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Effects of Aquatic Herbicides on Primary Productivity of Phytoplankton in the Laboratory

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In the United States herbicides must be tested for toxicity to selected aquatic organisms before being registered by USEPA for use in public waters. However, experiments to measure toxicity of herbicides on phytoplankton are not generally required. If we view the lake holistically, we should consider the potential effects of herbicides on phytoplankton, the basis of the aquatic food web. We conducted short term laboratory experiments to assess the toxicity of two widely used herbicides (Diquat® and Rodeo®) on photosynthesis of natural phytoplankton from a local lake. We compared the results to an algicide (copper sulfate) that is used specifically to control growth of phytoplankton in lakes and ponds.

The use of algicides and aquatic herbicides for control of excessive algae and aquatic macrophyte plant growth is a common lake and pond management tool (Cooke et al. 1986). Use of aquatic plant herbicides typically consists of direct application to surface waters for control of submergent species or direct application on emergent plant leaves. The primary target for most herbicides or algicides, such as diquat dibromide (Diquat®) and copper sulfate, is the electron transport system of photosystem I (Jager 1983). Glyphosate based herbicides, such as Rodeo®, interfere with biosynthesis of enzymes in the shikimate pathway (Coggins 1989). Both mechanisms are present in vascular plants and planktonic algae suggesting that herbicidal applications to control aquatic macrophytes may impact non-target phytoplankton. The source of copper sulfate was a commercially available copper sulfate pentahydrate (25% metallic Cu) product that was commonly used for algae control by lake managers and consultants.

Effects of herbicides on phytoplankton communities are of special concern since it is this group that provides the basis for the lacustrine food chain. Since phytoplankton possess photosystem I and the shikimate pathway which are targets for the commonly used algicides and herbicides (Coggins 1989), the susceptibility of phytoplankton to herbicidal effects is important. In this study, the effects of two aquatic herbicides, Rodeo® and Diquat®, used to control aquatic macrophytes and copper sulfate, were evaluated to test the effects upon net primary productivity (herein referred to as primary productivity) using the C^{14} assimilation method.

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MATERIALS AND METHODS

Glenwild Lake was chosen as the phytoplankton collection site, because it had been an ecological study area. The average total alkalinity of Glenwild Lake is 15 mg$CaCO_3$/L, the average conductivity is 186 µS/cm, and the pH range is 6.6-8.2 (Sebetich and Ferriero, 1977; Sebetich and Horner-Neufeld, 2000). A Wisconsin plankton net (300 µm mesh) was used to collect concentrated plankton samples from Glenwild Lake, Passaic County, New Jersey. A bulk water sample (4.0 liters) was also collected and filtered through 0.45 µm Millipore® filters for use in the serial dilutions of the herbicides and plankton samples. The concentrated plankton sample was uniformly diluted using filtered lake water to develop the phytoplankton innoculum for the testing trials. Final algal densities were determined for a sub-sample of the plankton solution for each testing trial. Algal densities were determined using a Sedgewick-Rafter counting chamber and the methodology described in Wetzel and Likens (1991). The species assemblage present in the concentrated plankton samples was dominated by three diatom genera: *Asterionella sp.* (2,000 cells/mL), *Tabellaria* sp. (350 cells/mL), *Fragilaria* (14 cells/mL) and the dinoflagellate *Dinobryon sp.* (1600 cells/mL).

Commercially available Rodeo® and Diquat® formulations were employed in volumetric serial dilutions to develop the concentration gradient to which the phytoplankton community would be exposed. Concentrations of active ingredients (i.e., cupric ion, glyphosate and ethylene 2,2 dipyridylium dibromide) were calculated as the product of the percent active ingredient present in the formulation concentration and the final dilution concentration. Copper concentrations in solution were calculated from the molar concentration of copper sulfate diluted by serial dilution. Effects on primary productivity were expressed relative to the primary productivity in untreated controls containing ^{14}C and no herbicide or algicide. The productivity quotient was calculated as the ratio of the observed productivity in treatment flasks to control flasks. The test concentrations for the evaluation were: 0.001, 0.01 and 0.1 mg Cu/ L ; 0.125, 1.25 and 12.5 mg glyphosate /L; and 0.118, 1.18 and 11.8 mg dibromide complex/L. Treatment concentrations were selected to capture the range for recommended treatment doses in lakes and ponds.

One hundred milliliters (100 mL) of the plankton solution was poured into pre-cleaned, 250 mL, wide mouthed Erlenmeyer flasks. The experimental design employed duplicate light flasks for each herbicide treatment for estimating photosynthesis and a single flask wrapped in aluminum foil for the dark bottle in estimating respiration. A stock solution [sodium bicarbonate ($NaH^{14}CO_3$)] of carbon-14 (^{14}C) was prepared to generate a solution containing 0.25 microcuries (µCi)/mL (0.556 x 10^6 disintegrations per min/mL). One milliliter of the ^{14}C solution was added to each flask containing plankton and lake water. Control

flasks received the same phytoplankton solution and ^{14}C, but no chemical treatment.

The flasks were placed in an environmental chamber at 24°C and 575.0 microamps of fluorescent light. During the seven hour incubation period, all flasks were shaken by hand at two-hour intervals. After incubation, the plankton-^{14}C solution in each flask was filtered through individual 0.45 μm Millipore® cellulose filters, which were placed in borosilicate glass counting vials and dissolved with Bray's liquid scintillation cocktail. Scintillation vials were placed in a Packard Tri-Carb liquid scintillation counter and counted twice for twenty minutes to derive an average activity count per minute (cpm) for each flask. Duplicate light bottle counts were averaged to derive the light bottle count for application in estimating primary photosynthesis.

The productivity values for all incubation levels were obtained from the following equation (Wetzel and Likens 1991): Productivity in mgC^{12}/Liter = (isotopic correction factor) (total carbon available) (count of light sample) bottle volume - count of dark sample bottle volume filtered - (^{14}C available) (efficiency of scintillation counter) where the isotopic correction factor is 1.06. The isotopic correction factor is used to estimate C^{12} uptake from ^{14}C. Total carbon available was calculated by multiplying the total alkalinity by a conversion factor obtained from the pH values as presented in Saunders et al. (1962); the radioactive counts of the light sample and dark sample are provided in cpm. The filtered volume was used to adjust for filtration of aliquots and ^{14}C availability was estimated by the relationship of 1 mL $NaH^{14}CO_3$ stock solution being equal to 1 μCi and 2.22×10^6 dpm of activity.

The value obtained from the equation above is in mgC^{12}/L. To convert to mgC^{12}/m^3/hr, the value obtained in mgC^{12}/L was multiplied by 10^3 and divided by the hours of incubation. To account for potential quenching effects on the scintillation counter estimates, serial dilutions of the herbicides and algicide were run independent of phytoplankton to derive a correction term for each treatment. Productivity estimates of control and treatment flasks were statistically compared using a one way analysis of variance (ANOVA) to determine if observed differences were significant (α=0.05).

RESULTS AND DISCUSSION

Effects of copper on primary productivity revealed observable inhibition relative to untreated controls in the 0.1 mg Cu/L treatment (Table 1). Primary productivity in this treatment was reduced to 78% of that in the untreated controls. Phytoplankton exposed to 0.01 and 0.001 mg/L treatments displayed enhanced primary productivity relative to untreated controls. Primary productivity in these treatments was equivalent to 102% and 115% of primary productivity observed in the untreated controls. Effler et al. (1980) found that treatment with 0.013 mg

Cu/L was sufficient to suppress ^{14}C uptake in a phytoplankton assemblage dominated by green algae and diatoms from a hard water lake in New York. Additionally, Hedtke (1984) found that gross primary production in a phytoplankton assemblage dominated by green algae and diatoms held in moderately hard water (200 mg $CaCO_3$/L) mesocosms remained unaffected at 0.004 mg Cu/L and was significantly reduced at treatments of 0.009, 0.03, 0.09 and 0.42 mg Cu/L. However, copper tends to be more soluble in softer water than in harder waters.

The effects of 0.125, 1.25 and 12.5 mg glyphosate/L resulted in stimulation in primary productivity relative to untreated controls at all treatment concentrations. The degree of stimulation was consistent across all three treatment concentrations (Table 1). The 12.5 and 0.125 mg glyphosate/L treatments were observed to enhance primary productivity equivalent to 161% of untreated controls. The 1.25 mg glyphosate/L treatment corresponded to an enhanced primary productivity equivalent to 168% of experimental controls. Concentrations in the range of 0.125 to 12.5 mg/L enhance productivity of the phytoplankton assemblage evaluated.

Phytoplankton treated with 1.18 and 11.8 mg dibromide complex/L displayed an inhibition of primary productivity equivalent to 45% and 19% of that observed in untreated experimental controls (Table 1). Primary productivity in the presence of the lowest concentration of Diquat® (0.118 mg dibomide complex/L) exhibited a stimulatory effect equivalent to 117 % of the primary productivity observed in untreated experimental controls. The 1.18 mg dibromide complex/L Diquat® treatment is within the application range noted for normal control of aquatic submergent vegetation. Diquat® has been found to be effective in weed control at application concentrations of 1 to 3 mg/L (Leung et al., 1983). These observations suggest that treatment applications used to control vascular submergent plants would also have the potential to affect primary productivity in phytoplankton assemblages at the time of application. The lowest treatment of 0.118 mg dibromide complex/L from our study was the only treatment which stimulated primary productivity in phytoplankton. Birmingham and Colman (1983) found that growth (defined as increased cellular division) of the diatom *Navicula pelliculosa* was inhibited at Diquat® concentrations greater than 0.3 mg/L.

Rodeo treatments of 0.125, 1.25 and 12.5 mg glyphosate/L all resulted in significant stimulation of primary productivity of phytoplankton in our study. The observed degree of stimulation remained about the same for all three treatments suggesting that Rodeo had a consistent effect on primary productivity which remained independent of concentration within the range of 0.125 to 12.5 mg/L. One explanation for the observed stimulation in the 0.125 to 12.5 mg/L treatments in this study could be the use of the nitrogen and phosphorus released through the degradation process for glyphosate. Sullivan et al. (1981) found slight trends of stimulation in the growth of the diatoms *Tabellaria* and *Navicula* when exposed

to glyphosate (as Roundup® herbicide). However, the observed stimulation could not be solely attributed to glyphosate availability.

Our results support the findings postulated by Ritter et al. (2000) that non-target receptor species such as phytoplankton may be subject to herbicidal effects following treatments for control of vascular aquatic plants. Additionally, the results demonstrated that phytoplankton primary productivity could be both negatively affected or stimulated by the application of herbicides to control aquatic macrophytes.

Table 1. Herbicide treatments and effects on net primary productivity in phytoplankton assemblages.

Herbicide/ Algicide	**Treatment Concentration (mg/L)**	**Net Primary Productivity (mg C^{12}/m^3 hr^{-1})**	**Percent Net Primary Productivity of Controls (%)**
Copper Sulfate (as Cu)	0.001	0.012	115
	0.01	0.010	102
	0.1	0.007812	78*
Rodeo® (as glyphosate)	0.125	0.016	161*
	1.25	0.017	168*
	12.5	0.016	161*
Diquat® (as dibromide complex)	0.118	0.0019	117*
	1.18	0.0045	45*
	11.8	0.013	19*

*Treatments found to be statistically different from untreated controls $p \leq 0.05$

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