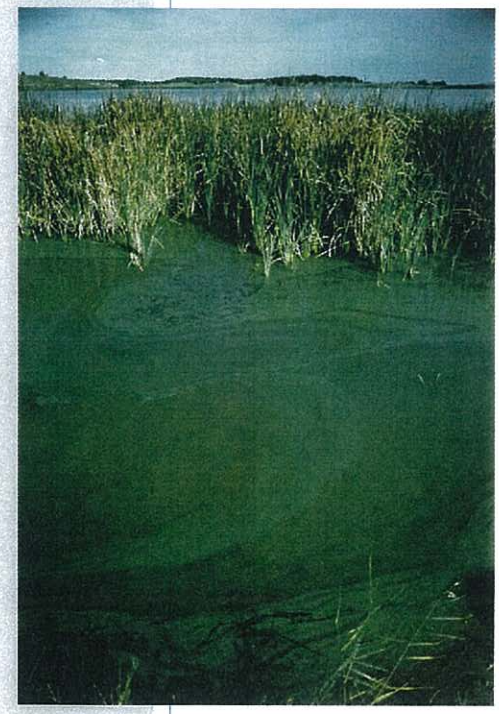
RESIDUAL ACTIVITY OF COPPER-BASED ALGICIDES AGAINST THE CYANOBACTERIUM *Microcystis aeruginosa*

20th March 2001



AUSTRALIAN WATER QUALITY CENTRE

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INTRODUCTION

Copper-based algicides are used widely in Australia for control of algal growth in both water and waste-water situations. The range of compounds that are available are generally effective, although there are circumstances where re-treatment is required after short time intervals. The reasons for this may relate to a combination of a number of factors, such as an inadequate initial dose and loss of activity of the agent by complexation, precipitation, or degradation, etc. In any case there is very little systematic information available on residual toxicity for these algicides in natural waters.

The purpose of this investigation was to establish the residual activity of four copper-based algicides against the cyanobacterium *Microcystis aeruginosa*. The project involved laboratory testing of CUPRICIDE 110®, KUPRAMINE®, KUPRAMINE 500®, and also copper sulphate and for their algicidal affect on the cyanobacterium *Microcystis aeruginosa* in a trial designed to test their toxicity over an extended time in natural water. *Microcystis aeruginosa* was chosen as the test organism because it has been identified as a problem in water supplies due to the production of hepatotoxins, which pose a hazard to human and animal health and also aesthetic problems of bad tastes and odours.

Test Design

The principal of the residual activity test involved treating a sample of natural reservoir water with a range of concentrations of the different algicides and then following the toxicity in that water sample over 11 days using the cultured cells of the cyanobacterium as the exposed test organism. The test was in effect a series of four successive and separate 2-day bioassays over the 11-day period. This was achieved by placing freshly-prepared cells of *Microcystis aeruginosa* into the dosed solution as it aged, at time intervals of 3 days, ie a fresh inoculum of cells was added at times 0,3,6 and 9 days. The cells were exposed for 2 days (48h) in the solution, examined for injury, and then filtered out of the test water, before a fresh inoculum was added to the same solution after a subsequent 24 hours and so on, to determine change in toxicity to the fresh actively growing cells as the solution aged.

A detailed description of the design and methods is as follows. On day 0 an inoculum culture was added to Myponga Reservoir water to produce a cell concentration of approximately 5 x 10^4 cells/mL. The Myponga Reservoir water was filtered through a 2 μm filter to remove any competing algal species. Total Cell Counts and % Living Cells were then measured. 100 mL volumes of this culture were distributed to 15 flasks for each algicide. The algicides were then added to these flasks at copper concentrations of **0, 0.5, 1.0, 2.0 and 4.0 mg Cu/L** and incubated for 2 days at 25°C and 30 μmol photons m^{-2} s^{-1}. After 2 days Total Cell Counts and % Living Cells were measured. Cell material was then removed from all cultures using a 2 μm filter and the filtered media stored overnight under test conditions. A 2 μm filter retained *M.aeruginosa* cells but allowed copper algicides to pass through. On the following day a fresh inoculum was added to the filtrate to produce a cell count of approximately $5x10^4$ cells/mL. Total Cell Counts and the % Living Cells were again measured. This process was continued until day 11. The pH of all cultures was measured throughout the experiment.

Culture Preparation

The test organism was a cultured strain (MIC 338)of *Microcystis aeruginosa* from the AWQC Culture Collection. The strain is a known producer of the hepatotoxic peptide microcystin.

Inoculum cultures were grown in ASM-1 media and subcultured to maintain them in exponential growth phase. Inoculum cultures were grown at 25°C under a continuous irradiance of 30 μmol photons m^{-2} s^{-1}.

Bioassay Protocol

Effective toxicity was assessed 2 days (48h) after treatment. This was determined by microscope cell counting and assessment of cell viability using vital stains (Fluorescein diacetate (FDA) and Propidium iodide (PI)). FDA passes through cell membranes and is hydrolysed by intracellular esterases to produce fluorescein (a fluorescent product). Fluorescein passes slowly through living cell membranes and accumulates inside the cell. It exhibits a green fluorescence when excited with blue light. PI only passes through the membranes of dead or dying cells and stains DNA in the cells to an orange colour.

Effectiveness was judged as the "Percentage of Living Cells" counted after the prescribed time interval using a Lund Cell. "Total Cell Counts" were measured using a Sedgewick-Rafter chamber and the "% Living Cells" were then used to calculate "Total Live Cells". % Total Live Cells compared to the Control was also calculated which allowed for a better measure of the effectiveness of each algicide at the copper concentrations studied. Test end-point data was used to derive the test statistic – MLD_{100} or "Minimum Lethal Dose to 100% of individuals" after 48 hours. For this investigation it was "Minimum Lethal Dose to 100% of Viable Cells" This statistic is equivalent to the EC_{100}, or "Effective Concentration to 100% of individuals".

Growth response of *Microcystis aeruginosa* exposed to the four algicides is presented in figures 1 to 4. Each figure provides results for Total Cell Count, Total Live Cells and % Total Live Cells compared to the Control for the four exposure periods. Figure 5 shows pH measurements for all cultures throughout the 11-day experiment. Data used to construct these graphs is presented in Appendix 1. The raw data for all experimental work can be found in Appendix 2.

Total copper concentrations were measured for cultures on day 0 and results are presented in Table 1. Results for chemical analysis of Myponga Reservoir water is presented in Table 2. 100 mg Cu/L stock solutions were prepared for each algicide according to concentration data on label and were analysed using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) to obtain an accurate measurement of copper levels (See Table 3 for results). This stock was used in subsequent experiments.

Figure 1: CUPRICIDE 110®

Total Cell Count, Total Live Cells and % Total Live Cells compared to the Control for *Microcystis aeruginosa* cultures exposed to CUPRICIDE 110®. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to CUPRICIDE 110® at various copper concentrations. (n=3)

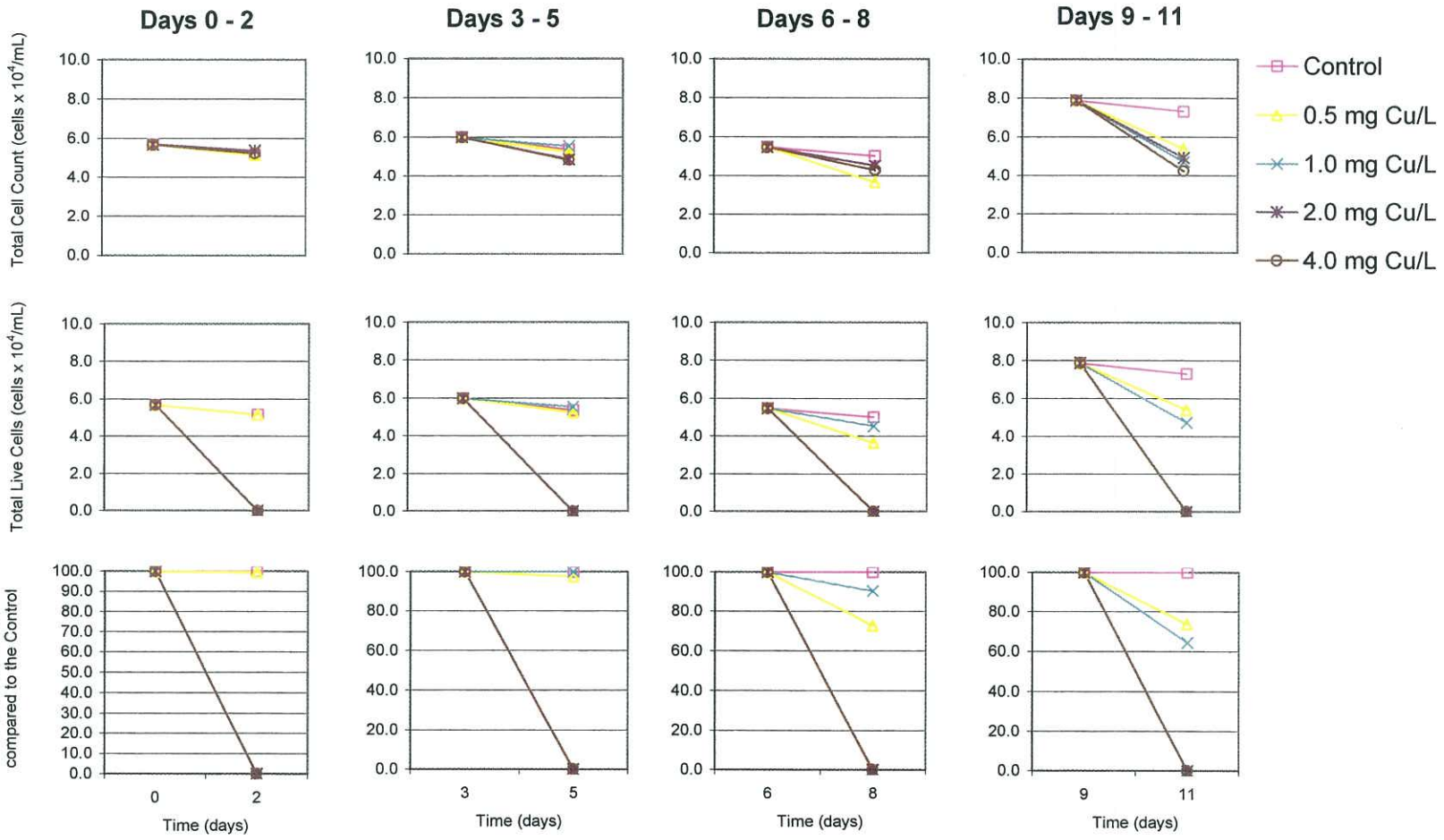

Figure 2: KUPRAMINE®

Total Cell Count, Total Live Cells and % Total Live Cells compared to the Control for *Microcystis aeruginosa* cultures exposed to KUPRAMINE®. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to KUPRAMINE® at various copper concentrations. (n=3)

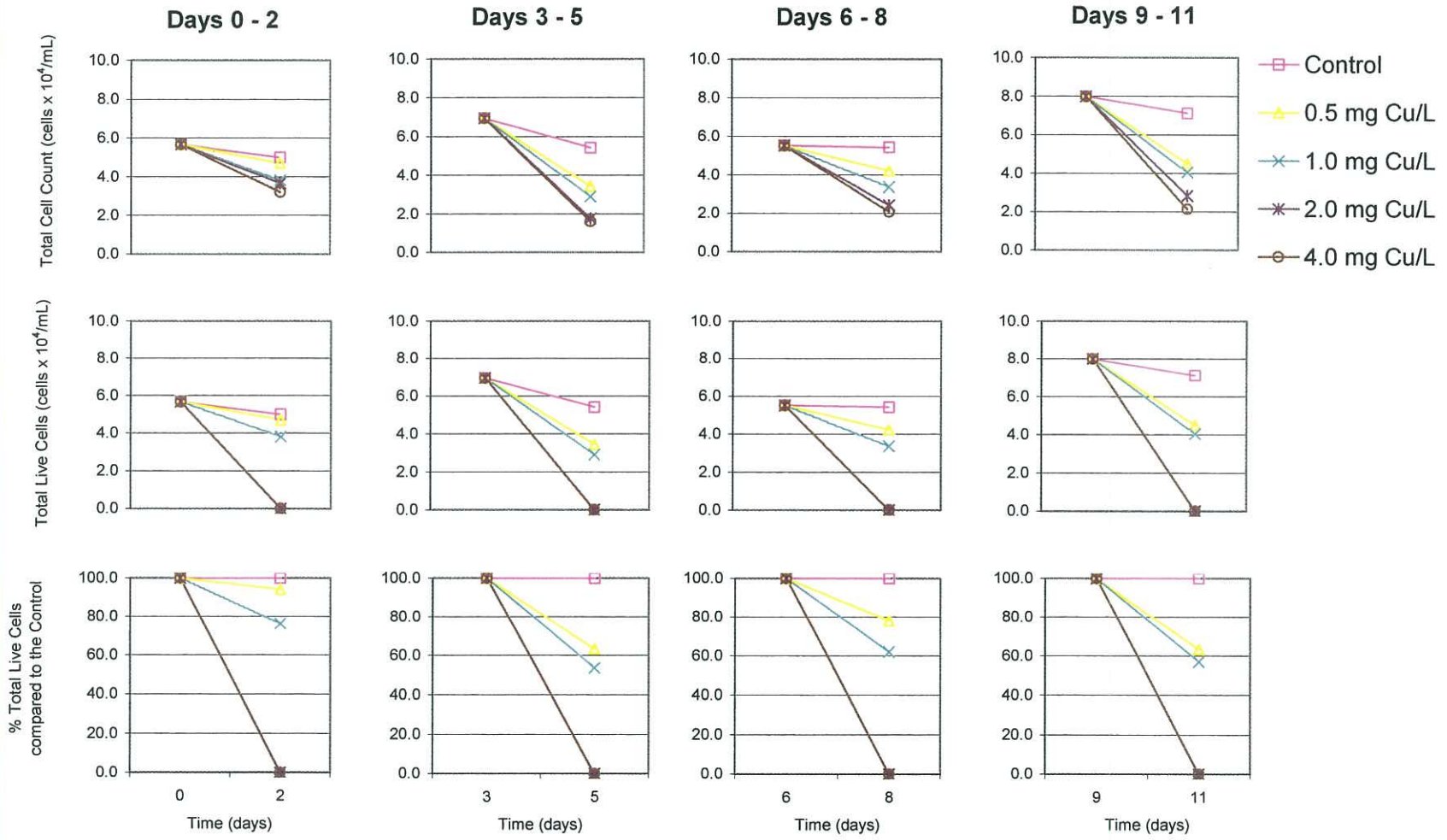

Figure 3: KUPRAMINE 500®

Total Cell Count, Total Live Cells and % Total Live Cells compared to the Control for *Microcystis aeruginosa* cultures exposed to KUPRAMINE 500®. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to KUPRAMINE 500® at various copper concentrations. (n=3)

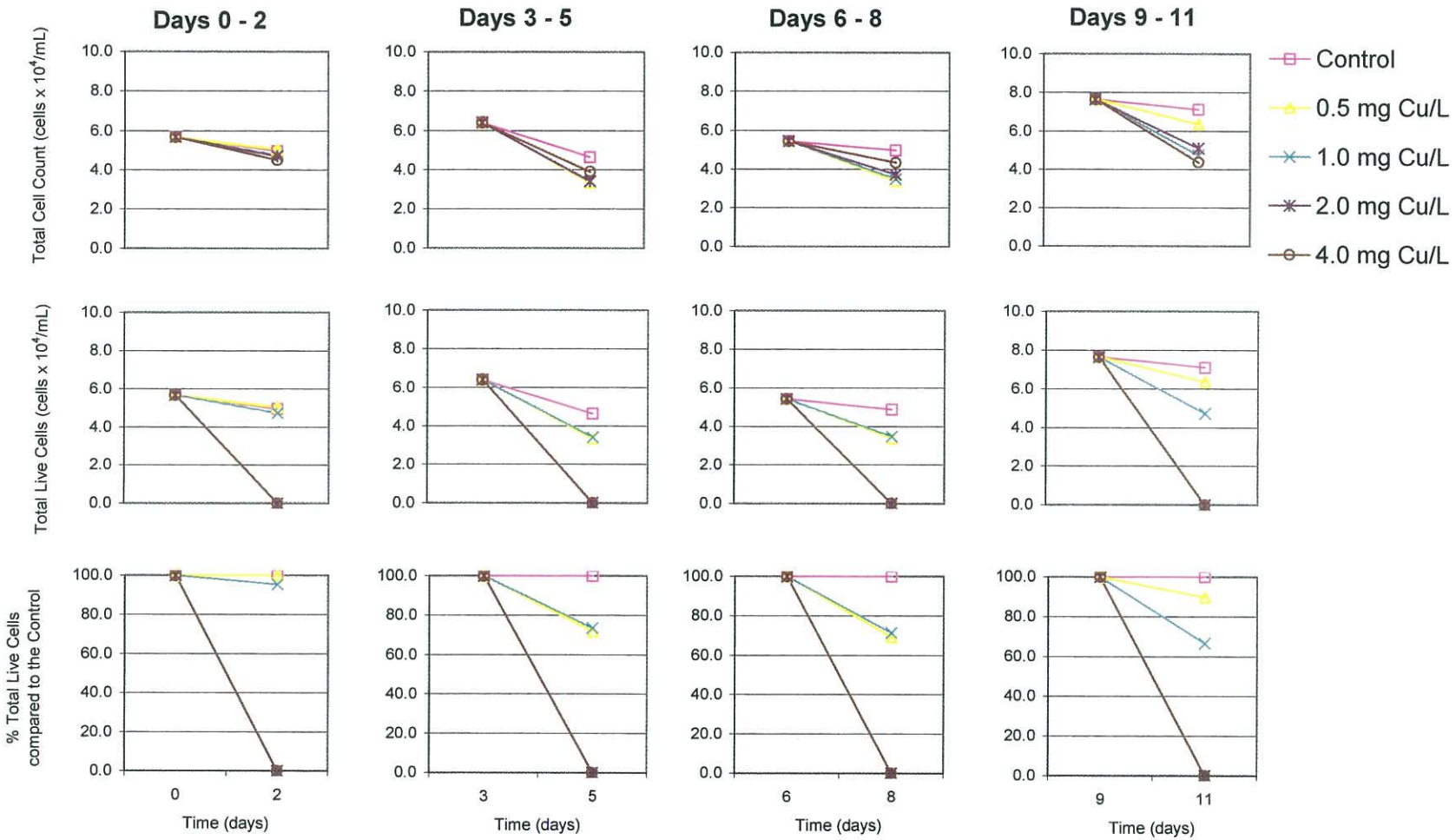

Figure 4: Copper Sulphate

Total Cell Count, Total Live Cells and % Total Live Cells compared to the Control for *Microcystis aeruginosa* cultures exposed to Copper Sulphate. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to Copper Sulphate at various copper concentrations. (n=3)

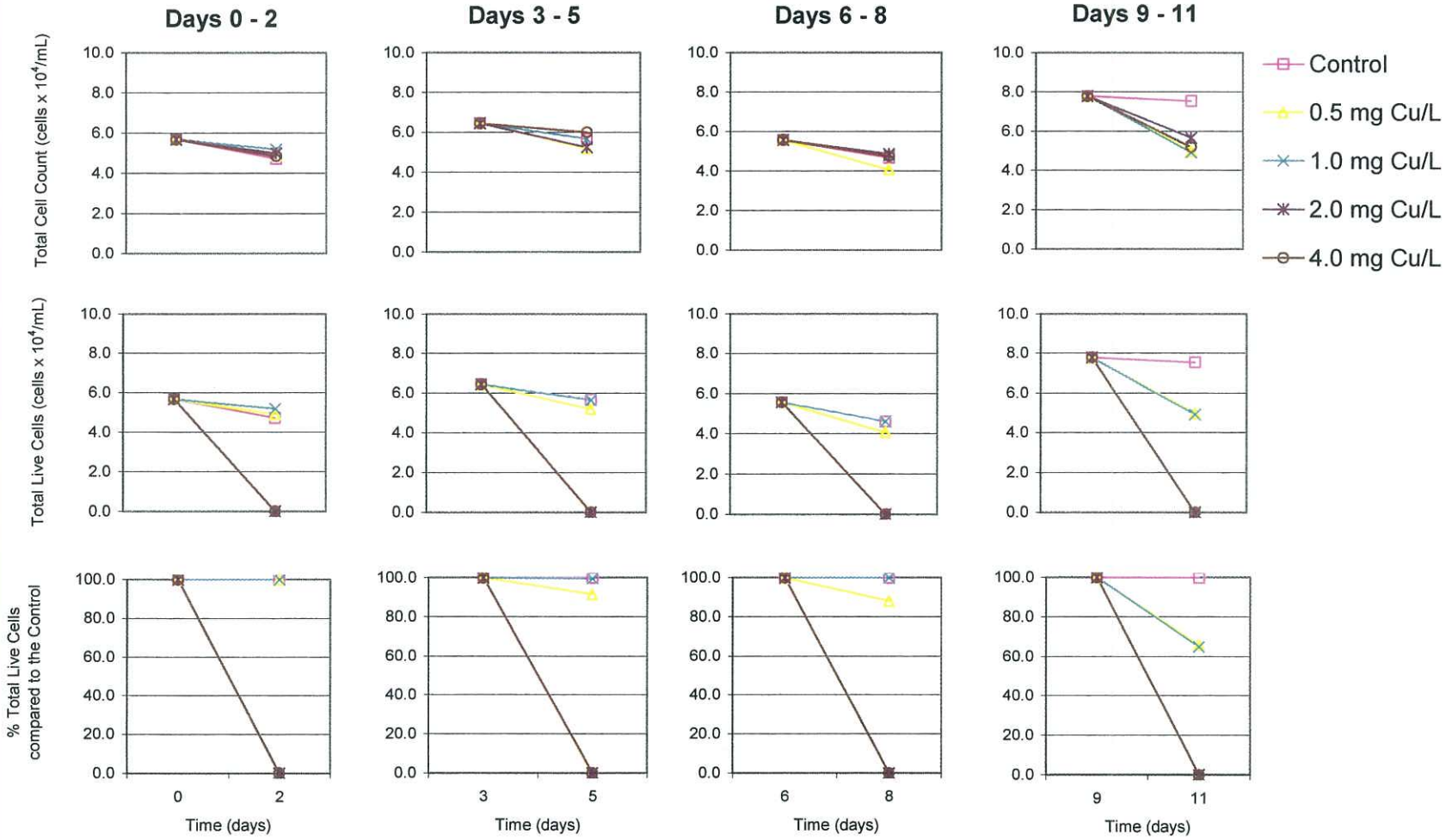

Figure 5:

pH of *Microcystis aeruginosa* cultures exposed to the four copper based algicides. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to each of the algicides at various copper concentrations. (n=3)

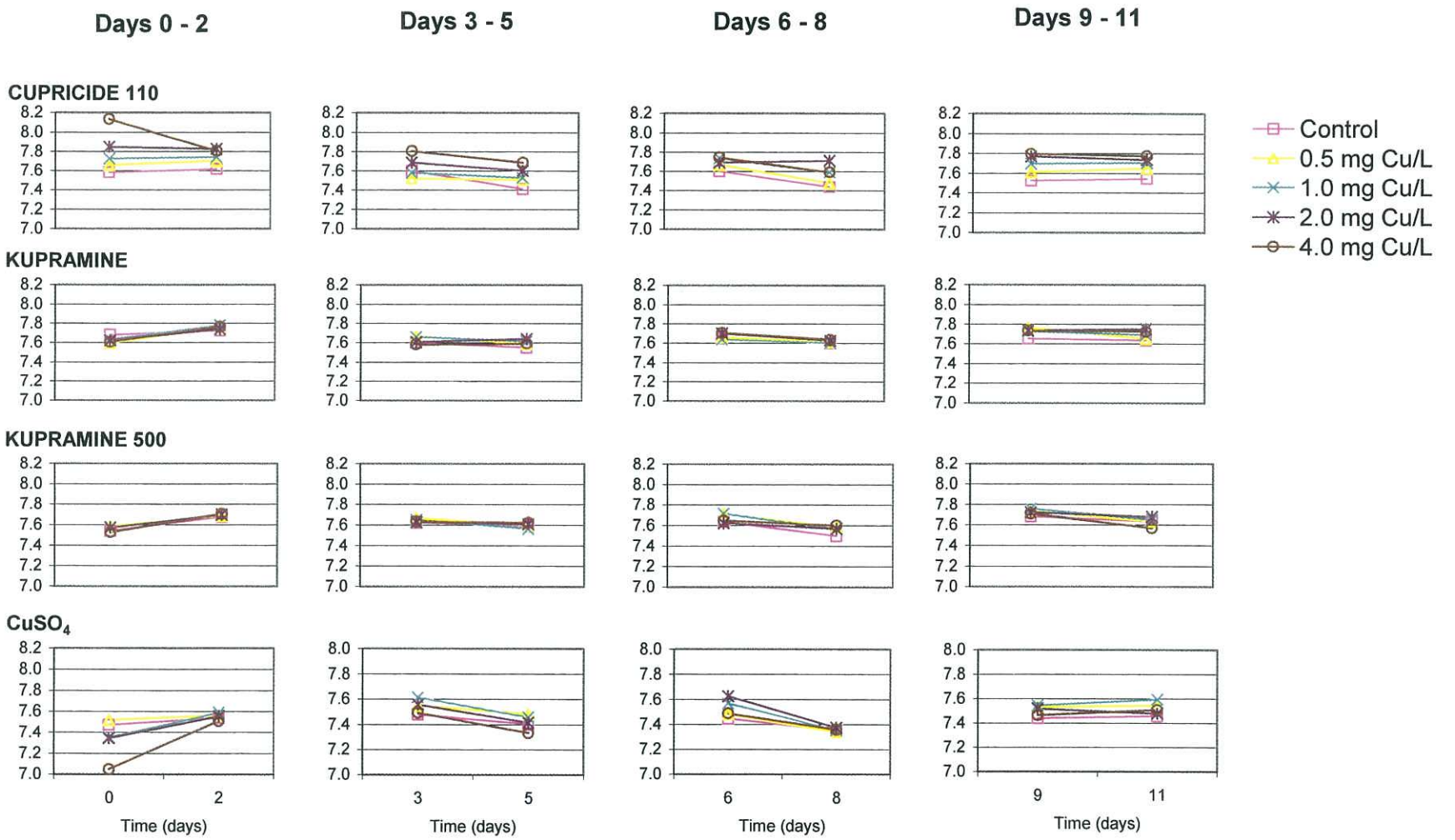

Table 1

Total copper concentrations for test cultures on day 0.

Algicide	Expected Copper Concentration	Actual Copper Concentration
CUPRICIDE 110®	0.5	0.569
	1.0	1.03
	2.0	2.00
	4.0	3.86
KUPRAMINE®	0.5	0.596
	1.0	1.09
	2.0	2.16
	4.0	4.07
KUPRAMINE 500®	0.5	0.599
	1.0	1.12
	2.0	2.19
	4.0	4.12
Copper Sulphate	0.5	0.581
	1.0	1.06
	2.0	2.07
	4.0	3.89

Table 2

Water Chemistry Results

Analysis	Result
Dissolved Organic Carbon (DOC)	11.7 mg/L
Total Dissolved Solids	310 mg/L
Total Organic Carbon	12.2 mg/L
Conductivity	560 µS/cm
Calcium	18.9 mg/L
Magnesium	13.6 mg/L
Potassium	6.6 mg/L
Sodium	62.0 mg/L
Bicarbonate	67 mg/L
Carbonate	0 mg/L
Chloride	127 mg/L
Sulphate	18.4 mg/L
Alkalinity as calcium carbonate	55 mg/L
Ion Balance	-1.32 %

Table 3

Copper concentrations for test algicide stock solutions.

Algicide	Copper Concentration (mg Cu/L)
CUPRICIDE 110®	99.2
KUPRAMINE®	105.0
KUPRAMINE 500®	101.0
Copper Sulphate	96.5

Results for controls showed that cultures were not in exponential growth phase but dropped slightly in number over the 2-day exposure period. This was consistent for each of the four exposure periods, 0 –2 days, 3 – 5 days, 6 – 8 days and 9 – 11 days. This trend was observed for the controls of all four algicides tested. This may have been due to the fact that inoculum cultures were grown in ASM-1 medium under optimum nutrient conditions. When added to the Myponga Reservoir water a long lag time resulted, along with some cell loss, as cultures became accustomed to the new medium. However, a comparison between controls and copper treatments is still possible.

Copper sulphate appeared to be the least effective algicide (Figure 4). There was no difference in % Total Live Cells compared to the Control between cultures grown in 0.5 and 1.0 mg Cu/L for the first three exposure periods ie. 0 – 2 days, 3 – 5 days and 6 - 8 days. For the 9 – 11 days exposure period a reduction in % Total Live Cells compared to the Control was observed for 0.5 and 1.0 mg Cu/L.

CUPRICIDE 110® had slightly better algicidal properties than copper sulphate (Figure 1). % Total Live Cells compared to the Control showed a similar trend to copper sulphate with no difference observed between cultures grown in copper concentrations of 0.5 and 1.0 mg Cu/L for exposure periods 0 – 2 days and 3 – 5 days. However, at 6 – 8 days a slight reduction in % Total Live Cells compared to the Control for cultures grown at 0.5 and 1.0 mg Cu/L was observed. This became more pronounced for the growth period 9 – 11 days.

KUPRAMINE 500® was the next most effective algicide (Figure 3). No reduction in % Total Live Cells compared to the Control was observed for cultures grown at copper concentrations of 0.5 and 1.0 mg Cu/L for the first exposure period, 0 – 2 days. At days 3 – 5, 6 – 8 and 9 – 11 a reduction in % Total Live Cells compared to the Control was measured for cultures grown at 0.5 and 1.0 mg Cu/L.

The most effective algicide appears to be KUPRAMINE® (Figure 2). A reduction in % Total Live Cells compared to the Control was observed for all four exposure periods at concentrations ≥ 0.5 mg Cu/L.

No Live Cells were measured at copper concentrations ≥ 2.0 mg Cu/L for all four algicides at all four exposure periods. Therefore, the MLD_{100} for all four algicides was 2.0 mg Cu/L. While this appears to be relatively high, the actual effective value may be lower. This is due to the large gap between the test concentrations of 1.0 and 2.0 mg Cu/L. The actual MLD_{100} would lie somewhere between 1.0 and 2.0 mg Cu/L. In the case of algicides such as KUPRAMINE® and KUPRAMINE 500® this may be very close to 1.0 mg Cu/L due to the strong activity shown by these algicides at copper concentrations of 0.5 and 1.0 mg Cu/L.

It is interesting to note that the algicidal activity of Copper Sulphate, CUPRICIDE 110® and KUPRAMINE 500® increased over the exposure periods. The algicidal activity was expected to reduce over time. When cells are removed after each exposure period some copper would be lost due to binding of copper to cellular material. It is possible that the filtering process has in someway removed organic ligands that could have been affecting the Cu^{2+} activity. This is

especially true for Copper Sulphate and CUPRICIDE 110®. After these organic ligands are removed the algicidal properties of Cu^{2+} return. This same effect was not seen in the KUPRAMINE® treatment, which showed algicidal activity for all four growth periods.

The pH of cultures were shown to be high with values ranging from 7.4 to 7.8. They remained relatively constant throughout the exposure periods. However, a large increase in pH from 7 to 7.6 was recorded for cultures exposed to copper sulphate at 4.0 mg Cu/L during the 0 – 2 days period. A large decrease in pH was also recorded during the 0 – 2 days period for cultures grown at 4.0 mg Cu/L when CUPRICIDE 110® was used as the algicide.

In summary, the testing showed that KUPRAMINE® was the most effective against *Microcystis aeruginosa* with an MLD_{100} of 2.0 mg Cu/L. While all algicides also showed an MLD_{100} of 2.0 mg Cu/L, KUPRAMINE® had the strongest algicidal activity at copper concentrations of ≥ 0.5 mg Cu/L. This algicide also had the best residual activity compared to the other three with algicidal properties at ≥ 0.5 mg Cu/L for all four exposure periods.

The differences shown between the toxicity of the algicides tested may be due to the water chemistry conditions of Myponga Reservoir. This could be either due to the relatively high pH (7.4 – 7.8) or DOC (11.7 mg/L). Inorganic ligands such as OH^-, HCO_3^-, CO_3^{2-} and SO_4^{2-} combine with Cu^{2+} ions to form soluble inorganic complexes. As pH rises the amount of free Cu^{2+} decreases as inorganic complexes are produced. The high DOC levels could also result in formation of organic complexes with ionic Cu^{2+}. This is particularly true for Copper Sulphate. However, the use of copper in a complexed form should largely avoid the rapid formation of inorganic and organic copper complexes in alkaline and humic rich natural water and lead to greater expression and retention of the algicidal activity under these conditions.

Total Cell Count, Total Live Cells, %Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to CUPRICIDE 110®. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to CUPRICIDE 110® at various copper concentrations. Includes standard deviation (s.d.), n=3.

Total Cell Count (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	5.18	0.21	5.16	0.45	5.37	0.66	5.38	0.69	5.24	0.76
3	5.98	0.27	5.98	0.27	5.98	0.27	5.98	0.27	5.98	0.27
5	5.37	0.27	5.24	0.54	5.55	0.29	4.87	0.23	4.84	0.50
6	5.48	0.26	5.48	0.26	5.48	0.26	5.48	0.26	5.48	0.26
8	5.03	0.26	3.66	0.20	4.54	0.17	4.53	0.07	4.30	0.31
9	7.89	0.40	7.89	0.40	7.89	0.40	7.89	0.40	7.89	0.40
11	7.33	0.31	5.41	0.60	4.73	0.30	4.94	0.30	4.25	0.49

Total Live Cells (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	5.18	0.39	5.16	0.45	0.00	0.00	0.00	0.00	0.00	0.00
3	5.98	0.27	5.98	0.27	5.98	0.27	5.98	0.27	5.98	0.27
5	5.37	0.55	5.24	0.54	5.55	0.29	0.00	0.00	0.00	0.00
6	5.48	0.26	5.48	0.26	5.48	0.26	5.48	0.26	5.48	0.26
8	5.03	0.18	3.66	0.20	4.54	0.17	0.00	0.00	0.00	0.00
9	7.89	0.40	7.89	0.40	7.89	0.40	7.89	0.40	7.89	0.40
11	7.33	0.31	5.41	0.60	4.73	0.30	0.00	0.00	0.00	0.00

%Total Live Cells compared to the Control

Time (day)	Copper Concentration (mg Cu/L)				
	Control	0.5	1.0	2.0	4.0
0	100.00	100.00	100.00	100.00	100.00
2	100.00	99.61	0.00	0.00	0.00
3	100.00	100.00	100.00	100.00	100.00
5	100.00	100.00	97.70	0.00	0.00
6	100.00	100.00	100.00	100.00	100.00
8	100.00	72.90	90.41	0.00	0.00
9	100.00	100.00	100.00	100.00	100.00
11	100.00	73.85	64.57	0.00	0.00

pH

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	7.58	0.05	7.66	0.04	7.73	0.03	7.85	0.06	8.13	0.08
2	7.62	0.07	7.70	0.07	7.74	0.07	7.83	0.07	7.81	0.06
3	7.60	0.06	7.52	0.09	7.58	0.02	7.69	0.10	7.81	0.04
5	7.41	0.06	7.51	0.03	7.53	0.03	7.60	0.03	7.69	0.03
6	7.60	0.10	7.67	0.04	7.74	0.02	7.69	0.05	7.75	0.05
8	7.44	0.05	7.48	0.05	7.59	0.02	7.71	0.03	7.59	0.12
9	7.53	0.03	7.61	0.03	7.70	0.04	7.77	0.04	7.80	0.02
11	7.55	0.03	7.65	0.04	7.71	0.01	7.74	0.02	7.78	0.02

Total Cell Count, Total Live Cells, %Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE 500®. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to KUPRAMINE 500® at various copper concentrations. Includes standard deviation (s.d.), n=3.

Total Cell Count (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	4.96	0.21	5.05	0.48	4.74	0.57	4.70	0.60	4.51	0.27
3	6.41	0.62	6.41	0.62	6.41	0.62	6.41	0.62	6.41	0.62
5	4.65	0.62	3.35	0.20	3.43	0.32	3.41	0.44	3.92	0.05
6	5.44	0.21	5.44	0.21	5.44	0.21	5.44	0.21	5.44	0.21
8	4.97	0.21	3.38	0.40	3.49	0.15	3.71	0.19	4.33	0.18
9	7.64	0.65	7.64	0.65	7.64	0.65	7.64	0.65	7.64	0.65
11	7.11	0.65	6.38	1.49	4.74	0.23	5.11	0.78	4.38	0.19

Total Live Cells (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	4.96	0.61	5.05	0.48	4.74	0.57	0.00	0.00	0.00	0.00
3	6.41	0.62	6.41	0.62	6.41	0.62	6.41	0.62	6.41	0.62
5	4.65	0.27	3.35	0.20	3.43	0.32	0.00	0.00	0.00	0.00
6	5.44	0.21	5.44	0.21	5.44	0.21	5.44	0.21	5.44	0.21
8	4.89	0.28	3.38	0.40	3.49	0.15	0.00	0.00	0.00	0.00
9	7.64	0.65	7.64	0.65	7.64	0.65	7.64	0.65	7.64	0.65
11	7.11	0.93	6.38	1.49	4.74	0.23	0.00	0.00	0.00	0.00

%Total Live Cells compared to the Control

Time (day)	Copper Concentration (mg Cu/L)				
	Control	0.5	1.0	2.0	4.0
0	100.00	100.00	100.00	100.00	100.00
2	100.00	100.00	95.50	0.00	0.00
3	100.00	100.00	100.00	100.00	100.00
5	100.00	71.92	73.71	0.00	0.00
6	100.00	100.00	100.00	100.00	100.00
8	100.00	69.24	71.35	0.00	0.00
9	100.00	100.00	100.00	100.00	100.00
11	100.00	89.78	66.62	0.00	0.00

pH

Time (day)	Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	7.54	0.04	7.59	0.03	7.57	0.05	7.57	0.03	7.53	0.07
2	7.68	0.07	7.69	0.09	7.70	0.06	7.70	0.05	7.71	0.09
3	7.62	0.03	7.67	0.03	7.65	0.01	7.63	0.03	7.64	0.06
5	7.59	0.04	7.60	0.04	7.57	0.04	7.61	0.02	7.62	0.05
6	7.64	0.03	7.71	0.06	7.72	0.07	7.62	0.02	7.65	0.03
8	7.50	0.04	7.59	0.04	7.57	0.02	7.57	0.03	7.60	0.03
9	7.69	0.03	7.74	0.01	7.76	0.02	7.72	0.02	7.71	0.03
11	7.64	0.02	7.64	0.04	7.65	0.03	7.68	0.03	7.57	0.02

Total Cell Count, Total Live Cells, %Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to Copper Sulphate. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to Copper Sulphate at various copper concentrations. Includes standard deviation (s.d.), n=3.

Total Cell Count (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	4.72	0.21	4.90	0.23	5.19	0.15	5.00	0.06	4.86	0.14
3	6.45	0.67	6.45	0.67	6.45	0.67	6.45	0.67	6.45	0.67
5	5.68	0.67	5.19	0.27	5.66	0.40	5.26	0.13	6.00	0.33
6	5.57	0.11	5.57	0.11	5.57	0.11	5.57	0.11	5.57	0.11
8	4.67	0.11	4.07	0.26	4.76	0.34	4.86	0.34	4.77	0.33
9	7.79	0.96	7.79	0.96	7.79	0.96	7.79	0.96	7.79	0.96
11	7.55	0.96	4.98	0.54	4.91	0.31	5.67	0.22	5.20	0.16

Total Live Cells (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	4.72	0.48	4.90	0.23	5.19	0.15	0.00	0.00	0.00	0.00
3	6.45	0.67	6.45	0.67	6.45	0.67	6.45	0.67	6.45	0.67
5	5.68	0.45	5.19	0.27	5.66	0.40	0.00	0.00	0.00	0.00
6	5.57	0.11	5.57	0.11	5.57	0.11	5.57	0.11	5.57	0.11
8	4.62	0.41	4.07	0.26	4.62	0.33	0.00	0.00	0.00	0.00
9	7.79	0.96	7.79	0.96	7.79	0.96	7.79	0.96	7.79	0.96
11	7.55	1.33	4.98	0.54	4.91	0.31	0.00	0.00	0.00	0.00

%Total Live Cells compared to the Control

Time (day)	Copper Concentration (mg Cu/L)				
	Control	0.5	1.0	2.0	4.0
0	100.00	100.00	100.00	100.00	100.00
2	100.00	100.00	100.00	0.00	0.00
3	100.00	100.00	100.00	100.00	100.00
5	100.00	91.43	99.65	0.00	0.00
6	100.00	100.00	100.00	100.00	100.00
8	100.00	88.10	100.00	0.00	0.00
9	100.00	100.00	100.00	100.00	100.00
11	100.00	65.98	65.05	0.00	0.00

pH

Time (day)	Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	7.47	0.04	7.52	0.03	7.35	0.02	7.35	0.04	7.05	0.07
2	7.54	0.06	7.56	0.06	7.59	0.06	7.56	0.05	7.51	0.03
3	7.48	0.04	7.55	0.05	7.62	0.03	7.56	0.02	7.49	0.02
5	7.40	0.03	7.48	0.05	7.46	0.03	7.42	0.01	7.33	0.02
6	7.45	0.02	7.49	0.07	7.57	0.03	7.63	0.06	7.49	0.04
8	7.35	0.05	7.34	0.03	7.36	0.07	7.38	0.02	7.36	0.03
9	7.44	0.06	7.53	0.04	7.55	0.02	7.52	0.04	7.47	0.01
11	7.46	0.04	7.55	0.05	7.60	0.02	7.48	0.06	7.51	0.04

Total Cell Count, Total Live Cells, %Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE®. Cultures were added at days 0, 3, 6 and 9 and exposed for 2 days to KUPRAMINE® at various copper concentrations. Includes standard deviation (s.d.), n=3.

Total Cell Count (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	5.01	0.21	4.72	0.25	3.82	0.47	3.67	0.38	3.22	0.56
3	6.95	0.72	6.95	0.72	6.95	0.72	6.95	0.72	6.95	0.72
5	5.43	0.72	3.45	0.10	2.92	0.15	1.76	0.12	1.61	0.12
6	5.52	0.54	5.52	0.54	5.52	0.54	5.52	0.54	5.52	0.54
8	5.44	0.54	4.24	0.49	3.37	0.45	2.43	0.29	2.08	0.22
9	7.99	0.06	7.99	0.06	7.99	0.06	7.99	0.06	7.99	0.06
11	7.13	0.06	4.51	0.34	4.06	0.01	2.84	0.29	2.15	0.18

Total Live Cells (cells x 10^4/mL)

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21	5.68	0.21
2	5.01	0.23	4.72	0.25	3.82	0.47	0.00	0.00	0.00	0.00
3	6.95	0.72	6.95	0.72	6.95	0.72	6.95	0.72	6.95	0.72
5	5.43	0.44	3.45	0.10	2.92	0.15	0.00	0.00	0.00	0.00
6	5.52	0.54	5.52	0.54	5.52	0.54	5.52	0.54	5.52	0.54
8	5.44	0.49	4.24	0.49	3.37	0.45	0.00	0.00	0.00	0.00
9	7.99	0.06	7.99	0.06	7.99	0.06	7.99	0.06	7.99	0.06
11	7.13	0.43	4.51	0.34	4.06	0.01	0.00	0.00	0.00	0.00

%Total Live Cells compared to the Control

Time (day)	Copper Concentration (mg Cu/L)				
	Control	0.5	1.0	2.0	4.0
0	100.00	100.00	100.00	100.00	100.00
2	100.00	94.15	76.20	0.00	0.00
3	100.00	100.00	100.00	100.00	100.00
5	100.00	63.56	53.68	0.00	0.00
6	100.00	100.00	100.00	100.00	100.00
8	100.00	78.05	61.99	0.00	0.00
9	100.00	100.00	100.00	100.00	100.00
11	100.00	63.25	56.94	0.00	0.00

pH

Time (day)	Copper Concentration (mg Cu/L)									
	Control	s.d.	0.5	s.d.	1.0	s.d.	2.0	s.d.	4.0	s.d.
0	7.68	0.05	7.60	0.05	7.63	0.04	7.62	0.04	7.61	0.05
2	7.73	0.09	7.76	0.06	7.78	0.07	7.74	0.06	7.77	0.03
3	7.60	0.04	7.67	0.02	7.66	0.03	7.61	0.02	7.58	0.04
5	7.55	0.06	7.59	0.01	7.62	0.03	7.64	0.01	7.59	0.02
6	7.65	0.01	7.66	0.04	7.64	0.07	7.70	0.03	7.71	0.03
8	7.60	0.02	7.61	0.02	7.60	0.03	7.63	0.04	7.64	0.03
9	7.66	0.03	7.77	0.03	7.73	0.02	7.74	0.02	7.74	0.02
11	7.64	0.04	7.66	0.03	7.69	0.01	7.75	0.04	7.73	0.04

Total Cell Count, Total Live Cells, % Living Cells and % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to Copper Sulphate from day 0 to 2 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	% Total Live Cells compared to the Control	pH	mean	s.d.
Day 0	0	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.43	7.47	0.04
	0	5.44			5.44					7.48		
	0	5.83			5.83					7.51		
	0.5	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.49	7.52	0.03
	0.5	5.44			5.44					7.54		
	0.5	5.83			5.83					7.53		
	1	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.37	7.35	0.02
	1	5.44			5.44					7.34		
	1	5.83			5.83					7.35		
	2	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.39	7.35	0.04
	2	5.44			5.44					7.34		
	2	5.83			5.83					7.31		
	4	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.13	7.05	0.07
	4	5.44			5.44					7.02		
	4	5.83			5.83					6.99		
Day 2	0	5.24	4.72	0.48	5.24	4.72	0.48	100.00	100.00	7.48	7.54	0.06
	0	4.61			4.61					7.55		
	0	4.30			4.30					7.59		
	0.5	5.04	4.90	0.23	5.04	4.90	0.23	100.00	100.00	7.49	7.56	0.06
	0.5	5.03			5.03					7.59		
	0.5	4.64			4.64					7.61		
	1	5.26	5.19	0.15	5.26	5.19	0.15	100.00	100.00	7.54	7.59	0.06
	1	5.01			5.01					7.58		
	1	5.29			5.29					7.65		
	2	5.01	5.00	0.06	0.00	0.00	0.00	0.00	0.00	7.50	7.56	0.05
	2	5.05			0.00					7.58		
	2	4.94			0.00					7.59		
	4	4.74	4.86	0.14	0.00	0.00	0.00	0.00	0.00	7.48	7.51	0.03
	4	4.83			0.00					7.52		
	4	5.01			0.00					7.54		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to Copper Sulphate from day 3 to 5 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 3	0	5.91	6.45	0.67	5.91	6.45	0.67	100.00	100.00	7.46	7.48	0.04
	0	6.23			6.23					7.45		
	0	7.20			7.20					7.53		
	0.5	5.91	6.45	0.67	5.91	6.45	0.67	100.00	100.00	7.53	7.55	0.05
	0.5	6.23			6.23					7.51		
	0.5	7.20			7.20					7.61		
	1	5.91	6.45	0.67	5.91	6.45	0.67	100.00	100.00	7.60	7.62	0.03
	1	6.23			6.23					7.65		
	1	7.20			7.20					7.60		
	2	5.91	6.45	0.67	5.91	6.45	0.67	100.00	100.0	7.58	7.56	0.02
	2	6.23			6.23					7.56		
	2	7.20			7.20					7.54		
	4	5.91	6.45	0.67	5.91	6.45	0.67	100.00	100.00	7.48	7.49	0.02
	4	6.23			6.23					7.49		
	4	7.20			7.20					7.51		
Day 5	0	6.19	5.68	0.45	6.19	5.68	0.45	100.00	100.00	7.44	7.40	0.03
	0	5.34			5.34					7.39		
	0	5.50			5.50					7.38		
	0.5	5.35	5.19	0.27	5.35	5.19	0.27	100.00	91.43	7.43	7.48	0.05
	0.5	5.34			5.34					7.51		
	0.5	4.88			4.88					7.51		
	1	5.99	5.66	0.40	5.99	5.66	0.40	100.00	99.65	7.43	7.46	0.03
	1	5.77			5.77					7.49		
	1	5.21			5.21					7.46		
	2	5.19	5.26	0.13	0.00	0.00	0.00	0.00	0.00	7.41	7.42	0.01
	2	5.41			0.00					7.43		
	2	5.18			0.00					7.41		
	4	5.91	6.00	0.33	0.00	0.00	0.00	0.00	0.00	7.33	7.33	0.02
	4	6.37			0.00					7.35		
	4	5.73			0.00					7.32		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to Copper Sulphate from day 6 to 8 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 6	0	5.44	5.57	0.11	5.44	5.57	0.11	100.00	100.00	7.45	7.45	0.02
	0	5.64			5.64					7.43		
	0	5.63			5.63					7.47		
	0.5	5.44	5.57	0.11	5.44	5.57	0.11	100.00	100.00	7.43	7.49	0.07
	0.5	5.64			5.64					7.46		
	0.5	5.63			5.63					7.57		
	1	5.44	5.57	0.11	5.44	5.57	0.11	100.00	100.00	7.58	7.57	0.03
	1	5.64			5.64					7.54		
	1	5.63			5.63					7.59		
	2	5.44	5.57	0.11	5.44	5.57	0.11	100.00	100.00	7.56	7.63	0.06
	2	5.64			5.64					7.67		
	2	5.63			5.63					7.65		
	4	5.44	5.57	0.11	5.44	5.57	0.11	100.00	100.00	7.45	7.49	0.04
	4	5.64			5.64					7.53		
	4	5.63			5.63					7.49		
Day 8	0	4.74	4.67	0.42	4.69	4.62	0.41	99.00	100.00	7.40	7.35	0.05
	0	4.22			4.18					7.31		
	0	5.05			5.00					7.35		
	0.5	4.02	4.07	0.26	4.02	4.07	0.26	100.00	88.10	7.35	7.34	0.03
	0.5	4.36			4.36					7.31		
	0.5	3.84			3.84					7.36		
	1	4.41	4.76	0.34	4.28	4.62	0.33	97.12	100.00	7.28	7.36	0.07
	1	5.09			4.94					7.39		
	1	4.79			4.65					7.40		
	2	5.20	4.86	0.34	0.00	0.00	0.00	0.00	0.00	7.39	7.38	0.02
	2	4.86			0.00					7.38		
	2	4.53			0.00					7.36		
	4	4.71	4.77	0.33	0.00	0.00	0.00	0.00	0.00	7.34	7.36	0.03
	4	4.47			0.00					7.36		
	4	5.13			0.00					7.39		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to Copper Sulphate from day 9 to 11 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 9	0	8.58	7.79	0.96	8.58	7.79	0.96	100.00	100.00	7.45	7.44	0.06
	0	6.72			6.72					7.38		
	0	8.06			8.06					7.50		
	0.5	8.58	7.79	0.96	8.58	7.79	0.96	100.00	100.00	7.49	7.53	0.04
	0.5	6.72			6.72					7.55		
	0.5	8.06			8.06					7.56		
	1	8.58	7.79	0.96	8.58	7.79	0.96	100.00	100.00	7.55	7.55	0.02
	1	6.72			6.72					7.56		
	1	8.06			8.06					7.53		
	2	8.58	7.79	0.96	8.58	7.79	0.96	100.00	100.00	7.55	7.52	0.04
	2	6.72			6.72					7.48		
	2	8.06			8.06					7.53		
	4	8.58	7.79	0.96	8.58	7.79	0.96	100.00	100.00	7.46	7.47	0.01
	4	6.72			6.72					7.48		
	4	8.06			8.06					7.46		
Day 11	0	9.08	7.55	1.33	9.08	7.55	1.33	100.00	100.00	7.43	7.46	0.04
	0	6.93			6.93					7.45		
	0	6.65			6.65					7.50		
	0.5	4.37	4.98	0.54	4.37	4.98	0.54	100.00	65.98	7.49	7.55	0.05
	0.5	5.20			5.20					7.56		
	0.5	5.38			5.38					7.59		
	1	5.26	4.91	0.31	5.26	4.91	0.31	100.00	65.05	7.58	7.60	0.02
	1	4.66			4.66					7.60		
	1	4.82			4.82					7.61		
	2	5.79	5.67	0.22	0.00	0.00	0.00	0.00	0.00	7.41	7.48	0.06
	2	5.41			0.00					7.50		
	2	5.80			0.00					7.53		
	4	5.35	5.20	0.16	0.00	0.00	0.00	0.00	0.00	7.47	7.51	0.04
	4	5.04			0.00					7.51		
	4	5.20			0.00					7.55		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to CUPRICIDE 110® from day 0 to 2 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 0	0	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.53	7.58	0.05
	0	5.44			5.44					7.60		
	0	5.83			5.83					7.62		
	0.5	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.63	7.66	0.04
	0.5	5.44			5.44					7.65		
	0.5	5.83			5.83					7.70		
	1	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.70	7.73	0.03
	1	5.44			5.44					7.73		
	1	5.83			5.83					7.75		
	2	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.79	7.85	0.06
	2	5.44			5.44					7.85		
	2	5.83			5.83					7.90		
	4	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	8.05	8.13	0.08
	4	5.44			5.44					8.15		
	4	5.83			5.83					8.20		
Day 2	0	5.19	5.18	0.39	5.19	5.18	0.39	100.00	100.00	7.54	7.62	0.07
	0	5.56			5.56					7.67		
	0	4.79			4.79					7.65		
	0.5	5.25	5.16	0.45	5.25	5.16	0.45	100.00	99.61	7.63	7.70	0.07
	0.5	4.67			4.67					7.72		
	0.5	5.56			5.56					7.76		
	1	4.82	5.37	0.66	0.00	0.00	0.00	0.00	0.00	7.67	7.74	0.07
	1	5.20			0.00					7.75		
	1	6.10			0.00					7.81		
	2	4.80	5.38	0.69	0.00	0.00	0.00	0.00	0.00	7.75	7.83	0.07
	2	6.14			0.00					7.88		
	2	5.21			0.00					7.85		
	4	4.68	5.24	0.76	0.00	0.00	0.00	0.00	0.00	7.74	7.81	0.06
	4	4.93			0.00					7.83		
	4	6.10			0.00					7.85		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to CUPRICIDE 110® from day 3 to 5 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 3	0	5.80	5.98	0.27	5.80	5.98	0.27	100.00	100.00	7.54	7.60	0.06
	0	6.29			6.29					7.60		
	0	5.86			5.86					7.66		
	0.5	5.80	5.98	0.27	5.80	5.98	0.27	100.00	100.00	7.51	7.52	0.09
	0.5	6.29			6.29					7.44		
	0.5	5.86			5.86					7.62		
	1	5.80	5.98	0.27	5.80	5.98	0.27	100.00	100.00	7.58	7.58	0.02
	1	6.29			6.29					7.56		
	1	5.86			5.86					7.60		
	2	5.80	5.98	0.27	5.80	5.98	0.27	100.00	100.00	7.59	7.69	0.10
	2	6.29			6.29					7.68		
	2	5.86			5.86					7.79		
	4	5.80	5.98	0.27	5.80	5.98	0.27	100.00	100.00	7.76	7.81	0.04
	4	6.29			6.29					7.83		
	4	5.86			5.86					7.83		
Day 5	0	5.99	5.37	0.55	5.99	5.37	0.55	100.00	100.00	7.35	7.41	0.06
	0	5.13			5.13					7.42		
	0	4.98			4.98					7.46		
	0.5	5.26	5.24	0.54	5.26	5.24	0.54	100.00	100.00	7.49	7.51	0.03
	0.5	5.77			5.77					7.49		
	0.5	4.70			4.70					7.54		
	1	5.21	5.55	0.29	5.21	5.55	0.29	100.00	97.70	7.50	7.53	0.03
	1	5.73			5.73					7.54		
	1	5.70			5.70					7.55		
	2	5.06	4.87	0.23	0.00	0.00	0.00	0.00	0.00	7.57	7.60	0.03
	2	4.93			0.00					7.63		
	2	4.61			0.00					7.61		
	4	4.28	4.84	0.50	0.00	0.00	0.00	0.00	0.00	7.66	7.69	0.03
	4	5.23			0.00					7.69		
	4	5.01			0.00					7.71		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to CUPRICIDE 110® from day 6 to 8 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 6	0	5.77	5.48	0.26	5.77	5.48	0.26	100.00	100.00	7.50	7.60	0.10
	0	5.40			5.40					7.61		
	0	5.28			5.28					7.70		
	0.5	5.77	5.48	0.26	5.77	5.48	0.26	100.00	100.00	7.62	7.67	0.04
	0.5	5.40			5.40					7.69		
	0.5	5.28			5.28					7.70		
	1	5.77	5.48	0.26	5.77	5.48	0.26	100.00	100.00	7.75	7.74	0.02
	1	5.40			5.40					7.74		
	1	5.28			5.28					7.72		
	2	5.77	5.48	0.26	5.77	5.48	0.26	100.00	100.00	7.64	7.69	0.05
	2	5.40			5.40					7.70		
	2	5.28			5.28					7.74		
	4	5.77	5.48	0.26	5.77	5.48	0.26	100.00	100.00	7.69	7.75	0.05
	4	5.40			5.40					7.76		
	4	5.28			5.28					7.79		
Day 8	0		5.03	0.18		5.03	0.18	100.00	100.00	7.39	7.44	0.05
	0	4.90			4.90					7.45		
	0	5.15			5.15					7.48		
	0.5	3.87	3.66	0.20	3.87	3.66	0.20	100.00	72.90	7.42	7.48	0.05
	0.5	3.47			3.47					7.52		
	0.5	3.65			3.65					7.50		
	1	4.36	4.54	0.17	4.36	4.54	0.17	100.00	90.41	7.57	7.59	0.02
	1	4.57			4.57					7.60		
	1	4.70			4.70					7.61		
	2	4.60	4.53	0.07	0.00	0.00	0.00	0.00	0.00	7.72	7.71	0.03
	2	4.46			0.00					7.74		
	2	4.53			0.00					7.68		
	4	4.30	4.30	0.31	0.00	0.00	0.00	0.00	0.00	7.72	7.59	0.12
	4	4.60			0.00					7.57		
	4	3.99			0.00					7.49		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to CUPRICIDE 110® from day 9 to 11 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 9	0	7.82	7.89	0.40	7.82	7.89	0.40	100.00	100.00	7.49	7.53	0.03
	0	8.32			8.32					7.54		
	0	7.52			7.52					7.55		
	0.5	7.82	7.89	0.40	7.82	7.89	0.40	100.00	100.00	7.59	7.61	0.03
	0.5	8.32			8.32					7.61		
	0.5	7.52			7.52					7.64		
	1	7.82	7.89	0.40	7.82	7.89	0.40	100.00	100.00	7.65	7.70	0.04
	1	8.32			8.32					7.71		
	1	7.52			7.52					7.73		
	2	7.82	7.89	0.40	7.82	7.89	0.40	100.00	100.00	7.73	7.77	0.04
	2	8.32			8.32					7.80		
	2	7.52			7.52					7.78		
	4	7.82	7.89	0.40	7.82	7.89	0.40	100.00	100.00	7.81	7.80	0.02
	4	8.32			8.32					7.80		
	4	7.52			7.52					7.78		
Day 11	0	9.08	7.55	1.33	9.08	7.55	1.33	100.00	100.00	7.51	7.55	0.03
	0	6.93			6.93					7.56		
	0	6.65			6.65					7.57		
	0.5	4.37	4.98	0.54	4.37	4.98	0.54	100.00	65.98	7.62	7.65	0.04
	0.5	5.20			5.20					7.65		
	0.5	5.38			5.38					7.69		
	1	5.26	4.91	0.31	5.26	4.91	0.31	100.00	65.05	7.70	7.71	0.01
	1	4.66			4.66					7.72		
	1	4.82			4.82					7.70		
	2	5.79	5.67	0.22	0.00	0.00	0.00	0.00	0.00	7.74	7.74	0.02
	2	5.41			0.00					7.75		
	2	5.80			0.00					7.72		
	4	5.35	5.20	0.16	0.00	0.00	0.00	0.00	0.00	7.76	7.78	0.02
	4	5.04			0.00					7.77		
	4	5.20			0.00					7.80		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE® from day 0 to 2 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 0	0	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.63	7.68	0.05
	0	5.44			5.44					7.69		
	0	5.83			5.83					7.73		
	0.5	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.56	7.60	0.05
	0.5	5.44			5.44					7.59		
	0.5	5.83			5.83					7.65		
	1	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.59	7.63	0.04
	1	5.44			5.44					7.64		
	1	5.83			5.83					7.67		
	2	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.58	7.62	0.04
	2	5.44			5.44					7.65		
	2	5.83			5.83					7.64		
	4	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.56	7.61	0.05
	4	5.44			5.44					7.61		
	4	5.83			5.83					7.66		
Day 2	0	5.23	5.01	0.23	5.23	5.01	0.23	100.00	100.00	7.63	7.73	0.09
	0	5.03			5.03					7.76		
	0	4.78			4.78					7.79		
	0.5	4.90	4.72	0.25	4.90	4.72	0.25	100.00	94.15	7.70	7.76	0.06
	0.5	4.54			4.54					7.78		
	0.5									7.81		
	1	4.17	3.82	0.47	4.17	3.82	0.47	100.00	76.20	7.70	7.78	0.07
	1	4.00			4.00					7.81		
	1	3.29			3.29					7.83		
	2	3.57	3.67	0.38	0.00	0.00	0.00	0.00	0.00	7.67	7.74	0.06
	2	3.35			0.00					7.77		
	2	4.09			0.00					7.79		
	4	3.77	3.22	0.56	0.00	0.00	0.00	0.00	0.00	7.73	7.77	0.03
	4	3.22			0.00					7.78		
	4	2.66			0.00					7.79		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE® from day 3 to 5 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 3	0	7.63	6.95	0.72	7.63	6.95	0.72	100.00	100.00	7.59	7.60	0.04
	0	6.20			6.20					7.57		
	0	7.02			7.02					7.64		
	0.5	7.63	6.95	0.72	7.63	6.95	0.72	100.00	100.00	7.65	7.67	0.02
	0.5	6.20			6.20					7.67		
	0.5	7.02			7.02					7.69		
	1	7.63	6.95	0.72	7.63	6.95	0.72	100.00	100.00	7.64	7.66	0.03
	1	6.20			6.20					7.70		
	1	7.02			7.02					7.65		
	2	7.63	6.95	0.72	7.63	6.95	0.72	100.00	100.00	7.59	7.61	0.02
	2	6.20			6.20					7.61		
	2	7.02			7.02					7.63		
	4	7.63	6.95	0.72	7.63	6.95	0.72	100.00	100.00	7.55	7.58	0.04
	4	6.20			6.20					7.57		
	4	7.02			7.02					7.63		
Day 5	0	5.25	5.43	0.44	5.25	5.43	0.44	100.00	100.00	7.49	7.55	0.06
	0	5.94			5.94					7.55		
	0	5.11			5.11					7.61		
	0.5	3.46	3.45	0.10	3.46	3.45	0.10	100.00	63.56	7.58	7.59	0.01
	0.5	3.55			3.55					7.59		
	0.5	3.35			3.35					7.60		
	1	2.96	2.92	0.15	2.96	2.92	0.15	100.00	53.68	7.59	7.62	0.03
	1	3.04			3.04					7.65		
	1	2.75			2.75					7.61		
	2	1.90	1.76	0.12	0.00	0.00	0.00	0.00	0.00	7.63	7.64	0.01
	2	1.72			0.00					7.65		
	2	1.67			0.00					7.65		
	4	1.73	1.61	0.12	0.00	0.00	0.00	0.00	0.00	7.59	7.59	0.02
	4	1.50			0.00					7.58		
	4	1.61			0.00					7.61		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE® from day 6 to 8 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 6	0	4.91	5.52	0.54	4.91	5.52	0.54	100.00	100.00	7.64	7.65	0.01
	0	5.91			5.91					7.66		
	0	5.75			5.75					7.66		
	0.5	4.91	5.52	0.54	4.91	5.52	0.54	100.00	100.00	7.71	7.66	0.04
	0.5	5.91			5.91					7.65		
	0.5	5.75			5.75					7.63		
	1	4.91	5.52	0.54	4.91	5.52	0.54	100.00	100.00	7.56	7.64	0.07
	1	5.91			5.91					7.67		
	1	5.75			5.75					7.69		
	2	4.91	5.52	0.54	4.91	5.52	0.54	100.00	100.00	7.69	7.70	0.03
	2	5.91			5.91					7.68		
	2	5.75			5.75					7.74		
	4	4.91	5.52	0.54	4.91	5.52	0.54	100.00	100.00	7.68	7.71	0.03
	4	5.91			5.91					7.73		
	4	5.75			5.75					7.72		
Day 8	0	6.00	5.44	0.49	6.00	5.44	0.49	100.00	100.00	7.62	7.60	0.02
	0	5.10			5.10					7.61		
	0	5.21			5.21					7.58		
	0.5	4.70	4.24	0.49	4.70	4.24	0.49	100.00	78.05	7.59	7.61	0.02
	0.5	4.31			4.31					7.62		
	0.5	3.72			3.72					7.63		
	1	3.09	3.37	0.45	3.09	3.37	0.45	100.00	61.99	7.57	7.60	0.03
	1	3.89			3.89					7.63		
	1	3.13			3.13					7.61		
	2	2.69	2.43	0.29	0.00	0.00	0.00	0.00	0.00	7.59	7.63	0.04
	2	2.48			0.00					7.64		
	2	2.12			0.00					7.66		
	4	2.22	2.08	0.22	0.00	0.00	0.00	0.00	0.00	7.66	7.64	0.03
	4	1.83			0.00					7.65		
	4	2.20			0.00					7.61		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE® from day 9 to 11 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 9	0	8.02	7.99	0.06	8.02	7.99	0.06	100.00	100.00	7.63	7.66	0.03
	0	7.92			7.92					7.66		
	0	8.04			8.04					7.68		
	0.5	8.02	7.99	0.06	8.02	7.99	0.06	100.00	100.00	7.79	7.77	0.03
	0.5	7.92			7.92					7.78		
	0.5	8.04			8.04					7.73		
	1	8.02	7.99	0.06	8.02	7.99	0.06	100.00	100.00	7.75	7.73	0.02
	1	7.92			7.92					7.72		
	1	8.04			8.04					7.72		
	2	8.02	7.99	0.06	8.02	7.99	0.06	100.00	100.00	7.75	7.74	0.02
	2	7.92			7.92					7.72		
	2	8.04			8.04					7.75		
	4	8.02	7.99	0.06	8.02	7.99	0.06	100.00	100.00	7.72	7.74	0.02
	4	7.92			7.92					7.75		
	4	8.04			8.04					7.75		
Day 11	0	6.77	7.13	0.43	6.77	7.13	0.43	100.00	100.00	7.60	7.64	0.04
	0	7.60			7.60					7.65		
	0	7.02			7.02					7.67		
	0.5	4.13	4.51	0.34	4.13	4.51	0.34	100.00	63.25	7.62	7.66	0.03
	0.5	4.61			4.61					7.68		
	0.5	4.79			4.79					7.67		
	1		4.06	0.01		4.06	0.01	100.00	56.94	7.68	7.69	0.01
	1	4.05			4.05					7.70		
	1	4.07			4.07					7.70		
	2	2.96	2.84	0.29	0.00	0.00	0.00	0.00	0.00	7.72	7.75	0.04
	2	2.51			0.00					7.75		
	2	3.04			0.00					7.79		
	4	1.96	2.15	0.18	0.00	0.00	0.00	0.00	0.00	7.69	7.73	0.04
	4	2.16			0.00					7.73		
	4	2.32			0.00					7.77		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE 500® from day 0 to 2 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 0	0	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.54	7.54	0.04
	0	5.44			5.44					7.50		
	0	5.83			5.83					7.58		
	0.5	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.55	7.59	0.03
	0.5	5.44			5.44					7.60		
	0.5	5.83			5.83					7.61		
	1	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.52	7.57	0.05
	1	5.44			5.44					7.58		
	1	5.83			5.83					7.62		
	2	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.55	7.57	0.03
	2	5.44			5.44					7.57		
	2	5.83			5.83					7.60		
	4	5.76	5.68	0.21	5.76	5.68	0.21	100.00	100.00	7.46	7.53	0.07
	4	5.44			5.44					7.53		
	4	5.83			5.83					7.59		
Day 2	0	4.44	4.96	0.61	4.44	4.96	0.61	100.00	100.00	7.60	7.68	0.07
	0	4.81			4.81					7.70		
	0	5.64			5.64					7.74		
	0.5	4.51	5.05	0.48	4.51	5.05	0.48	100.00	100.00	7.59	7.69	0.09
	0.5	5.40			5.40					7.71		
	0.5	5.25			5.25					7.77		
	1	4.56	4.74	0.57	4.56	4.74	0.57	100.00	95.50	7.64	7.70	0.06
	1	5.38			5.38					7.71		
	1	4.28			4.28					7.75		
	2	5.28	4.70	0.60	0.00	0.00	0.00	0.00	0.00	7.65	7.70	0.05
	2	4.08			0.00					7.70		
	2	4.74			0.00					7.75		
	4	4.44	4.51	0.27	0.00	0.00	0.00	0.00	0.00	7.61	7.71	0.09
	4	4.80			0.00					7.73		
	4	4.28			0.00					7.78		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE 500® from day 3 to 5 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 3	0	5.90	6.41	0.62	5.90	6.41	0.62	100.00	100.00	7.62	7.62	0.03
	0	7.10			7.10					7.60		
	0	6.23			6.23					7.65		
	0.5	5.90	6.41	0.62	5.90	6.41	0.62	100.00	100.00	7.68	7.67	0.03
	0.5	7.10			7.10					7.69		
	0.5	6.23			6.23					7.64		
	1	5.90	6.41	0.62	5.90	6.41	0.62	100.00	100.00	7.65	7.65	0.01
	1	7.10			7.10					7.66		
	1	6.23			6.23					7.64		
	2	5.90	6.41	0.62	5.90	6.41	0.62	100.00	100.00	7.60	7.63	0.03
	2	7.10			7.10					7.63		
	2	6.23			6.23					7.66		
	4	5.90	6.41	0.62	5.90	6.41	0.62	100.00	100.00	7.58	7.64	0.06
	4	7.10			7.10					7.64		
	4	6.23			6.23					7.69		
Day 5	0	4.62	4.65	0.27	4.62	4.65	0.27	100.00	100.00	7.61	7.59	0.04
	0	4.94			4.94					7.62		
	0	4.40			4.40					7.55		
	0.5	3.55	3.35	0.20	3.55	3.35	0.20	100.00	71.92	7.56	7.60	0.04
	0.5	3.33			3.33					7.63		
	0.5	3.16			3.16					7.60		
	1	3.06	3.43	0.32	3.06	3.43	0.32	100.00	73.91	7.53	7.57	0.04
	1	3.66			3.66					7.56		
	1	3.57			3.57					7.61		
	2	2.94	3.41	0.44	0.00	0.00	0.00	0.00	0.00	7.61	7.61	0.02
	2	3.48			0.00					7.59		
	2	3.81			0.00					7.63		
	4	3.97	3.92	0.05	0.00	0.00	0.00	0.00	0.00	7.57	7.62	0.05
	4	3.90			0.00					7.64		
	4	3.88			0.00					7.66		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE 500® from day 6 to 8 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 6	0	5.25	5.44	0.21	5.25	5.44	0.21	100.00	100.00	7.60	7.64	0.03
	0	5.66			5.66					7.65		
	0	5.41			5.41					7.66		
	0.5	5.25	5.44	0.21	5.25	5.44	0.21	100.00	100.00	7.64	7.71	0.06
	0.5	5.66			5.66					7.74		
	0.5	5.41			5.41					7.76		
	1	5.25	5.44	0.21	5.25	5.44	0.21	100.00	100.00	7.66	7.72	0.07
	1	5.66			5.66					7.79		
	1	5.41			5.41					7.70		
	2	5.25	5.44	0.21	5.25	5.44	0.21	100.00	100.00	7.61	7.62	0.02
	2	5.66			5.66					7.65		
	2	5.41			5.41					7.61		
	4	5.25	5.44	0.21	5.25	5.44	0.21	100.00	100.00	7.62	7.65	0.03
	4	5.66			5.66					7.65		
	4	5.41			5.41					7.68		
Day 8	0	4.67	4.97	0.30	4.61	4.89	0.28	98.08	100.00	7.45	7.50	0.04
	0	5.26			5.16					7.52		
	0	4.99			4.89					7.53		
	0.5	3.40	3.38	0.40	3.40	3.38	0.40	100.00	69.24	7.54	7.59	0.04
	0.5	3.77			3.77					7.60		
	0.5	2.98			2.98					7.62		
	1	3.48	3.49	0.15	3.48	3.49	0.15	100.00	71.35	7.55	7.57	0.02
	1	3.34			3.34					7.56		
	1	3.64			3.64					7.59		
	2	3.93	3.71	0.19	0.00	0.00	0.00	0.00	0.00	7.54	7.57	0.03
	2	3.59			0.00					7.59		
	2	3.62			0.00					7.58		
	4	4.19	4.33	0.18	0.00	0.00	0.00	0.00	0.00	7.58	7.60	0.03
	4	4.27			0.00					7.60		
	4	4.54			0.00					7.63		

Total Cell Count, Total Live Cells, % Living Cells, % Total Live Cells compared to the Control and pH of *Microcystis aeruginosa* cultures exposed to KUPRAMINE 500® from day 9 to 11 at various copper concentrations. Includes standard deviation (s.d.), n=3.

Time	Cu conc. (mg Cu/L)	Total Cell Count (cells x 10^4/mL)	mean	s.d.	Total Live Cells (cells x 10^4/mL)	mean	s.d.	%Living Cells	%Total Live Cells compared to the Control	pH	mean	s.d.
Day 9	0	7.17	7.64	0.65	7.17	7.64	0.65	100.00	100.00	7.69	7.69	0.03
	0	7.38			7.38					7.71		
	0	8.38			8.38					7.66		
	0.5	7.17	7.64	0.65	7.17	7.64	0.65	100.00	100.00	7.73	7.74	0.01
	0.5	7.38			7.38					7.73		
	0.5	8.38			8.38					7.75		
	1	7.17	7.64	0.65	7.17	7.64	0.65	100.00	100.00	7.76	7.76	0.02
	1	7.38			7.38					7.78		
	1	8.38			8.38					7.74		
	2	7.17	7.64	0.65	7.17	7.64	0.65	100.00	100.00	7.70	7.72	0.02
	2	7.38			7.38					7.74		
	2	8.38			8.38					7.73		
	4	7.17	7.64	0.65	7.17	7.64	0.65	100.00	100.00	7.74	7.71	0.03
	4	7.38			7.38					7.68		
	4	8.38			8.38					7.72		
Day 11	0	6.68	7.11	0.93	6.68	7.11	0.93	100.00	100.00	7.62	7.64	0.02
	0	6.47			6.47					7.65		
	0	8.18			8.18					7.65		
	0.5	7.98	6.38	1.49	7.98	6.38	1.49	100.00	89.78	7.59	7.64	0.04
	0.5	5.03			5.03					7.67		
	0.5	6.14			6.14					7.66		
	1	4.47	4.74	0.23	4.47	4.74	0.23	100.00	66.62	7.63	7.65	0.03
	1	4.83			4.83					7.65		
	1	4.91			4.91					7.68		
	2	5.43	5.11	0.78	0.00	0.00	0.00	0.00	0.00	7.65	7.68	0.03
	2	5.67			0.00					7.69		
	2	4.22			0.00					7.70		
	4	4.20	4.38	0.19	0.00	0.00	0.00	0.00	0.00	7.55	7.57	0.02
	4	4.58			0.00					7.58		
	4	4.37			0.00					7.59		