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Comparative Algicide Evaluations Using Laboratory And Field Algae

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ABSTRACT

Swimming pool algicides were evaluated under standard conditions using *Chlorella pyrenoidosa* (Wis. 2005) and *Phormidium inundatum* (Wis. 1093) ("Black Algae"). Four-hour treatments were used for testing algicidal properties. Results of algistatic and algicidal tests showed that commercial copper products are not suitable for swimming pool use as algicides, but would kill lake and pond problem algae. Algimycin 400 (an organic product) was adequate for swimming pools. Short term algicide tests (10 minute contact) with pond algae indicated that Algimycin PLL-C (copper chelated with citrate and gluconate) was the most effective copper containing product tested. Some products required as much as 10 times more copper than others. Tests of algicides demonstrated detoxification by their reactions with algae.

INTRODUCTION

The methods used to evaluate the efficacy of chemicals for killing or preventing obnoxious algal growths will vary depending whether standard laboratory cultures of problem species are available or if only algae from the field are available. The purpose of this article is to demonstrate a laboratory approach to the evaluation of algicides for control of algae in swimming pools using standard cultures and local problem-causing algae and to evaluate algistatic and algicidal properties of chemicals using field-collected, problem algae from specific ponds and canals. A great deal of information on the factors that affect the efficacy of chemical treatments can be obtained by the use of either approach.

The evaluation of chemicals for the control of algae of swimming pools and the planktonic blue-green algae of lakes has evolved to the stage where algae and conditions have been standardized. However, enough experience has not been accumulated on the evaluation of chemicals for the control of the filamentous algae causing problems in ponds and canals. Some of these algae are available in culture collections and have been used in algicide tests (5, 6, 8), but there are many species that are not readily available in culture. The ease with which algae can be collected from ponds make their use in algicide evaluations very simple. Once the kinds of algae and the medium in which to run the tests have been selected to represent the problem to be solved, the most important question is to determine which product would be best suited for solving the problem. Since no practical algicide has been found that is not detoxified or lost from the environment of the algae (10) we consider the product that kills the algae with the lowest amount of active ingredient after the shortest treatment time to be the product of choice.

The biodegradation of practical algicides or pesticides in general is a study of prime importance to anyone interested in protecting aquatic environments for normal multiple uses without interference from obnoxious growths of plants or animals. There is a definite need to demonstrate that chemicals used for the control of algae or aquatic weeds are safe for use in the environment.

One of the easiest methods of evaluating whether a chemical will have secondary harmful ecological considerations is to use the target species that the chemical is to be used to control and to determine if that organism concentrates and biodegrades the toxic fraction of the product (10). Simple modifications of algistatic or algicidal tests can demonstrate whether a practical toxic chemical is effectively removed from a body of water by its reactions with the target organism.

METHODS AND MATERIALS

Swimming Pool Algicides: The algae used in standard tests were those approved for EPA registration of swimming pool algicides, the green alga, *Chlorella pyrenoidosa* (Wis. 2005) and the blue-green algae, *Phormidium inundatum* (Wis. 1093) ("Black Algae" of swimming pools). Concentrations of chemicals to be tested were added to Allen's neutral medium (1, 5, 13) containing the equivalent of 300,000 cells/ml of the test alga. This initial concentration of algae in a swimming pool would make the bottom invisible at depths of more than 2 meters. The concentration of *Chlorella* in stock cultures was determined by cell counts using a haemocytometer slide and microscope. Optical densities (absorbance) of stock cultures of *Chlorella* adjusted to convenient cell counts for inoculation of treatment flasks were used as reference cultures to adjust the absorbance of stock cultures of any other algae to be tested. Cultures of *Phormidium* were homogenized in a sterile Waring Blender before measuring their absorbance. Filamentous and clumpy algal growths must be maintained evenly dispersed for uniform distribution to treatment flasks (6). Tests with algae other than the standard species were also carried out using the same inoculation rates and medium. A collection of wild algae was used from the Podgor swimming pool in

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Miami, Florida, which after culture in Allen's medium in the laboratory was a mixture made up mainly of *Coccochloris* sp., a green alga, and a mixture of diatoms. The common problem-causing "mustard algae", of swimming pools, a green alga, previously isolated from a swimming pool and maintained as a unialgal culture was also used in testing swimming pool algicides.

In swimming pool product evaluations the concentration of product that is required to prevent the growth of the test alga for 7 or more days is the algistatic concentration. The concentration of product which kills the test alga (no growth within 7 days incubation of subcultures made 4 hours after treatment of the original cultures) is the algicidal concentration (12, 13). The products to be evaluated for their algistatic and algicidal properties towards swimming pool algae were either organic algicides or products containing copper. Two formulations of organic algicides, Algimycin 400 and Algimycin 400 E, containing two or more chemicals toxic to swimming pool algae were evaluated against two products based on the toxicity of copper to algae, Swimfree and Swimtrine. A description of copper containing products used is presented in Table 1.

TABLE 1. SOURCES OF COPPER TESTED AS ALGICIDES AGAINST SWIMMING POOL AND POND ALGAE.

Product	Percentage Cu (%)	Chelator	Company
1. Copper Sulfate	25	none	Mallinckrodt, Inc. St. Louis, Mo.
2. Swimfree	7.1	Triethanolamine	Hydrology Labs, Inc. Smithtown, N.Y.
3. Swimtrine	7.41	Triethanolamine	Applied Biochemists, Inc. Mequon, Wis.
4. Algimycin PLL-C	5.0	Citrate and gluconate	Great Lakes Biochemical Co., Inc. Milwaukee, Wis.
5. Mariner A	7.0	Triethanolamine	New Bus. Ventures Div., 3 M, St. Paul, Minn.
6. Cutrine Plus	9.0	Alkanolamine	Applied Biochemists, Inc. Mequon, Wis.

Lake and Pond Algicides: The planktonic algae used in the laboratory evaluations of chemicals intended for application to lakes and ponds were the blue-greens, *Microcystis aeruginosa* (Wis. 1036) and *Oscillatoria rubescens* (Wis. 2000), common non-nitrogen-fixing bloom producing species, and the nitrogen-fixing blue-green, *Gloeotrichia echinulata* (Wis. 1052) (11). Algistatic tests for the control of lake planktonic algae were carried out with 1,000,000 cells/ml concentrations of the algae in Gorham's Medium (14) which had $FeCl_3$ substituted for ferric citrate and EDTA since EDTA has been shown to detoxify copper (4, 5). Copper containing products were evaluated for preventing the growth of lake planktonic blue-green algae by determining the concentration of copper (as active ingredient) required from the different sources. The copper sources tested were inorganic copper sulfate and four chelated forms of copper. The commercial products containing copper are described in Table 1. These were two formulations of copper chelated as citrate and gluconate, the Algimycin PLL-C products. New Algimycin PLL-C was a formulation which does not contain sulfates. Sulfates interfere with the effective formulation of invert mixtures of herbicides, such as diquat and chelated copper which have been found to be more effective when combined than when applied separately (19). The other commercial copper products were alkanolamines, such as triethanolamine. These latter products were effective sources of copper when long enough treatment times are used (9).

Evaluations of copper containing chemicals for controlling problem causing filamentous or mat-forming pond algae were carried out by employing procedures for obtaining relatively uniform amounts of field collected algae. The tested species were *Spirogyra, Ulothrix, Zygnema, Chara,* and various mat-forming blue-greens, such as *Lyngbya.* Collections of these algae were made from the ponds to be treated. Sufficient pond water was taken as media for the tests. Algae were stored in open beakers at room temperatures for a few days or in a refrigerator for a week or more.

A reasonably uniform inoculum is essential to compare tests of all chemicals and concentrations. Ten milligram (dry weight) samples have been found to be optimum for these tests. To determine a suitable amount of algae, duplicate piles of field-collected algae were placed on a flat surface with increasing amounts of algae in each pair. One of each pair were weighed after drying for one hour at 110°C, then 20-40 piles were made that appeared to have about the same quantity of algae as the pile closest to 10 mg. These were used for the algicide evaluation, and three or four extra piles were made to measure the amount of algae actually used per test. The amount of chemicals to be compared were added to the treatment flasks on the basis of their copper content. All chemical stock solutions were made in the water to be treated so the effect of that particular water on the efficacy of the product would be most evident.

A uniform pile of algae was added to each treatment flask at a rate of about every 15 to 20 seconds. The treatments were of short duration so the efficacy of different products could be differentiated. We used a treatment time of 10 minutes duration when comparing copper from different commercial products and the testing of 0.025 to 1.6 mg of copper per flask (10 mg algae).

After the treatment periods, which were uniform for all chemicals, the algae were collected by using a forceps or pouring the water through a 10 cm diameter plankton net. The algae were washed in running tap water for about five seconds and transferred to test tubes or Erlenmeyer flasks for incubation. The incubation medium was pond water (10 ml per test tube or 25 ml per 50 ml Erl. flask) to which 1 to 2 mg PO_4-P was added per liter of solution (7).

After a few days, the algae that had been killed by the treatments were distinguished from unaffected algae by having turned from green to brown or black or having sunk to the bottom of the flask. The amount of copper required to kill a specific alga with a 10-minute contact time was

compared among different chemicals or sources of copper. These methods have been adapted from tests with aquatic weeds (18).

The filamentous algae from ponds on the Florida International University campus were phosphorus-limited [0.006 to 0.05 mg extractable PO_4-P/100 mg algae (dry)][2]. In order to determine if this condition had any effect on the response to copper treatments tests also were carried out with sampes of *Zygnema* and *Spirogyra* which had been incubated for several days in pond water supplemented with PO_4-P (1.2 mg P/L). Such algae had surplus phosphorus (0.3 mg PO_4-P/100 mg algae) (7).

Detoxification of Algicides: Tests to determine if algae treated with toxic chemicals detoxified or removed the toxic material from the treated water were simple modifications of algicidal or algistatic test procedures (10). The pond alga, *Spirogyra,* was tested against two sources of copper, Algimycin PLL-C and Swimfree. In these tests samples of the alga were treated for 10 minutes with six to ten concentrations of copper, washed and cultured in pond water fertilized with 1 to 2 mg PO_4-P/L. Then a second similar sample of *Spirogyra* was added to each treatment flask, held for 10 minutes, washed and incubated. Another pond alga, *Zygnema,* was used with two organic products used for long term algae control, Algimycin GLB-X and Algimycin GLB-Y. These tests were carried out by adding a second 5 mg inoculum of *Zygnema* to treatment flasks 10 days after the treatment of the first inoculum of *Zygnema* without removing the debris from the first inoculum. Reinoculation of a treatment vessel with the target species following an initial algicide or algistatic test was used to demonstrate whether that concentration of chemical that killed the first inoculum is still present in a toxic form. Comparison was made between the lowest effective concentration against the second inoculum and the first inoculum.

RESULTS AND DISCUSSION

Swimming Pool Algicides: The concentrations of four commercial products required to prevent the growth for 14 days of the test green alga, *Chlorella,* and "Black Algae" (*Phormidium*), commonly used to evaluate swimming pool products, are summarized in Table 2.

The organic products, Algimycin 400 and Algimycin 400E prevented the growth of *Chlorella* and *Phormidium* at 1 and 2 mg/l, respectively. *Chlorella* was prevented from growing by concentrations of 3 and 6 mg/l of the copper containing products, Swimfree and Swimtrine, respectively. A concentration of 8 mg/l of Swimtrine prevented *Phormidium* from growing, but 8 mg/l of Swimfree was not effective. Thus, the two Algimycin products, 400 and 400E, and Swimtrine were shown to be algistats for either alga, but Swimfree was shown to be an algistat only towards *Chlorella.*

There is little practicality to the use of chemicals in swimming pools that are only algistatic since algistatic chemicals must be present at effective concentrations continuously and most chemicals are lost from the pool waters by reactions with organic compounds found to accumulate in swimming pools and on pool filters. It is more practical to use chemicals that will kill algae with reasonably short contact times. Therefore, algicidal tests for swimming pool chemicals are of prime importance.

TABLE 2. ALGISTATIC PROPERTIES OF SWIMMING POOL PRODUCTS ON ALGAE INOCULATED AT 300,000 CELLS/ML IN ALLEN'S MEDIUM AND INCUBATED 14 DAYS.

	Effectiveness of Products to Prevent Growth			
	Wis. 2005 *Chlorella pyrenoidosa*		Wis. 1093 *Phormidium inundatum*	
Product[1]	Conc. (mg/L)	Inhibition (%)	Conc. (mg/L)	Inhibition (%)
Algimycin-400	1/2	0	1	0
	1	100	2	100
Algimycin-400E	1/2	0	1/2	0
	1	100	1	75
			2	100
Swimfree	1-1/2	0	2	0
	2	75	3	50
	3	100	4	75
			6	90
			8	90
Swimtrine	2	0	3	0
	3	90	4	50
	4	90	6	75
	6	100	8	100

[1] Products used as received. Concentrations are on total product basis.

Preliminary algicidal tests indicated that only the organic products, Algimycin 400 and Algimycin 400E, killed *Chlorella* when tested for 4 hours treatment, the usual time used in the evaluation of swimming pool algicides (13). Therefore, longer treatment times were tested to determine effective levels of the products containing copper. The two Algimycin 400 products were effective algicides at 0.5 to 2 mg/l, whereas the copper products, Swimfree and Swimtrine were not algicidal at concentrations as high as 8 mg/l and treatment times as long as 10 days (Table 3). This confirms previous results (8, 11).

TABLE 3. ALGICIDAL PROPERTIES OF SWIMMING POOL CHEMICALS ON ALGAE INOCULATED AT 300,000 CELLS/ML IN ALLEN'S MEDIUM.

	Concentrations Required to Kill			
	Wis. 2005 *Chlorella pyrenoidosa*		Wis. 1093 *Phormidium inundatum*	
Product	Treatment (days)	Concentration (mg/L)	Treatment (days)	Concentration (mg/L)
Algimycin-400	1	1.0	10	2.0
Algimycin-400E	1	1.0	10	0.5
Swimfree	9	>8	9	>8
Swimtrine	9	>8	10	>8

Inasmuch as the usual test algae that are used to evaluate swimming pool algicides were not killed by the copper products at excessively high concentrations, even after 9 or 10 days treatment, it was felt that perhaps copper might be effective against wild algae from swimming pools. The results of tests with algae collected from the Podgor swimming pool as the concentrations of products required to

[2]Fitzgerald, G. P. and D. F. Jackson, in preparation.

prevent these algae from growing or to kill them with the standard 4 hour treatment are summarized in Table 4.

TABLE 4. ALGISTATIC AND ALGICIDAL PROPERTIES OF SWIMMING POOL CHEMICALS AGAINST PODGOR SWIM. POOL ALGAE (WILD) INOCULATED AT 300,000/ML IN ALLEN'S MEDIUM.

Product	Concentration of product (mg/L)	
	To prevent growth of algae for 10 days[1]	To kill algae after 4 hours treatment[2]
Algimycin-400	2	2
Swimfree	4	>8
Swimtrine	6	>8

[1] Algistatic concentration
[2] Algicidal concentration

All 3 products prevented the mixture of *Coccochloris* and diatoms from growing at concentrations of 2 to 6 mg/l. However, only Algimycin 400 killed the algae with a 4 hour contact time. The problem-causing "Mustard Algae" (a green alga) was isolated from a swimming pool and treated for one day before subculturing to give the copper products more time to be effective. The results of these tests are summarized in Table 5.

TABLE 5. ALGISTATIC AND ALGICIDAL PROPERTIES OF SWIMMING POOL CHEMICALS AGAINST MUSTARD ALGAE (WIS. 1178)[1] INOCULATED AT 300,000/ML IN ALLEN'S MEDIUM.

Product	Concentration of product (mg/l)	
	To prevent growth of algae for 7 days[2]	To kill algae after 1 day treatment[3]
Algimycin-400	0.25	0.5
Swimfree	2	>8
Swimtrine	1	>8

[1] Isolated by Dr. R. M. Stern, Great Lakes Biochemical Co., Inc., Milwaukee, Wisconsin
[2] Algistatic concentration
[3] Algicidal concentration

The "Mustard Algae" of swimming pools can be prevented from growing with as little as 1/4 to 2 mg/l of all the products tested but only Algimycin 400 was algicidal. Even 24 hours exposure to the other products was ineffective. Thus, these copper products cannot be used to kill existing algal problems in swimming pools.

The general results of tests of swimming pool products against algae show that the organic products, Algimycin 400 and Algimycin 400E were both algistatic and algicidal to the usual test algae and Algimycin 400 was also algicidal to wild and mustard algae of swimming pools. The copper products, Swimfree and Swimtrine were at most only algistatic. It has been repeatedly shown that copper acts only as an algistat towards the algae of swimming pools (5, 11, 12, 13), a result confirmed by experiments reported herein. The possible mechanisms by which some plants are resistant to the toxic effects of copper have been reviewed by Watson and Bollen (20) and Dykeman and DeSousa (2). Other organic products, quaternary ammonium compounds, which are known to be algicidal towards swimming pool algae (3, 12, 13, 17), were not tested in the persent studies because the potential human pathogenic bacteria, *Pseudomonas aeruginosa,* was found to increase in Florida swimming pools treated with these types of products (15). There is thus some doubt as to the advisability of using quaternary ammonium products in swimming pools even under conditions in which they could be effective algicides.

The simplicity of the swimming pool algistat and algicide testing procedures presented here demonstrates how readily toxic chemicals can be evaluated. The results of the tests are visually obvious in a few days. Cultures containing concentrations of chemicals that prevent the growth of the tested algae remain colorless whereas controls and sublethal treatments become dense green. The differentiation between no growth and partially inhibited growth becomes more obvious with increased incubation time since the desired concentration prevents any growth and cultures with less effective concentrations continue to grow at very low rates.

Lake and Pond Algicides: Copper products are particularly effective against planktonic bloom forming blue-green algae. To determine the comparative efficacy of different sources of copper, three bloom-forming algae were tested against five sources of copper on an active ingredient basis. These tests were only to prevent growth because it has been shown that relatively the same concentrations of copper that are algistatic to planktonic bloom-producing blue-green algae also kill those algae (8, 11). The results are summarized in Table 6.

TABLE 6. COMPARATIVE ALGISTATIC PROPERTIES OF DIFFERENT SOURCES OF COPPER TO PLANKTONIC BLUE-GREEN ALGAE INOCULATED AT 1,000,000 CELLS/ML IN GORHAM'S ($FeCl_3$) MEDIUM.

Source of Copper	Concentrations of Copper Required to Prevent Growth (mg Cu/L)			
	Oscillatoria rubescens (Wis. 2000)	*Microcystis aeruginosa* (Wis. 1036)	*Gloeotrichia echinulata* (Wis. 1052)	
			Test 1	Test 2
Copper Sulfate (25% Cu)	0.05	>0.04	0.04	0.1
Algimycin PLL-C (5% Cu)	0.06	0.015	0.06	0.12
New Algimycin PLL-C (5% Cu)	0.06	0.015	0.06	0.12
Mariner A (7% Cu)	0.03	0.04	>0.08	0.17
Cutrine Plus (9% Cu)	0.14	0.05	>0.11	0.14

These tests indicate that all the products tested will prevent the growth of planktonic blue-green algae when sufficient copper is present. The advantage of the four commercial products over copper sulfate is that they are formulated so the copper does not precipitate in alkaline lake or pond waters. When copper sulfate is added to alkaline waters it immediately precipitates and a great percentage of it is lost from the environment of the algae, thus requiring excessively large amounts to be used in order to get effective results as an algicide. Therefore, economically and ecologically, a product that is only removed from the environment by the reactions with the target organisms is preferable.

Since these copper products are also sold as algicides for

the control of pond algae, a series of tests were made of the comparative ability of the products on an active ingredient basis (copper) to kill problem causing species of pond algae. The results of these tests are briefly summarized in Table 7 as the amounts of copper required to kill 10 mg of algae with a 10 minute treatment.

Some of the products were much more successful in killing problem-causing algae than others. *Chara* and *Ulothrix* were susceptible to copper regardless of the product source. However, the two sources of *Spirogyra* and the *Lyngbya* and *Zygnema* differentiated among algicides. Copper from Algimycin PLL-C was more effective than that from the other sources for these latter algae.

Both phosphorus-limited *Spirogyra* and *Zygnema* and these algae with surplus phosphorus were killed by the same range of concentrations of copper from several sources when treated for 10 minutes as in the tests summarized in Table 7. These results confirm previous studies with *Cladophora* sp. from Lake Michigan which showed that algae from environments with surplus available phosphorus had the same susceptibility to copper as algae from areas where they were P-limited (9). Therefore, the phosphorus nutritional status of algae does not seem to have any effect on the toxicity of copper to algae.

Detoxification of Algicides: The first evaluation of whether algae remove toxic materials from treated water was carried out using *Spirogyra* and two sources of copper. Two tests using 20 mg and 3 mg samples of *Spirogyra* were made with different concentrations of copper from Algimycin PLL-C. One test with 8 mg samples of *Spirogyra* was made with copper from Swimfree. The amount of copper required to kill the first inoculum and the second inoculum, after removal of the first inoculum, is summarized in Table 8.

The initial treatment of *Spirogyra* with copper for 10 minutes removed 0.1 to more than 0.2 mg of copper since the second inoculum of algae required higher amounts of copper than the first inoculum. These data did not indicate whether the copper removed from solution was detoxified by the algae, but other tests with copper reported earlier had indicated that copper absorbed by algae was detoxified (10).

In order to demonstrate directly how algae detoxify toxic solutions, two organic products, Algimycin GLB-X and Algimycin GLB-Y, were tested against *Zygnema* using the usual algistat procedure, except that after 10 days, when

TABLE 8. THE DETOXIFICATION OF ALGICIDAL AND ALGISTATIC CHEMICALS.

Date (1978)	Algae	Product	Mg of Cu. to Kill[1] First inoculum	Mg of Cu. to Kill[1] Second inoculum
Jan. 13	*Spirogyra* sp. (20 mg/Test)	Algimycin PLL-C	0.1	0.2
Jan. 24	*Spirogyra* sp. (3 mg/Test)	Algimycin PLL-C	0.05	0.2
Jan. 13	*Spirogyra* sp. (8 mg/Test)	Swimfree	0.2	>0.4
			mg/l of Cu to prevent growth[2] First inoculum	mg/l of Cu to prevent growth[2] Second inoculum
Jan. 16	*Zygnema* sp. (5 mg/Test)	Algimycin-GLBX	10	30
Jan. 16	*Zygnema* sp. (5 mg/Test)	Algimycin-GLBY	10	20

[1] Sorption or detoxification by added and removed algae. The first inoculum was treated for 10 minutes, removed and the second inoculum added to the same treatment flask.
[2] Detoxification in algistatic tests. The first inoculum was not removed from the treatment flask. The second inoculum was added 10 days after the first.

TABLE 7. COMPARATIVE ALGICIDAL PROPERTIES OF DIFFERENT COPPER FORMULATIONS AGAINST ALGAE FROM F.I.U. PONDS. NUMERICAL VALUES REPRESENT THE AMOUNT OF COPPER REQUIRED TO KILL 10 MG OF ALGAE IN A 10 MINUTE TREATMENT PERIOD. ALL TESTS WERE CONDUCED IN DECEMBER 1977 AND JANUARY 1978.

Algae	Copper sulfate	Swimfree	Swimtrine	Algimycin PLL-C	Mariner A	Cutrine plus
Blue-Green (*Lyngbya* sp.)	0.2	0.4	0.2	0.075	—	—
Ulothrix sp.[1]	0.2	0.1	0.1	0.1	—	—
	0.2	0.1	0.1	0.1	—	—
Chara sp.	0.05	0.05	0.1	0.05	—	—
Green[2]	0.1	0.1	0.4	0.02	—	—
(*Spirogyra* sp.)	—	0.2	—	0.05	—	—
	—	—	1.8	0.15	>4.8	2.4
Yellow[3]	—	—	>3.2	0.6	1.2	>3.2
(*Spirogyra* sp.)	—	—	—	0.4	0.8	—
Zygnema sp[4]	2.0	1.0	>3.6	0.2	—	—
	0.8	>1.5	>1.6	0.2	—	—
	0.6	0.3	>1.8	0.1	—	—
	—	—	0.8	0.3	0.6	0.6

[1] Tests were conducted Dec. 12, 1977 and repeated on Jan. 13, 1978.
[2] Tests were conducted Dec. 30, 1977 and Jan. 13 and 24, 1978.
[3] Tests were conducted Dec. 20, 1977 and Jan. 26, 1978.
[4] Tests were conducted Dec. 26 and 28, 1977 and Jan. 13 and 23, 1978.

visual results indicated which concentrations of chemicals had actually killed the algae (loss of color and decomposition), an additional inoculation with *Zygnema* was made to the treatment vessels. If the chemical that had killed the initial inoculum of algae had been released in a toxic form, it would have been available to kill the second inoculum. However, the data (Table 8) indicates that 2 to 3 times as much chemical was required to kill the second inoculum. Thus, Algimycin GLB-X and Algimycin GLB-Y are biodegradable when used to kill this alga.

One of the best ways of demonstrating detoxification is by using the target species. If a chemical is no longer toxic to the kind of plant it is used to control, it is unlikely to be toxic to other organisms. This was demonstrated when the copper accumulated in lake bottom muds (450 mg Cu/Kg., dry weight) from algicide applications was found to be less than 1/100 as toxic to bloodworms (*Tendipes plumosus*) or fingernail clams (*Pisidium Idahoense*) (16) as was freshly applied inorganic copper.

The results of tests with *Spirogyra* demonstrate how target organisms remove toxic sources of copper from solution. In tests reported by Fitzgerald (10) it was demonstrated how several different kinds of toxic chemicals were not only removed from solution by reactions with algae, but were also detoxified so that when the killed algae decomposed there was no release of toxic material. The test, with *Zygnema* and the two Algimycin products, GLB-X and GLB-Y, also show that the chemicals that killed the first inoculum were not available in toxic form for the second inoculum. In contrast to the previous detoxification tests with sources of chelated copper, the Algimycins, GLB-X and GLB-Y, are organic products which have long lasting algistatic properties. They were formulated to have longer residuals in water by being resistant to physical losses and chemical degradation. The data presented here indicate that they can be degraded by actions against target species of algae, however. Thus, it is apparent that mere chemical analyses for the presence of pesticides must be tempered with bioassays for actual toxic materials. This type of information is very important when considering the safety of adding a new chemical to the aquatic environment and all chemicals to be used for algae or weed control should be tested for biodegradation before they are allowed to be used in open aquatic environments.

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