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AN ABSTRACT OF THE THESIS OF Gerald Eugene Schulz for the Master of Science in Biology presented August 8, 1973.

Title: Growth of Selenastrum capricornutum Printz in Natural Waters Treated with Copper Sulfate.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Richard Petersen, Chairman

B. E. Lippert

Richard Tocher

Robert Rempfer

The green alga, Selenastrum capricornutum Printz, was used in an algal bioassay to determine the toxic properties of copper in various natural lake waters of Oregon. Lake water was collected and brought back to the laboratory where it was treated with various copper concentrations and inoculated with a cell suspension of the test alga.

Growth of the alga was determined by direct cell counts. The copper ion activity, pH, and absorbance of the test cultures were also determined. The growth of the alga was found to depend upon the water tested and also upon the copper concentration used.

GROWTH OF SELENASTRUM CAPRICORNUTUM PRINTZ IN
NATURAL WATERS TREATED WITH COPPER SULFATE

by
GERALD EUGENE SCHULZ

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

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in
BIOLOGY

Portland State University
1973

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of
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August 10, 1973

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INTRODUCTION

In a fresh water environment such as a lake or reservoir, algal growth of some species may develop to the extent that it causes fishy tastes and odors, or it may contribute to unsightly scum (Palmer 1962). The development of excess growth is dependent upon a number of factors. To understand this process fully, Fruh (1966) stated the need to monitor the nutrients, productivity, and biota of the water for a number of years. However, the algal problem is often acute and must be dealt with promptly.

To combat the unwanted growths of problem-causing algae, the algicide copper sulfate has been employed for many years with great success. However, the chemistry of the water and the algal species involved vary widely from lake to lake, and no set amount of the algicide can be universally applied with similar results. Monie (1944) reported a test to determine the concentration of copper to be applied based on the changes of alkalinity in the water caused by the addition of the algicide. This method has been used successfully for over 15 years (Monie 1956). However, there are many factors which can influence the effectiveness of the algicide.

Monie (1956) listed six factors controlling the effectiveness of the copper. These included: (1) the algal species involved, (2) the amount of organic matter present, (3) the carbonic acid content, (4) the hardness of the water, (5) the temperature of the water, and (6) the amount of water to be treated. Still other controlling factors had been

noted which included: (1) the method of application (Toth and Reimer 1968), (2) the presence of suspended silt (Reimer and Toth 1970), (3) the presence of bacteria (Fitzgerald and Faust 1965), (4) the occurrence of aerobic or anaerobic conditions (McBrien et al. 1965), (5) the light intensity (Steeman-Nielsen et al. 1969 and Cedeno-Maldonado et al. 1972), and (6) the concentration of algae present (Knaus 1954 and Steeman-Nielsen et al. 1969). The period of time that the algae were in contact with the copper was also noted as being an important factor (Steeman-Nielsen et al. 1969). Therefore, each algal problem must be studied individually for complete understanding of what corrective measures should be followed.

As a means of studying problems related to algal growth in natural waters, standard algal bioassay techniques have been proposed (Johnson et al. 1970 and Anon. 1971). By combining the techniques of the algal bioassay and the algicide copper sulfate, I plan to show a useful method for determining the algicidal properties of copper in various natural waters as these properties apply to the test algal species Selenastrum capricornutum Printz. This method might also prove useful in determining these properties for other algal species.

COPPER

Copper has been shown to be a necessary element for growth of plants and algae. Two of the first investigators who studied the requirements of copper by these organisms were Sonner and Walker. Sonner (1945) demonstrated that small amounts of copper, when applied to the soil, resulted in an increase of plant growth. But he noted that toxic

conditions arose when larger applications of copper were made. Walker (1953) showed that a species of the green alga Chlorella required copper. He was unable to grow the alga in a copper-free medium, and he found no other element capable of replacing the missing copper. Later, Gross (1970) found that a Chlorella species grew well in a medium containing a copper concentration of about 1 micro molar. At concentrations higher than this, copper became toxic to the organism.

A deficiency of copper reduces respiration in algae (Wiessner 1961), and excess amounts of the metal also affect respiration. Haberman (1969) found copper to be a specific inhibitor for the photooxidation of diketo-gulonic acid, a process that requires manganese in photosystem II. This was further substantiated by Cedeno-Maldonado et al. (1972) who found that applications of excess manganese reduced the toxic affects of copper.

Excess copper was also shown to affect cell permeability. McBrien et al. (1965) found that cells of Chlorella vulgaris, when suspended in a medium containing a high copper concentration, showed a decrease of cellular potassium which showed up in the external medium. An increase of cell permeability is thought to be the main toxic effect of copper. In a more recent paper, it was suggested that the copper bound to the disulfide linkages in the algal cell wall (McBrien et al. 1967), and this lead to the increased permeability.

The use of copper sulfate as an algicide in public water supplies was first proposed in 1904. It had been found to be an effective algicide eliminating many of the problem-causing algal species. It was also observed to be an effective bactericidal agent toxic to many forms of pathogenic bacteria. The algicide was also low in toxicity to fish and

higher animals (Kellerman and Moore 1904). These factors made the use of copper sulfate very appealing, and since that time it has been the most widely used method for dealing with algal problems. However, as was mentioned earlier, there are a number of factors which can influence the effectiveness of the algicide in natural waters.

Copper sulfate has usually been applied to waters in crystalline form (Kellerman and Moore 1904, Monie 1944, Tarlton 1949, Monie 1956, and Antonie et al. 1963). It was assumed that the crystals dissolved shortly after application, creating the desired concentration of copper throughout the lake. However, Toth and Reimer (1968) found that much of the copper actually dissolved once it reached the bottom sediments where much of the copper was adsorbed by clay particles and dissolved organic matter. Their study showed that a theoretical concentration of 0.50 mg./l. copper, when applied to a lake in crystalline form, actually produced a concentration of only 0.09 mg./l. copper. But when the same theoretical concentration was applied in liquid form, a concentration of 0.42 mg./l. copper resulted. The copper in both instances was found to be evenly distributed throughout the lake. These facts substantiate Hasler's argument that the most effective means of applying the algicide was as a spray solution (Hasler 1949).

In hard waters, copper is thought to precipitate as the insoluble compound copper hydrate (CuO_2H_2) (Ellms 1905). In an effort to keep the copper in solution, copper-citrate combinations have been proposed (Palmer 1956). However, organic compounds acting as chelating agents, such as citrate, pose different problems. Chelators do keep the copper in solution, but they also bind the copper in a manner that makes the

copper less toxic to the algae (Palmer 1956 and Fitzgerald and Faust 1963). Bacteria present in the water can further affect the chelation process. The bacteria can attack the chelator, free the metal, and eventually precipitate the copper (Fitzgerald and Faust 1965). Many naturally occurring chelating agents for copper have been demonstrated (Albert 1947 and Albert 1953), and these could create further problems when using the algicide in some natural waters.

McBrien et al. (1965) showed copper to be more toxic under anaerobic conditions than under aerobic conditions. This may be of importance in well stratified lakes or in lakes deficient in dissolved oxygen content.

Knaus (1954) found the concentration of algal cells to affect drastically the toxicity of the copper. Using large numbers of algal cells, he found a normally toxic dose of copper to be adsorbed by the cells, and relatively normal growth continued.

Light intensity has also been shown to be an important factor to consider when applying the algicide. In a study conducted by Cedeno-Maldonado et al. (1972), copper was found to bind irreversibly to non-inhibitory sites on the cell membrane with no apparent toxic effect when applied in dim light or in the dark. However, when the same concentration of copper was applied in the light, it became incorporated in the chloroplast membrane, and the algae were destroyed.

Due to some of the previously mentioned factors, the effect of each application of copper sulfate is short-lived. Thus to be effective, continuous applications of the algicide must be made. This causes the greatest problem involved with the use of copper sulfate:

that is, the effects of the applications are cumulative (Hasler 1949). Copper, a heavy metal, becomes incorporated in the bottom sediments in an amount proportional to that applied (Antonie et al. 1963, Toth and Reimer 1968, Reimer and Toth 1970, and Sanchez et al. 1973). Mackenthun and Cooley (1952) reported copper concentrations in the sediments of some lakes where the algicide had been applied for many years reached 480 mg. copper per kilogram of mud on a dry weight basis. However, they also found this concentration to be well below the toxic level of copper on bottom dwelling organisms which they determined to be about 9000 mg. copper per kilogram mud on a dry weight basis. The natural sedimentation process within lakes would eventually cover the copper containing sediments (Reimer and Toth 1970). As a result, over a period of time less toxic conditions would prevail.

Because of the problems associated with the use of copper sulfate, many new algicides have been proposed and tested (Fitzgerald et al. 1952, Fitzgerald 1959 and 1964, Palmer and Maloney 1955, Maloney 1958, and Sladekova 1968). However, copper sulfate continues to be widely used in water treatment, and Palmer (1956) reviewed the favorable aspects of the algicide. These include: (1) cost, (2) low toxicity to fish and higher animals, and (3) toxicity to a wide variety of algae in low concentrations. These factors seem to outweigh the problems involved with the algicide, for no other method of algal control is as widely employed.

ALGAL BIOASSAY

Algal bioassays as proposed by Johnson et al. (1970) and Anon. (1971) offer standardized test procedures for the monitoring of waters. The bioassay employs specific algal species which can be cultured easily in the laboratory. Maintaining the test organisms in the laboratory permits the study of their growth in natural waters. If all parameters remain constant, the growth of the algae in the natural waters will depend upon: (1) the availability of the nutrients and (2) the presence of inhibitory or toxic substances in the water.

Johnson et al. (1970) found the alga Selenastrum gracilli to be the test organism of choice from a number of algal species examined. This species grew well in the laboratory, and it was also quite sensitive to slight changes in the nutrients in an artificial medium. The latter point was evidenced by growth changes of the alga.

Anon. (1971) suggested the use of four different algal species to accurately assess the productivity potential of natural waters. The test organisms suggested were thought to be representative of the types of algae which might be encountered in a variety of habitats. The test algal species suggested were: (1) Selenastrum capricornutum Printz, (2) Microcystis aeruginosa Kutz, (3) Anabaena flos-aquae (Lyngb) De Brebisson, and (4) a diatom which had not yet been selected.

Algal bioassays have been used by other researchers for a number of years, but each researcher usually used a different algal species. A few of these investigators included Potash (1956) who used Kirchneriella subsolitaria as a test organism, MacPhee (1961) who used a species of

Chlorella for his study, and Skulberg (1964) who used the alga Selenastrum capricornutum Printz in an algal bioassay of the Netelv River, Norway. Still other investigators have suggested algal bioassays using the naturally occurring algae in the water to be studied (Gerloff et al. 1957 and Shapiro et al. 1965).

From studies done by these investigators and by others, the algal bioassay has proved to be an effective tool in the study of productivity of natural waters. It may also prove useful as a parameter for comparison and as a supplement to physical and chemical measurements. Finally, the standardized algal bioassay techniques may be useful in assessing the toxic qualities of copper or other algicides in natural waters.

TEST ALGAL SPECIES

The test alga used in this study was Selenastrum capricornutum Printz. It was first described by Printz in 1914 in Norway where it was found to be widely distributed. Since that time much controversy has arisen as to the exact classification of the alga. Some authors have placed it into the genus Selenastrum, while others have placed it into the genus Ankistrodesmus. Other names given to this alga include Selenastrum bibraianum Reinsch, Ankistrodesmus selenastrum G. S. West, and Ankistrodesmus bibraianum Kors (Fott 1969).

Selenastrum is a genus of fresh water green algae in the Division Chlorophyta, Order Chlorococcales, and Family Oocystaceae (Smith 1950). This species of Selenastrum is characterized by being nonmotile, solitary, symmetrically crescent shaped, and not enclosed in a gelatinous matrix. The apices of the cells are acutely pointed. In the young cells, the

chloroplast is a single laminate parietal body, but in the older cells the chloroplast fills the entire cell. Some authors report the presence of a single pyrenoid while others report the lack of such a structure within the cell. Cell size varies from 10 to 48 microns in length and from 3 to 9 microns in width (Fott 1969 and Anon. 1971).

This species of Selenastrum was first shown to be useful in bioassays by Skulberg (1964). Anon. (1971) also suggested the use of this algal species in algal bioassays. The alga is easy to grow, it is easy to identify, and it is sensitive to slight changes in the nutrient medium in which it was grown. Because this alga is solitary, changes in its growth are easily determined by direct cell count. Therefore, this alga might prove useful in determining the algicidal properties of copper in natural waters.

MATERIALS AND METHODS

The test alga, Selenastrum capricornutum Printz, was obtained from the Pacific Northwest Water Laboratory of the Environmental Protection Agency in Corvallis, Oregon. Axenic cultures were then obtained by streaking the alga on agar plates containing the artificial nutrient medium. The plates were then left in an algal culture room until isolated algal colonies appeared. Isolated colonies were then removed and placed in culture tubes containing the sterilized culture medium. This method of purification of the alga was similar to one proposed by James (1971).

All chemicals used in the preparation of the nutrient medium and the copper solutions were of reagent grade. All weights were determined with a Mettler Analytical Balance model H 10, and all volumes were measured with volumetric pipets or volumetric flasks of the appropriate size.

The artificial medium was proposed by Anon. (1971) (Table I). The macronutrients were made up and stored in separate containers at concentrations 1000 times the final desired concentration. The micronutrients were also made up at concentrations of 1000 times the desired amount, but they were stored in a single container. The medium was then prepared by adding one ml. from each of the individual containers to one liter of distilled and deionized water. The nutrient K_2HPO_4 was the last to be added to prevent the precipitation of the phosphate. The solution was well mixed, placed in the appropriate flasks, stoppered, and autoclaved

TABLE I
ARTIFICIAL NUTRIENT MEDIUM

MACRONUTRIENTS

<u>Compound</u>	<u>Concentration mg./l.</u>	<u>Element</u>	<u>Concentration mg./l.</u>
NaNO_3	25.500	N	4.208
$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	1.368	P	0.186
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	12.164	Mg	2.930
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	14.700	S	1.911
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	4.410	Ca	1.200
NaHCO_3	15.000	C	2.145
		Na	11.558
		K	0.468

MICRONUTRIENTS

<u>Compound</u>	<u>Concentration mg./l.</u>	<u>Element</u>	<u>Concentration mg./l.</u>
H_3BO_3	185.520	B	32.466
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	415.447	Mn	115.494
ZnCl_2	32.709	Zn	15.624
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	1.428	Ca	0.354
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	0.011	Cu	0.004
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	7.260	Mo	3.200
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	159.882	Fe	32.936
$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	300.000		233.400

for 15 minutes at 19 psi and 120°C to 130°C. After the medium had cooled, sterile techniques were employed to inoculate the flasks with the alga.

The copper solutions were made from a solution of copper sulfate with a copper concentration of 1000 mg./l. Dilutions of this solution were made with distilled and deionized water to produce copper solutions with copper concentrations of 0.625, 1.000, 1.500, 2.500, 4.000, and 6.250 mg./l. One ml. of each solution was added to the culture tubes to produce final copper concentrations of 0.025, 0.040, 0.060, 0.100, 0.160, and 0.250 mg./l.

All glassware was washed with labtone and 10% HCl. Between each wash, the glassware was rinsed six times with distilled and deionized water. This number of rinses was found to remove all effects of a pH change caused by the acid wash (Johnson et al. 1970). All glassware was sterilized before use.

The culture tubes were 30 ml. Pyrex culture tubes and were fitted with plastic screw caps. These culture tubes were used to maintain fresh cultures of the test alga, and they were used as reaction vessels in which the growth tests were conducted.

The lakes chosen for this study are indicative of some of the types of lakes found in Oregon. They were: Cullaby Lake, Blue Lake, Lake Oswego, and Clear Lake. Cullaby Lake represented the coastal environment. Water here is colored yellow-brown as is the water of other lakes in the region. The color indicates a probable high iron content and the possibility of much dissolved organic material. The lake is highly productive and would be considered eutrophic. Blue Lake and Lake Oswego are highly productive lakes in the Willamette valley. Blue

Lake, receiving much of its water from the Columbia River through underground seepage, produces unwanted weeds and algal blooms, and to combat this condition sodium arsenate was used until about six years ago (McHugh 1973). Now the weeds are harvested by a mechanical harvester, and no algicides are used to control the algae. Lake Oswego is currently, and has been in the past, treated with copper sulfate to eliminate the problem-causing algae in the lake (McHugh 1973). Finally, by way of contrast, Clear Lake, characteristic of the lakes found in the Mt. Hood area, is an oligotrophic lake.

Lake water from the above mentioned lakes was collected in five gallon plastic Jerry Jugs. The Jerry Jugs were rinsed six times with lake water before they were brought back to the laboratory for testing. The water was filtered through type H A Millipore filters with a pore size of .45 microns. It was then distributed to culture tubes and treated with the alga and the copper solutions. This was all done within one day after collection of the water sample. The major ion concentrations were determined at each lake with a Portable Water Engineer's Laboratory model DR-EL manufactured by the Hach Chemical Co., Ames, Iowa.

The test procedure involved placing 20 ml. of the test water into each of 21 culture tubes. A suspension of algal cells was prepared by placing the alga in the test water to produce a concentration of 6.25×10^3 cells per ml. Four ml. of this algal suspension was then placed into each culture tube. Next, the culture tubes were treated with one ml. of the appropriate copper solution. After the copper had been added, the algal cell concentration was 1×10^3 cells/ml., and the final volume of water in each culture tube was 25 ml. Each treatment

was run in triplicate, and six different copper concentrations were used. Three cultures served as a control and received no added copper. To each of the control cultures, 21 ml. of the test water were used, to which four ml. of the algal cell suspension were added. In the test with the artificial medium, the medium and the copper were mixed and autoclaved for sterilization. After the solutions had cooled, they were inoculated with four ml. of the algal suspension.

The same day that the test cultures were prepared, cell counts, optical density, copper ion activity, and the pH of each culture were determined. Cell counts were made with a Spencer hemacytometer as viewed through a phase-contrast microscope at 400 X. Optical density was determined with a Bausch and Lomb Spectronic 20 at wave lengths of 600, 680, and 750 m μ as proposed by Anon. (1971). Copper ion activity was determined with an Orion Specific Ion Analyser model 404 with a copper electrode. The millivolt values were recorded and were compared to a standard curve to determine the actual copper ion activity in the growth medium. The pH of the cultures was determined with a Coleman model 28 B pH meter.

The cultures were incubated in a culture room at 20°C. Lighting was provided by cool-white fluorescent lights at an intensity of approximately 4360 lux. The culture room was on a cycle of 16 hours light and 8 hours dark. The culture tubes were stoppered lightly to allow for gas exchange. They were also shaken daily to facilitate gas exchange and to keep the algal cells in suspension.

Cell counts were performed twice weekly by the direct count method. The optical density, copper ion activity, and pH were determined

at the beginning of the test and then every other week until the end of the test period. The end of the test period was determined when the algal growth reached the stationary phase in all culture tubes. At the end of the growth period, small amounts of each culture that showed no growth were transferred to flasks containing sterile artificial nutrient medium. Growth in the artificial medium was determined by visual observation. If no growth appeared after one week, the copper treatment was considered to be toxic or algicidal, but if growth did appear, the treatment was considered to be only inhibitory or algistatic (Fitzgerald and Faust 1963).

RESULTS

The test alga, Selenastrum capricornutum Printz, grew quite well in the artificial nutrient medium (Figure 1). Growth curves of the test alga in this medium provided the basis of comparison for growth of the alga in the natural waters tested (Figures 2, 3, 4, and 5). The greatest growth occurred in the medium not treated with copper, and the maximum growth attained decreased with an increase in the copper concentration. The copper treatments of 0.160 and 0.250 mg./l. proved to be toxic to the test alga.

The growth curves were determined from the direct cell counts of the algal cultures. The precision of the cell counts improved greatly with higher cell concentrations. Thus, at low algal cell concentrations (1×10^3 cells/ml.), the greatest deviation in cell counts occurred. This deviation of the cell counts can be attributed as the cause of the apparent irregular growth curves of the alga when small concentrations of algal cells are present.

The copper ion activity measurements (Table II), as determined by the Orion Specific Ion Analyser, indicate that some of the added copper is chelated in the medium. This is probably due to the EDTA that is present in the micronutrient composition. The copper ion activity also shows a decrease with increased algal growth and with an increase in the age of the algal cultures.

Growth of the test alga and an increase in the age of the algal culture were also responsible for an increase of the pH in the medium

Figure I. Growth of the test alga in the artificial nutrient medium.

Figure 2. Growth of the test alga in Cullaby Lake.

Figure 3. Growth of the test alga in Blue Lake.

Figure 4. Growth of the test alga in Lake Oswego.

Figure 5. Growth of the test alga in Clear Lake.

TABLE II
 IONIC COPPER IN THE
 TEST WATERS (MG./L.)

<u>Artificial Nutrient Medium</u>				
<u>Copper Treatment</u>	<u>Initial</u>	<u>10 Days</u>	<u>20 Days</u>	<u>Final 38 Days</u>
0.000	<.0006	<.0006	<.0006	<.0006
0.025	0.0150	<.0006	<.0006	<.0006
0.040	0.0300	0.0100	<.0006	<.0006
0.060	0.0550	0.0150	0.0050	<.0006
0.100	0.0900	0.0800	0.0600	0.0600
0.160	0.1400	0.1300	0.1300	0.1000
0.250	0.2200	0.2000	0.2000	0.1500

<u>Cullaby Lake</u>			
<u>Copper Treatment</u>	<u>Initial</u>	<u>10 Days</u>	<u>Final 18 Days</u>
0.000	<.0006	<.0006	<.0006
0.025	0.0200	<.0006	<.0006
0.040	0.0350	0.0100	0.0110
0.060	0.0500	0.0200	0.0100
0.100	0.0950	0.0400	0.0100
0.160	0.1500	0.0600	0.0500
0.250	0.2200	0.1000	0.1000

TABLE II (continued)

<u>Blue Lake</u>		
<u>Copper Treatment</u>	<u>Initial</u>	<u>Final 14 Days</u>
0.000	< .0006	< .0006
0.025	0.0250	0.0200
0.040	0.0350	0.0350
0.060	0.0600	0.0600
0.100	0.0900	0.0900
0.160	0.1500	0.1000
0.250	0.2500	0.2000

<u>Lake Oswego</u>			
<u>Copper Treatment</u>	<u>Initial</u>	<u>10 Days</u>	<u>Final 18 Days</u>
0.000	< .0006	< .0006	< .0006
0.025	0.0200	0.0060	< .0006
0.040	0.0300	0.0150	0.0100
0.060	0.0550	0.0500	0.0500
0.100	0.0980	0.0900	0.0500
0.160	0.1500	0.1200	0.1000
0.250	0.2500	0.2200	0.2000

TABLE II (continued)

<u>Copper Treatment</u>	<u>Clear Lake</u>	
	<u>Initial</u>	<u>Final 14 Days</u>
0.000	< .0006	< .0006
0.025	0.0200	< .0006
0.040	0.0350	0.0100
0.060	0.0600	0.0500
0.100	0.0900	0.0500
0.160	0.1400	0.1000
0.250	0.2200	0.1500

(Table III).

Finally, optical density measurements (Table IV) were used as a supplement to the algal cell counts. These measurements showed an increase that corresponded to an increase in the algal cell concentration. However, these measurements were not sensitive enough to register any optical density until the cell concentrations reached about 1×10^4 cells/ml. Thus, at low algal cell concentrations, this method for determining algal growth is of little use when working with this particular algal species.

Of the natural waters tested, Blue Lake, Lake Oswego, and Cullaby Lake had similar ion concentrations of those ions tested for with the use of the Hach Kit (Table V). The calcium concentration in Cullaby Lake was lower than the calcium concentration in Lake Oswego or Blue Lake, and in Blue Lake the nitrate concentration was much lower than the nitrate concentration in either Lake Oswego or Cullaby Lake. Clear Lake, on the other hand, proved to be a much softer water with quite low concentrations of magnesium, nitrate, and ortho-phosphate.

Growth of the test alga in Cullaby Lake was not inhibited by any of the copper treatments used (Figure 2). The copper treatments did reduce the growth of the alga to some extent, but substantial growth occurred in each culture tube. The copper chelating ability of this lake water was greater than that for any other water tested except the artificial nutrient medium (Table VII), and this may account for some of the algal growth in the copper treated water. However, some other factor must be involved in rendering the copper less toxic to the test alga in this water. From the results obtained, it is evident that a

TABLE III
pH OF TEST WATERS

<u>Artificial Nutrient Medium</u>				
<u>Copper Treatment (mg./l.)</u>	<u>Initial</u>	<u>10 Days</u>	<u>20 Days</u>	<u>Final 38 Days</u>
0.000	7.50	8.20	8.60	8.45
0.025	7.50	8.15	8.40	8.35
0.040	7.40	7.90	7.90	8.15
0.060	7.40	7.60	7.70	8.15
0.100	7.30	7.50	7.50	7.65
0.160	7.20	7.50	7.50	7.45
0.250	7.15	7.40	7.40	7.35

<u>Cullaby Lake</u>			
<u>Copper Treatment (mg./l.)</u>	<u>Initial</u>	<u>10 Days</u>	<u>Final 18 Days</u>
0.000	6.80	8.20	8.50
0.025	6.70	8.00	8.30
0.040	6.70	7.60	7.90
0.060	6.70	7.50	7.90
0.100	6.70	7.50	7.90
0.160	6.70	7.50	7.90
0.250	6.70	7.40	7.90

TABLE III (continued)

<u>Blue Lake</u>		
<u>Copper Treatment (mg./l.)</u>	<u>Initial</u>	<u>Final 14 Days</u>
0.000	7.65	8.05
0.025	7.60	8.03
0.040	7.50	8.00
0.060	7.40	7.95
0.100	7.40	7.85
0.160	7.25	7.80
0.250	6.90	7.75

<u>Lake Osewgo</u>			
<u>Copper Treatment (mg./l.)</u>	<u>Initial</u>	<u>10 Days</u>	<u>Final 18 Days</u>
0.000	8.00	8.10	8.00
0.025	8.00	8.10	8.00
0.040	8.00	8.10	7.95
0.060	8.00	7.90	7.90
0.100	7.90	7.90	7.85
0.160	7.90	7.90	7.85
0.250	7.90	7.90	7.85

TABLE III (continued)

<u>Clear Lake</u>		
<u>Copper Treatment (mg./l.)</u>	<u>Initial</u>	<u>Final 14 Days</u>
0.000	6.50	7.50
0.025	6.45	7.40
0.040	6.35	7.20
0.060	6.35	7.00
0.100	6.30	6.90
0.160	6.30	6.90
0.250	6.20	6.90

TABLE IV
ABSORBANCE

Artificial Nutrient Medium

[illegible]

Cullaby Lake

[illegible]

TABLE IV (continued)

Blue Lake

Copper	Initial			Final 14 Days		
<u>Tmt. (mg./l.)</u>	<u>600mM</u>	<u>680mM</u>	<u>750mM</u>	<u>600mM</u>	<u>680mM</u>	<u>750mM</u>
0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.025	0.000	0.000	0.000	0.000	0.000	0.000
0.040	0.000	0.000	0.000	0.000	0.000	0.000
0.060	0.000	0.000	0.000	0.000	0.000	0.000
0.100	0.000	0.000	0.000	0.000	0.000	0.000
0.160	0.000	0.000	0.000	0.000	0.000	0.000
0.250	0.000	0.000	0.000	0.000	0.000	0.000

Lake Oswego

[illegible]

TABLE IV (continued)

Copper Tmt. (mg./l.)	<u>Clear Lake</u> Initial			Final 14 Days		
	<u>600mM</u>	<u>680mM</u>	<u>750mM</u>	<u>600mM</u>	<u>680mM</u>	<u>750mM</u>
0.000	0.000	0.000	0.000	0.010	0.030	0.015
0.025	0.000	0.000	0.000	0.000	0.000	0.000
0.040	0.000	0.000	0.000	0.000	0.000	0.000
0.060	0.000	0.000	0.000	0.000	0.000	0.000
0.100	0.000	0.000	0.000	0.000	0.000	0.000
0.160	0.000	0.000	0.000	0.000	0.000	0.000
0.250	0.000	0.000	0.000	0.000	0.000	0.000

TABLE V
MAJOR IONS OF THE
LAKE WATERS TESTED

Nutrient (mg./l.)	Lake			
	<u>Cullaby Lake</u>	<u>Blue Lake</u>	<u>Lake Oswego</u>	<u>Clear Lake</u>
Alkalinity	30	55	50	15
Total Hardness	30	50	50	5
Mg ⁺⁺	25	20	20	0
Ca ⁺⁺	5	30	30	5
NO ₃ ⁼	0.32	0.05	1.10	0.04
Ortho PO ₄ ⁼	0.25	0.28	0.25	0.06
Meta PO ₄ ⁼	0.76	0.39	1.70	0.60
Silica	4.80	0.05	12.50	22.50
SO ₄ ⁼	45	5	5	3
Cl ⁻	12.5	2.5	10	2.5

much higher concentration of copper must be applied to this lake to prevent growth of the test alga.

Blue Lake water did not sustain any growth of the test alga. In an effort to rule out any contamination from the Jerry Jugs used to collect the water sample, another water sample was collected in a five gallon Pyrex carboy. Still, no growth of the test alga was obtained. Some of the cultures were then spiked with nitrogen, phosphorous, the micronutrients found in the artificial medium, and EDTA; again no growth of the test alga was obtained.

When the cultures were tested for the algicidal properties of the water and the copper treatments (Table VI), growth was observed in the flasks containing algae from the control cultures and from the cultures treated with 0.025 and 0.040 mg./l. copper. Thus, there was nothing present in Blue Lake water that was toxic to the alga, but for some undetermined reason growth of the test alga in the water from Blue Lake was inhibited.

The results obtained suggest an oligotrophic condition, but Blue Lake is actually an eutrophic lake which supports a variety of algal species. Growth of the test alga was inhibited, however, and misleading information about the potential productivity within the lake results. This study was limited in that only one algal species was used, but by using a variety of algal species as proposed by Anon. (1971), a more reliable determination of the potential productivity might be obtained. However, as a method for determining the algicidal capabilities of copper to a particular algal species, this method proved useful.

The water from Lake Oswego sustained growth of the alga in the

TABLE VI
ALGICIDAL PROPERTIES OF THE
COPPER TREATMENTS IN
THE WATERS TESTED

Copper	Artificial	Cullaby	Blue	Lake	Clear
<u>Concentration (mg./l.)</u>	<u>Medium</u>	<u>Lake</u>	<u>Lake</u>	<u>Oswego</u>	<u>Lake</u>
0.000	*	*	I	*	*
0.025	*	*	I	*	I
0.040	*	*	I	A	A
0.060	*	*	A	A	A
0.100	*	*	A	A	A
0.160	A	*	A	A	A
0.250	A	*	A	A	A

Key: * Not algicidal-alga grew in this medium at this copper concentration.

I Inhibitory-alga was inhibited in this medium at this copper concentration.

A Algicidal-alga was killed by the copper concentration in the medium.

TABLE VII
COPPER CHELATING ABILITY
OF THE TEST WATERS

Test	Volume	Volume Of 0.02 mg./l.	Copper
<u>Water</u>	<u>Tested (ml.)</u>	<u>Copper Titrated (ml.)</u>	<u>Chelated (mg./l.)</u>
Artificial			
Nutrient	25	13	0.0068
Medium			
Cullaby	25	4	0.0028
Lake			
Blue	50	5	0.0018
Lake			
Lake	50	4	0.0015
Oswego			
Clear	50	2.5	0.0010
Lake			

control cultures and the cultures treated with 0.025 mg./l. copper (Figure 4). The remaining cultures showed no growth, and the copper treatments were found to be algicidal (Table VI).

The algal growth in Lake Oswego water was not as great as that obtained in either the artificial medium or in Cullaby Lake. This indicates the possible presence of some inhibitory substance or the depletion of essential nutrients by the slight growth of the alga.

Clear Lake water supported a limited amount of growth of the test alga in the control cultures (Figure 5). However, none of the cultures treated with copper sustained any algal growth. The copper concentration of 0.025 mg./l. proved to be only inhibitory to the test alga, but all other copper concentrations used were toxic to the alga (Table VI). The low copper level toxic to the alga might be attributed to the low chelating ability of the lake water (Table VII), and the fact that the lake water is very soft water. The copper level toxic to the alga was also the same for Lake Oswego.

STATISTICAL ANALYSIS

The factorial analysis of the growth of the test alga in the waters tested indicates that the copper level toxic to the alga depended upon the water tested and the concentration of copper used. This is demonstrated by the large F value (Table VIII) obtained for the interaction between the copper treatments used, the lake waters tested, and the toxicity of the copper to the alga.

The greatest growth attained by the test alga was in the artificial nutrient medium and in Cullaby Lake. The apparent lack of growth

of the alga in the other waters tested could be due to nutrient limitation or to inhibitory substances already present in the water.

TABLE VIII
FACTORIAL ANALYSIS

<u>Sum of Squares</u>	
I. $Y^2=209.983$	Total S.S.=I-II=14.516
II. $G^2=195.467$	Treatment S.S.=III-II=13.931
III. $\frac{T^2}{3}=209.398$	Copper S.S.=IV-II=4.850
IV. $\frac{Cu^2}{15}=200.317$	Lake S.S.=V-II=6.083
V. $\frac{Lake^2}{21}=201.550$	Interaction S.S.=III-IV-V+II=2.998
	Error S.S.=Tot. S.S.-Tmt. S.S.=0.585
<u>Mean Squares</u>	
Error M.S.= $\frac{0.585}{70}=0.0084$	Interaction M.S.= $\frac{2.998}{24}=0.1249$

F TEST

$$F_{\frac{24}{70}}^{\frac{0.1249}{0.0084}}=14.869$$

SUMMARY

Copper, a widely used algicide, is toxic to many algal species in low concentrations. To determine the algicidal effects of copper in natural waters, a bioassay test procedure was used involving the test alga, Selenastrum capricornutum Printz. The growth of the alga varied with the water tested and the copper concentration used. In some instances the copper concentration of 0.040 mg./l. proved to be toxic to the alga, but in other instances the concentration of 0.250 mg./l. failed to inhibit growth of the test alga. When algal growth occurred at the higher copper concentrations, the total amount of algal growth was less than that obtained in the control cultures, and the total amount of algal growth decreased with an increase in the copper concentration used. This study proved useful for determining the algicidal properties of copper as they apply to the test alga used. This type of study might further prove useful for determining these properties for other algal species.

CONCLUSION

The test alga, Selenastrum capricornutum Printz, grew well under laboratory conditions, and fresh cultures were easily maintained in the artificial nutrient medium used. This study proved useful for determining the algicidal properties of copper in the natural waters tested as they apply to the test alga used. However, as pointed out in the study of Blue Lake, the potential productivity of a lake is not adequately assessed by the use of only one algal species.

When utilizing this procedure with other algal species, two problems may arise. First, some algal species may not grow well in the laboratory environment. And second, it may be difficult to accurately determine the growth of filamentous or colonial algal species by either direct cell counts or by optical density measurements.

REFERENCES CITED

- Albert, A. And W. S. Gledhill. 1947. The Choice of a Chelating Agent for Inactivating Trace Metals. Biochem. J., 41:529-533.
- _____. 1953. Quantitative Studies of the Avidity of Naturally Occurring Substances for Trace Metals: Pteridines, Riboflavin, and Purines. Biochem. J., 54:646-654.
- Anon. August 1971. Algal Assay Procedure Bottle Test. National Eutrophication Research Program, Environmental Protection Agency.
- Antonie, C. Joseph and Wayne H. Osness. 1963. Distribution and Accumulation of Copper Following Copper Sulfate Application on Lakes. Trans. Wis. Acad. Sci. Arts Lett., 52:1969-1975.
- Cedeno-Maldonado, Arturo, J. A. Swader, and Robert L. Heath. 1972. The Cupric Ion as an Inhibitor of Photosynthesis Electron Transport in Isolated Chloroplasts. Plant Physiol., 50:698-701.
- Ellms, J. 1905. Behavior and Uses of Copper Sulfate in the Purification of Hard and Turbid Waters. New England Water Works Ass. J., 19:496-503.
- Fitzgerald, George P., George C. Gerloff, and Folke Skogg. 1952. Studies on Chemicals with Selective Toxicity to Blue-Green Algae. Sewage Ind. Wastes, 24:888-896.
- _____. 1959. Bactericidal and Algicidal Properties of Some Algicides for Swimming Pools. Appl. Microbiol., 7:205-211.
- _____ and S. L. Faust. 1963. Factors Affecting the Algicidal and Algistatic Properties of Copper. Appl. Microbiol., 11:345-351.
- _____. 1964. Factors in the Testing and Application of Algicides. Appl. Microbiol., 12:247-253.
- _____ and S. L. Faust. 1965. Effect of Bacteria on the Solubility of Copper Algicides. Water Sewage Works, 112:271-275.
- Fott, Bohuslav. 1969. Studies in Phychology. Stuttgart, Germany. PP. 83-85.
- Fruh, E. G., K. M. Stewart, G. F. Lee, and G. A. Rohlic. 1966. Measurements of Eutrophication and Trends. J. Water Pollut. Contr. Fed., 38:1237-1258.

- Gerloff, G. C. and F. Skogg. 1957. Nitrogen as a Limiting Factor for the Growth of Microcystis aeruginosa in Southern Wisconsin Lakes. Ecology, 38:556.
- Gross, R. E., P. Pugno, and W. M. Dugger. 1970. Observations on the Mechanism of Copper Damage in Chlorella. Plant Physiol., 46:183-185.
- Haberman, H. M. 1969. Reversal of Copper Inhibition in Chloroplast Reactions by Magnesium. Plant Physiol., 44:331-336.
- Hasler, Arthur D. 1949. Antibiotic Aspects of Copper Treatment of Lakes. Trans. Wis. Acad. Sci. Arts Lett., 39:97-103.
- James, Daniel E. September 1, 1971. Isolation and Purification of Algae. Carolina Tips, 34:34-36.
- Johnson, J. M., T. O. Odlaug, T. A. Olson, and O. R. Ruschmeyer. 1970. The Potential Productivity of Freshwater Environments as Determined by an Algal Bioassay Technique. Water Resources Research Center, Univ. Minn. Grad. School. Bull. No. 20. PP. 1-77.
- Kellerman, Karl F. and George T. Moore. May 7, 1904. A Method of Destroying or Preventing the Growth of Algae and Certain Pathogenic Bacteria in Water Supplies. U.S.D.A. Bureau of Plant Industry, Bull. No. 64. PP. 1-44.
- Knaus, H. J. and J. W. Porter. 1954. The Absorbtion of Inorganic Ions by Chlorella pyrenoidosa. Plant Physiol., 29:229-234.
- McBrien, David C. H. and Kenneth A. Hassall. 1965. Loss of Cell Potassium by Chlorella vulgaris after Contact with Toxic Amounts of Copper Sulfate. Physiol. Plant., 18:1059-1065.
- _____, H. Hassall, and K. E. Hassall. 1967. The Effect of Toxic Doses of Copper Upon Respiration, Photosynthesis, and Growth of Chlorella vulgaris. Physiol. Plant., 20:113-117.
- McHugh, Robert. Feb. 15, 1963. Oregon State Department of Environmental Quality. Personal Communication.
- Mackenthun, Kenneth M. and Harold L. Cooley. 1952. The Biological Effect of Copper Sulfate Treatment on Lake Ecology. Trans. Wis. Acad. Sci. Arts Lett., 41:177-187.
- MacPhea, C. 1961. Bioassay of Algal Production in Chemically Altered Waters. Limnol. Oceanogr., 6:416-422.
- Maloney, T. E. 1958. Control of Algae with Chlorophenyl Dimethyl Urea. J. Amer. Water Works Ass., 50:417-422.

- Monie, William D. 1944. A Copper Sulfate Test for Algal Control. Taste Odor Contr. J., 10:1-8.
- _____. 1956. Algal Control with Copper Sulfate. Water Sewage Works, 103:392-397.
- Palmer, C. Mervin and Thomas E. Maloney. 1955. Preliminary Screening for Potential Algicides. Ohio J. Sci., 55:1-8.
- _____. 1956. Evaluation of New Algicides for Water Supply Purposes. J. Amer. Water Works Ass., 48:1133-1137.
- _____. 1962. Algae in Water Supplies. U.S. Dept. of H.E.W. Public Health Service Publication No. 657. 88 PP.
- Potash, Milton. 1956. A Biological Test for Determining the Potential Productivity of Water. Ecology, 37:631-639.
- Reimer, D. N. and S. J. Toth. 1970. Adsorption of Copper by Clay Minerals, Humic Acid, and Bottom Muds. J. Amer. Water Works Ass., 62:195-197.
- Sanchez, Isaac and G. Fred Lee. 1973. Sorption of Copper on Lake Monona Sediments-Effect of NTA on Copper Release from Sediments. Water Res., 4:587-593.
- Shapiro, J. and R. Ribeiro. 1965. Algal Growth and Sewage Effluent in the Potomac Estuary. J. Water Pollut. Contr. Fed., 37:1034-1043.
- Skulberg, Olav M. 1964. Algal Problems Related to the Eutrophication of European Water Supplies and a Bioassay Method to Assess Fertilization Influences of Pollution on Inland Waters. In Algae and Man-A NATO Advanced Study Institute-Louisville, Ky. Held 7/22-8/11, 1962. PP. 262-299.
- Sladeckova, Alena and Vladimir Sladecok. 1968. Algicides-Friends or Foes? In Algae, Man, and the Environment. Proceedings of an International Symposium, 6/18-6/30, 1967. Syracuse University Press. Ch. 22. PP. 441.
- Smith, Gilbert M. 1950. The Fresh-Water Algae of the United States. Second Edition. McGraw-Hill Book Co., New York. PP. 266-267.
- Sonner, A. L. 1945. Copper and Plant Growth. Soil Sci., 60:71-79.
- Steeman-Nielsen, E., L. Kamp-Nielsen, and S. Wium-Andersen. 1969. Influence of Deleterious Concentrations of Copper on the Photosynthesis of Chlorella pyrenoidosa. Physiol. Plant., 22:1122-1133.
- Tarlton, Ellis A. 1949. Algae Control: Experiences and Practices at Danbury, Conn. Water Sewage Works, 96:221-224.

- Toth, S. J. and D. N. Reimer. 1968. Precise Chemical Control of Algae in Ponds. J. Amer. Water Works Ass., 60:367-371.
- Walker, J. B. 1953. Inorganic Micronutrient Requirements of Chlorella I: Requirements for Calcium (or Strontium), Copper, and Molybdenum. Arch. Biochem. Biophys., 46:I-II.
- Wiessner, W. 1962. Inorganic Micronutrients. In Physiology and Biochemistry of Algae. Ralph Lewin Ed. Academic Press. New York. Ch. 17. PP. 267-286.