AUSTRALIAN WATER QUALITY CENTRE

BIOASSAY TESTING OF "CUPRICIDE 110®" FOR EFFECTIVENESS AGAINST ALGAL SPECIES IDENTIFIED IN RICE FIELDS OF THE MURRUMBIDGEE IRRIGATION AREA

AUSTRALIAN WATER QUALITY CENTRE

TABLE OF CONTENTS

INTRODUCTION

Algae sometimes pose a risk to water quality in drinking water supplies and can also pose problems for rice growers using flood irrigation practices. Noble and Happeywood, 1987 explain that in temperate rice growing areas, such as those found in the Murray River region, algal populations may grow very rapidly and become a serious problem, interfering with rice seedling development. They observed three physical effects that algal development had on rice seedlings. Firstly leaves were shaded by algae and secondly a barrier was formed between the leaf and stem and the surrounding water. Thirdly the algal biomass dragged down delicate leaf blades preventing emergence through the water surface. Other arguments for the effect of algal growth on rice include immobilisation of fertiliser nitrogen and an increase in pH of water due to growth of algae encouraging nitrogen loss through ammonia volatilisation.

The main purpose of this project was to establish the effectiveness of the chelated copper algicide, CUPRICIDE 110, against algal species found in rice growing areas located in the Murrumbidgee Irrigation Area (MIA).

MATERIALS AND METHODS

The assessment involved laboratory bioassay tests with CUPRICIDE 110® using a number of algal species identified in soil and water used for production of rice crops. These algal species were isolated from various locations as identified in Table 1. Tests were carried out in representative test water from the MIA which was supplied to AWQC by AGMIN Chelates Pty Ltd. The tests were carried out at 25°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 2 to 5 x 10^4 cells/mL depending on the activity of algal cultures. Each test was carried out at a range of dose concentrations, plus a control (0 mg Cu/L). The nominal dose rates were: **0, 0.2, 0.5, 0.7, 1.0 and 2.0 mg Cu/L**. This range covered the current label recommendations. All dose concentrations were performed in triplicate (ie. x3). A summary of test conditions is provided in Table 1.

A 100 mg Cu/L stock solution was prepared of the algicide according to concentration data on label and was analysed using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) to obtain an accurate measurement of copper levels. This stock was then used in subsequent experiments.

Effective toxicity was assessed at 48 hours 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 membrane 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" was then used to calculate "Total Live Cells". 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".

Copper uptake was investigated by measuring filterable copper levels at 0 and 48 hours using copper concentrations of **0.2 and 1.0 mg Cu/L** for several of the algal species.

TABLE 1: TEST CONDITIONS

Test Organism	Scenedesmus	Ankistrodesmus	Spirogyra	Aulacoseira granulata (Melosira)	Nitschia	Chlamydomonas
Type	non-motile green alga	non-motile green alga	filamentous green alga	diatom	diatom	green flagellate alga
Source	Myponga Reservoir, S.A.	Paskerville Reservoir, S.A.	South Road Wetlands, Adelaide, S.A.	N.S.W.	"Wandilibi", Tocumwal, N.S.W.	"Wandilibi", Tocumwal, N.S.W.
Temperature	25°C	25°C	25°C	25°C	25°C	25°C
Irradiance	150 $\mu mol\ m^{-2}\ s^{-1}$	150 $\mu mol\ m^{-2}\ s^{-1}$	150 $\mu mol\ m^{-2}\ s^{-1}$	150 $\mu mol\ m^{-2}\ s^{-1}$	150 $\mu mol\ m^{-2}\ s^{-1}$	150 $\mu mol\ m^{-2}\ s^{-1}$
Time	48 hours	48 hours	48 hours	48 hours	48 hours	48 hours
Water Type	Rice irrigation water, collected from "Wandilibi", Tocumwal, N.S.W., 3 µm filtered	Rice irrigation water, collected from "Wandilibi", Tocumwal, N.S.W., 3 µm filtered	Rice irrigation water, collected from "Wandilibi", Tocumwal, N.S.W., 3 µm filtered	Rice irrigation water, collected from "Wandilibi", Tocumwal, N.S.W., 3 µm filtered	Rice irrigation water, collected from "Wandilibi", Tocumwal, N.S.W., 3 µm filtered	Rice irrigation water, collected from "Wandilibi", Tocumwal, N.S.W., 3 µm filtered
Water Characteristics	Dissolved Organic Carbon (DOC): 3.5 mg/L Total Dissolved Solids: 36 mg/L Total Organic Carbon (TOC): 3.8 mg/L Conductivity: 66 µS/cm Calcium: 4.5 mg/L Magnesium: 2.5 mg/L Potassium: 2.1 mg/L Sodium: 7.9 mg/L			Bicarbonate: 28 mg/L Carbonate: 0 mg/L Chloride: 8.0 mg/L Sulphate: 2.6 mg/L Alkalinity as calcium carbonate: 23 mg/L Ion balance: 5.54% pH: 7.15		
Replication	3x	3x	3x	3x	3x	3x
Dose Rates	0, 0.2,0.5, 1.0, 2.0 mg Cu/L	0, 0.2,0.5, 1.0, 2.0 mg Cu/L	0, 0.2,0.5, 1.0, 2.0 mg Cu/L	0, 0.2,0.5, 0.7, 1.0, 2.0 mg Cu/L	0, 0.2,0.5, 0.7, 1.0, 2.0 mg Cu/L	0, 0.2,0.5, 0.7, 1.0, 2.0 mg Cu/L

RESULTS

Figure 1 shows the algal species used in this study.

Growth response of *Scenedesmus*, *Spirogyra*, *Ankistrodesmus*, *Melosira*, *Nitschia* and *Chlamydomonas* to CUPRICIDE 110® are shown in Figures 2 - 7 respectively (see APPENDIX for raw data). Each figure gives the following information:

(a) Total Cell Counts after 48 hours
(b) % Living Cells after 48 hours
(c) Total Live Cells after 48 hours, and
(d) pH change over the 48 hour exposure period.

Figure 8 shows soluble and total copper levels for algal cultures tested at times 0 and 48 hours (see APPENDIX for raw data).

A stock algicide solution of CUPRICIDE 110 ®, 100 mg Cu/L, was made up using copper concentration provided on label. Analysis by ICP-MS identified a copper concentration of 99.2 mg Cu/L for stock solution.

FIGURE 1:
Various algal species used in this study.

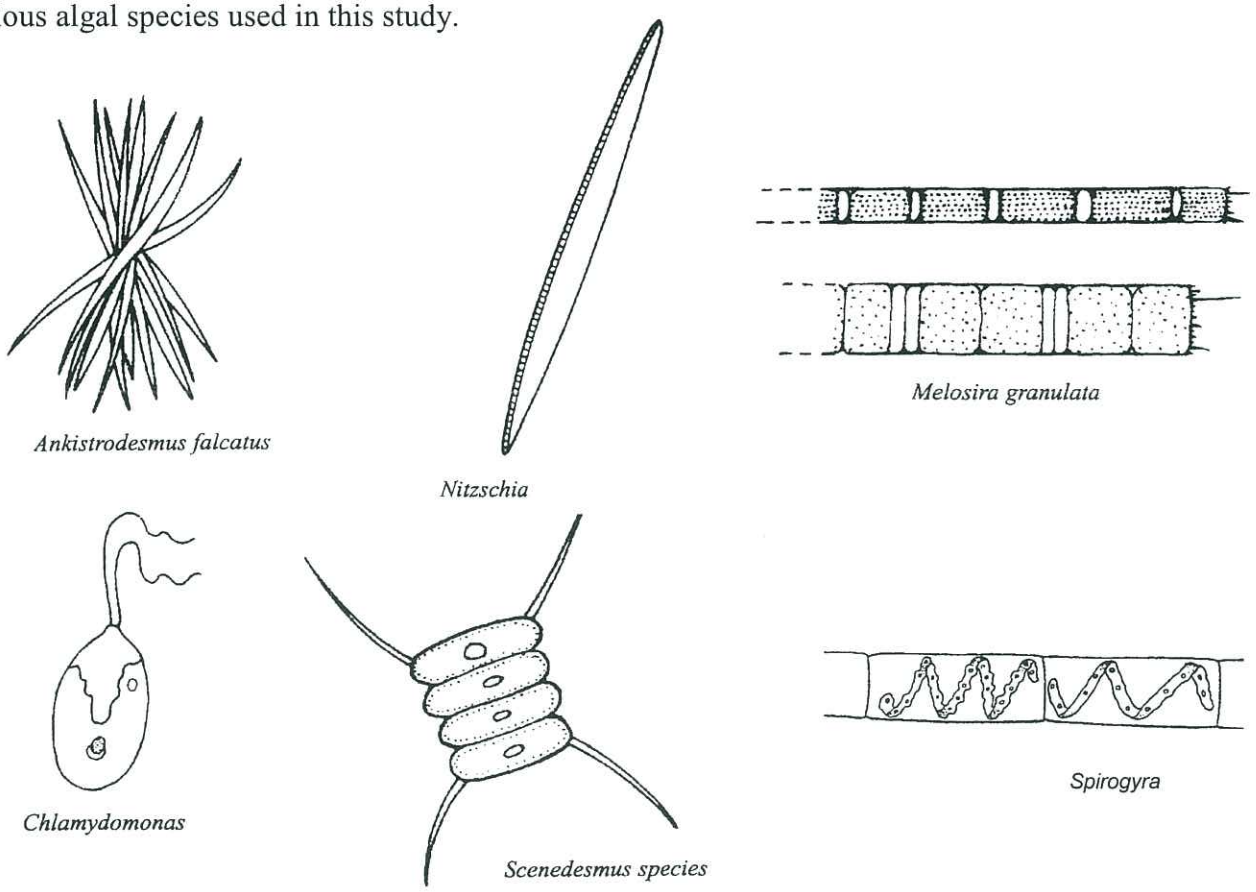


FIGURE 2:

(a) Total Cell Count, (b) % Living Cells (c) Total Live Cells and (d) pH of Scenedesmus cultures after 48 hours exposure in rice field irrigation water containing Cupricide 110 at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

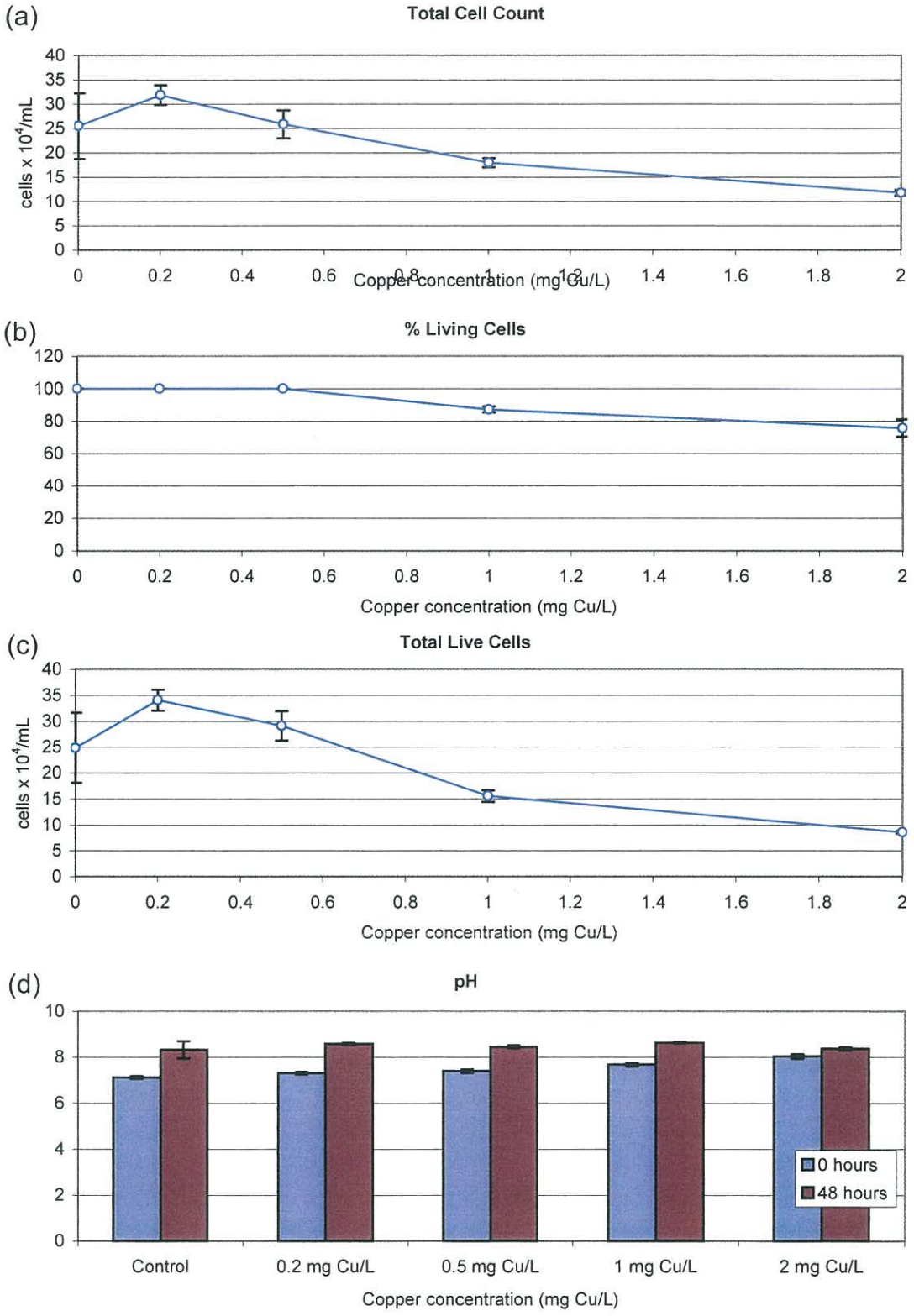


FIGURE 3:

(a) Total Cell Count, (b) % Living Cells (c) Total Live Cells and (d) pH of Spirogyra cultures after 48 hours exposure in rice field irrigation water containing Cupricide 110 at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

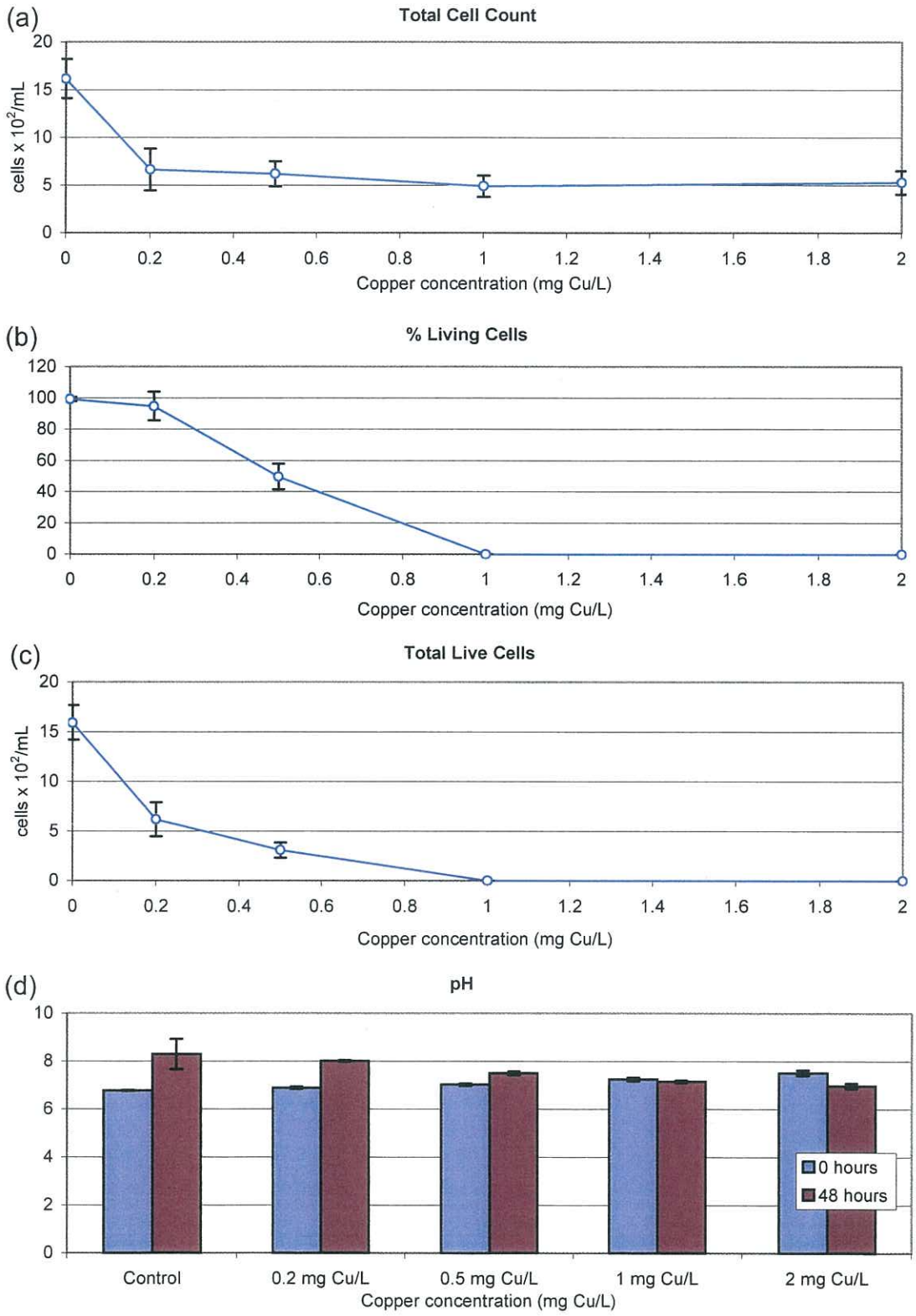


FIGURE 4:

(a) Total Cell Count, (b) % Living Cells (c) Total Live Cells and (d) pH of Ankistrodesmus cultures after 48 hours exposure in rice field irrigation water containing Cupricide 110 at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

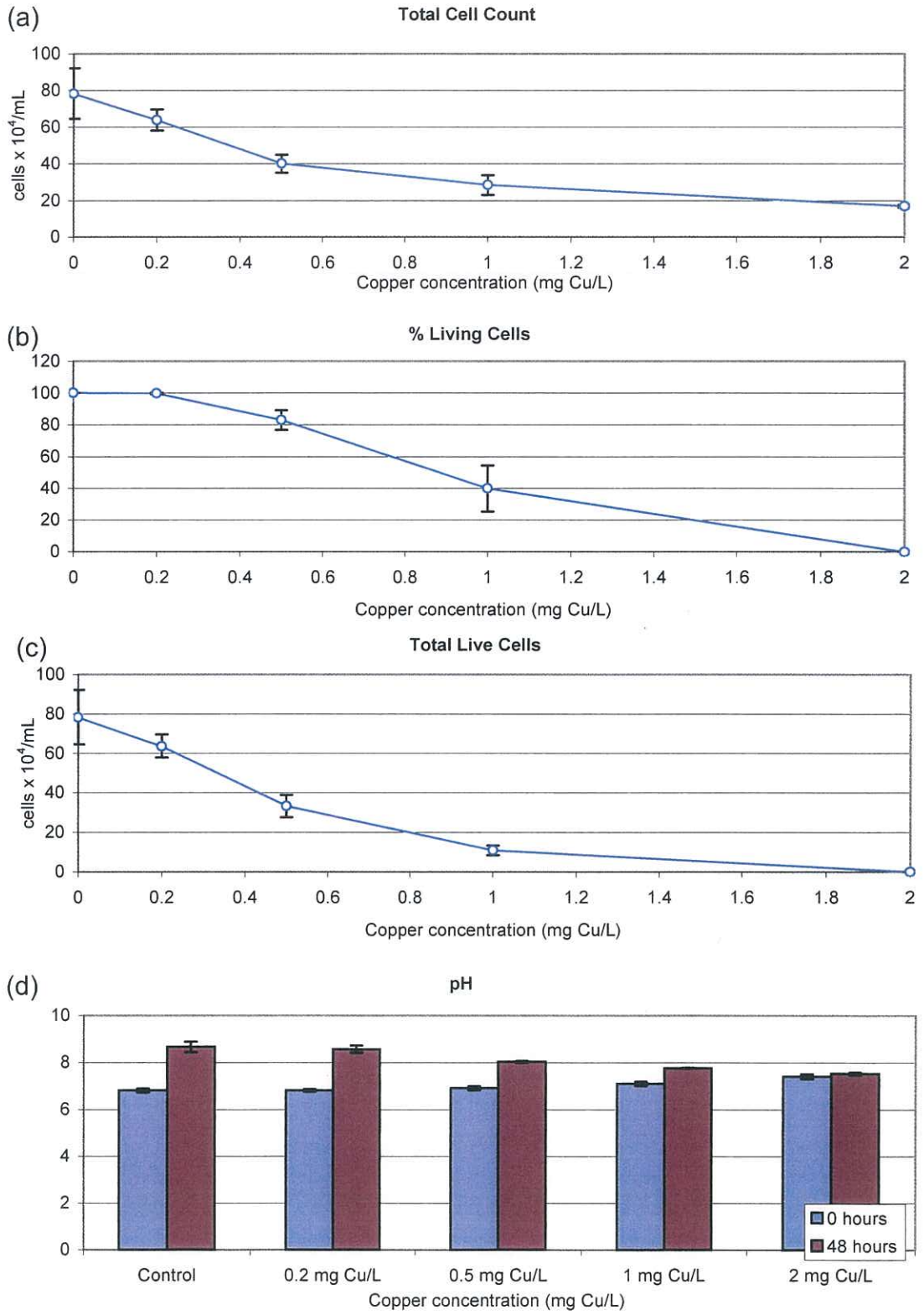


FIGURE 5:

(a) Total Cell Count, (b) % Living Cells (c) Total Live Cells and (d) pH of Melosira cultures after 48 hours exposure in rice field irrigation water containing Cupricide 110 at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

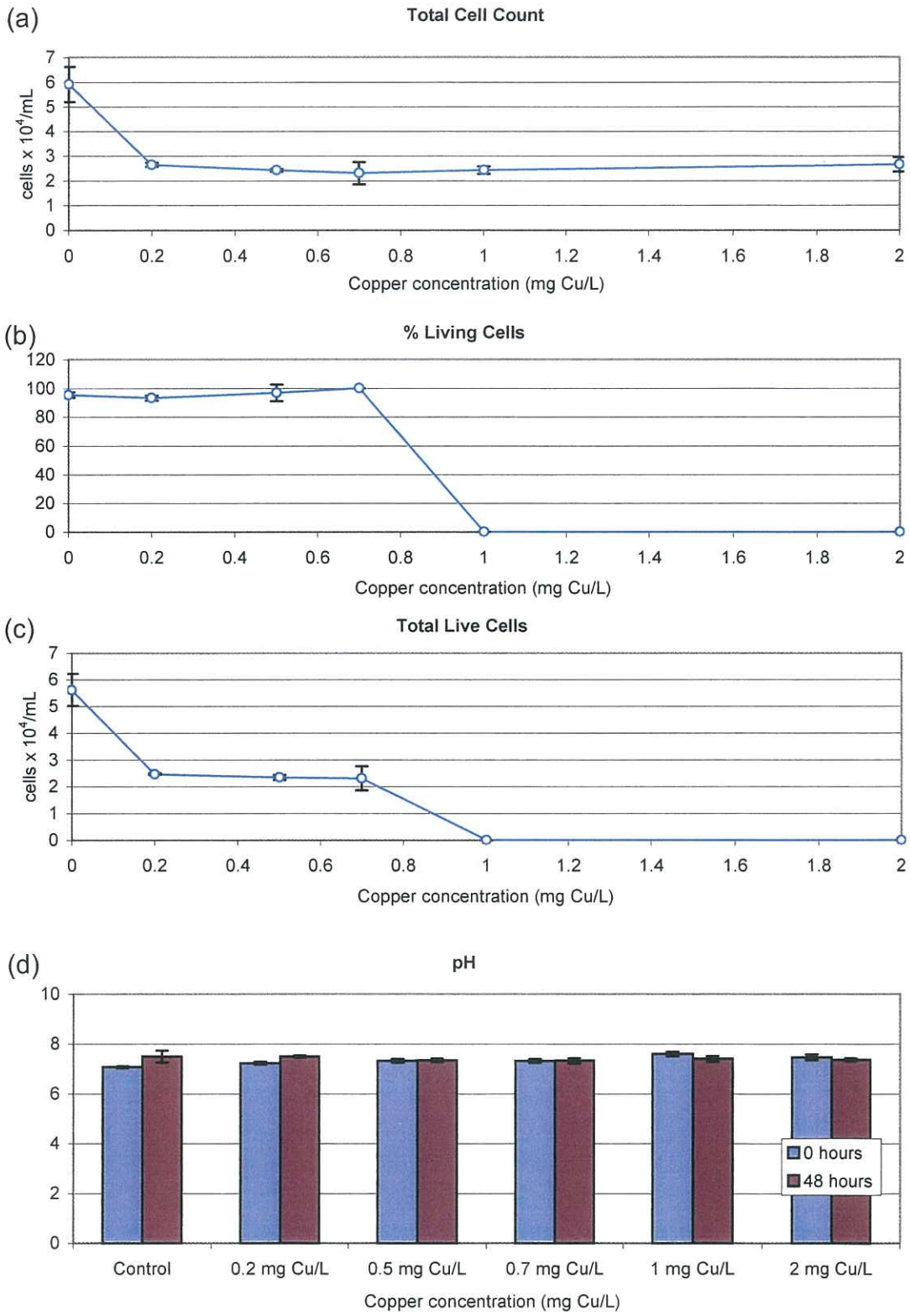


FIGURE 6:

(a) Total Cell Count, (b) % Living Cells (c) Total Live Cells and (d) pH of Nitschia cultures after 48 hours exposure in rice field irrigation water containing Cupricide 110 at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

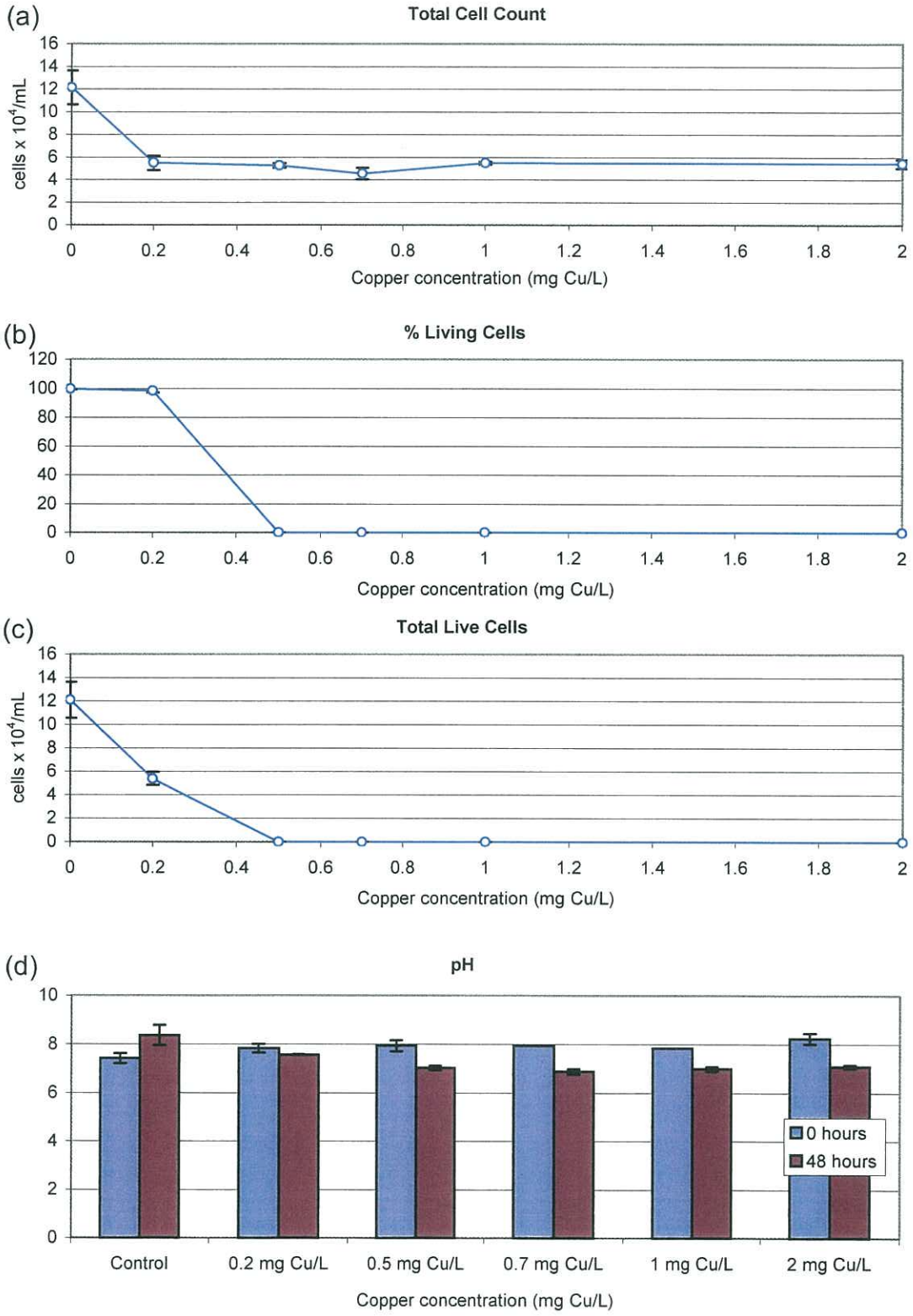


FIGURE 7:

(a) Total Cell Count, (b) % Living Cells (c) Total Live Cells and (d) pH of Chlamydomonas cultures after 48 hours exposure in rice field irrigation water containing Cupricide 110 at various copper (Cu) concentrations. Values are mean ± S.D., n=3.

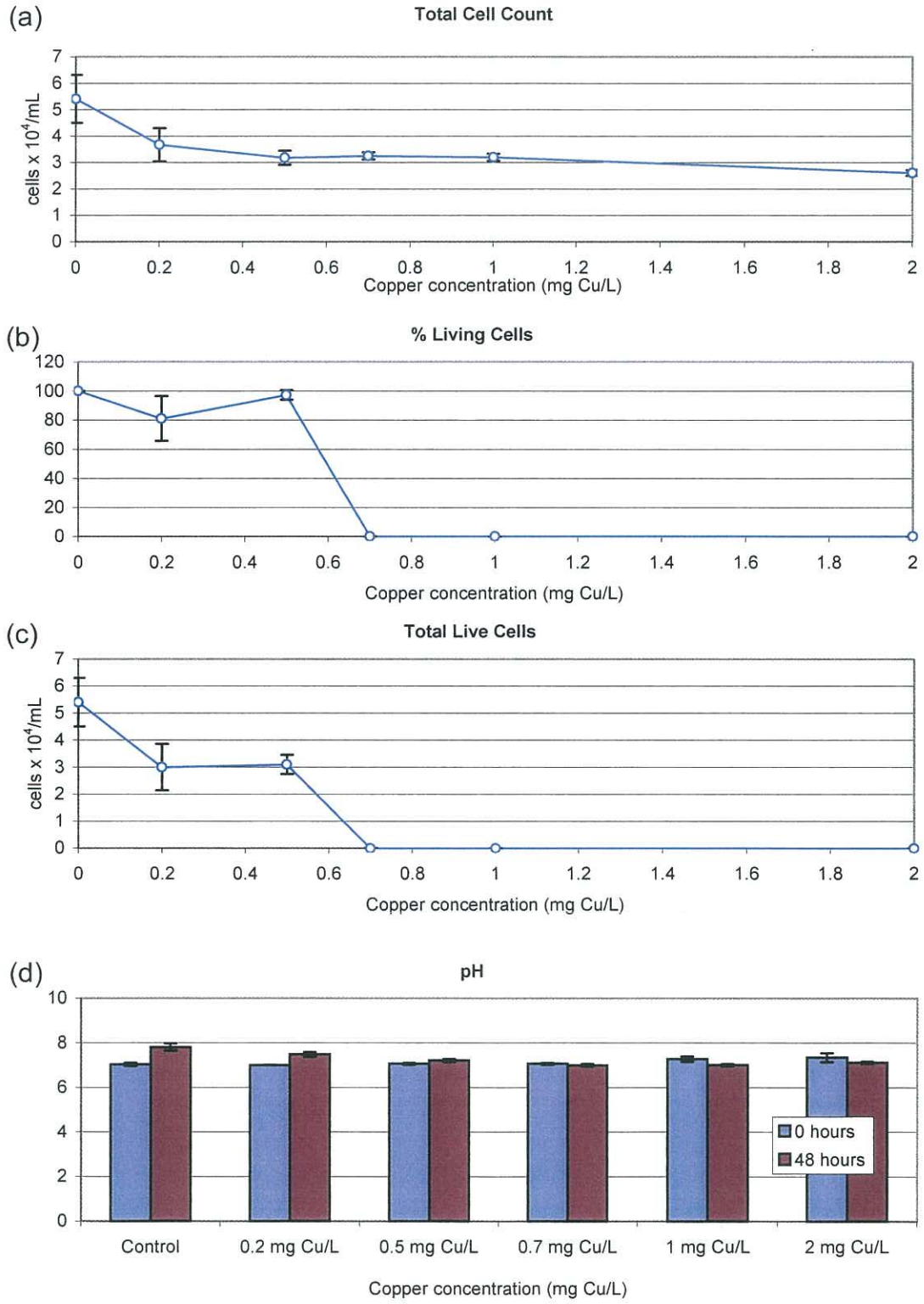
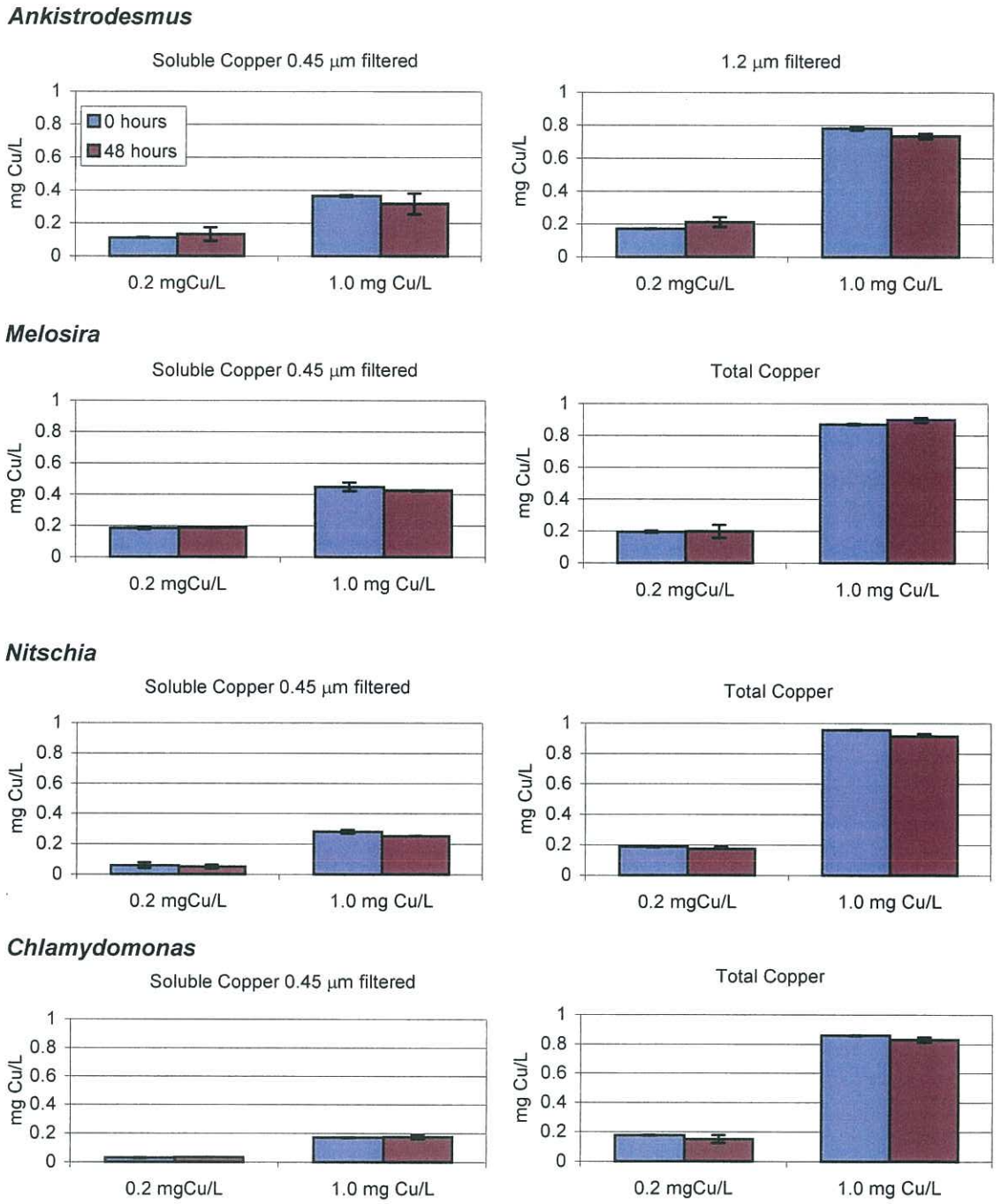


FIGURE 8:

Soluble and Total copper concentration in cultures of various algal species grown in rice field irrigation water containing Cupricide 110 at 0.2 and 1.0 mgCu/L at 0 and 48 hours. Values are mean ± S.D., n=3.



DISCUSSION

Results for controls showed that cultures for all 6 algal types were in exponential growth phase for the duration of the experiment, 48 hours, identified by a large increase in cell count after 48 hours.

The first three algal species, *Scenedesmus*, *Spirogyra* and *Ankistrodesmus* reacted with the bioassay stains as expected. An increase in copper concentration resulted in a steady decrease in cells stained green (living) and a steady increase in dead cells which stained orange.

However, the two diatoms, *Nitschia* and *Melosira*, and flagellate, *Chlamydomonas*, showed a different response in relation to their reactivity with bioassay stains compared to the results observed with the other three algal species. The two diatoms reacted with the FDA stain up to a copper concentration of 0.2 mg Cu/L for *Nitschia* and 0.5 mg Cu/L for *Melosira*. Some of the cells in these cultures also absorbed the PI stain with orange cells identified at the same copper concentrations. However, at copper concentrations greater than 0.2 mg Cu/L for *Nitschia* and 1.0 mg Cu/L for *Melosira* no auto-flourescence was observed even though cells could be seen under normal light conditions. The presence of red auto-fluorescence by the green pigment chlorophyll in algal cells when they are observed under UV light microscopy is usually an indicator of active healthy cells. It was concluded that the cells of Nitschia and Melosira that were not flourescing at the higher copper concentrations (> 0.2 and 1.0 mg Cu/L respectively) were physiologically inactive and were therefore also unable to take up either the FDA or PI stains.

Cultures of *Chlamydomonas* were found to be reproducing sexually and so both vegetative cells and zygotes were observed. *Chlamydomonas* can carry out both asexual and sexual reproduction. In asexual reproduction the nuclei of an individual vegetative cell undergoes mitoses, splits, resulting in the formation of two or more vegetative cells within the parental wall. Eventually the parental wall ruptures releasing the young cells which grow and eventually repeat the asexual cycle. In sexual reproduction sexually compatible cells are attracted to each other and combine to form one cell, a zygote. The zygote matures and vegetative cells develop within the cell wall. After a period of dormancy the zygote germinates releasing the vegetative cells which can then go on and repeat the sexual cycle. Vegetative cells of *Chlamydomonas* were identified at all copper concentrations, however, not all of them were able take up the stains. At copper concentrations up to and including 0.5 mg Cu/L the vegetative cells were able to absorb the FDA and were stained green. However, no orange vegetative cells were observed indicating that either the PI stain was not absorbed or that no dead cells were present in the culture. At copper concentrations equal to and greater than 0.7 mg Cu/L, the vegetative cells were still present but did not take up either the FDA or PI stains. Therefore, it was concluded that at copper concentrations greater than 0.5 mg Cu/L all the vegetative cells were dead. Zygotes were also observed at all copper concentrations. However, they absorbed the PI stain but not the FDA and so appeared as either red or orange. It was decided to look at the affect of the algicide on vegetative cells only. Therefore, the results presented in Figure 6 have been extrapolated and represent the affect of the algicide on vegetative cells only.

Total Cell Count measurements showed cells present for all 6 algal species after 48 hours growth even at the highest copper concentration of 2.0 mg Cu/L. However, % Living Cells varied between the algal species. *Scenedesmus* recorded a high percentage of living cells, 75%, at the highest copper concentration of 2.0 mg Cu/L. *Ankistrodesmus* was more susceptible to the algicide with none present at a copper concentration of 2.0 mg Cu/L. *Spirogyra* was also significantly affected with none present at copper concentrations of 1.0 and 2.0 mg Cu/L. *Chlamydomonas* and *Melosira* were also significantly affected with no Living Cells present at a copper concentration of 0.7 mg Cu/L and 1.0 mg Cu/L respectively. The most sensitive species was *Nitschia*, which showed no living cells at a copper concentration of 0.5 mg Cu/L.

Total Live Cell count for *Scenedesmus* showed a slight increase from 0 mg Cu/L, control, to a copper concentration of 0.2 mg Cu/L. At greater than 0.2 mg Cu/L the Total Live Cell count for *Scenedesmus* reduced with increasing copper concentration. However, live cells were still present after 48 hours exposure at the highest copper concentration of 2 mg Cu/L. Total Live Cell count for *Ankistrodesmus* also reduced with increasing copper concentration with no live cells left after 48 hours exposure to a copper concentration of 2 mg Cu/L. The Total Live Cell count for both *Spirogyra* and *Melosira* also showed a reduction with no live cells present after 48 hours exposure to 1 mg Cu/L. No live cells of *Chlamydomonas* were present after 48 hours exposure to 0.7 mg Cu/L. The most dramatic reduction in Total Live Cell count was recorded for *Nitschia* with no live cells recorded after 48 hours exposure to a copper concentration of 0.5 mg Cu/L.

In summary, the testing showed that the green alga *Scenedesmus* was the most resistant to CUPRICIDE 110®. Extrapolation of Total Live Cell count results using linear regression identified an MLD_{100} of 2.70 mg Cu/L for *Scenedesmus* (Table 2). *Ankistrodesmus*, also a green alga, was the next most resistant with an MLD_{100} of 2.0 mg Cu/L (Table 2). This was followed by *Spirogyra* (filamentous green) and *Melosira* (filamentous diatom) which both had an MLD_{100} of 1.0 mg Cu/L. *Chlamydomonas*, the green flagellate, had an MLD_{100} of 0.7 mg Cu/L and *Nitschia* (diatom) was the most affected with an MLD_{100} of 0.5 mg Cu/L (Table 2).

TABLE 2: COPPER CONCENTRATION FOR MLD_{100}

Algal Species	Type	Copper Concentration for MLD_{100} (mg Cu/L)
Scenedesmus	Non-motile Green	2.7
Ankistrodesmus	Non-motile Green	2.0
Spirogyra	Filamentous Green	1.0
Melosira	Diatom	1.0
Chlamydomonas	Flagellate	0.7
Nitschia	Diatom	0.5

The change in pH of cultures over the 48 hour growth period was minimal for all 6 algal species. This low pH change would appear to have had little affect on the activity of the algicide.

No significant difference in copper concentrations was observed between samples at 0 and 48 hours for both total and soluble copper measurements. Total copper levels for *Melosira*, *Nitschia* and *Chlamydomonas* cultures were close to the nominal concentrations of 0.2 and 1.0 mg Cu/L. However, soluble copper levels were approximately 50% of total copper levels. This suggests that a large proportion of the copper is bound to particulate matter with a size greater than 0.45 μm. While not the main aim of the project, *Ankistrodesmus* cultures were filtered through a 1.2 μm filter before copper analysis was carried out. Interestingly the copper levels were close to the nominal copper concentrations of both 0.2 and 1.0 mg Cu/L. However, soluble copper levels, 0.45 μm filtered, were approximately 50% of 1.2 μm filtered cultures. Therefore, it could be concluded that a large proportion of the copper was bound to particulate matter ranging from 0.45 to 1.2 μm. This could be an area for further investigation to determine what substances the copper is bound to and whether the copper is still active as an algicide.

REFERENCES

Noble J.C. and Happey-Wood C.M. (1987) Some aspects of the ecology of algal communities in ricefields and rice irrigation systems of Southern New South Wales. The Journal of the Australian Institute of Agricultural Science 53 No. 3, 170-184.

APPENDIX

Table 1. (a) Total Cell Count, (b) % Living Cells, (c) Total Live Cells and (d) pH for *Scenedesmus* cultures exposed to rice field irrigation water containing CUPRICIDE 110® at copper concentrations of 0 (Control), 0.2, 0.5, 1.0 and 2.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

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

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	9.32	8.93	0.34	9.32	8.93	0.34	9.32	8.93	0.34	9.32	8.93	0.34	9.32	8.93	0.34
	8.78			8.78			8.78			8.78			8.78		
	8.70			8.70			8.70			8.70			8.70		
48	24.85	25.49	6.76	34.05	31.87	2.03	29.10	25.82	2.85	17.63	17.95	0.93	12.37	11.78	0.57
	19.07			30.05			23.95			19.00			11.23		
	32.55			31.50			24.40			17.23			11.73		

(b) % Living Cells

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	97.13	99.04	1.66	97.13	99.04	1.66	97.13	99.04	1.66	97.13	99.04	1.66	97.13	99.04	1.66
	100.00			100.00			100.00			100.00			100.00		
	100.00			100.00			100.00			100.00			100.00		
48	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	88.09	87.20	1.93	69.53	75.67	5.35
	100.00			100.00			100.00			88.52			79.34		
	100.00			100.00			100.00			84.98			78.15		

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

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	9.05	8.84	0.18	9.05	8.84	0.18	9.05	8.84	0.18	9.05	8.84	0.18	9.05	8.84	0.18
	8.78			8.78			8.78			8.78			8.78		
	8.70			8.70			8.70			8.70			8.70		
48	24.85	25.49	6.76	34.05	31.87	2.03	29.10	25.82	2.85	15.53	15.66	1.09	8.60	8.89	0.28
	19.07			30.05			23.95			16.82			8.91		
	32.55			31.50			24.40			14.64			9.17		

(d) pH

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.05	7.12	0.06	7.28	7.30	0.06	7.33	7.39	0.08	7.60	7.68	0.08	7.92	8.03	0.10
	7.14			7.26			7.37			7.68			8.08		
	7.16			7.37			7.48			7.75			8.10		
48	7.92	8.32	0.37	8.55	8.57	0.04	8.38	8.45	0.07	8.65	8.62	0.03	8.32	8.37	0.06
	8.38			8.54			8.45			8.62			8.34		
	8.65			8.62			8.51			8.59			8.44		

Table 2. (a) Total Cell Count, (b) % Living Cells, (c) Total Live Cells and (d) pH for *Spirogyra* cultures exposed to rice field irrigation water containing CUPRICIDE 110® at copper concentrations of 0 (Control), 0.2, 0.5, 1.0 and 2.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

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

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.59	6.93	0.62	7.59	6.93	0.62	7.59	6.93	0.62	7.59	6.93	0.62	7.59	6.93	0.62
	6.37			6.37			6.37			6.37			6.37		
	6.84			6.84			6.84			6.84			6.84		
48	17.60	16.15	2.05	4.21	6.63	2.19	6.51	6.19	1.32	4.37	4.89	1.11	4.12	5.28	1.23
	14.70			7.20			4.74			6.17			5.15		
				8.48			7.32			4.14			6.57		

(b) % Living Cells

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00
	100.00			100.00			100.00			100.00			100.00		
	100.00			100.00			100.00			100.00			100.00		
48	97.45	99.15	1.47	100.00	94.68	9.21	58.75	49.63	8.25	0.00	0.00	0.00	0.00	0.00	0.00
	100.00			100.00			47.46			0.00			0.00		
	100.00			84.05			42.69			0.00			0.00		

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

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.59	6.93	0.62	7.59	6.93	0.62	7.59	6.93	0.62	7.59	6.93	0.62	7.59	6.93	0.62
	6.37			6.37			6.37			6.37			6.37		
	6.84			6.84			6.84			6.84			6.84		
48	17.15	15.93	1.73	4.21	6.18	1.71	3.82	3.07	0.79	0.00	0.00	0.00	0.00	0.00	0.00
	14.70			7.20			2.25			0.00			0.00		
				7.13			3.12			0.00			0.00		

(d) pH

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	6.80	6.77	0.03	6.83	6.89	0.05	6.97	7.02	0.05	7.16	7.25	0.08	7.41	7.52	0.12
	6.75			6.90			7.06			7.29			7.64		
	6.76			6.93			7.03			7.30			7.50		
48	7.57	8.29	0.64	7.98	8.02	0.03	7.43	7.50	0.08	7.10	7.15	0.06	6.92	6.97	0.11
	8.52			8.04			7.50			7.21			6.90		
	8.78			8.03			7.58			7.15			7.10		

Table 3. (a) Total Cell Count, (b) % Living Cells, (c) Total Live Cells and (d) pH for *Ankistrodesmus* cultures exposed to rice field irrigation water containing CUPRICIDE 110® at copper concentrations of 0 (Control), 0.2, 0.5, 1.0 and 2.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

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

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.07	7.25	0.15	7.07	7.25	0.15	7.07	7.25	0.15	7.07	7.25	0.15	7.07	7.25	0.15
	7.35			7.35			7.35			7.35			7.35		
	7.32			7.32			7.32			7.32			7.32		
48	93.00	78.33	13.80	70.40	63.80	5.72	41.30	39.97	4.94	34.60	28.40	5.38	16.00	16.77	0.80
	65.60			60.60			34.50			25.60			17.60		
	76.40			60.40			44.10			25.00			16.70		

(b) % Living Cells

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00
	100.00			100.00			100.00			100.00			100.00		
	100.00			100.00			100.00			100.00			100.00		
48	100.00	100.00	0.00	100.00	99.68	0.55	89.86	82.89	6.24	23.92	39.77	14.54	0.00	0.00	0.00
	100.00			99.04			77.83			42.92			0.00		
	100.00			100.00			80.98			52.48			0.00		

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

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	7.07	7.25	0.15	7.07	7.25	0.15	7.07	7.25	0.15	7.07	7.25	0.15	7.07	7.25	0.15
	7.35			7.35			7.35			7.35			7.35		
	7.32			7.32			7.32			7.32			7.32		
48	93.00	78.33	13.80	70.40	63.61	5.89	37.11	33.23	5.56	8.28	10.79	2.43	0.00	0.00	0.00
	65.60			60.02			26.85			10.99			0.00		
	76.40			60.40			35.71			13.12			0.00		

(d) pH

Time (hours)	Copper concentration (mg Cu/L)														
	Control			0.2			0.5			1.0			2.0		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
0	6.71	6.81	0.09	6.75	6.81	0.06	6.83	6.91	0.09	7.3	7.10	0.09	7.01	7.41	0.11
	6.84			6.83			6.91			7.41			7.11		
	6.88			6.86			7			7.51			7.19		
48	8.50	8.65	0.22	8.38	8.56	0.16	8.06	8.03	0.04	7.77	7.76	0.02	7.53	7.53	0.06
	8.55			8.66			8.04			7.74			7.48		
	8.91			8.64			7.98			7.77			7.59		

Table 4. (a) Total Cell Count, (b) % Living Cells, (c) Total Live Cells and (d) pH for *Melosira* cultures exposed to rice field irrigation water containing CUPRICIDE 110® at copper concentrations of 0 (Control), 0.2, 0.5, 1.0 and 2.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

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

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	2.23	2.67	0.86	2.23	2.67	0.86	2.23	2.67	0.86	2.23	2.67	0.86	2.23	2.67	0.86	2.23	2.67	0.86
	2.13			2.13			2.13			2.13			2.13			2.13		
	3.66			3.66			3.66			3.66			3.66			3.66		
48	5.12	5.91	0.71	2.63	2.65	0.08	2.49	2.43	0.06	2.40	2.31	0.45	2.29	2.43	0.15	2.38	2.66	0.29
	6.09			2.74			2.37			2.70			2.42			2.64		
	6.51			2.58			2.42			1.82			2.59			2.96		

(b) % Living Cells

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	98.50	99.34	0.76	98.50	99.34	0.76	98.50	99.34	0.76	98.50	99.34	0.76	98.50	99.34	0.76	98.50	99.34	0.76
	100.00			100.00			100.00			100.00			100.00			100.00		
	99.51			99.51			99.51			99.51			99.51			99.51		
48	96.50	95.33	2.02	93.00	93.17	1.76	90.00	96.67	5.77	100.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	96.50			91.50			100.00			100.00			0.00			0.00		
	93.00			95.00			100.00			100.00			0.00			0.00		

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

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	2.20	2.66	0.85	2.20	2.66	0.85	2.20	2.66	0.85	2.20	2.66	0.85	2.20	2.66	0.85	2.20	2.66	0.85
	2.13			2.13			2.13			2.13			2.13			2.13		
	3.64			3.64			3.64			3.64			3.64			3.64		
48	4.94	5.62	0.60	2.45	2.47	0.03	2.24	2.34	0.09	2.40	2.31	0.45	0.00	0.00	0.00	0.00	0.00	0.00
	5.88			2.51			2.37			2.70			0.00			0.00		
	6.05			2.45			2.42			1.82			0.00			0.00		

(d) pH

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	7.05	7.08	0.04	7.18	7.24	0.06	7.25	7.33	0.08	7.33	7.45	0.14	7.52	7.61	0.09	7.39	7.47	0.11
	7.06			7.23			7.33			7.43			7.60			7.42		
	7.12			7.30			7.40			7.60			7.70			7.60		
48	7.24	7.50	0.24	7.51	7.50	0.04	7.28	7.35	0.07	7.22	7.34	0.10	7.33	7.41	0.11	7.32	7.37	0.06
	7.56			7.46			7.37			7.38			7.36			7.36		
	7.70			7.54			7.41			7.41			7.54			7.44		

Table 5. (a) Total Cell Count, (b) % Living Cells, (c) Total Live Cells and (d) pH for *Nitschia* cultures exposed to rice field irrigation water containing CUPRICIDE 110® at copper concentrations of 0 (Control), 0.2, 0.5, 1.0 and 2.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

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

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	4.82	5.15	0.49	4.82	5.15	0.49	4.82	5.15	0.49	4.82	5.15	0.49	4.82	5.15	0.49	4.82	5.15	0.49
	4.92			4.92			4.92			4.92			4.92			4.92		
	5.71			5.71			5.71			5.71			5.71			5.71		
48	13.80	12.13	1.50	5.43	5.48	0.62	5.45	5.26	0.20	5.14	4.55	0.51	5.63	5.48	0.13	4.97	5.42	0.39
	11.70			4.89			5.28			4.22			5.41			5.65		
	10.90			6.13			5.06			4.28			5.39			5.64		

(b) % Living Cells

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	99.38	0.70	100.00	99.38	0.70	100.00	99.38	0.70	100.00	99.38	0.70	100.00	99.38	0.70	100.00	99.38	0.70
	98.62			98.62			98.62			98.62			98.62			98.62		
	99.52			99.52			99.52			99.52			99.52			99.52		
48	100.00	99.56	0.42	99.51	98.28	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	99.16			98.14			0.00			0.00			0.00			0.00		
	99.52			97.20			0.00			0.00			0.00			0.00		

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

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	4.82	5.12	0.49	4.82	5.12	0.49	4.82	5.12	0.49	4.82	5.12	0.49	4.82	5.12	0.49	4.82	5.12	0.49
	4.85			4.85			4.85			4.85			4.85			4.85		
	5.68			5.68			5.68			5.68			5.68			5.68		
48	13.80	12.08	1.53	5.40	5.39	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	11.60			4.80			0.00			0.00			0.00			0.00		
	10.85			5.96			0.00			0.00			0.00			0.00		

(d) pH

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	7.20	7.41	0.21	7.65	7.82	0.18	7.68	7.93	0.23	7.55	7.87	0.28	7.48	7.83	0.33	8.18	8.24	0.06
	7.42			7.80			7.98			7.95			7.90			8.24		
	7.61			8.01			8.13			8.10			8.12			8.29		
48	7.88	8.36	0.42	7.54	7.56	0.02	6.93	7.02	0.09	6.77	6.88	0.11	6.88	6.99	0.09	7.00	7.08	0.07
	8.55			7.57			7.04			6.88			7.05			7.11		
	8.65			7.57			7.10			6.98			7.03			7.13		

Table 6. (a) Total Cell Count, (b) % Living Cells, (c) Total Live Cells and (d) pH for *Chlamydomonas* cultures exposed to rice field irrigation water containing CUPRICIDE 110® at copper concentrations of 0 (Control), 0.2, 0.5, 1.0 and 2.0 mg Cu/L. Includes value for each measurement, mean and standard deviation (s.d.).

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

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27
	2.04			2.04			2.04			2.04			2.04			2.04		
	1.78			1.78			1.78			1.78			1.78			1.78		
48	4.50	5.41	0.91	3.42	3.67	0.63	3.33	3.18	0.27	3.14	3.25	0.13	3.05	3.20	0.13	2.73	2.61	0.11
	6.32			3.20			3.34			3.40			3.29			2.53		
	5.41			4.38			2.87			3.21			3.26			2.57		

(b) % Living Cells

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00	100.00	100.00	0.00
	100.00			100.00			100.00			100.00			100.00			100.00		
	100.00			100.00			100.00			100.00			100.00			100.00		
48	100.00	99.85	0.27	93.88	80.99	15.29	100.00	97.17	3.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	99.54			64.10			97.97			0.00			0.00			0.00		
	100.00			85.00			93.55			0.00			0.00			0.00		

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

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27	2.32	2.05	0.27
	2.04			2.04			2.04			2.04			2.04			2.04		
	1.78			1.78			1.78			1.78			1.78			1.78		
48	4.50	5.40	0.90	3.21	2.99	0.86	3.33	3.10	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6.29			2.05			3.27			0.00			0.00			0.00		
	5.41			3.72			2.68			0.00			0.00			0.00		

(d) pH

Time (hours)	Copper concentration (mg Cu/L)																	
	Control			0.2			0.5			0.7			1.0			2.0		
	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	6.95	7.03	0.07	6.99	7.00	0.01	7.02	7.06	0.04	7.13	7.21	0.07	7.14	7.27	0.12	7.12	7.34	0.20
	7.05			7.01			7.07			7.25			7.30			7.40		
	7.09			7.00			7.10			7.26			7.38			7.51		
48	7.75	7.80	0.17	7.37	7.48	0.11	7.20	7.21	0.07	7.05	7.00	0.05	6.95	7.01	0.05	7.07	7.12	0.05
	7.66			7.48			7.15			6.95			7.04			7.12		
	7.98			7.58			7.28			7.00			7.03			7.16		

Table 7a: Soluble copper concentration in cultures of various algal species grown in rice field irrigation water containing CUPRICIDE 110® at 0.2 and 1.0 mg Cu/L at 0 and 48 hours. Includes value for each measurement, mean and standard deviation (s.d.).

Organism	Soluble Copper 0.45 µm filtered											
	0.2 mg/L copper						1.0 mg/L copper					
	0 hours			48 hours			0 hours			48 hours		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
Ankistrodesmes	0.109	0.112	0.003	0.134	0.133	0.006	0.337	0.366	0.041	0.245	0.318	0.063
	0.112			0.138						0.354		
	0.114			0.127			0.395			0.354		
Melosira	0.188	0.184	0.007	0.186	0.186	0.001	0.470	0.448	0.028	0.423	0.424	0.003
	0.176			0.187			0.458			0.422		
	0.188			0.186			0.416			0.427		
Nitschia	0.081	0.058	0.020	0.065	0.050	0.013	0.294	0.281	0.012	0.252	0.253	0.001
	0.045			0.042			0.278					
	0.049			0.043			0.270			0.254		
Chlamydomonas	0.027	0.029	0.003	0.034	0.034	0.002	0.168	0.170	0.003	0.180	0.176	0.014
	0.028			0.033			0.174			0.187		
	0.032			0.036			0.169			0.160		

Table 7b: Total copper concentration in cultures of various algal species grown in rice field irrigation water containing CUPRICIDE 110® at 0.2 and 1.0 mg Cu/L at 0 and 48 hours. Includes value for each measurement, mean and standard deviation (s.d.). Note: *Ankistrodesmus* cultures were filtered through a 1.2 µm GF/C filter pad.

Organism	Total Copper											
	0.2 mg/L copper						1.0 mg/L copper					
	0 hours			48 hours			0 hours			48 hours		
	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.	value	mean	s.d.
Ankistrodesmes	0.170	0.171	0.001	0.216	0.212	0.012	0.748	0.782	0.030	0.731	0.736	0.016
	0.172			0.221			0.794			0.753		
	0.171			0.198			0.803			0.723		
Melosira	0.200	0.195	0.006	0.197	0.198	0.040	0.907	0.870	0.004	0.913	0.897	0.014
	0.188			0.203			0.876			0.890		
	0.197			0.195			0.827			0.888		
Nitschia	0.185	0.187	0.004	0.171	0.173	0.014	0.965	0.955	0.002		0.916	0.016
	0.185			0.174			0.939			0.927		
	0.192			0.174			0.961			0.904		
Chlamydomonas	0.177	0.177	0.003	0.149	0.151	0.027	0.861	0.858	0.003	0.842	0.829	0.017
	0.179			0.154			0.883			0.836		
	0.174			0.150			0.830			0.810		