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Enhancement of Pigment Concentrations in *Dunaliella tertiolecta* as a Result of Copper Toxicity

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The addition of copper to coastal waters through the disposal of complex effluents has been increasing. Copper is a well known algicide. It binds to the cell membrane, effects permeability mechanisms and attaches to SH groups on vital enzymes (O'Kelley 1974). In sublethal concentrations it retards log phase of growth (Steemann-Nielsen and Wium-Anderson 1970; Hawkins and Griffiths 1982). The ability of algae to withstand copper toxicity varies greatly. The green flagellate, Dunaliella tertiolecta has previously been shown to be highly resistant to copper (Erickson et al. 1970; Lustigman et al. 1985).Its ability to withstand high concentrations of copper promotes growth of Dunaliella in coastal waters contaminated with increased copper concentrations. Dunaliella tertiolecta, possesses chlorophylls a and b, small amounts of alpha- and greater amounts of beta-carotene as well as neoxanthin,zeaxanthin and lutein (Strain 1966).The beta-carotene content of the algae varies with the species and growth conditions (Ben-Amotz and Avron 1983).

This study was undertaken to determine the effect of lethal and sublethal concentrations of copper on the growth and production of chlorophyll and carotenoids in Dunaliella tertiolecta.

MATERIALS AND METHODS

Dunaliella tertiolecta was obtained from Dr. A. D'Agostino, Montauk, L.I., New York. The algae were grown on 0.2 μ millipore filtered modified DC medium of 3.2‰S, pH 7.8 (Provasoli et al. 1957)(Table 1). This is considered to be isotonic to sea water. The cultures were tested periodically by plating on bacteriological media and were found to be free of bacterial contamination. Test tubes and other glassware were acid washed, sterilized , filled with 15 ml of medium and incubated 24 hours prior to inoculation. Cell counts were made to establish an inoculum of 10^6 cells. Cultures were maintained in an incubator at 22-25^0 C, continuously illuminated with 2000 lux from GE cool white 30 watt fluorescent bulbs. Cultures were maintained for 21 days.

Copper was supplied as $CuSO_4 \cdot 5H_2O$ from 1‰ Cu^{++} stock solution to achieve concentrations of 0,5,10,20,30,40,50 ppm Cu^{++}. These values were tested by atomic absorption analysis of uninoculated samples and found to be accurate. In addition, the Cu^{++} concentrations of the cells and the medium at the end of the experimental period were measured. The concentration and solubility of Cu^{++} are determined by pH and chelation. In addition to buffering pH, TRIS (tris-hydroxymethyl- aminomethane) chelates copper.

Table 1. Composition of modified DC medium (artificial seawater medium) after Provasoli et al.(1957)

NaCl		2.5 g%
KCl		60.0 mg%
$MgSO_4 \cdot 7H_2O$		500.0 mg%
Ca (as Cl^-)		10.0 mg%
$NaNO_3$		50.0 mg%
KH_2PO_4		3.0 mg%
PII metal mix		5 .0 ml / L
H_3BO_3	114.0 mg%	
$FeCl_3 \cdot 6H_2O$	4.9 mg%	
$MnSO_4 \cdot 4H_2O$	16.4 mg%	
$ZnSO_4 \cdot 7H_2O$	2.2 mg%	
Vitamin mix 8 A		1.0 ml/ L
Thiamine·HCl	20.0 mg%	
Biotin	50.0 mg%	
B_{12}	5.0 µg%	
TRIS (Tris hydroxy-methyl-aminomethane)		100.0 mg%
Final pH 7.8		

It regulates Cu^{++} activity so that it remains constant. A total of 18 cultures were tested for each experimental value.At fourteen days, 5 ml samples containing appproximately 4 X 10^6 cells /ml were removed from the cultures and the number of cells counted, using a Neubauer hemocytometer. The samples were then centrifuged at 1000 Xg for 10 minutes. The supernatant was retained for atomic absorption analysis. The pellet was washed twice in medium of the proper salinity. The pellet was treated with 5 ml of absolute ethyl alcohol and kept in complete darkness for 2 hours at ambient temperature. The sample was then recentrifuged at 1000 Xg for 10 minutes. Absorption spectra were registered on a Beckman DB-G spectrophotometer and showed peaks at 665, 650, and 452 nm for chlorophyll a, chlorophyll b, and carotenoids respectively.Calculations were made according to the formulae devised by Senger (1970) for total chlorophyll, chlorophyll a, chlorophyll b and carotenoids.

RESULTS AND DISCUSSION

The reproduction rate of Dunaliella tertiolecta decreased in medium containing copper sulfate (Table 2). The growth of the cultures was severely retarded which was apparent at 5 ppm Cu^{++}. The greatest effect on population growth was at concentrations up to 30 ppm Cu^{++}. There then appears to be a leveling off up to 50 ppm. Copper concentrations above 50 ppm were uniformly lethal.

Increased copper concentrations produced an increase in the pigment content /cell which was apparent by 5 ppm Cu^{++}. At 50 ppm there were almost twice as much carotenoids/ cell as in the 0 ppm controls (Table 3). Copper also increased the amount of chlorophyll/ cell so that there was more than 1.5 times as much chlorophyll/ cell at 50 ppm Cu^{++}.

Table 2. Number of cells (10^6/ml with increasing copper at 7, 14, and 21 days

Cu^{++} ppm	7 days	14 days	21 days
0	1.52±0.24	2.84±0.56	3.63±0.47
5	0.72±0.38	1.89±0.22	3.16±0.59
10	0.56±0.21	1.71±0.57	2.53±0.83
20	0.47±0.16	1.05±0.35	2.01±0.27
30	0.43±0.17	1.18±0.33	2.04±0.98
40	0.41±0.23	1.46±0.31	2.70±0.69
50	0.42±0.09	1.40±0.30	2.76±0.89

According to F test and T test analysis, copper was statistically significant in determining chlorophyll and carotenoid concentration. Copper reduced the rate of division in Dunaliella tertiolecta leading to fewer cells/ ml throughout the experimental period. In the course of normal log phase of growth, rapid cell division reduces the concentration of pigments so that they do not accumulate in greater amounts within the individual cells. Due to the delay in log phase with copper treatment, there was a significantly greater concentration of both carotenoids and chlorophyll per cell. Total yield/ ml,however, did not increase with copper treatment because of the decrease in the number of cells /ml. The ratio of carotenoids/ chlorophyll also remained relatively constant.

Table 3. [Carotenoid] and [chlorophyll]/ cell at 14 days with increasing copper given as pg /cell

Cu^{++} (ppm)	Carotenoids	Chlorophyll
0	0.54±0.12	1.73±0.41
5	0.74±0.11	2.20±0.25
10	0.73±0.10	2.50±0.27
20	0.80±1.07	3.10±0.09
30	0.98±0.09	3.62±0.41
40	0.94±0.16	3.71±0.37
50	0.87±0.21	2.82±0.55

Previous studies have shown that copper retards log phase of growth(Pace et al. 1977; Steemann-Nielsen and Wium-Anderson 1970). The increased amount of both carotenoids and chlorophyll/cell found here is probably due to accumulation of both pigments as a result of delayed cell division.

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