Anabaena circinalis* to CUPRICIDE 110® for a broader concentration range: 0.02-1.0 mg Cu/L, is shown in Figure 2 (test 2). The data used to construct this figure are presented in Table 3 (see Appendix 1 for raw data).

Figures 4(a) - 4(c) show pH change of *A. circinalis* cultures grown in both CUPRICIDE 110® and KUPRAMINE® for the three tests. Values for pH are given in Table 5.

Stock algicide solutions, 100 mg Cu/L, were made up using copper concentration provided on labels. Analysis by ICP-MS identified copper concentration of 108 mg Cu/L for CUPRICIDE 110 ® stock solution and 111 mg Cu/L for KUPRAMINE ® stock solution.

Figure 1 (Test 1) Growth in terms of (a) Total Cell Number, (b) % Viable Cells and (c) Number of Viable Cells for Anabaena circinalis cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

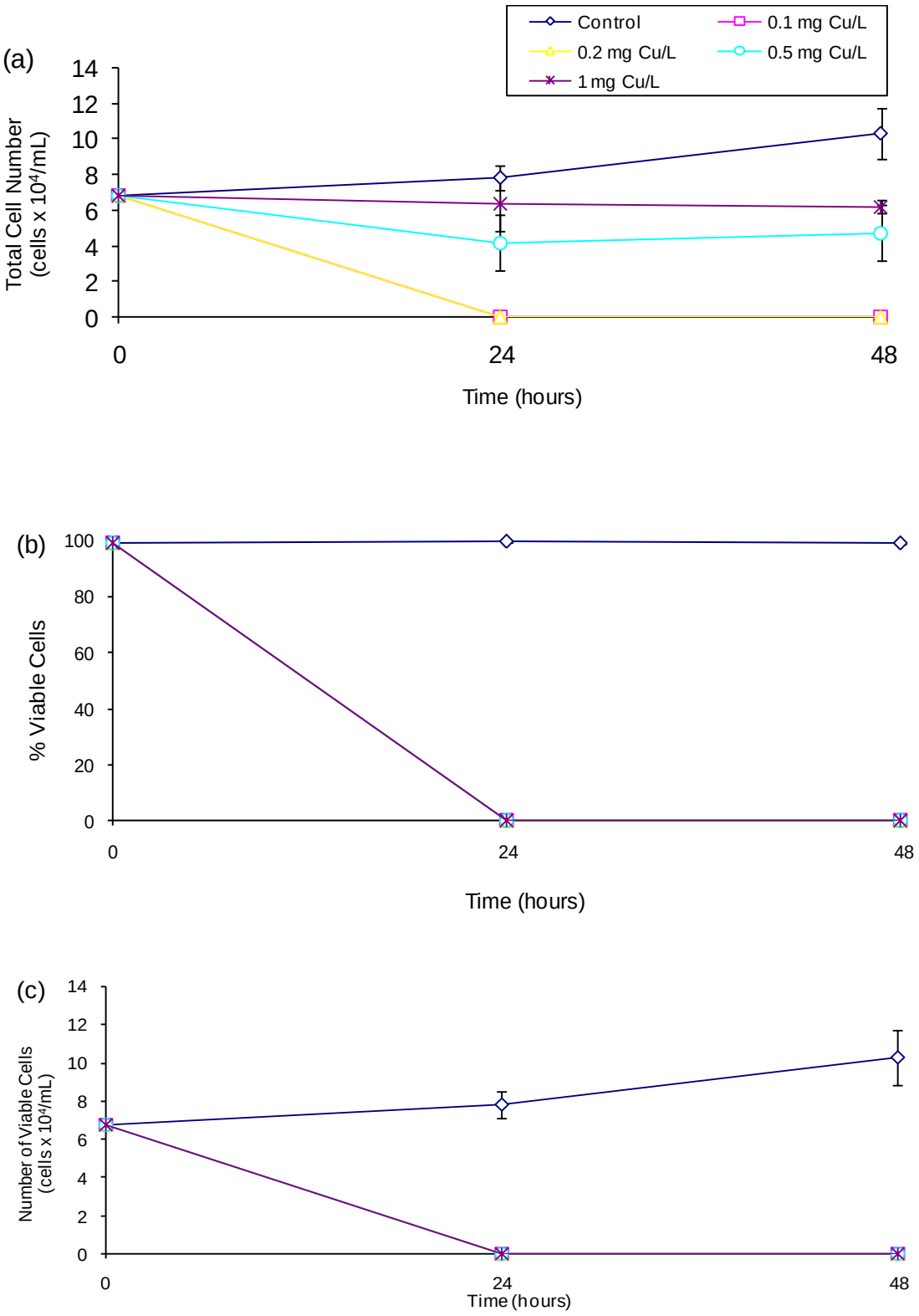


Figure 2 (Test 2) Growth in terms of (a) Total Cell Number, (b) % Viable Cells and (c) Number of Viable Cells for Anabaena circinalis cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

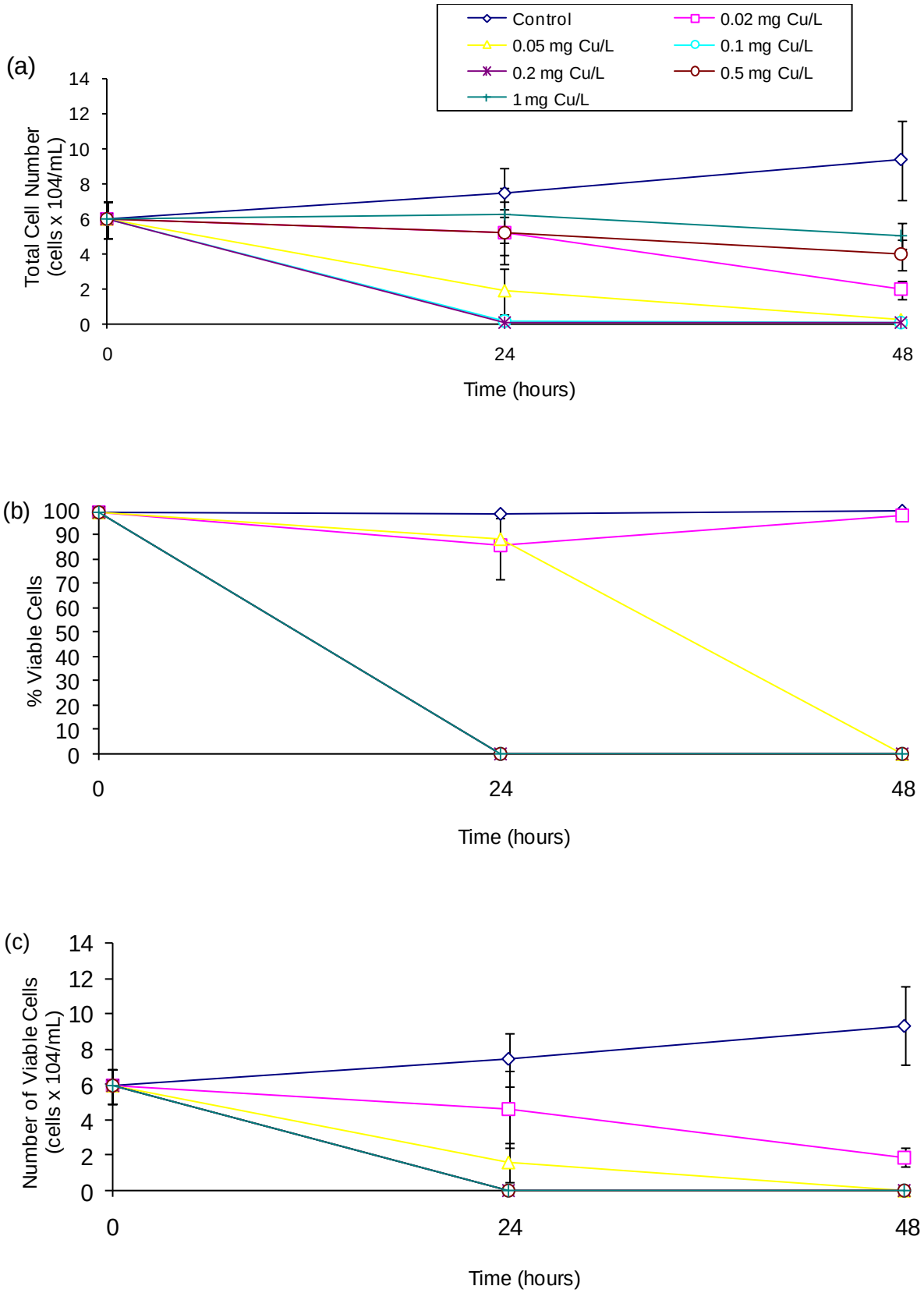


Figure 3 (Test 3) Growth in terms of (a) Total Cell Number, (b) % Viable Cells and (c) Number of Viable Cells for Anabaena circinalis cultures grown in Hope Valley Reservoir water containing KUPRAMINE® at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

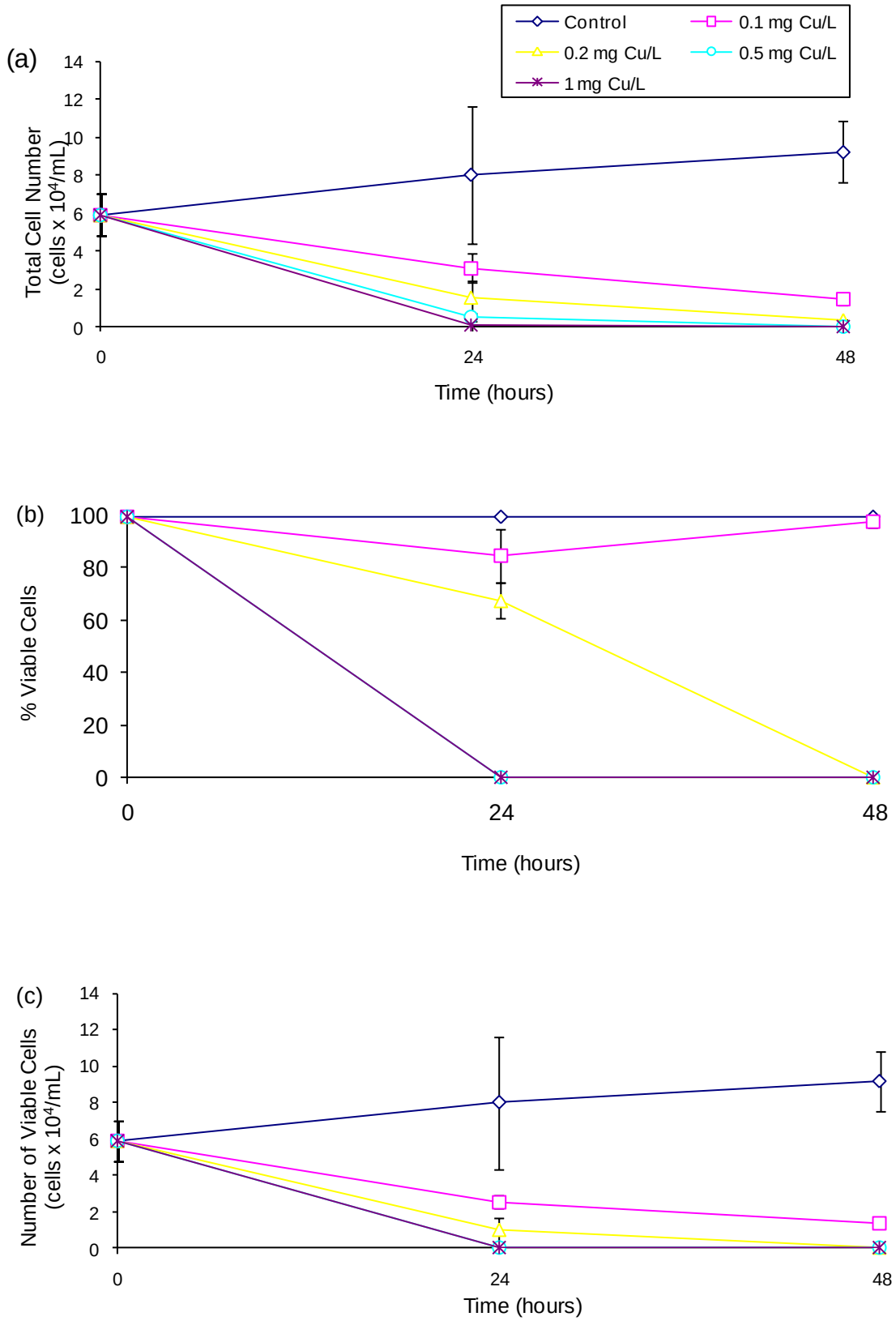
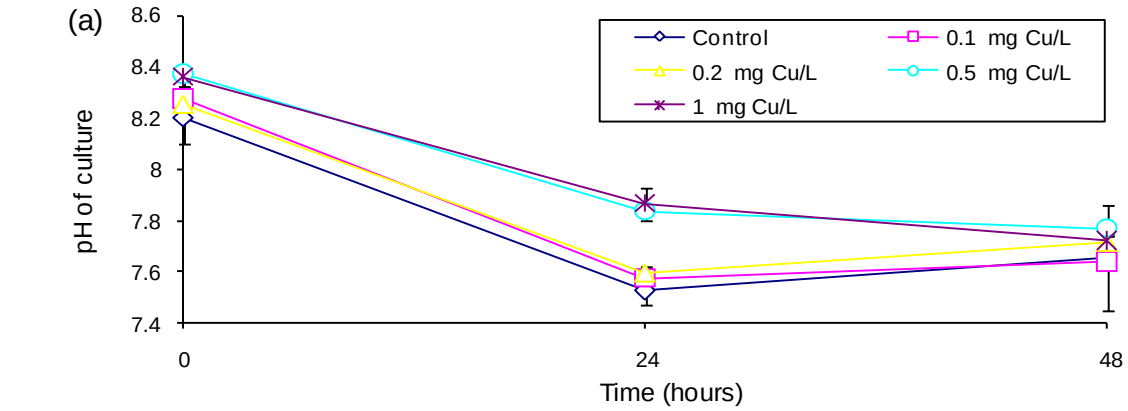
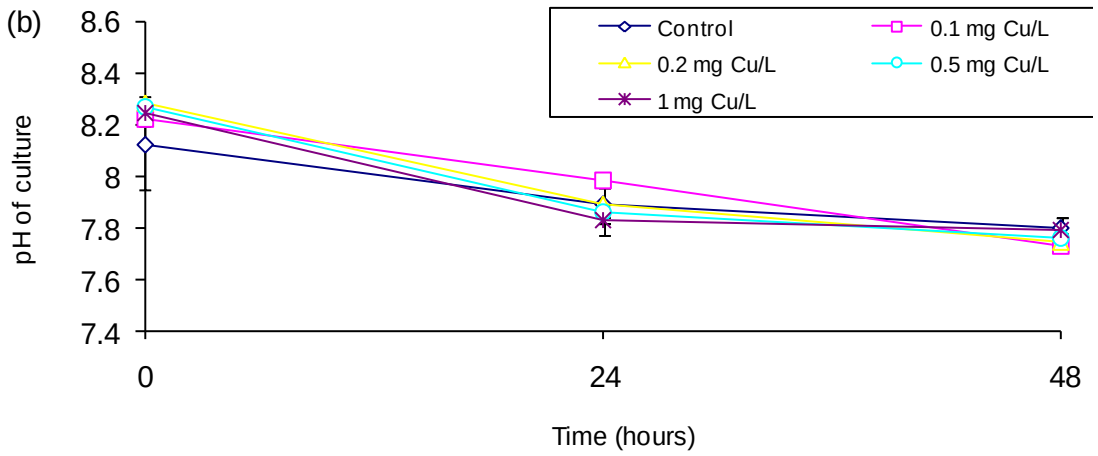


Figure 4. pH of Anabaena circinalis cultures grown in Hope Valley Reservoir water containing (a) & (b) CUPRICIDE 110® and (c) KUPRAMINE® at various copper (Cu) concentrations. Values are mean ± S.D., n=3.





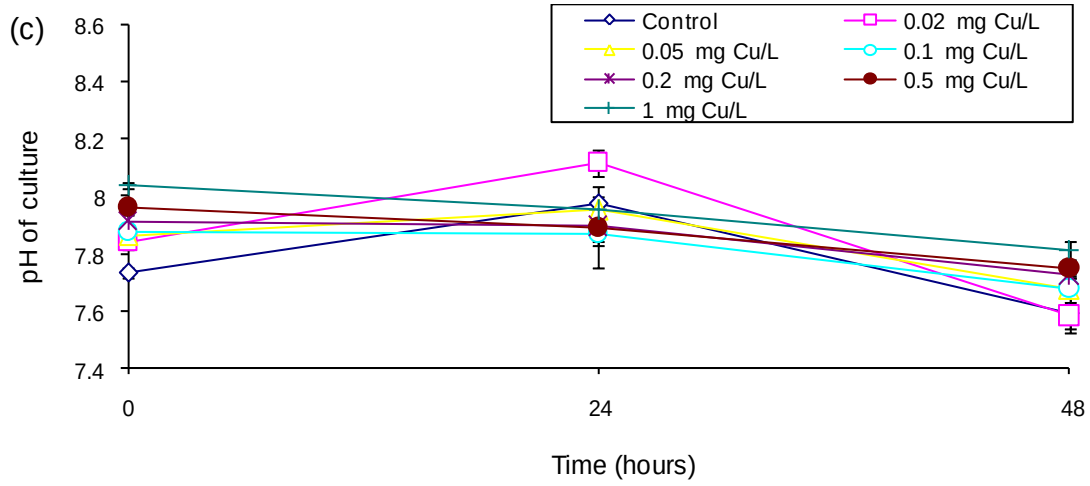


Table 2 (Test 1). Growth in terms of (a) total cell count, (b) % viable cells and (c) total viable cell count for *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® at copper concentrations of 0, 0.1, 0.2, 0.5 and 1.0 mg Cu/L. Includes mean value (0) and standard deviation (s.d.).

(a) Total Cell Number (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	6.77	0.23	6.77	0.23	6.77	0.23	6.77	0.23	6.77	0.23
24	7.84	0.71	0.001	0.001	0.00	0.00	4.18	1.57	6.39	1.55
48	10.35	1.44	0.02	0.04	0.01	0.02	4.74	1.59	6.21	0.39

(b) % Viable Cells

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	99.22	1.35	99.22	1.35	99.22	1.35	99.22	1.35	99.22	1.35
24	100.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	99.41	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(c) Number of Viable Cells (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	6.71	0.15	6.72	0.15	6.72	0.15	6.72	0.15	6.72	0.15
24	7.84	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	10.28	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3 (Test 2). Growth in terms of (a) total cell count, (b) % viable cells and (c) total viable cell count for *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® at copper concentrations of 0, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 mg Cu/L. Includes mean value (0) and standard deviation (s.d.).

(a) Total Cell Number (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)													
	0		0.02		0.05		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	5.95	1.03	5.95	1.03	5.95	1.03	5.95	1.03	5.95	1.03	5.95	1.03	5.95	1.03
24	7.49	1.43	5.16	1.78	1.85	1.29	0.15	0.04	0.03	0.00	5.23	1.28	6.19	1.53
48	9.34	2.24	1.93	0.56	0.24	0.06	0.02	0.01	0.01	0.01	3.94	0.90	5.02	0.76

(b) % Viable Cells

Time (hours)	Copper concentration (mg Cu/L)													
	Control		0.02		0.05		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	99.37	1.09	99.37	1.09	99.37	1.09	99.37	1.09	99.37	1.09	99.37	1.09	99.37	1.09
24	98.89	1.55	85.89	14.15	88.64	4.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	100.0	0.00	98.36	1.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(c) Number of Viable Cells (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)													
	Control		0.02		0.05		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	5.90	0.96	5.90	0.96	5.90	0.96	5.90	0.96	5.90	0.96	5.90	0.96	5.90	0.96
24	7.42	1.49	4.60	2.17	1.61	1.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	9.34	2.24	1.89	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4 (Test 3). Growth in terms of (a) total cell count, (b) % viable cells and (c) total viable cell count for *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing KUPRAMINE® at copper concentrations of 0, 0.1, 0.2, 0.5 and 1.0 mg Cu/L. Includes mean value (0) and standard deviation (s.d.).

(a) Total Cell Number (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	5.89	1.12	5.89	1.12	5.89	1.12	5.89	1.12	5.89	1.12
24	8.01	3.64	3.08	0.75	1.49	0.88	0.48	0.19	0.02	0.01
48	9.23	1.61	1.39	0.27	0.28	0.08	0.00	0.00	0.00	0.00

(b) % Viable Cells

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	99.86	0.24	99.86	0.24	99.86	0.24	99.86	0.24	99.86	0.24
24	99.83	0.29	84.50	10.27	67.60	6.65	0.00	0.00	0.00	0.00
48	99.70	0.30	97.64	2.04	0.00	0.00	0.00	0.00	0.00	0.00

(c) Number of Viable Cells (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	5.89	1.12	5.89	1.12	5.89	1.12	5.89	1.12	5.89	1.12
24	8.00	3.65	2.56	0.38	1.04	0.66	0.00	0.00	0.00	0.00
48	9.21	1.64	1.36	0.26	0.00	0.00	0.00	0.00	0.00	0.00

Table 5. pH of *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® (a) & (b) and KUPRAMINE® (c) at various copper concentrations. Includes mean value (0) and standard deviation (s.d.).

(a) Test 1

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	8.20	0.11	8.27	0.01	8.25	0.04	8.37	0.03	8.36	0.04
24	7.52	0.05	7.57	0.04	7.60	0.02	7.84	0.02	7.86	0.07
48	7.66	0.21	7.64	0.02	7.72	0.02	7.77	0.02	7.72	0.02

(b) Test 2

Time (hours)	Copper concentration (mg Cu/L)													
	Control		0.02		0.05		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	7.73	0.02	7.84	0.03	7.86	0.02	7.88	0.02	7.91	0.02	7.96	0.02	8.04	0.01
24	7.98	0.03	8.12	0.05	7.95	0.03	7.87	0.04	7.89	0.14	7.89	0.05	7.95	0.01
48	7.59	0.07	7.58	0.04	7.67	0.05	7.68	0.02	7.72	0.01	7.75	0.03	7.81	0.04

(c) Test 3

Time (hours)	Copper concentration (mg Cu/L)									
	0		0.1		0.2		0.5		1.0	
	0	s.d.	0	s.d.	0	s.d.	0	s.d.	0	s.d.
0	8.12	0.17	8.23	0.03	8.28	0.03	8.27	0.01	8.24	0.01
24	7.89	0.11	7.99	0.03	7.89	0.07	7.86	0.04	7.83	0.01
48	7.80	0.04	7.73	0.01	7.75	0.02	7.76	0.02	7.79	0.05

DISCUSSION

Results for controls used in these tests indicated that the cultures of *A.circinalis* were maintained in exponential growth phase for the duration of the experiment (48 hours) when grown in natural reservoir water. This indicated that the cultured cells were healthy and the test design was appropriate to determine toxic effects in natural water in a reproducible manner.

The microscopic observations of the test cultures of *A.circinalis* in TEST 1 with CUPRICIDE 110® indicated that the cells were both lysed and eliminated after 24 hours exposure to concentrations of 0.1 and 0.2 mg Cu/L. However, at the two higher dose concentrations of 0.5 and 1.0 mg Cu/L, the cells were killed but remained apparently intact in the test medium and could be counted after 24 and 48 hours.

This observation was unexpected and was confirmed with a repeat experiment. The second test with CUPRICIDE 110® (TEST 2), involved a new culture and a wider concentration range (0.02 – 1.0 mg Cu/L), with the inclusion of two lower concentrations (0.02 and 0.05 mg Cu/L). The results were similar in TEST 2. Cells were lysed and disappeared progressively from the lowest concentration of 0.02 mg Cu/L up to 0.1 mg Cu/L, where no intact cells could be found after 24 hours. At the higher concentrations of 0.5 – 1.0 mg Cu/L, cells were visible and apparently intact, but were clearly dead as determined by the viability staining assay.

The reason for the dose-dependent lysis and loss of *Anabaena* cells is unclear. It may reflect dose-dependent transport or exclusion mechanisms across membranes. Notwithstanding the causes, it is important to note that for practical interpretation of field treatment, that higher dose treatments will be effective in killing cells but this may not be immediately apparent, as cells may still be visible under simple microscopic examination of samples.

The above observations indicate the limitations of interpreting toxicity from direct cell counts, which record both living and dead cells, whereas the cell viability assay allows identification of both viable and non-viable cells. The determination of an MLD_{100} using viable cells provides a more accurate representation of the effectiveness of the algicides. This can be regarded as the effective or critical dose required for the particular water conditions. The MLD_{100} for the two algicides under these two test conditions are given in Table 6. Using results for "Number of Viable Cells" the MLD_{100} for CUPRICIDE 110® after 24 hours was 0.1 mg Cu/L but after 48 hours was slightly lower at 0.05 mg Cu/L. This shows that while cells were identified at highest copper concentrations (0.5 and 1.0 mg Cu/L), by direct cell counting, none of these cells were viable.

The effect of KUPRAMINE ® on Total Cell number and cell viability increased in a dose-dependent manner over 48 hours. The MLD_{100} for KUPRAMINE® was 0.5 mg Cu/L after 24 hours and 0.2 mg Cu/L after 48 hours.

These tests indicated that CUPRICIDE 110® was the more effective of the two algicides against *A. circinalis*, with an MLD_{100} of 0.1 mg Cu/L after 24 hours and 0.05 mg Cu/L after 48 hours. KUPRAMINE® had an MLD_{100} of 0.5 mg Cu/L after 24 hours and 0.2 mg Cu/L after 48 hours. However, under test conditions both CUPRICIDE 110® and KUPRAMINE® performed well, and in accordance with dilutions stipulated in the directions for use. It is important to note that the effective or minimum doses may be slightly lower than those given in Table 6, indicating that CUPRICIDE 110® in particular has more effective algicidal action than indicated on the current label. The actual MLD_{100} values will be between the value given and the next lowest concentration used in this test range.

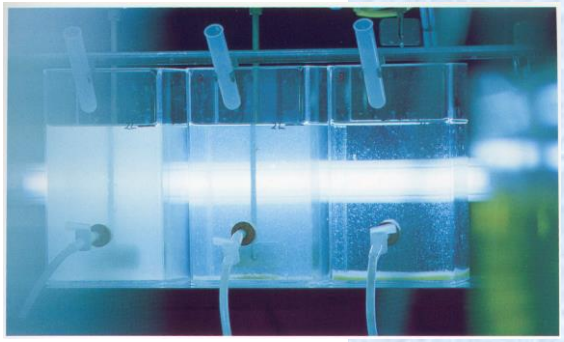
Table 6

MLD_{100} for *Anabaena circinalis* grown in hard alkaline water using the algicides CUPRICIDE 110® and KUPRAMINE® .

	CUPRICIDE 110®	KUPRAMINE®
MLD_{100} after 24 hours	0.10	0.50
MLD_{100} after 48 hours	0.05	0.20

Results show that the pH reduced over the 48 hour growth period for treatment with both CUPRICIDE 110® and KUPRAMINE® respectively. The pH of cultures grown in KUPRAMINE® steadily reduced from approximately 8.2 to 7.8 over 48 hours. The pH of cultures grown in CUPRICIDE 110® also decreased over the 48 hour period from approximately 8.3 to 7.7 in Test 1. The reduction was less dramatic in Test 2, and pH declined from approximately 7.9 to 7.7 across the concentration range for the treatments.

Results from ICP-MS on stock solutions of the batch of algicides indicated that copper concentrations appear to be approximately 10% higher than that reported on labels. We have no immediate explanation for this discrepancy, and do not believe it to be analytical error.



REFERENCES

Burch, M.D. (1990). Algicidal control of algal blooms. In *Water Board cyanobacteria workshop. Blue-green algae in drinking and receiving waters. Proceedings of the Water Board Cyanobacterial workshop November 1990*. pp. 21-23. Sydney Water Board, Sydney.

McKnight, D.M., Chisholm, S.W., & Harleman, D.R.F. (1983). $CuSO_4$ treatment of nuisance algal blooms in drinking water reservoirs. *Environ. Management*. 7, 311-320.

Raman, R.K. (1985). Controlling algae in water supply impoundments. *J. AWWA*. 77, 41-43.

Rotman, B. & Papermaster, B.W. (1966). Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. *Proc. Natl. Acad. Sci. USA*. 55, 134-141.

Table 2-1A (Test 1). Growth in terms of (a) Total Cell Number, (b) % Viable Cells and (c) Number of Viable Cells for *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® at copper concentrations of 0, 0.1, 0.2, 0.5 and 1.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

(a) Total Cell Number (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.01	6.77	0.23	7.01	6.770	0.226	7.01	6.77	0.23	7.01	6.77	0.23	7.01	6.77	0.23
	6.74			6.74			6.74			6.74			6.74		
	6.56			6.56			6.56			6.56			6.56		
24	8.66	7.84	0.71	0.002	0.001	0.001	0.000	0.000	0.000	4.79	4.18	1.57	7.07	6.39	1.55
	7.48			0.000			0.000			5.35			4.62		
	7.39			0.000			0.000			2.40			7.48		
48	8.74	10.35	1.44	0.063	0.022	0.036	0.033	0.011	0.019	5.89	4.74	1.59	6.58	6.21	0.39
	10.80			0.000			0.000			2.92			5.81		
	11.50			0.003			0.000			5.40			6.24		

(b) % Viable Cells

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	97.66	99.22	1.35	97.66	99.22	1.35	97.66	99.22	1.35	97.66	99.22	1.35	97.66	99.22	1.35
	100.00			100.00			100.00			100.00			100.00		
	100.00			100.00			100.00			100.00			100.00		
24	100	100	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100			0.00			0.00			0.00			0.00		
	100			0.00			0.00			0.00			0.00		
48	99.22	99.41	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100			0.00			0.00			0.00			0.00		
	99.02			0.00			0.00			0.00			0.00		

(c) Number of Viable Cells (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	6.85	6.72	0.15	6.85	6.72	0.15	6.85	6.72	0.15	6.85	6.72	0.15	6.85	6.72	0.15
	6.74			6.74			6.74			6.74			6.74		
	6.56			6.56			6.56			6.56			6.56		
24	8.66	7.84	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7.48			0.00			0.00			0.00			0.00		
	7.39			0.00			0.00			0.00			0.00		
48	8.67	10.28	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10.80			0.00			0.00			0.00			0.00		
	11.38			0.00			0.00			0.00			0.00		

Table 3-1A (Test 2). Growth in terms of (a) Total Cell Number, (b) % Viable Cells and (c) Number of Viable Cells for *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing CUPRICIDE 110® at copper concentrations of 0, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

(a) Total Cell Number (cells x 10^4/mL)

Time hours	Copper concentration (mg Cu/L)																				
	0			0.02			0.05			0.10			0.20			0.50			1.00		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	5.30	5.95	1.03	5.30	5.95	1.03	5.30	5.95	1.03	5.30	5.95	1.03	5.30	5.95	1.03	5.30	5.95	1.03	5.30	5.95	1.03
	5.40			5.40			5.40			5.40			5.40			5.40			5.40		
	7.14			7.14			7.14			7.14			7.14			7.14			7.14		
24	9.02	7.49	1.43	6.75	5.16	1.78	0.40	1.85	1.29	0.17	0.15	0.04	0.03	0.03	0.00	6.71	5.23	1.28	7.76	6.19	1.53
	7.26			3.24			2.29			0.17			0.03			4.49			6.12		
	6.19			5.49			2.87			0.10			0.03			4.49			4.70		
48	10.89	9.34	2.24	2.07	1.93	0.56	0.18	0.24	0.06	0.01	0.02	0.01	0.01	0.01	0.01	4.27	3.94	0.90	5.60	5.02	0.76
	10.36			1.31			0.29			0.02			0.02			4.63			4.16		
	6.77			2.40			0.26			0.02			0.00			2.93			5.30		

(b) % Viable Cells

Time hours	Copper concentration (mg Cu/L)																				
	0			0.02			0.05			0.10			0.20			0.50			1.00		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	100.00	99.37	1.09	100.00	99.37	1.09	100.00	99.37	1.09	100.00	99.37	1.09	100.00	99.37	1.09	100.00	99.37	1.09	100.00	99.37	1.09
	100.00			100.00			100.00			100.00			100.00			100.00			100.00		
	98.12			98.12			98.12			98.12			98.12			98.12			98.12		
24	99.54	98.89	1.55	97.28	85.89	14.15	91.67	88.64	4.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100.00			70.05			90.91			0.00			0.00			0.00			0.00		
	97.12			90.34			83.33			0.00			0.00			0.00			0.00		
48	100.00	100.00	0.00	96.77	98.36	1.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100.00			100.00			0.00			0.00			0.00			0.00			0.00		
	100.00			98.31			0.00			0.00			0.00			0.00			0.00		

(c) Number of Viable Cells (cells x 10^4/mL)

Time hours	Copper concentration (mg Cu/L)																				
	0			0.02			0.05			0.10			0.20			0.50			1.00		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	5.30	5.90	0.96	5.30	5.90	0.96	5.30	5.90	0.96	5.30	5.90	0.96	5.30	5.90	0.96	5.30	5.90	0.96	5.30	5.90	0.96
	5.40			5.40			5.40			5.40			5.40			5.40			5.40		
	7.01			7.01			7.01			7.01			7.01			7.01			7.01		
24	8.98	7.42	1.49	6.57	4.60	2.17	0.37	1.61	1.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7.26			2.27			2.08			0.00			0.00			0.00			0.00		
	6.01			4.96			2.39			0.00			0.00			0.00			0.00		
48	10.89	9.34	2.24	2.00	1.89	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10.36			1.31			0.00			0.00			0.00			0.00			0.00		
	6.77			2.36			0.00			0.00			0.00			0.00			0.00		

Table 4-1A (Test 3). Growth in terms of (a) Total Cell Number, (b) % Viable Cells and (c) Number of Viable Cells for *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing KUPRAMINE® at copper concentrations of 0, 0.1, 0.2, 0.5 and 1.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

(a) Total Cell Number (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	5.15	5.89	1.12	5.15	5.89	1.12	5.15	5.89	1.12	5.15	5.89	1.12	5.15	5.89	1.12
	7.18			7.18			7.18			7.18			7.18		
	5.34			5.34			5.34			5.34			5.34		
24	5.60	8.01	3.64	2.24	3.08	0.76	0.49	1.50	0.88	0.30	0.48	0.19	0.0211	0.018	0.012
	12.20			3.71			1.92			0.46			0.0289		
	6.23			3.3			2.08			0.67			0.0051		
48	7.46	9.23	1.61	1.34	1.40	0.27	0.35	0.280	0.08	0.0051	0.0119	0.0089	0.00	0.00	0.00
	9.63			1.69			0.29			0.0221			0.00		
	10.61			1.16			0.20			0.0085			0.00		

(b) % Viable Cells

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	99.58	99.86	0.24	99.58	99.86	0.24	99.58	99.86	0.24	99.58	99.86	0.24	99.58	99.86	0.24
	100.00			100.00			100.00			100.00			100.00		
	100.00			100.00			100.00			100.00			100.00		
24	100.00	99.83	0.29	94.40	84.50	10.27	61.70	67.60	6.65	0.00	0.00	0.00	0.00	0.00	0.00
	100.00			73.90			66.30			0.00			0.00		
	99.50			85.20			74.80			0.00			0.00		
48	99.40	99.70	0.30	100.00	97.64	2.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	99.70			96.43			0.00			0.00			0.00		
	100.00			96.49			0.00			0.00			0.00		

(c) Number of Viable Cells (cells x 10^4/mL)

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	5.15	5.89	1.12	5.15	5.89	1.12	5.15	5.89	1.12	5.15	5.89	1.12	5.15	5.89	1.12
	7.18			7.18			7.18			7.18			7.18		
	5.34			5.34			5.34			5.34			5.34		
24	5.60	7.99	3.65	2.12	2.56	0.38	0.30	1.04	0.66	0.00	0.00	0.00	0.00	0.00	0.00
	12.20			2.74			1.27			0.00			0.00		
	6.19			2.81			1.56			0.00			0.00		
48	7.41	9.21	1.64	1.34	1.36	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	9.60			1.63			0.00			0.00			0.00		
	10.61			1.12			0.00			0.00			0.00		

Table 5-1A. pH of *Anabaena circinalis* cultures grown in Hope Valley Reservoir water containing (a) & (b) CUPRICIDE 110® and (c) KUPRAMINE® at various copper concentrations. Includes value for each measurement, mean and standard deviation (s.d.).

(a) Test 1

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	8.08	8.20	0.11	8.26	8.27	0.01	8.21	8.25	0.04	8.36	8.37	0.03	8.40	8.36	0.04
	8.26			8.28			8.28			8.35			8.35		
	8.27			8.28			8.27			8.40			8.33		
24	7.49	7.52	0.05	7.54	7.57	0.04	7.58	7.60	0.02	7.84	7.84	0.02	7.88	7.86	0.07
	7.50			7.56			7.59			7.82			7.92		
	7.58			7.62			7.62			7.85			7.79		
48	7.43	7.66	0.21	7.61	7.64	0.02	7.70	7.72	0.02	7.76	7.77	0.02	7.72	7.72	0.02
	7.71			7.65			7.74			7.75			7.74		
	7.83			7.65			7.71			7.79			7.70		

(b) Test 2

Time hours	Copper concentration (mg Cu/L)																				
	0			0.02			0.05			0.10			0.20			0.50			1.00		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.73	7.73	0.02	7.82	7.84	0.03	7.84	7.86	0.02	7.86	7.88	0.02	7.90	7.91	0.02	7.95	7.96	0.02	8.03	8.04	0.01
	7.75			7.83			7.88			7.88			7.90			7.96			8.05		
	7.72			7.87			7.86			7.89			7.94			7.98			8.03		
24	7.95	7.98	0.03	8.06	8.12	0.05	7.98	7.95	0.03	7.91	7.87	0.04	7.77	7.89	0.14	7.84	7.89	0.05	7.95	7.95	0.01
	7.98			8.15			7.93			7.83			8.05			7.89			7.94		
	8.00			8.14			7.94			7.86			7.86			7.94			7.96		
48	7.52	7.59	0.07	7.54	7.58	0.04	7.62	7.67	0.05	7.66	7.68	0.02	7.72	7.72	0.01	7.78	7.75	0.03	7.78	7.81	0.04
	7.59			7.58			7.70			7.68			7.73			7.74			7.80		
	7.66			7.62			7.70			7.69			7.72			7.72			7.85		

(c) Test 3

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.1			0.2			0.5			1.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.93	8.12	0.17	8.19	8.23	0.03	8.32	8.28	0.03	8.27	8.27	0.01	8.24	8.24	0.01
	8.18			8.25			8.27			8.28			8.25		
	8.25			8.24			8.26			8.26			8.24		
24	7.81	7.89	0.11	8.01	7.99	0.03	7.94	7.89	0.07	7.9	7.86	0.04	7.83	7.83	0.01
	7.84			7.99			7.92			7.86			7.82		
	8.02			7.96			7.81			7.82			7.84		
48	7.78	7.80	0.04	7.74	7.73	0.01	7.76	7.75	0.02	7.74	7.76	0.02	7.73	7.79	0.05
	7.85			7.73			7.73			7.78			7.82		
	7.77			7.72			7.76			7.76			7.82		