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The Combined Effect of Cu and Zn on *Selenastrum capricornutum*

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It has been demonstrated that the toxicity of heavy metals depends on their chemical speciation and can be related to their free ion activities, rather than the total metal concentrations (Steemann-Nielsen and Wium-Andersen, 1970; Sunda and Guillard, 1976; Andrew, Biesinger, and Glass, 1977; Anderson, Morel and Guillard, 1978). The objective of this study was to determine the effect of combined free ions of zinc and copper on the toxicity to the green alga

Selenastrum capricornutum. This alga was grown in a defined medium, under controlled laboratory conditions, with a varying range of zinc and copper concentrations. The growth rate of the alga was inhibited at pZn = 5.93 or at pCu = 7.24. The results suggest that when the chemical speciation of combined zinc and copper is taken into account, there is little or no toxic interaction between the two metal ions.

THE COMBINED EFFECT OF CU AND ZN ON

SELENASTRUM CAPRICORNUTUM

by

HELMER COLONIA-ROQUE

A thesis submitted in partial fulfillment of the requirements for the degree of

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TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

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INTRODUCTION

There is abundant information on the toxicity of simple heavy metals to cultures or natural aggregations of aquatic organisms. Copper, a heavy metal, has been extensively studied because of its wide use in the control of obnoxious algae. Steemann-Nielsen, Kamp-Nielsen, and Wium-Andersen (1969) determined that the effect of Cu on the photosynthesis of cultures of *Chlorella pyrenoidosa* is inversely related to both the cell concentration and the pH of the medium. Copper inhibited the growth and chlorophyll concentration of marine dinoflagellates (Saifullah, 1978). Andros and Garton (1980) reported on the acute toxicity of copper to the squawfish *Ptychoceilus oregonensis*.

Zinc, another heavy metal, also has been extensively studied for its toxic effects to a wide variety of aquatic organisms. The growth rate of *Chlorella vulgaris*, exposed to selected concentrations of Zn, was reduced by 50 percent with a Zn concentration of $2.4 \pm .02$ ppm (3.67×10^{-5} M) (Rachlin and Farran, 1974). Hendricks (1978) reported a reduction of oxygen production by *Selenastrum capricornutum* after exposure of cultures of the alga to selected concentrations of Zn; oxygen production was decreased by 50 percent at 1.0 mg l^{-1} of Zn. Experiments also showed that Zn affected the adhesiveness and fragility of the eggs of fathead minnows *Pimephales promelas*. The eggs were significantly affected at 145 ug (2.22×10^{-6} M) Zn l^{-1}, and hatchability and survival of the larvae were reduced at 295 ug (4.5×10^{-6} M) Zn l^{-1} (Benoit and Holcombe, 1978). Sprague and Ramsay (1965) reported lethal levels of Zn to juvenile salmon at 420 ug l^{-1} (6.42×10^{-6} M). Recent studies show Zn concentrations as low as 15 ug l^{-1} (2.29×10^{-7} M) can inhibit photosynthesis in natural populations of phytoplankton collected from the English Channel (Davies and Sleep, 1979). In summary, copper and zinc are toxic to aquatic organisms, but the concentration of copper or zinc which produces a toxic effect depends upon the chemical composition of the water in which the toxicity tests are conducted.

Recent studies have demonstrated that the toxicity of a heavy metal depends on its chemical speciation and may be related to its free ion activity, rather than the total metal concentration (Steemann-Nielsen and Wium-Andersen, 1970; Sunda and Guillard, 1976; Andrew, Biesinger, and Glass, 1977; Anderson, Morel and Guillard, 1978). Sunda and Guillard (1976) demonstrated that the growth rate inhibition by copper of the marine diatom *Thalassiosira pseudonana* is functionally related to the copper ion activity irrespective of the total copper concentration in the medium. Studies of the effect of copper to the water flea *Daphnia magna* over a wide range of total copper and chelator concentrations showed a linear relationship between acute toxicity to *Daphnia magna* and ionic copper activities (Andrew et al., 1977). In a study by Zevenhuizen, Dolfing, Eshuis, and Scholten-Koerselman (1979) the growth rate of the bacterium *Klebsiella aerogenes* was inhibited in the range of 10^{-8} to 10^{-6} M of cupric ion concentration. Anderson et al. (1978) demonstrated that the zinc concentration limited the growth rate of the marine diatom

Thalassiosira weisflogii. These results indicate that when chemical speciation is taken into account, a toxic effect is apparent at approximately the same concentration of copper ion or zinc ion for water or nutrient media from a wide variety of sources. For this reason it is important to consider chemical speciation when assessing the toxicity of metals to aquatic organisms.

Since heavy metals seldom exist in isolation in natural aquatic environments, there has been increased interest in the study of mixtures of metals and their combined effect on aquatic organisms. Sprague (1970) described a scheme to predict toxicity of mixtures of pollutants on the basis of chemical measurements, and presented a system of nomenclature for the interaction, or antagonism. Hutchinson (1973) measured the toxicity of mixtures of Cu and Ni, and Se and Cd to phytoplankton; he reported a joint action of the two first metals and antagonistic effect in the second pair of metals. Lead was found to ameliorate the toxicity of Cu to Selenastrum capricornutum while Mg enhanced the toxic effect of Cu in the same organism (Christensen, Scherfig, and Dixon, 1979). Combined effects of Cu and Zn free ions on cultures of four common species of marine phytoplankton were reported by Braek and Jensen (1976); the ions acted synergistically on Skeletonema costatum, Thalassiosira pseudonana, and Amphidinium carteri while zinc reduced the inhibition effect of copper on Phaeodactylum tricornutum. Lewis (1978) determined the synergistic effect of both mixtures of Cu and Zn, and Cu and Mg which produced acute toxicity on juvenile longfin dace, Agosia chrysogaster; the Cu-Zn mixture exhibited a more than additive toxicity while the Cu-Mg toxicity was additive. Thomas, Hollibaugh, Seibert, and Wallace (1980) studied the effects of a mixture of ten heavy metals on phytoplankton from Saanich Inlet, B.C., Canada, and on cultures of the diatom Thalassiosira aestivalis. They attributed the inhibition in the growth of the experimental organisms to the combined toxicities of Cu and Hg. Working with Selenastrum capricornutum, Bartlett, Rabe and Funk (1974) found that combinations of Cu, Zn, and Cd were similar in toxicity to equal concentrations of Zn. However, to date, all of the research on the combined effect of metals has been concerned with the total amounts of these metals, and not with the interaction of free ions.

Although the mechanism of the toxic effects of various combinations of metals is complex, attention in experimental research must be focused on this area. Combinations, rather than single heavy metals, are most often found in the natural environment, and their interactions may be detrimental to algae. The purpose of this investigation was to determine if the free ions of copper and zinc have an additive or competitive interaction on the toxicity to the green alga Selenastrum capricornutum in a defined artificial medium, under controlled laboratory conditions. The results illustrate why it is important, in order to understand the effects of metals on aquatic organisms, to consider both chemical speciation and the combined effect of several metals.

MATERIALS AND METHODS

The test alga Selenastrum capricornutum is a unicellular green alga (Chlorophyta) of the order Chlorococcales, family Oocystaceae (Smith, 1950). The strain of this species used in the study was obtained from the Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, Oregon. It was maintained in modified artificial EPA-NAAM medium (Table I) in polycarbonate flasks (250 ml). Transfer to fresh medium was made every week.

The tests were conducted in batch cultures following the general guidelines of the Algal Assay Bottle Test (Miller, Greene, and Shiroyama, 1971), with some modifications: zinc was reduced from 2.4×10^{-7} M to 1.0×10^{-8} M to avoid possible zinc toxicity; iron was reduced from 5.92×10^{-7} M to 1.00×10^{-8} M to prevent iron precipitation; and EDTA was increased from 8.06×10^{-7} M to 3.00×10^{-6} M to prevent chemical precipitation. For the copper and zinc experiments, either copper or zinc, or both, were deleted from the medium, and later added in the course of each experiment. Free ion concentrations of zinc and copper were estimated by the computer program MINEQL (Westall, Zachary, and Morel, 1976).

Stock solutions were prepared from reagent grade chemicals (Mallinckrodt Chemical Works). The solutions were made with deionized glass distilled water. Once the stock solutions were prepared, they were stored in 1 liter linear polyethylene bottles, and replaced every six months. The medium was prepared from the stock

TABLE I

MODIFIED ALGAL ASSAY MEDIUM

component	concentration moles l^{-1}	component	concentration moles l^{-1}
Ca^{+2}	3.00×10^{-5}	SO_4^{-2}	5.96×10^{-5}
Mg^{+2}	5.98×10^{-5}	Cl^{-}	1.84×10^{-4}
K^{+}	1.20×10^{-5}	PO_4^{-3}	5.99×10^{-6}
Na^{+}	4.85×10^{-4}	EDTA	3.00×10^{-6}
Fe^{+3}	1.00×10^{-8}	$B(OH)_4^{-}$	3.00×10^{-6}
Mn^{+2}	2.10×10^{-6}	MoO_4^{-2}	3.00×10^{-8}
Zn^{+2}	1.00×10^{-8}	NO_3^{-}	3.00×10^{-4}
CO_2^{+2}	6.00×10^{-9}	H^{+}	1.94×10^{-4}
CO_3^{-2}	1.79×10^{-4}	Cu^{+2}	6.29×10^{-11}

solutions each time in a 2000 ml volumetric flask. Copper and zinc solutions were made from copper sulfate and zinc chloride stock solutions, respectively.

Once the medium was ready, 100 ml aliquots were dispensed into 250 ml teflon^R flasks. The medium was then autoclaved at 120° C for 15 minutes. After autoclaving, the medium was allowed to sit to equilibrate for 24 hours. Each flask was then inoculated with an actively growing culture with a volume which would introduce an initial cell density of 10^3 cells per ml. The cultures were grown on a rotary shaker under constant light at 24° C. Two days after the introduction of the inoculum, the cultures were counted and spiked with a different concentration of the experimental metal solution.

Cell numbers were counted each day in an AO Brightline Hemocytometer. The pH of the medium was measured with an Orion Model 404 ion analyzer.

All glassware was acid washed in 10 percent HNO_3 and rinsed with deionized distilled water prior to use.

RESULTS AND DISCUSSION

Results of recent research demonstrate the toxicity of heavy metals to aquatic organisms is due to metal ion activity (Sunda and Guillard, 1976; Anderson et al., 1978). This toxicity is independent of the dissolved or total metal concentrations (Andrew et al., 1977). The purpose of this study was to determine the level of concentration of ionic zinc or ionic copper required to inhibit the cell growth of Selenastrum capricornutum, and to compare the effects of the combination of zinc and copper ions upon its cell growth.

A total of thirteen toxicity experiments was reported (Appendices A to M); a modified EPA-NAAM medium was utilized for the cultures. All the assays were spiked with differing concentrations of either zinc or copper solutions. While one of the metal concentrations in each bioassay was varied, the other was kept fixed at a predetermined amount.

EFFECT OF ZINC

Total zinc concentrations ranging from 1×10^{-13} M to 2×10^{-6} M were added to two-day-old cultures of Selenastrum capricornutum (Figure 1). The total zinc and total copper normally present in the EPA-NAAM medium were not included in the experiment to avoid background levels of the metals that would interfere with the results. As shown in Figure 1, there was a direct correlation between total ionic zinc concentration and inhibition of growth.

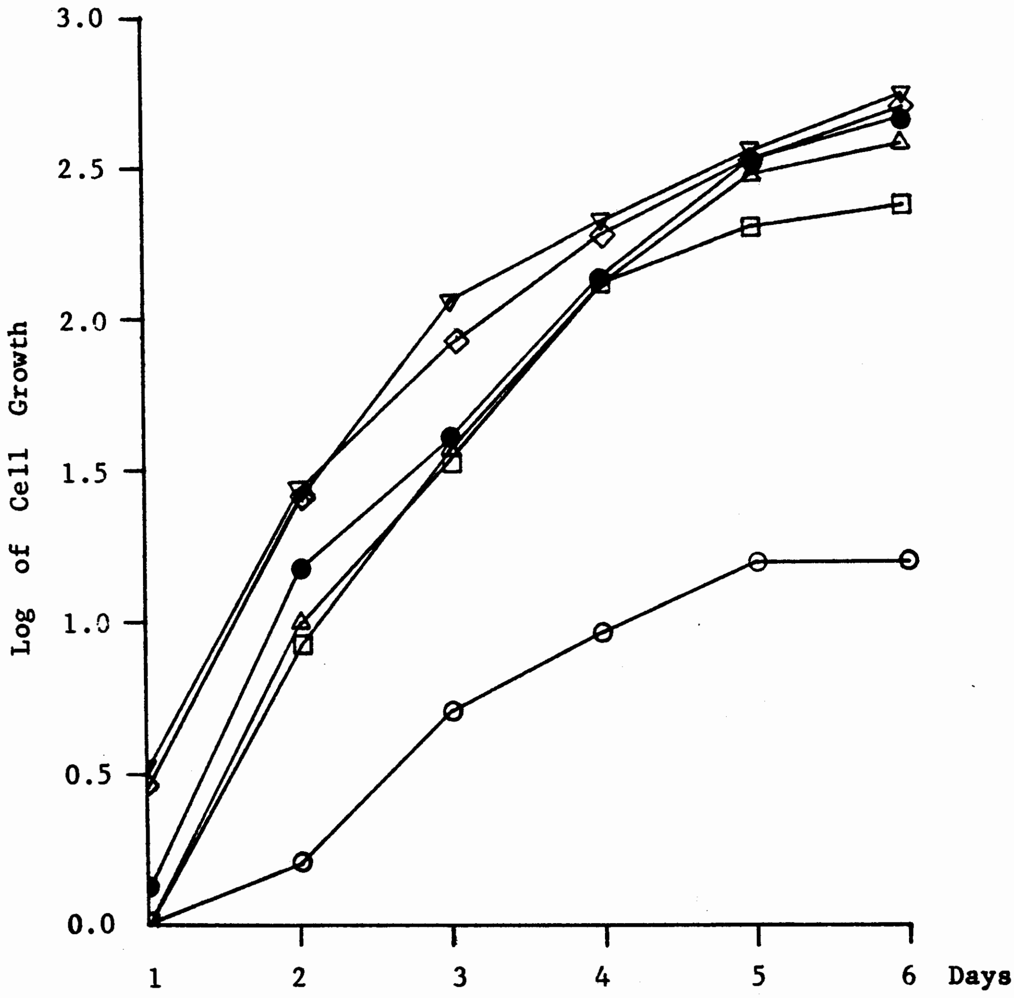

Figure 1. Growth curves of Selenastrum capricornutum at various concentrations of pZn:
▽ pZn = 15.2; ◇ pZn = 8.45; ● pZn = 8.02;
△ pZn = 7.18; □ pZn = 6.35; ○ pZn = 5.93.

The point of inhibition of cell growth occurred at pZn = 5.93, as calculated by the program MINEQL (Westall et al., 1976). By definition pZn is equal to the negative logarithm of the zinc ion activity (Sunda and Guillard, 1976).

EFFECT OF COPPER

Growth rates of cultures in modified EPA-NAAM medium treated with various concentrations of free copper ions are presented in Figure 2. Selenastrum capricornutum cultured cells were counted daily throughout the six days of incubation. The range of total copper added in this bioassay was from 1.00×10^{-13} M to 2×10^{-5} M. The point of inhibition of cell growth occurred at pCu = 7.24, as calculated by the program MINEQL (Westall et al., 1976). pCu is defined as equal to the negative logarithm of the cupric ion activity (Sunda and Guillard, 1976).

In summary, the level of copper required to inhibit cell growth was pCu = 7.24, and the level of zinc required to inhibit cell growth was pZn = 5.93. Results indicate ionic copper is more toxic to Selenastrum capricornutum than zinc.

EFFECT OF ZINC AND COPPER

A total of thirteen bioassays was carried out in which the total amount of $ZnCl_2$ or $CuSO_4$ added was the same. In one set of bioassays the $CuSO_4$ concentration was constant while the $ZnCl_2$ concentration was varied; in the other set the reverse was true. Using the least squares linear regression, the point at which the

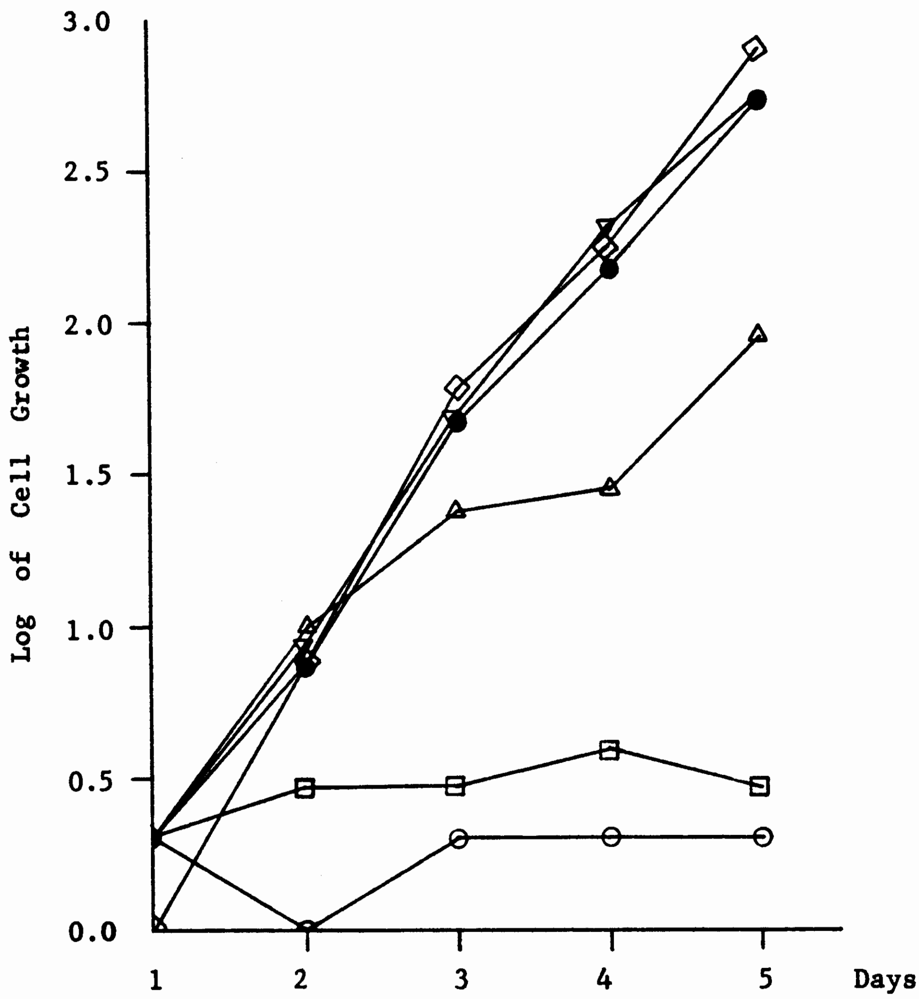

Figure 2. Growth curves of *Selenastrum capricornutum* at various concentrations of pCu: ▽ pCu = 20.0; ◇ pCu = 10.65; ● pCu = 9.69; △ pCu = 8.71; □ pCu = 7.24; ○ pCu = 7.10.

concentration of combined copper and zinc produced a 50 percent reduction in the growth rate was determined for each bioassay. The total copper, total zinc, and pCu and pZn values which produced 50 percent maximum growth rate for each bioassay are presented in Table II. The pCu and pZn values which produced 50 percent maximum growth rate for each bioassay are presented in Figure 3. Results indicate there was no interaction between zinc and copper ions in their toxicity to Selenastrum capricornutum.

I noted that there were morphological changes in the alga, depending upon the metal used. With the addition of copper, the alga appeared to enlarge and become yellowish, while with zinc additions the alga appeared to decrease in size and darken. Further investigation of these apparent morphological differences is needed.

It appears, therefore, that the common observation that toxic metals are additive in their effect on aquatic organisms may be an artifact which results from considering only the total metal concentrations. When toxicity is compared only on the basis of total metal added, results appear to be additive (Sprague, 1970), but if chemical speciation is considered, it may be, as observed in this study, that the results are better described as non-interactive.

TABLE II

CONCENTRATION OF ZINC AND COPPER THAT PRODUCED A 50 PERCENT REDUCTION IN GROWTH RATE ON *SELENASTRUM CAPRICORNUTUM*

bioassay	total copper moles l^{-1}	pCu	total zinc moles l^{-1}	pZn
1	6.30×10^{-11}	11.75	1.75×10^{-6}	6.06
2	1.39×10^{-6}	7.40	2.40×10^{-7}	6.68
3	6.30×10^{-11}	12.58	1.50×10^{-6}	6.17
4	5.09×10^{-7}	8.28	1.00×10^{-6}	6.17
5	6.26×10^{-7}	7.87	1.00×10^{-6}	6.05
6	6.26×10^{-7}	8.10	7.70×10^{-7}	6.25
7	6.30×10^{-11}	13.46	5.50×10^{-6}	6.26
8	4.67×10^{-6}	8.74	2.40×10^{-7}	7.99
9	2.73×10^{-6}	8.90	2.00×10^{-7}	7.94
10	2.75×10^{-6}	7.55	3.00×10^{-7}	6.73
11	2.94×10^{-6}	7.49	4.00×10^{-7}	6.56
12	3.03×10^{-6}	7.20	5.00×10^{-7}	6.36
13	2.90×10^{-6}	8.47	1.11×10^{-7}	7.80

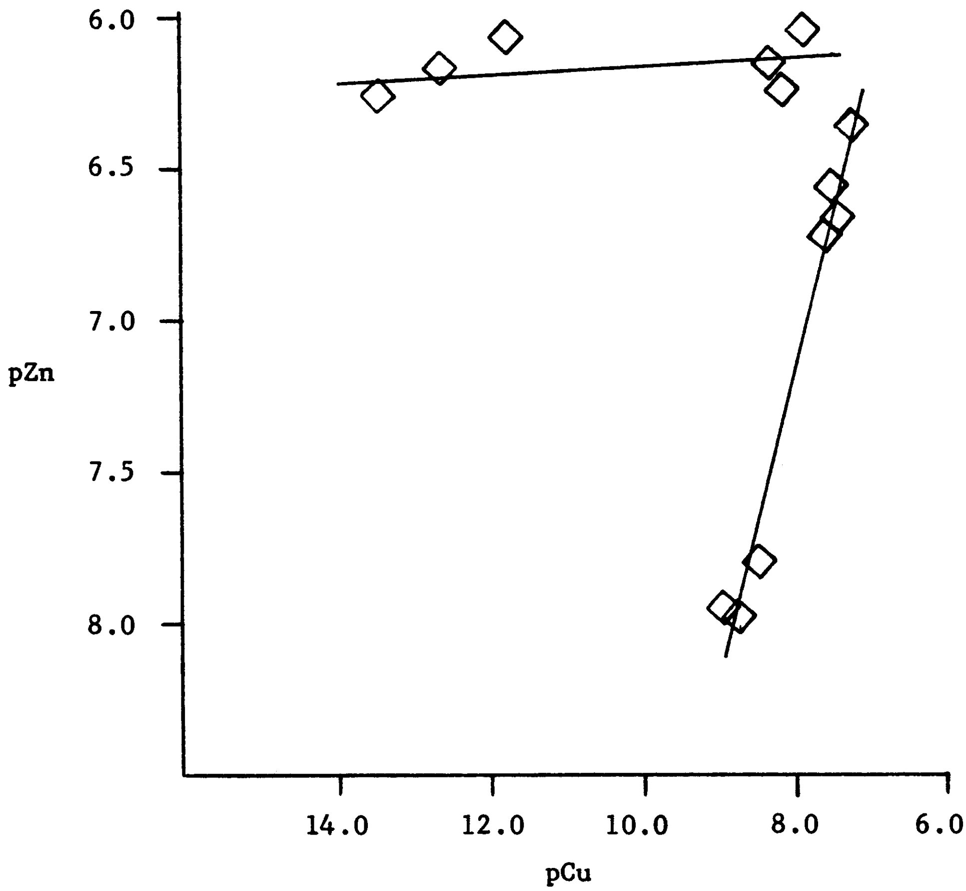

Figure 3. Combinations of pZn and pCu which reduced the growth of *Selenastrum capricornutum* to 50 percent of its maximum growth.

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APPENDICES

APPENDIX A

TABLE III

BIOASSAY 1: CELL COUNTS OF S. CAPRICORNUTUM GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	6	total Zn	pZn	total Cu	pCu
2	14	56	175	376	428				
-	9	25	111	296	418	1.0×10^{-13} M	15.2	6.3×10^{-11} M	14.7
-	6	21	97	239	281				
1	17	57	159	411	478				
2	16	41	143	302	462	3.2×10^{-7} M	8.45	6.3×10^{-11} M	14.5
1	12	28	125	308	468				
3	22	95	182	318	426				
3	28	64	185	338	474	5.0×10^{-7} M	8.02	6.3×10^{-11} M	14.2
4	34	93	205	357	597				
2	21	139	211	385	562				
3	38	122	215	373	576	7.9×10^{-7} M	7.18	6.3×10^{-11} M	13.5
4	23	86	221	338	544				
2	9	30	151	195	229				
1	7	35	108	211	225	1.3×10^{-6} M	6.35	6.3×10^{-11} M	12.8
1	9	43	137	207	267				
-	1	7	13	13	15				
-	-	3	8	18	18	2.0×10^{-6} M	5.93	6.3×10^{-11} M	12.4
-	4	6	7	17	14				

APPENDIX B

TABLE IV

BIOASSAY 2: CELL COUNTS OF _S._ _CAPRICORNUTUM_ GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	6	total Zn	pZn	total Cu	pCu
32	72	173	329	541	545				
38	88	190	338	433	443	2.4×10^{-7} M	8.64	1.0×10^{-13} M	14.5
28	85	183	338	424	544				
34	82	179	329	403	498				
29	79	183	351	465	522	2.4×10^{-7} M	7.14	6.3×10^{-7} M	8.86
40	92	200	328	384	471				
35	69	163	268	422	510				
28	86	138	337	482	453	2.4×10^{-7} M	6.76	7.8×10^{-7} M	7.97
30	85	174	396	512	681				
10	28	112	322	365	563				
18	36	139	337	397	480	2.4×10^{-7} M	6.66	9.9×10^{-7} M	7.29
7	30	113	293	389	401				
3	8	20	58	190	295				
4	6	28	87	217	333	2.4×10^{-7} M	6.66	1.3×10^{-6} M	7.25
4	12	27	60	211	274				

APPENDIX C

TABLE V

BIOASSAY 3: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	6	total Zn	pZn	total Cu	pCu
18	96	383	511	557	538				
25	107	347	502	512	541	1.0×10^{-13} M	15.2	6.3×10^{-11} M	14.7
17	108	338	485	539	520				
11	58	232	363	484	475				
6	29	178	311	423	512	1.0×10^{-6} M	6.66	6.3×10^{-11} M	13.1
15	41	206	354	472	590				
5	42	137	212	259	364				
2	25	79	156	206	193	1.3×10^{-6} M	6.35	6.3×10^{-11} M	12.8
1	25	84	183	238	276				
1	7	26	28	48	74				
2	3	26	37	40	42	1.6×10^{-6} M	6.12	6.3×10^{-11} M	12.5
1	6	16	25	24	41				
1	4	3	6	2	4				
1	1	1	2	2	8	2.0×10^{-6} M	5.93	6.3×10^{-11} M	12.4
-	7	5	4	6	7				
-	-	3	-	1	1				
-	-	1	-	-	1	2.5×10^{-6} M	5.78	6.3×10^{-11} M	12.2
-	-	-	1	-	-				

APPENDIX D

TABLE VI

BIOASSAY 4: CELL COUNTS OF _S. CAPRICORNUTUM_ GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	total Zn	pZn	total Cu	pCu
7	20	70	213	345				
1	12	74	272	294	1.0×10^{-13} M	15.2	1.0×10^{-13} M	17.5
1	14	51	198	271				
1	9	44	81	154				
3	5	37	58	126	1.0×10^{-6} M	6.18	5.0×10^{-7} M	8.31
1	10	32	63	181				
5	5	15	6	8				
6	6	7	9	11	1.0×10^{-6} M	6.12	6.3×10^{-7} M	7.99
4	6	9	9	10				
2	10	7	13	11				
1	6	7	13	12	1.0×10^{-6} M	6.06	7.8×10^{-7} M	7.60
4	4	5	9	7				
2	4	4	9	6				
2	1	3	1	1	1.0×10^{-6} M	6.04	1.0×10^{-6} M	7.25
9	8	5	5	4				
1	2	1	3	3				
2	2	4	5	5	1.0×10^{-6} M	6.04	1.3×10^{-6} M	7.25
3	8	6	8	4				

APPENDIX E

TABLE VII

BIOASSAY 5: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	total Zn	pZn	total Cu	pCu
17	92	388	492	478				
26	144	457	511	481	1.0×10^{-13} M	15.2	1.0×10^{-13} M	17.5
34	139	405	473	509				
12	38	232	286	414				
25	108	241	308	369	1.0×10^{-6} M	6.49	1.2×10^{-7} M	9.52
35	82	243	367	367				
8	47	168	277	431				
14	52	201	270	424	1.0×10^{-6} M	6.40	2.0×10^{-7} M	9.19
14	43	181	269	382				
11	29	93	144	237				
23	68	232	298	454	1.0×10^{-6} M	6.29	3.1×10^{-7} M	8.77
8	52	200	220	407				
42	71	271	326	388				
31	85	264	349	392	1.0×10^{-6} M	6.16	5.0×10^{-7} M	8.31
10	41	156	259	308				
9	17	23	26	34				
11	15	39	29	45	1.0×10^{-6} M	6.12	6.3×10^{-7} M	8.00
26	32	119	163	259				

APPENDIX F

TABLE VIII

BIOASSAY 6: CELL COUNTS OF S. CAPRICORNUTUM GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	total Zn	pZn	total Cu	pCu
17	94	472	519	568				
33	196	438	467	478	1.0×10^{-13} M	15.2	1.0×10^{-13} M	17.5
52	163	408	472	529				
23	108	286	472	533				
42	142	353	451	424	3.8×10^{-7} M	6.71	6.3×10^{-7} M	8.49
25	125	324	491	452				
39	141	368	448	481				
12	53	205	379	353	4.8×10^{-7} M	6.56	6.3×10^{-7} M	8.36
37	142	306	438	501				
31	113	297	475	428				
28	81	133	324	362	6.1×10^{-7} M	6.38	6.3×10^{-7} M	8.21
29	129	264	408	445				
38	45	142	334	387				
28	59	154	326	354	7.7×10^{-7} M	6.26	6.3×10^{-7} M	8.10
17	47	99	247	335				
19	25	63	80	159				
25	70	107	189	294	9.6×10^{-7} M	6.14	6.3×10^{-7} M	8.01
29	89	162	241	296				

APPENDIX H

TABLE X

BIOASSAY 8: CELL COUNTS OF S. CAPRICORNUTUM GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day					total		total	
1	2	3	4	5	Zn	pZn	Cu	pCu
1	4	24	177	427				
1	8	58	187	595	1.0×10^{-13} M	17.7	1.0×10^{-13} M	20.0
2	13	68	269	701				
1	6	52	148	507				
1	10	70	188	974	2.4×10^{-7} M	9.44	3.0×10^{-6} M	10.7
1	7	57	214	891				
1	5	40	116	480				
2	10	63	207	591	2.4×10^{-7} M	8.64	4.2×10^{-6} M	9.69
2	8	41	136	626				
2	11	29	25	104				
2	8	19	25	82	2.4×10^{-7} M	7.74	4.7×10^{-6} M	8.71
2	10	23	35	80				
2	3	4	3	3				
2	3	2	5	5	2.4×10^{-7} M	6.78	6.0×10^{-6} M	7.24
1	2	4	4	3				
2	1	1	3	2				
2	1	3	2	3	2.4×10^{-7} M	6.74	2.0×10^{-5} M	7.10
2	2	2	2	2				

APPENDIX I

TABLE XI

BIOASSAY 9: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day					total		total	
1	2	3	4	5	Zn	pZn	Cu	pCu
5	24	82	237	320				
7	27	74	225	286	1.0×10^{-13} M	16.9	1.0×10^{-13} M	19.2
14	35	94	184	385				
3	12	51	68	153				
6	22	46	114	194	2.0×10^{-7} M	8.69	2.3×10^{-6} M	9.92
8	16	99	119	249				
3	19	27	49	74				
11	27	87	100	188	2.0×10^{-7} M	8.42	2.5×10^{-6} M	9.61
9	22	78	85	151				
8	15	36	48	74				
10	17	24	55	60	2.0×10^{-7} M	7.93	2.7×10^{-6} M	9.07
8	17	37	54	59				
3	7	10	13	12				
4	12	21	22	26	2.0×10^{-7} M	7.04	2.9×10^{-6} M	7.92
6	13	20	21	23				
11	21	15	25	14				
14	20	15	19	23	2.0×10^{-7} M	6.78	3.1×10^{-6} M	7.13
8	13	10	12	9				

APPENDIX J

TABLE XII

BIOASSAY 10: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day					total		total	
1	2	3	4	5	Zn	pZn	Cu	pCu
2	2	27	33	85				
2	5	17	28	72	1.0×10^{-13} M	16.9	1.0×10^{-13} M	19.2
1	6	8	28	77				
1	6	33	141	260				
3	3	29	152	316	3.0×10^{-7} M	8.39	2.3×10^{-6} M	9.80
5	2	50	138	297				
4	12	35	152	222				
3	5	19	90	131	3.0×10^{-7} M	8.05	2.5×10^{-6} M	9.42
5	9	51	183	290				
2	5	17	60	163				
4	3	29	58	143	3.0×10^{-7} M	7.35	2.7×10^{-6} M	8.62
1	1	11	62	155				
3	7	12	23	107				
3	4	25	64	145	3.0×10^{-7} M	6.77	2.9×10^{-6} M	7.71
2	2	19	31	70				
2	3	10	21	27				
4	10	18	32	75	3.0×10^{-7} M	6.60	3.1×10^{-6} M	7.10
4	3	12	22	30				

APPENDIX K

TABLE XIII

BIOASSAY 11: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day 1	2	3	4	5	total Zn	pZn	total Cu	pCu
25	96	143	270	452				
8	34	84	112	280	1.0×10^{-13} M	16.9	1.0×10^{-13} M	19.2
5	33	43	98	158				
13	35	69	226	335				
7	24	58	197	259	4.0×10^{-7} M	8.12	2.3×10^{-6} M	9.65
41	116	210	348	458				
55	77	128	240	284				
23	57	151	276	358	4.0×10^{-7} M	7.66	2.5×10^{-6} M	9.14
14	47	116	148	282				
14	43	105	228	375				
68	153	228	305	359	4.0×10^{-7} M	6.94	2.7×10^{-6} M	8.26
40	112	148	269	330				
32	108	121	261	395				
43	111	140	222	404	4.0×10^{-7} M	6.59	2.9×10^{-6} M	7.59
11	27	41	85	153				
16	23	42	73	103				
39	63	107	160	186	4.0×10^{-7} M	6.47	3.1×10^{-6} M	7.06
18	39	46	68	110				

APPENDIX L

TABLE XIV

BIOASSAY 12: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day					total		total	
1	2	3	4	5	Zn	pZn	Cu	pCu
2	28	84	189	315				
3	16	70	144	268	1.0×10^{-13} M	16.9	1.0×10^{-13} M	19.2
2	17	44	105	219				
3	16	66	178	217				
4	23	88	162	210	5.0×10^{-7} M	7.84	2.3×10^{-6} M	9.46
6	47	88	187	198				
4	9	28	97	185				
5	21	30	139	202	5.0×10^{-7} M	7.23	2.5×10^{-6} M	8.78
4	19	43	139	207				
6	15	36	91	173				
4	11	21	46	108	5.0×10^{-7} M	6.70	2.7×10^{-6} M	8.05
5	20	33	78	155				
4	13	37	68	135				
1	21	34	35	76	5.0×10^{-7} M	6.46	2.9×10^{-6} M	7.50
3	18	40	51	98				
7	12	25	26	38				
5	12	31	28	59	5.0×10^{-7} M	6.37	3.1×10^{-6} M	7.03
6	13	23	27	50				

APPENDIX M

TABLE XV

BIOASSAY 13: CELL COUNTS OF *S. CAPRICORNUTUM* GROWN IN MODIFIED ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF ZINC AND COPPER

cell count per day					total		total	
1	2	3	4	5	Zn	pZn	Cu	pCu
5	14	57	120	212				
3	5	37	103	200	1.0×10^{-13} M	16.9	1.0×10^{-13} M	19.2
5	15	44	100	196				
8	10	35	107	157				
1	10	14	42	110	5.0×10^{-8} M	8.27	2.9×10^{-6} M	8.76
5	17	23	36	100				
6	18	10	10	10				
8	9	13	16	17	1.5×10^{-7} M	7.35	2.9×10^{-6} M	8.21
1	2	5	4	9				
1	1	2	3	1				
6	11	10	7	11	2.5×10^{-7} M	6.95	2.9×10^{-6} M	7.92
4	10	4	8	6				
3	2	1	1	2				
9	2	4	6	5	3.5×10^{-7} M	6.72	2.9×10^{-6} M	7.77
8	3	6	7	3				
7	6	5	2	3				
4	5	4	11	4	4.5×10^{-7} M	6.57	2.9×10^{-6} M	7.67
4	7	5	5	4				