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NOTES

Relative Tolerance of Mat-forming Algae to Copper

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INTRODUCTION

Mat-forming algae are major constituents of the aquatic weed flora. Excessive growths of these macrophytic algae, which occur alone or in association with vascular plants, can limit recreational activities, restrict culture and harvest activities in fish culture ponds, reduce flow and clog intakes in water conveyance systems, and reduce habitat diversity (Lembi et al. 1988). The major method used for the control of these and other types of algae is the application of copper, either as copper sulfate or copper chelates. Experiences of commercial applicators suggest that mat-forming algal genera differ in their susceptibility to copper, but to this point data to support or confirm these observations have not been available. This study documents the relative tolerance of the common, weedy mat-forming green algae *Hydrodictyon*, *Oedogonium*, *Pithophora*, *Rhizoclonium*, and *Spirogyra* to copper. In addition, the copper tolerance of the cyanobacterial (blue-green algal) mat-forming *Oscillatoria* was assessed. The frequency with which this organism occurs in Indiana and probably other parts of the midwest has increased over the past few years[2].

MATERIALS AND METHODS

Samples of *Hydrodictyon reticulatum* (L.) Lagerh., *Oedogonium* sp., *Pithophora oedogonia* (Mont.) Wittr., *Rhizoclonium hieroglyphicum* (C. A. Ag.) Kütz., and *Spirogyra setiformis* (Roth) Kütz. were collected from Surrey Lake near Columbus, IN. *Oscillatoria tenuis* Ag. was collected from a pond in Ft. Wayne, IN by Neil Gerber (Aquatic Management, Inc., Bluffton, IN). All algae were isolated into unialgal culture by either dragging single filaments through sticky 1% agar or pulling single filaments from diluted samples. Long term stock cultures were grown in Cl(II) medium (O'Neal and Lembi 1995) at 30 to 60 µmol photons m^{-2} s^{-1} and 16 C (*Hydrodictyon, Oedogonium*) or 23 to 25C (*Oscillatoria, Pithophora, Rhizoclonium, Spirogyra*). All cultures were maintained at a 16:8 h L:D photoperiod in a controlled environment chamber.

Tests were conducted in 250-ml flasks. Each flask contained 150 ml of sterile Cl(II) medium. The source of copper was Cutrine®-Plus, and the following concentrations of copper (Cu) were tested: 0.0 (control), 0.1, 0.25, 0.5, and 1.0 mg/L. Each flask was inoculated with similar amounts of filamentous algae. This amount was estimated by placing approximately equal clumps (as estimated by eye) of alga into the wells of a ceramic color reaction plate. At least five of these clumps were weighed to obtain an average initial dry weight. The other clumps were distributed to the test flasks. Initial amounts for the various species ranged from 1 to 8 mg dry wt per well, with a ± 10% variability within an experiment. These amounts are extremely small in comparison to the volume of liquid, insuring that all filaments came into contact with the copper solution.

Three replicate flasks of each species were placed at 80 to 100 µmol photons m^{-2} s^{-1} and 23 to 25C and harvested after 12 days of growth. For dry weights, the filaments were filtered through Whatman filter paper No. 1 or glass microfibre filters and dried for 24 h at 100C.

Analysis of the data was conducted by applying a probit transformation to the mean percent reduction in dry weight. The probit value was regressed on the log of copper concentration. The EC50 (concentration that causes a 50% reduction in dry weight) was calculated from the regression equation.

RESULTS AND DISCUSSION

The mean dry weight biomass of algae in the untreated controls after 12 days was 3.1 (*Oscillatoria*), 3.6 (*Hydrodictyon*), 4.3 (*Oedogonium*), 5.7 (*Rhizoclonium*), 6.7 (*Spirogyra*), and 11 (*Pithophora*) times greater than that of the initial inocula. These data indicate that growth during the incubation period was generally good for all species. Differences in growth rates are due to the fact that algal species have different environmental requirements for optimal growth. Placing the cultures under the same set of environmental conditions enhances the growth of some species but probably is less suitable for the growth of other species. Clearly *Pithophora* thrives at 23 to 25C and 100 µmol photons m^{-2} s^{-1}. Even though *Hydrodictyon* and *Oedogonium* are grown at lower temperatures for long term culture, they did grow reasonably well under the test conditions.

The algae fell into three general groups according to their EC50s (Table 1). *Oedogonium* and *Spirogyra* were very sensitive to copper with EC50s of 0.003 mg Cu/L or less. *Hydrodictyon*, *Pithophora*, and *Rhizoclonium* were more than 15 times more tolerant to copper than *Oedogonium* and *Spirogyra*. *Oscillatoria*

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TABLE 1. LEVEL OF COPPER (MG/L CU) THAT RESULTS IN 50% REDUCTION IN DRY WEIGHT BIOMASS (EC50) AFTER 12 DAYS OF CULTURE UNDER LABORATORY CONDITIONS. R^2 = COEFFICIENT OF VARIATION FOR PROBIT DRY WEIGHT VERSUS LOG CONCENTRATION.

Species	EC50	R^2
Spirogyra setiformis	<0.001	.372
Oedogonium sp.	0.003	.991
Pithophora oedogonia	0.046	.924
Rhizoclonium hieroglyphicum	0.053	.934
Hydrodictyon reticulatum	0.048	.981
Oscillatoria tenuis	0.290	.934

was approximately six times more tolerant to copper than *Pithophora*.

These relative differences in copper tolerance agree with observations made by commercial applicators (pers. comm.). For example, *Spirogyra* is considered relatively easy to control with copper. It is usually listed among the algae susceptible to the lowest recommended dose ranges of copper sulfate. The Copper Sulfate Fine Crystals product (Old Bridge Chemicals, Inc.) lists *Spirogyra* as being susceptible to 0.25 to 0.5 mg/L $CuSO_4$ (approximately 0.0625 to 0.125 mg/L Cu). The recommended dosage for *Pithophora*, on the other hand, is 1.0 to 1.5 mg/L $CuSO_4$ (approximately 0.25 to 0.375 mg/L Cu), which is approximately three to four times greater than that for *Spirogyra*. The relative tolerance of *Pithophora* to copper has been known for a long time. Even as early as 1924, Tiffany concluded that a concentration of copper sulfate at least four times greater than that used for *Spirogyra* was required for control of *Pithophora*. Eipper (1959) found *Pithophora* to survive copper sulfate at concentrations 15 to 20 times greater than the recommended use rate for other algae, and Pearlmutter and Lembi (1986) found 50% survival of *Pithophora* filaments when exposed to 0.8 mg/L Cu for 12 h.

This study confirms the differences in tolerance of *Pithophora* and *Spirogyra* to copper. In addition, it provides information on other filamentous algal species for which copper efficacy data are lacking or possibly misleading. Although *Hydrodictyon* is listed on the Copper Sulfate Fine Crystals label as having the same sensitivity to copper as *Spirogyra*, experiences of commercial applicators along with the data provided here suggest that the tolerance of *Hydrodictyon* to copper is similar to that of *Pithophora*. The similarity in tolerances of *Rhizoclonium* to *Pithophora* was not unexpected since the two algae are in the same taxonomic family (Cladophoraceae) and are characterized by thick cell walls, which may reduce the penetration of copper into the cells (Pearlmutter and Lembi 1986). The sensitivity of *Oedogonium* to copper is similar to that of *Spirogyra* and may be, at least in part, due to the relatively thin cell walls that are characteristic of both genera.

Of the species studied here, *Oscillatoria* showed the greatest tolerance to copper. Mat-forming *Oscillatoria* species have only been recorded by commercial applicators as being major problems in the midwest within the past 10 years, and they are considered to be extremely difficult to control with copper (pers. comm.). It is tempting to theorize that this organism has appeared as a result of high dosages of copper being applied to control green mat-forming algae such as *Pithophora* and *Hydrodictyon*, but this hypothesis requires confirmation. The high degree of tolerance exhibited by this mat-forming cyanobacterial species, however, seems unusual given the relatively low tolerances exhibited by planktonic cyanobacteria in relation to planktonic green algae. For example, a dose of 0.025 to 0.040 mg/L Cu effectively controlled the planktonic cyanobacterium *Aphanizomenon flos-aquae* (Whitaker et al. 1978); this is in contrast to a dosage as high as 0.5 mg/L Cu required for the control of some green phytoplankters (Copper Sulfate Fine Crystals label).

The EC50s listed here should not be used as guidelines for copper dosages to be used in the field. Laboratory conditions differ greatly from those in the field, and laboratory-derived EC50s for the same organism can vary depending on the specific light, temperature, nutrient, and other environmental factors that are used for the test. For example, the EC50 listed by Hallingse and Phlips (1996) for *Spirogyra communice* (0.88) is considerably higher than the EC50 listed here for *S. setiformis*. Although light and temperature conditions were similar in the two studies, differences in culture medium, amount of initial inoculum, species, harvest time (5 days for Hallingse and Phlips), growth rates of untreated controls, and even method of analysis could account for large differences in EC50s.

Another reason not to equate laboratory data with field concentrations is that our study insured that each filament was exposed to the copper medium so that relative tolerance could be established without the interference of extraneous factors such as growth form. This condition is seldom attained in the field. For example, the dense, thick mats produced by *Pithophora* appear to reduce the rate of penetration of copper into the interior of the mats (Lembi et al. 1984), thus making *Pithophora* even more difficult to control than suggested by our data. The thick slime and coating of sediment that is common to benthic mats of *Oscillatoria* are also potential factors in preventing adequate penetration of copper solutions. On the other hand, *Spirogyra* and *Oedogonium* appear to be sensitive to copper not only because of their inherent susceptibility but because they form relatively thin, dispersed mats of vegetation through which copper should easily penetrate. Whether the mat morphology of the net-like *Hydrodictyon* confers any protection to copper penetration is unknown, but the organism itself appears to have a relatively high degree of inherent tolerance.

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