

# Influence of Aquashade<sup>1</sup> on Growth, Photosynthesis, and Phosphorus Uptake of Microalgae

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## ABSTRACT

When five species of algae were grown in small volume batch cultures (50 ml medium per 250 ml flask) in direct contact with Aquashade, reduced growth rates were not observed until the Aquashade concentration was 5 ppm. In separate experiments photosynthetic rates of algal cultures receiving light passing through a 1 m column of water were reduced by 50% relative to control values when the Aquashade concentration in the water column was from 1 to 3 ppm. The ratio of gross photosynthesis to respiration displayed a similar pattern being less than one at 1 to 3 ppm Aquashade. Phosphorus uptake by *Anabaena flos-aquae* or *Ankistrodesmus falcatus* var. *acicularis* was not affected by 1 ppm Aquashade. These results are consistent with the hypothesis that Aquashade controls plant growth by competing with photosystem II pigments and that the compound itself is not directly toxic to the plants. Based on these results, green and blue-green algae appear to be equally affected.

*Key words:* Aquashade, plankton, algae, photosynthesis, phosphorus uptake, blue-green algae, *Anabaena*, *Ankistrodesmus*.

## INTRODUCTION

One approach to controlling growth of aquatic plants has been to reduce light penetration by the application of light attenuating dyes. Eicher (1947) reported that nigrosine was effective in controlling curly leaf pondweed (*Potamogeton crispus*). Buglewicz (1972) tested the effects of various brown and blue dyes in an eutrophic Nebraska pond. He reported that *Potamogeton* spp. were eliminated by application of either brown or blue dyes, whereas *Chara* spp. were only controlled by the blue dye. White et al. (1975) reported that the blue dye Aquashade decreased growth of 4 species of submersed plants, and Osborne (1979) observed that Aquashade prevented reinfestation of hydrilla following an initial treatment of endothall in a small Florida pond.

The influence of these light attenuating compounds on growth of microalgae which make up the phytoplankton community is less well known. Buglewicz (1972) reported that blue-dye treated enclosures were characterized by reduced phytoplankton productivity and by a shift in relative species abundances. Specifically, he noted that blue-green algae were less common in the blue-dye treated enclosures relative to controls. More recently Boyd and Noor (1982) concluded that Aquashade had little effect on the phytoplankton communities of several shallow catfish rearing ponds. This paper reports on experiments designed to

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determine the effects of Aquashade on growth, photosynthesis, and phosphorus uptake for species of microalgae, and to determine if the responses of blue-green algae might be more affected than those of green algae as suggested by the observations of Buglewicz (1972).

## MATERIALS AND METHODS

Algae used in this study were obtained from the University of Texas Culture Collection (Starr, 1978) growing in a variety of media and transferred to the Algal Assay Medium (AAM) (Miller et al., 1978). Growth experiments were performed using this medium. *Selenastrum capricornutum* was obtained from the U.S. Environmental Protection Agency, Corvallis, Oregon. One set of experiments tested 0.00, 0.25, 0.5, 1.0, 2.0, and 3.0 parts per million (ppm) Aquashade. A fifty ml portion of AAM was dispensed into each of several 250 ml conical flasks, autoclaved, and the appropriate amount of Aquashade added. Following inoculation with the test alga, flasks were incubated at 20 C with a 14:10 LD cycle and  $97 \pm 16 \mu\text{E m}^{-2} \text{s}^{-1}$  provided by 2 cool white fluorescent bulbs. Growth of *Selenastrum capricornutum* was estimated by withdrawing 1 ml aliquots at 2 day intervals and counting the number of cells using a hemocytometer (Guillard, 1975). In the case of *Anabaena flos-aquae* and *A. cylindrica*, growth was estimated by measuring *in vivo* chlorophyll content using a Turner Model 110 Fluorometer equipped with a Corning CS-5-60 excitation filter and Corning CS-2-64 emission filter (Weber, 1973). An additional set of experiments was performed using 0, 5, and 10 ppm Aquashade. Growth conditions were similar to those described above except that the light intensity was reduced to  $47 \pm 4 \mu\text{E m}^{-2} \text{s}^{-1}$ . Biomass was again measured as *in vivo* chlorophyll. The growth rate for each culture (3 flasks per treatment) was determined by converting biomass to  $\log_2$  and using this value in a linear regression vs. time. Growth rates were examined for an Aquashade effect by an analysis of variance (ANOVA). Scheffe's procedure was used for the separation of treatment means. A probability greater than 0.05 was not considered significant. Statistical analyses were performed using SPSS (Nie, et. al 1975).

In a second set of experiments, photosynthetic rates were determined by measuring oxygen evolution using an Orion model 97-08 oxygen electrode in conjunction with an Orion model 901 ionalyzer. A 300 ml glass BOD bottle was filled with a liquid suspension of the test alga. The BOD bottle was then placed into the measurement apparatus (Figure 1). Oxygen concentration was measured at the beginning and at 5 minute intervals for 30 minutes. The effect of Aquashade on photosynthetic rate was assessed by adding enough Aquashade to the deionized water in the PVC pipe (Figure 1), to result in final concentrations of 0, 1, 3, and 5 ppm. The light entering the PVC pipe was  $2300 \mu\text{E m}^{-2} \text{s}^{-1}$ . Light intensity after passing through 1 m of water containing 0, 1, 3, and 5 ppm was 10.2, 4.0, 2.3, and 1.4% of the entering value, respectively.

Respiration rate was estimated by covering the BOD bottle and oxygen electrode with a lightproof black plastic bag and again recording dissolved oxygen concentration at 5 minute intervals for 30 minutes. Following the measure-

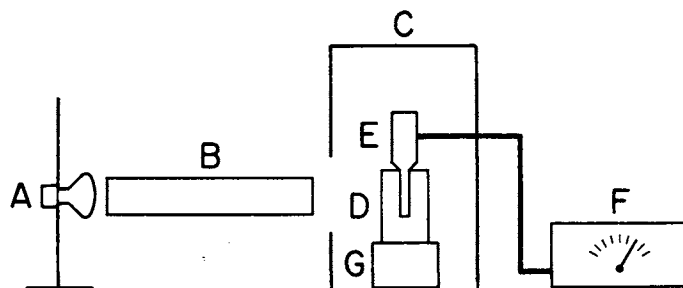


Figure 1. Experimental set up for measuring photosynthesis and respiration by algal cultures receiving the light which passes through a 1 m column of water. A. = 300 W flood lamp. B. = 1 m length of 0.1 m ID PVC pipe. C. = light proof box. D. = BOD bottle. E. = oxygen electrode. F. = pH meter. G. = magnetic stirrer.

ments, the contents of the BOD bottle were filtered using a glass fiber filter (effective pore size  $0.3 \mu\text{m}$ ; Millipore AP 400 4705). The filter was wrapped in aluminum foil and dropped into liquid nitrogen. After lyophilization of the filter and algae, the dry weight was determined. Photosynthetic and respiration rates were calculated by linear regression of oxygen concentration vs. time and normalized to dry weight. Results were expressed as  $\text{mg O}_2 \text{g}^{-1} \text{hr}^{-1}$ . Gross photosynthetic rate was determined by adding the respiration rate to the apparent photosynthetic rate.

Phosphorus uptake experiments were conducted by placing 100 ml aliquots of stationary phase batch cultures of either *A. falcatus* var. *acicularis* or *A. flos-aquae* in a 150 ml beaker which was then placed into the experimental apparatus. (Figure 1). Density was estimated to be  $1.399 \times 10^8 \text{ cells ml}^{-1}$  for *A. falcatus* var. *acicularis* and  $1.587 \times 10^7 \text{ filaments ml}^{-1}$  for *A. flos-aquae* using a Palmer-Maloney plankton cell (Wetzel and Likens, 1979). One ml of  $\text{K}_2\text{HPO}_4$  ( $50 \mu\text{g P ml}^{-1}$ ) was added. The beaker contents were stirred during the experiment. Five ml samples were removed at time 0 and at 20 minute intervals for 100 minutes. The samples were then passed through membrane filters ( $0.45 \mu\text{m}$ , Millipore HAMK 02412). Orthophosphate in the filtrate was determined as described by Wetzel and Likens (1979) except that 0.8 ml of combined reagent was added per 5 ml filtrate. Phosphorus uptake was determined by subtracting the amount of phosphate in the filtrate from the initial concentration. Measurements were made at 22-23 C using algal cultures which had been grown at low light ( $47 \mu\text{E m}^{-2} \text{sec}^{-1} \text{PAR}$ ) intensities.

## RESULTS

*Anabaena cylindrica* growth rates for cultures receiving 0 to 3 ppm Aquashade ranged from 1.087 to 1.409 doublings  $\text{day}^{-1}$  (Table 1). An analysis of variance indicated that Aquashade at the concentrations examined had no effect on the growth rate of this alga ( $F = 1.055$ ;  $df = 5, 12$ ;  $P = 0.43$ ). Similar results were obtained with *A. flos-aquae*, which exhibited growth rates ranging from 0.904 to 1.088 ( $F = 1.07$ ;  $df = 5, 12$ ;  $P = 0.42$ ). *Selenastrum capricornutum* grown with 0 to 3 ppm Aquashade displayed growth rates ranging from 0.627 through 0.726 doubling  $\text{day}^{-1}$ . The highest growth rates were for cultures receiving 2 ppm Aquashade. An analysis of variance indicated a significant increase in growth ( $F = 3.59$ ;  $df = 5, 12$ ;  $P = 0.032$ ).

TABLE 1. GROWTH RATES FOR THREE SPECIES OF ALGAE GROWN IN AAM WITH OF 0 TO 3 PPM AQUASHADE. VALUES ARE THE MEAN ( $\pm$ SD; N=3). THESE CULTURES WERE GROWN AT  $97 \mu\text{E m}^{-2} \text{s}^{-1}$ .

Species	0	0.25	0.5	1.0	2.0	3.0
<i>Selenastrum capricornutum</i> USEPA	0.680 (0.043)	0.627 (0.063)	0.599 (0.014)	0.659 (0.032)	0.726 (0.059)	0.697 (0.018)
<i>Anabaena flos aquae</i> UTEX 1444	0.976 (0.133)	1.088 (0.179)	0.912 (0.120)	1.039 (0.120)	0.904 (0.092)	1.024 (0.044)
<i>Anabaena cylindrica</i> UTEX 629	1.375 (0.30)	1.170 (0.198)	1.346 (0.139)	1.177 (0.074)	1.087 (0.187)	1.409 (0.329)

Results from cultures grown at higher Aquashade concentrations and at lower light intensities indicated that Aquashade reduced the growth rate of some species, but not others (Table 2). *Pediastrum tetras* and *Ankistrodesmus falcatus* var. *acicularis* were the most sensitive to treatment with Aquashade. Scheffe's test for equality among treatment means for these species indicated significant differences in growth rates between the control and 5 ppm treatments, but not between the 5 and 10 ppm treatments. Similar statistical analysis on the data for *Selenastrum capricornutum* demonstrated that this alga had a reduced growth rate at 10 ppm Aquashade, but not at 5 ppm when compared to control. *Anabaena flos-aquae* and *A. cylindrica* were unaffected by either 5 or 10 ppm Aquashade relative to the control.

Typical results for oxygen evolution (apparent photosynthesis) and uptake (respiration) are presented in Figure 2. Pearson correlation coefficients were typically  $> 0.95$ . Gross photosynthetic rates (respiration + apparent photosynthesis) for algae previously grown under high light conditions declined as the Aquashade concentration increased from 1 to 5 ppm (Figure 3). *Anabaena cylindrica*, *Pediastrum tetras*, and *Ankistrodesmus falcatus* var. *acicularis* each displayed  $> 50\%$  reduction in photosynthetic rate at 1 ppm Aquashade. *Anabena flos-aquae* and *Scenedesmus quadricauda* showed a smaller reduction at 1 ppm but were reduced by at least 50% at 3 ppm Aquashade. Gross

TABLE 2. GROWTH OF SIX SPECIES OF ALGAE AT THREE AQUASHADE CONCENTRATIONS. VALUES ARE THE MEAN ( $\pm$  SD; N=3). \*DENOTES THE FIRST TREATMENT MEAN THAT DIFFERS SIGNIFICANTLY FROM THE CONTROL AS DETERMINED BY SCHEFFE'S PROCEDURE WITH ALPHA = 0.05. THESE CULTURES WERE GROWN AT  $47 \mu\text{E m}^{-2} \text{s}^{-1}$ .

Species	Aquashade (ppm)		
	0	5	10
<i>Pediastrum tetras</i> UTEX 38	0.281 (0.025)	0.232* (0.007)	0.211 (0.019)
<i>A. falcatus</i> var. <i>acicularis</i> UTEX 101	0.447 (0.001)	0.424* (0.001)	0.419 (0.005)
<i>Selenastrum capricornutum</i> USEPA	0.390 (0.039)	0.323 (0.042)	0.228* (0.043)
<i>Anabaena flos-aquae</i> UTEX 1444	0.231 (0.005)	0.231 (0.0)	0.242 (0.0)
<i>Anabaena cylindrica</i> UTEX 629	0.149 (0.027)	0.127 (0.017)	0.170 (0.035)

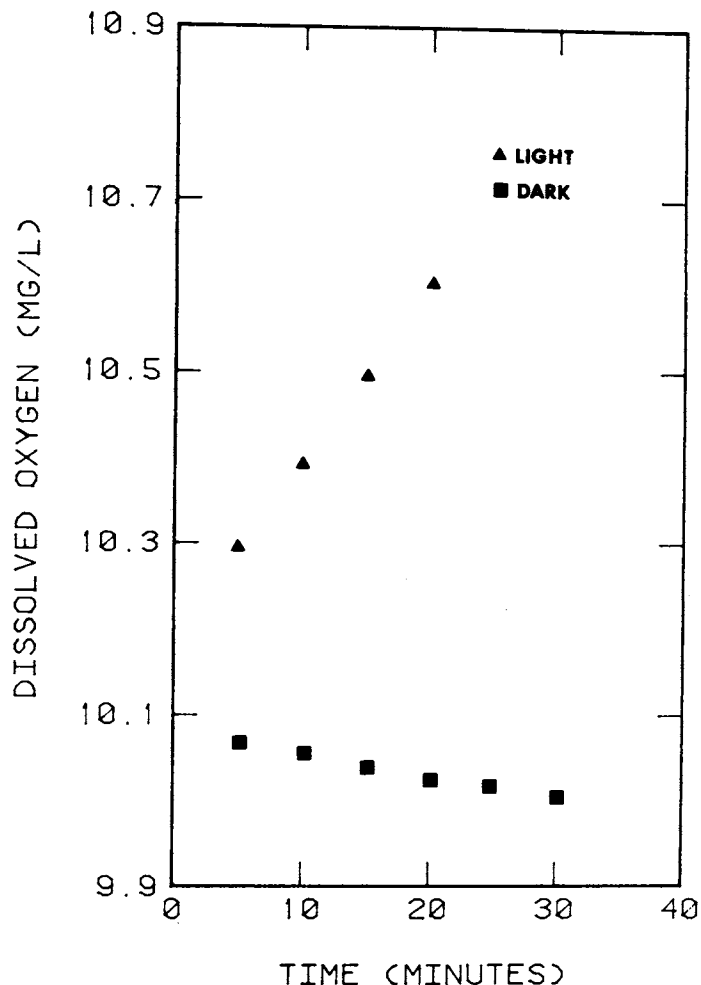


Figure 2. Light/dark technique for measuring photosynthesis and respiration for *Anabaena flos-aquae* culture.

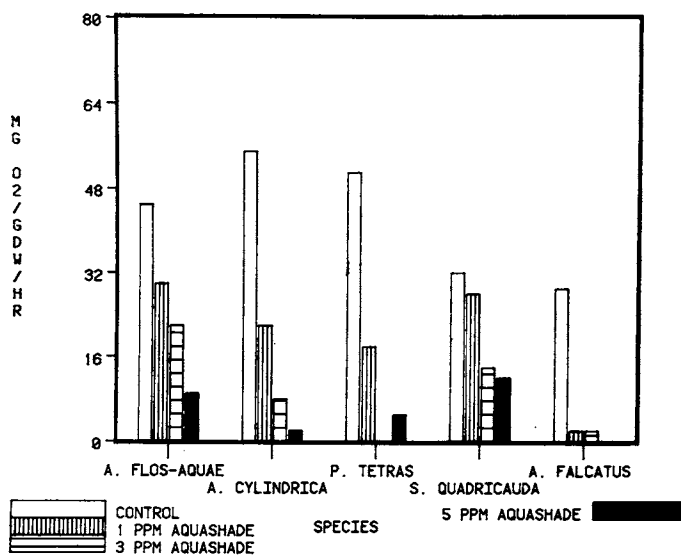


Figure 3. Gross photosynthetic rates for 5 algal species previously grown under high light and exposed to light passing through a 1 m column of water containing 0, 1, 3, 5, ppm Aquashade.

photosynthetic rates for control cultures of algae grown under low light conditions were higher than for controls grown under high light. This reflects the fact that algae grown under low light typically have more chlorophyll per unit mass. Each of the species examined however, exhibited > 50% reduction in photosynthetic rate at 1 ppm Aquashade (Figure 4).

The ratios of gross photosynthesis to respiration (P/R) for cultures initially grown at high light are presented in Figure 5. P/R ratios greater than 1 indicate that organic carbon is being fixed at a rate faster than it is being consumed through respiration. Values less than 1 indicate that the organism's respiration rate is greater than its photosynthetic rate. Algae can only survive under these conditions for extended periods by utilizing stored materials.

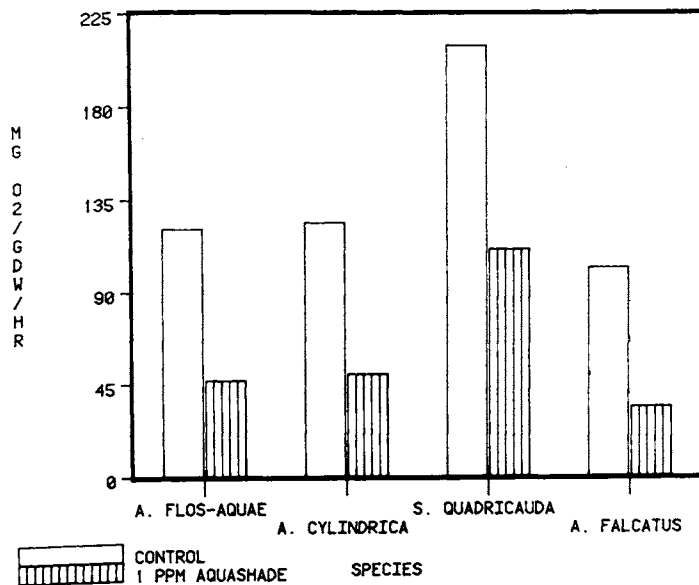


Figure 4. Gross photosynthetic rates for 4 algal species previously grown at low light and exposed to light passing through a 1m column of water containing 0 or 1 ppm Aquashade.

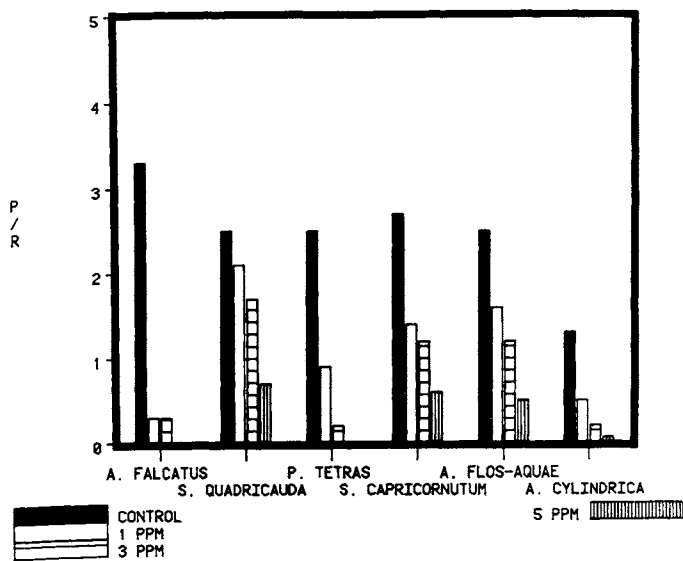


Figure 5. Ratio of gross photosynthesis to respiration for algae previously grown at high light and exposed to light passing through a 1 m column of water containing 0, 1, 3, 5 ppm Aquashade.

The P/R ratio decreased for all species examined as the Aquashade concentration increased. Values less than 1 were noted at concentrations of 3 ppm Aquashade or greater (except for *A. falcatus* var. *acicularis* which displayed a P/R ratio less than 1 at 1 ppm Aquashade).

Aquashade (1 ppm) had no effect on the phosphorus uptake rates by either *Anabena flos-aquae* or *Ankistrodesmus falcatus* var. *acicularis* (Figure 6). Phosphorus uptake rates for *A. flos-aquae* were  $0.13 \pm 0.06$  and  $0.18 \pm 0.03 \mu\text{g P min}^{-1}$  for the control and 1 ppm Aquashade treatment, respectively. Similarly, values for *A. falcatus* var. *acicularis* were  $0.53 \pm 0.10$  and  $0.50 \pm 0.07 \mu\text{g P min}^{-1}$ .

## DISCUSSION

The results of the batch culture experiments indicate that Aquashade does not reduce algal growth rates at concentrations less than 5 to 10 ppm in the culture medium. The manufacturer recommends an application rate of 1 ppm. In contrast, the photosynthetic measurements indicate that 1 to 3 ppm Aquashade resulted in reduced photosynthetic rates for all species tested. This apparent incongruity can be explained by examining the experimental protocols.

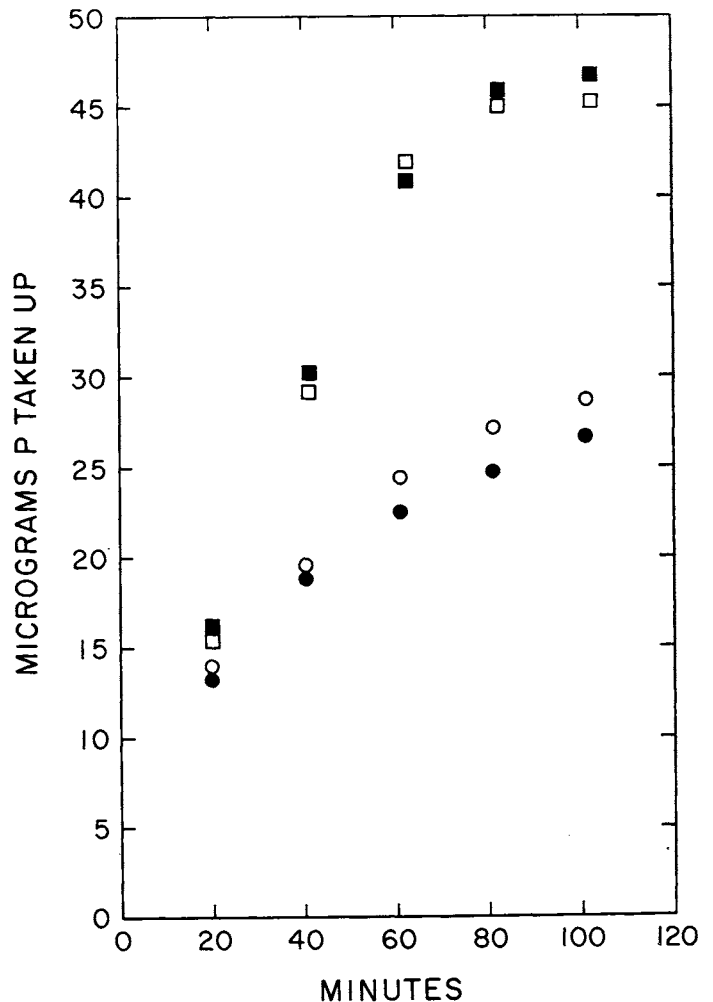


Figure 6. Phosphorus uptake by *Anabaena flos-aquae* (circle) and *Ankistrodesmus falcatus* (square). Open symbols = control; closed symbols = 1 ppm Aquashade.

The algae in the batch culture experiments were grown in 50 ml of medium in a 250 ml flask. The total depth of medium was less than 2 cm. On the other hand, the photosynthetic rates were determined using algae which received light which had passed through a 1 m column of Aquashade-treated water. This resulted in greater light attenuation even though the Aquashade concentration was lower. These results suggest that Aquashade reduces photosynthetic rates indirectly by competing with algae for available light and not by exerting some direct lethal effect on them. Further evidence consonant with this view is obtained from a comparison of the absorption spectra of Aquashade and algae. Osborne (1979) reported that the peak absorbance for Aquashade was 630 nm with an absorbance range of 550-650nm. Thus Aquashade absorbance overlaps with that of the pigments associated with photosystem II (Govendjee and Braun, 1974). Additional evidence that Aquashade competes with photosystem II pigments comes from the fact that phosphorus uptake was not affected by Aquashade. This is consistent with Smith (1966) who reported that DCMU and light filters that inhibited photosystem II did not influence phosphorus uptake.

Aquashade appears to inhibit photosynthesis of both blue-green algae and green algae about the same. Phosphorus uptake is unaffected, at least for the two species examined. These findings suggest that the shift in the relative abundance of green and blue-green algae observed by Buglewicz (1972) was probably not solely due to the addition of a blue dye.

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