Factors Affecting the Algicidal and Algistatic Properties of Copper

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ABSTRACT

FITZGERALD, G. P. (University of Wisconsin, Madison) AND S. L. FAUST. Factors affecting the algicidal and algistatic properties of copper. Appl. Microbiol. **11**:345–351. 1963.—Data from laboratory studies are presented to show that, whereas five different sources of copper appear to be equally effective as toxic agents for algae, the medium in which toxicity tests are carried out has a great influence on the toxicity of copper. A technique of subculture is described for determining whether a concentration of a chemical is algicidal, algistatic, or nontoxic in action against a specific alga, and demonstrations are given of tests with algae illustrating each class of action.

Concern about the use in many publications of the term "algicide" when dealing with copper sulfate has led to a short review of the algicidal versus algistatic properties of copper sulfate.

Copper sulfate and copper products have been used for the control of algae since the early 1900's (Moore and Kellerman, 1904) with apparent success (American Water Works Association, 1950; Derby and Townsend, 1953; Hale, 1954; Bartsch, 1954). However, one should be aware of the selectivity in the toxicity of copper sulfate to algae (Domogalla, 1926; Hale, 1954; Maloney and Palmer, 1956). The selectivity in the toxicity of copper sulfate is considered by some (Maloney and Palmer, 1956) to be due to the formation of insoluble copper complexes under certain culture conditions, but recent studies have indicated that copper is just as toxic to cultures of algae when the copper is in a soluble form as when it is precipitated.

This report will demonstrate the results of normal dilution toxicity tests (Fitzgerald, Gerloff, and Skoog, 1952; Maloney and Palmer, 1956) and some of the factors that might influence the toxicity of copper compounds in these tests. Also, tests with different types of algae will be used to demonstrate situations in which copper can be shown to kill algae (algicidal effect), prevent the growth of algae (algistatic effect), or be relatively nontoxic.

MATERIALS AND METHODS

The algae used are maintained on agar slants in the University of Wisconsin algae culture collection. This collection has algae representing the lake bloom-producing blue-green species and planktonic diatom species, as well as several blue-green and green algal species which were obtained from unsuccessfully chemically treated ponds or swimming pools. For use in toxicity tests, the alga to be studied was cultured in either Allen's (1952) or Gorham's (Hughes, Gorham, and Zehnder, 1958) liquid culture media. The amount of algae inoculated into toxicity test culture flasks was the approximate equivalent of 1,500,000 cells per ml of *Microcystis aeruginosa* Wis. 1036 for representatives of the planktonic blue-green algae and the approximate equivalent of 300,000 cells per ml of *Chlorella pyrenoidosa* Wis. 2005 for the testing of blue-green and green algae of swimming pools.

A bacteria-free isolation of one of the green algal species (Wis. 1125) from a swimming pool was obtained by using a modification of the technique suggested by McDaniel, Middlebrook, and Bowman (1962). In the present procedure, a sample of the algae from a dense culture was placed in sterile medium, treated for 1 hr with the algicide Exalgae L. C. (Inertol Co., Inc., Newark, N.J.) at a concentration of 10 mg per liter, centrifuged from the treating solution, washed with sterile medium, and then streaked on agar plates of Allen's (1952) culture medium plus 1% glucose. Isolated algal colonies that appeared to be free from bacteria after 7 days of incubation were then streaked on Tryptone glucose extract agar (American Public Health Association, 1960), and bacteria-free colonies were isolated for use in toxicity tests.

The selection of the medium in which toxicity tests are to be carried out is dependent upon the species of algae to be used and the factors of the environment to be investigated. Allen's (1952) medium is a very hard water medium of pH 7; Gorham's (Hughes et al., 1958) medium, a moderately hard water medium, normally has a pH of 9, but with suitable buffering (McLachlin and Gorham, 1961) or by aeration with 0.5% CO_2 in air can be maintained at pH 7.

Normal dilution toxicity tests determine the minimal concentration of a chemical which will inhibit or kill an algal culture. These tests were carried out by adding to a series of inoculated culture medium flasks (25 ml per 50-ml Erlenmeyer flask) increasing amounts of distilled water solutions of the chemical to be studied. Preliminary tests were usually carried out to determine the general range of concentrations of the chemical that are toxic to the algal species employed. The inoculated toxicity test flasks were maintained in a constant-temperature (24 ±

2 C) continuous-light (150 ft-c) culture room. The results of the tests can be recorded as visual estimates of the degree of inhibition of the growth of the algae after different periods of treatment (Palmer and Maloney, 1955) or the cultures can be filtered through glass-fiber pads (H. Reeve Angel & Co., Inc., New York, N.Y.), the pads dried, and photographs taken of comparative pads. The latter procedure was used in the present report.

Whether a toxic concentration of a chemical is algicidal or algistatic can be determined by removing a sample of the algae from the environment of the toxic chemical and placing it in a suitable culture medium (Fitzgerald and Faust, Water Sewage Works, *in press*). If the sampled algae have absorbed a toxic concentration of the chemical involved, subcultures of the alga will not revive, and the concentration of chemical tested can be considered to be algicidal after the treatment time tested. If the chemical acts only as an algistat, samples of the algae which have been placed in sterile culture medium will revive and start to grow within 1 or 2 weeks. In the tests reported here, 1-ml samples of toxicity test cultures were removed after different periods of treatment and placed in 25 ml of sterile culture medium; these subcultures were incubated in the culture room for suitable periods of time.

Analysis for the copper content of the stock solutions and some of the toxicity test flasks was carried out by the Cuprethol methods (American Public Health Association, 1960). The comparative amount of soluble copper in solutions was determined by analysing the amount of copper in unfiltered samples as compared with samples filtered through glass fiber pads (Fitzgerald and Faust, J. Environ. Health, *in press*).

RESULTS

Comparison of different sources of copper. A number of tests have indicated that the toxicity of copper is independent of the source of copper when inorganic salts are compared. In the field of commercial copper algicide preparations for swimming pools, the source of copper is usually an organic chelated form of copper in order to prevent the precipitation of the copper in hard waters (Fitzgerald, 1960; Fitzgerald and Faust, J. Environ. Health, *in press*). Tests were therefore carried out to determine the relative toxicity to *C. pyrenoidosa* Wis. 2005 in Allen's medium of copper sulfate, a mixture of 1 part copper sulfate and 2 parts citric acid, and three commercial sources of copper [Algeeclear, Wallace & Tiernam Inc., Belleville, N.J.; Cuprose (Texas), W. R. Henderson & Co., Houston, Texas; and Cuprose (Nalco), Nalco Chemical Co., Chicago, Ill.]. Analysis of stock solutions of the chemicals to be tested (25 mg per liter) showed the following actual copper concentrations present (in mg of Cu per liter); copper sulfate, 6.0; copper sulfate-citric acid mixture, 6.3; Algeeclear, 4.0; Cuprose (Texas), 4.2; and Cuprose (Nalco), 4.2. The results of a typical toxicity test with these sources of copper are presented in Fig. 1, a photograph of the algae from the toxicity test flasks which had been filtered through glass-fiber pads after 7 days of treatment or contact time.

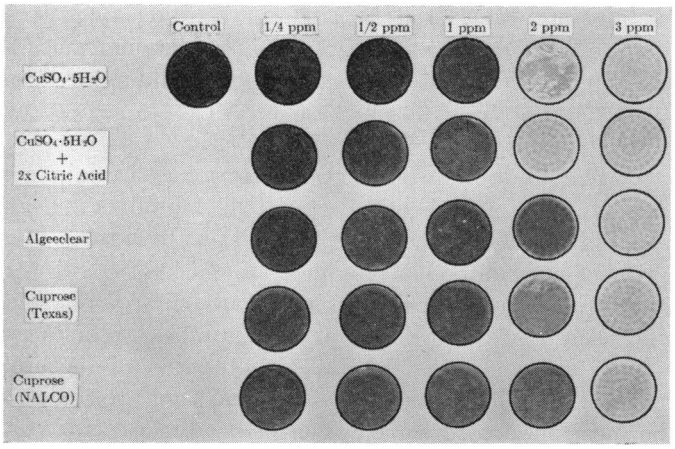

FIG. 1. *Relative toxicity of different copper products to Chlorella pyrenoidosa Wis. 2005 after 7 days of culture on Allen's medium.*

It is evident from these data the copper sulfate ($CuSO_4 \cdot 5H_2O$) is toxic to *Chlorella* at a concentration of 2 mg per liter (2 ppm) or more after 7 days of contact time. The presence of citric acid appears to have had no effect on the toxicity of the copper sulfate, since this mixture was also toxic at 2 mg per liter (2 ppm). The three commercial products were approximately equal in toxicity in this test, a concentration of 3 mg per liter (3 ppm) being required to prevent the growth of *Chlorella*.

When one compares the actual concentrations of copper in the stock solutions of the chemicals and the concentrations of the chemicals required to prevent the growth of *Chlorella* under these test conditions, it is evident that copper from these different sources appears to be equally toxic to *Chlorella*.

Inasmuch as the reason for the use of chelated forms of copper in algicides for swimming pools is to prevent the loss of the copper from solution and because tests have shown that a mixture of copper sulfate and citric acid and the commercial sources of copper tested are subject to attack by bacteria and a resulting loss in solubility of the copper in a few days time (Fitzgerald and Faust, J. Environ. Health, *in press*), comparative toxicity tests were carried out with a green algal species (Wis. 1125; isolated from a swimming pool) under conditions free from bacteria. The toxicities of autoclaved solutions of copper sulfate and of a mixture of 1 part copper sulfate and 2 parts citric acid were compared. A photograph of the algae filtered from these test solutions after 12 days of treatment is presented in Fig. 2.

Analyses of the comparative amounts of copper in unfiltered and filtered samples of the actual toxicity test solutions of Gorham's medium at pH 7 (± 0.3) indicated that at both the start of the test and after 12 days of treatment only 8% of the copper from copper sulfate was soluble, whereas 78% of the copper from the mixture of copper sulfate and citric acid was in a soluble form.

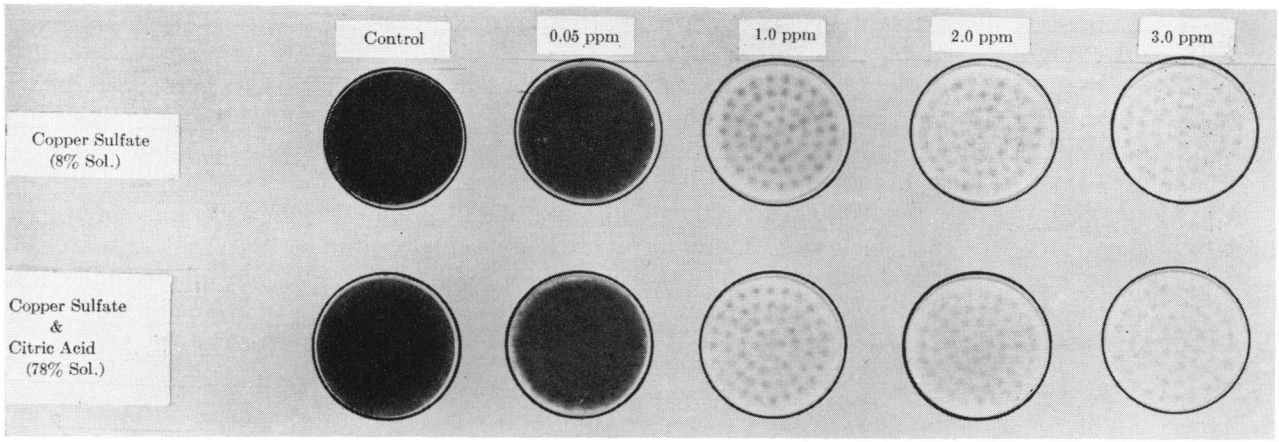

FIG. 2. *Comparison of the toxicity of insoluble and soluble copper to a green algal species (Wis. 1125; isolated from a swimming pool) cultured for 12 days under bacteria-free conditions on Gorham's medium (−Fe).*

Therefore, the results of the toxicity test (Fig. 2) indicate that when copper is present in the culture as a precipitate, it is just as toxic to this alga as when it is present in a soluble form.

Effect of the culture medium on the toxicity of copper. Toxicity tests were carried out in modifications of the dilute, alkaline medium of Gerloff, Fitzgerald, and Skoog (1950) by Fitzgerald et al. (1952), by Palmer and Maloney (1955), by Maloney and Palmer (1956), and by Fitzgerald (1959). Allen's (1952) neutral, but more concentrated, medium was used by Fitzgerald (1959, 1960). The choice of the medium to use for toxicity tests is usually limited by the species of algae to be tested inasmuch as some species do not grow as well in some media as in others. To demonstrate that the culture medium in which toxicity tests are carried out affects the results, comparative tests of the toxicity of copper sulfate were made with the green alga *C. pyrenoidosa* Wis. 2005 and the blue-green alga *M. aeruginosa* Wis. 1036.

The toxicity of copper sulfate ($CuSO_4 \cdot 5H_2O$) was compared in *Chlorella* cultures in Allen's (1952) medium, in Gorham's medium (Hughes et al., 1958), and in Allen's medium in which a chelated form of iron (McLachlin and Gorham, 1961; ASM medium) was added in place of the usual ferric chloride. The iron of the latter medium was chelated by the addition of 7.4 mg per liter of disodium ethylenediaminetetraacetate (EDTA). The iron of Gorham's medium is ferric citrate with the addition of 1 mg per liter of EDTA. The results of the tests are presented in Fig. 3 as a photograph of the filtered test solutions after 5 days of treatment.

It is evident from these data that 1 mg per liter (1 ppm) of copper sulfate is toxic to *Chlorella* in Allen's medium after 5 days, whereas 2 mg per liter are required in Gorham's medium, and 8 mg per liter in Allen's medium in which EDTA has been added. The 1 mg of EDTA per liter in Gorham's medium had only a slight effect on the toxicity of copper sulfate, increasing the required concentration for toxicity from 1 to 2 mg per liter. The 7.4 mg of disodium EDTA per liter in the Allen's medium with ASM-Fe increased the required concentration of copper sulfate from 1 to 8 mg per liter.

Similar results were obtained in other tests in which the toxicity of copper sulfate to *Chlorella* was determined in either Allen's or Gorham's media which had different sources of iron (ferric chloride, ferric citrate, ferric citrate plus 1 mg per liter of EDTA, or ferric chloride plus 7.4 mg per liter disodium EDTA) substituted for the usual sources of iron.

The results of tests comparing the toxicity of copper sulfate to *M. aeruginosa* Wis. 1036 in Gorham's medium which had the above four sources of iron substituted for the usual source of iron are presented in Fig. 4.

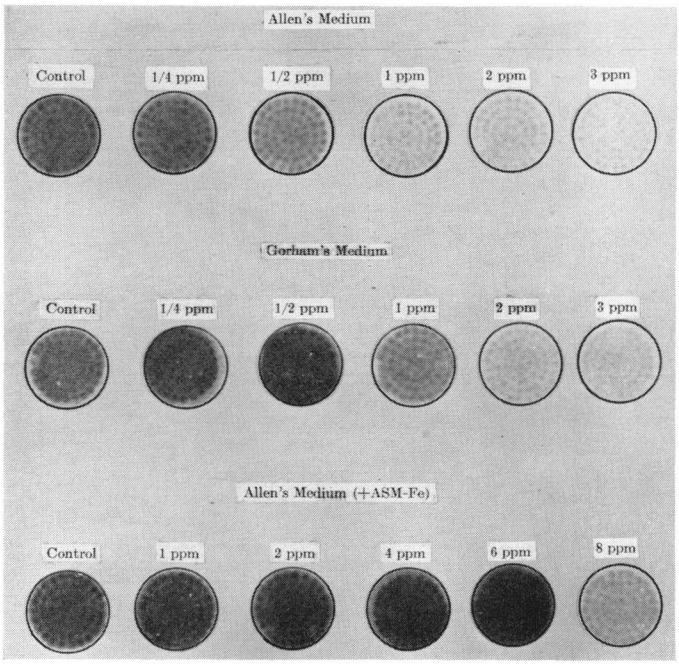

FIG. 3. *Effect of the culture medium on the toxicity of copper sulfate to Chlorella pyrenoidosa Wis. 2005 after culture for 5 days.*

These data indicate that considerably less copper sulfate is required for toxicity to *Microcystis* then to *Chlorella*. There appears to be no effect on the toxicity of copper sulfate when the iron of Allen's medium (ferric chloride) or Gerloff's medium (ferric citrate) is used, a concentration of 0.05 mg per liter (0.05 ppm) being toxic. However, the EDTA added when the iron of Gorham's medium (1 mg of EDTA per liter) or ASM medium (7.4 mg of disodium EDTA per liter) was employed was sufficient to neutralize the toxicity of at least 0.3 mg of copper sulfate per liter (0.3 ppm).

It is apparent from these data that careful consideration must be made in selecting a medium for toxicity tests, especially when the toxicity of copper compounds is to be tested.

Algicidal properties of copper sulfate. To be algicidal, a concentration of a chemical must kill the treated culture of algae. To determine whether the algae of the treated culture are actually dead or their growth is merely inhibited, subcultures into sterile medium should be made. The results of studies on the toxicity of copper sulfate to three bloom-forming blue-green algae, *M. aeruginosa* Wis.

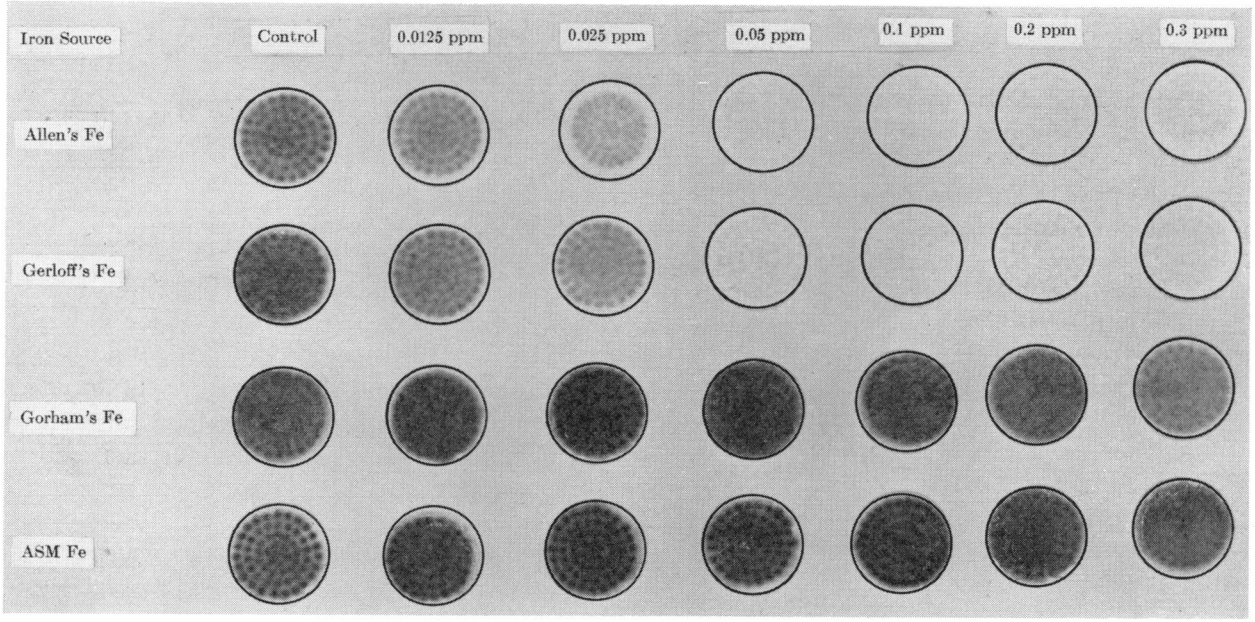

FIG. 4. *Effect of the culture medium on the toxicity of copper sulfate to Microcystis aeruginosa Wis. 1036 after culture for 5 days on Gorham's medium.*

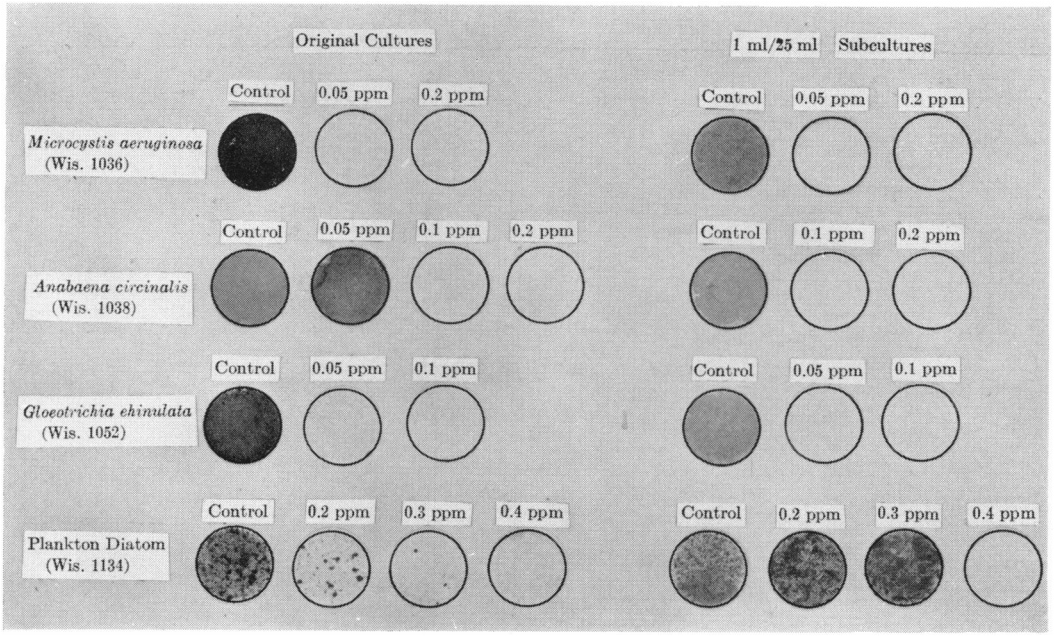

FIG. 5. *Algicidal properties of copper sulfate ($CuSO_4 \cdot 5H_2O$). Subcultures were made after treatment for 3 days. Results shown are after incubation for 14 days.*

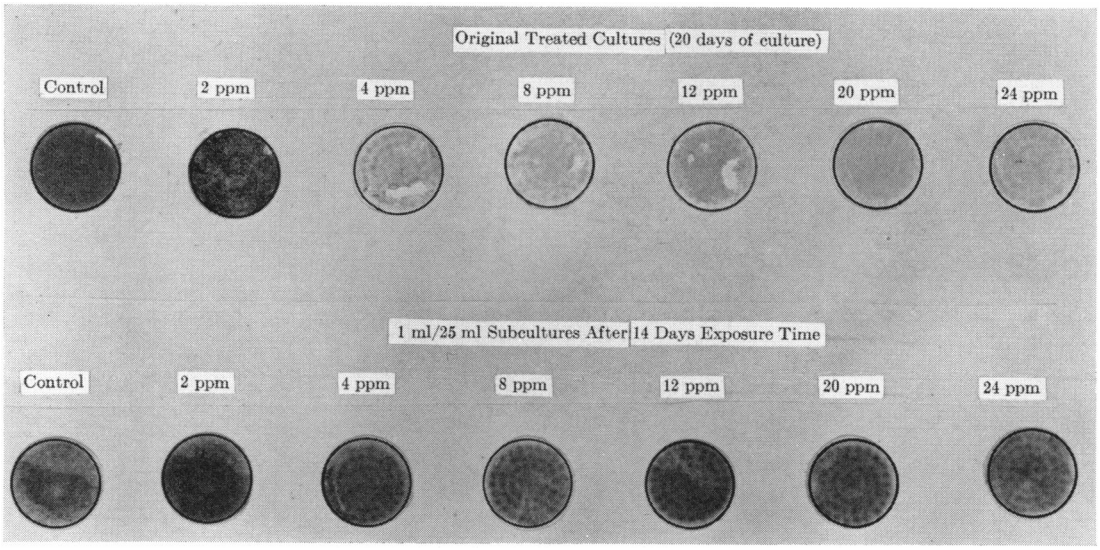

FIG. 6. *Algistatic effect of copper sulfate ($CuSO_4 \cdot 5H_2O$) on Chlorella pyrenoidosa Wis. 2005.*

1036, *Anabaena circinalis* Wis. 1038, and *Gloeotrichia echinulata* Wis. 1052, and to a planktonic diatom (Wis. 1134) are presented in Fig. 5.

The tests were carried out in Gorham's medium in which ferric chloride was substituted for the usual source of iron. After 3 days of treatment, 1-ml samples were placed in 25 ml of sterile medium. All cultures were harvested by filtration through glass-fiber pads after 14 days of incubation.

The pads from the originally treated cultures indicated that 0.05 mg per liter (0.05 ppm) of copper sulfate was toxic to *Microcystis* and *Gloeotrichia*, 0.1 mg per liter was toxic to *Anabaena*, and 0.4 mg per liter was toxic to the diatom. The results of the subcultures indicated that none of these algae grew up in subcultures from cultures that had been treated with apparently lethal concentrations of copper sulfate, although untreated control subcultures or subcultures from sublethal concentrations of copper sulfate did revive.

In the case of these algae, therefore, copper sulfate can be considered to be algicidal at the concentrations and contact times tested.

Algistatic properties of copper sulfate. It was noted by Palmer and Maloney (1955) and Fitzgerald (1959) that cultures of algae treated with certain chemicals appear to die at first, but then recover after further incubation. This has been frequently observed in toxicity tests with copper sulfate; as the incubation time increases, cultures that appeared to be dead recover and grow in time to much the same density as untreated control cultures. That this recovery of certain treated algal cultures is due to the fact that the cells of the algae are not dead but their growth is temporarily inhibited was demonstrated with tests on the toxicity of copper sulfate to Allen's medium cultures of *Chlorella* and a blue-green alga referred to as the "black algae" of swimming pools, *Phormidium inundatum* Wis. 1093. Subcultures were made of the cultures treated with different concentrations of copper sulfate after 14 and 5 days, respectively. The original cultures and subcultures were harvested for photographs after 20 days of culture (Fig. 6 and 7).

It is evident from the data presented that 4 and 8 mg of copper sulfate per liter (4 and 8 ppm) were sufficient to be apparently lethal to these two species of algae. The results of the subcultures, however, indicate that concentrations as high as 24 mg per liter were insufficient to prevent the growth of these algae when they had been treated for 14 or 5 days and then samples were removed to sterile, untreated medium.

Copper sulfate would be considered to be algistatic in effect in the case of algae represented by these species.

Algae resistant to copper sulfate. Bussy (1950) mentioned that some species of algae from swimming pools were found to be relatively resistant to treatments with copper sul-

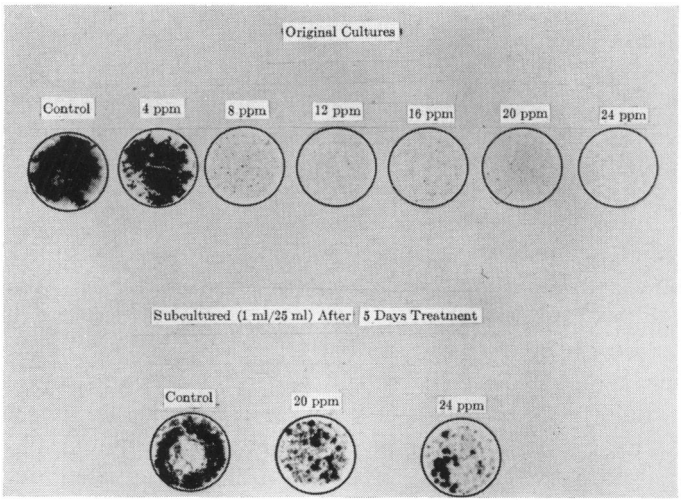

FIG. 7. *Algistatic properties of copper sulfate ($CuSO_4 \cdot 5H_2O$) to Phormidium inundatum Wis. 1093 after 20 days of culture.*

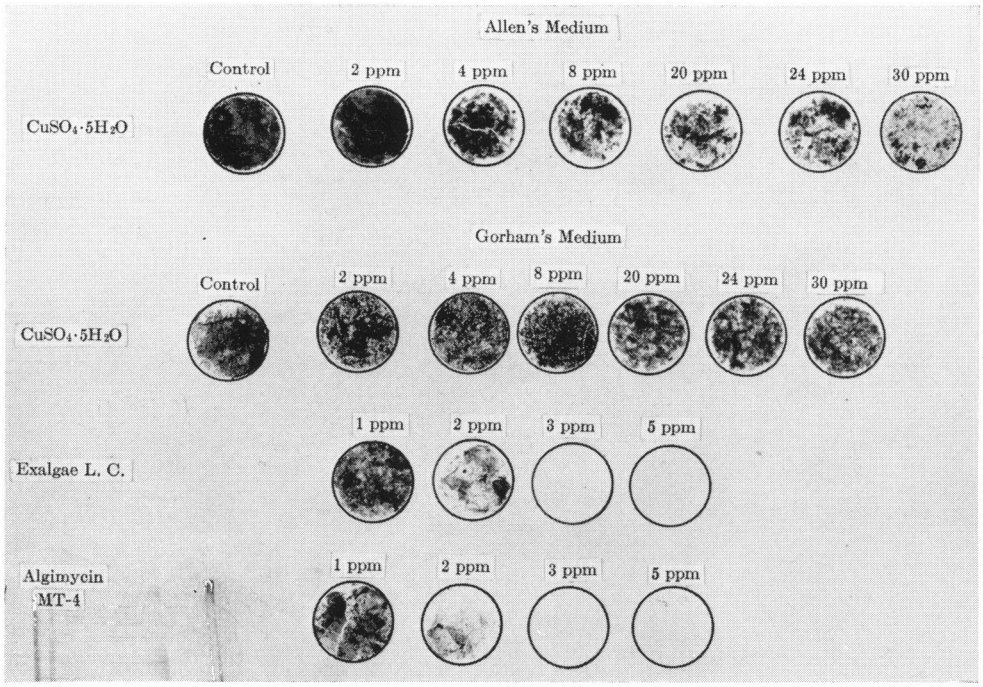

FIG. 8. *Resistance of a swimming-pool alga (Pearsall blue-green, Wis. 1129) to copper sulfate.*

fate. To demonstrate this fact, toxicity tests in Allen's medium and Gorham's medium (with ferric chloride as the source of iron) were carried out with a blue-green alga (Wis. 1129) isolated from a swimming pool. Concentrations of copper sulfate ($CuSO_4 \cdot 5H_2O$) up to 30 mg per liter were tested in either medium, as well as concentrations of 1 to 5 mg per liter of the algicides, Exalgae L. C. and Algimycin MT-4 (Great Lakes Biochemical Co., Milwaukee, Wis.). After 13 days of treatment, the cultures were harvested for pictures (Fig. 8).

It can be seen from these data that a concentration of copper sulfate of 30 mg per liter (30 ppm) was not sufficient to do more than cause a slight inhibition of the growth of this alga in either Allen's or Gorham's medium. In contrast, a concentration of the two commercial algicides of 3 mg per liter (3 ppm) appeared to be lethal. It is therefore evident that whereas a species of algae may be resistant to the action of one type of toxic chemical it does not necessarily mean it will be resistant to other toxic chemicals.

DISCUSSION

Data presented in this report demonstrate that, whereas different sources of copper appear to be equally effective as toxic agents for algae, the constituents of the medium the tests are carried out in can alter the results of toxicity tests to a marked degree. The effect caused by different media on the toxicity of copper to algae is apparently related to the source of iron used in the media. The effect of EDTA on the toxicity of copper and chromium was discussed by Morgan and Lackey (1958). The results of the present tests seem to indicate that approximately each part of EDTA in a medium can neutralize the toxicity of one part of copper sulfate.

The importance of the use of chelated forms of copper for the control of the growth of algae should not be assumed to be nullified by the facts that the different sources of copper were approximately equal in toxicity and, as was pointed out by Fitzgerald and Faust (J. Environ. Health, *in press*), that bacteria could attack various sources of copper and cause the copper to be precipitated. By knowing how long a product will maintain sufficient copper in solution for the prevention of the growth of algae, one could make additions of the chemical frequently enough to replace that which was lost from solution.

It is interesting to note that the studies carried out in bacteria-free media indicated copper was just as toxic when in a precipitated form as when soluble. This fact seems to be in contradiction to the suggestion of Maloney and Palmer (1956) that the selectivity in the toxicity of copper to algae is due to the formation of insoluble forms of copper in certain solutions. The fact that the species of algae most sensitive to the toxicity of copper, the bloom-forming blue-green algae, appear to be affected by copper under the most ideal conditions for the precipitation of copper also tends to lead one to believe that the selectivity of toxic action of copper for different algal species is probably a function of processes within the algal cells and not due to the chemistry of the surrounding water.

The need to know whether a concentration of a chemical will be algicidal or algistatic in action is of prime importance if the chemical involved can be readily removed from the environment of the algae by precipitation or adsorption onto filter media, debris, etc. From the data presented, it is evident that the planktonic blue-green algae and the diatom tested were killed by the concentrations of copper tested. Thus, even if the excess of copper in an environ-

ment, such as a lake, becomes insoluble and settles out of the water body, the algae that had been treated would die and decompose, as has been noted to be the case in the successful treatments of lakes and reservoirs (Moore and Kellerman, 1904; American Water Works Association, 1950; Derby and Townsend, 1953; Hale, 1954; Bartsch, 1954). Other algae that were either resistant to the concentration of chemical applied (Moyle, 1949; Domogalla, 1926) or were only inhibited in their rate of growth by the concentration of applied chemical (algistatic effect) would be able to grow as soon as the precipitated chemical was lost from their immediate environment.

In the case of swimming pools, in which insoluble products will be filtered from solution and products that are adsorbed onto the filter media will be lost from solution (Bussy, 1950; Fitzgerald, 1960, 1962, 1963), algistatic chemicals must be maintained at all times, whereas algicidal chemicals need be replaced only so often as required to kill the successive growths of algae.

The techniques described for determining whether a chemical is algicidal or algistatic in action against a specific kind of algae can be used to evaluate chemicals for such use and the conditions under which they are most effective.

It should be pointed out that in the case of copper compounds the range in toxic action can vary from algicidal activity at relatively low concentrations (0.05 to 0.4 mg of $CuSO_4 \cdot 5H_2O$ per liter), or algistatic activity at concentrations of 2 to 24 mg of $CuSO_4 \cdot 5H_2O$ per liter with certain algae, to situations in which the growth of algae is only slightly inhibited by a concentration of copper sulfate as high as 30 mg per liter. However, the relative resistance of the different algae to copper compounds has not been shown to be related to the amount of chemical necessary for toxic action in the case of other types of toxic chemicals.

Acknowledgment

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