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Effect of Atrazine on the Productivity of Artificial Stream Algal Communities

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Herbicides play a vital role in modern agriculture but, unfortunately, these chemicals enter aquatic environments through runoff and erosion. Concentrations in runoff and drainage are usually low, but increase when abundant rainfall closely follows herbicide application. Bulk runoff concentrations of one of the most common herbicides, atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine), as high as the low mg kg^{-1} range have been documented (Hall et al. 1972), and stream water concentrations in the μ g kg^{-1} range have been observed (Frank and Sirons 1979; Richard et al. 1975).

Although research concerning the effects of herbicides on algae has been extensive (Butler 1977) only a few studies have examined impacts on productivity and/or species composition at the community level. De Noyelles et al. (1982) reported detectable effects of 0.02 and 0.50 mg kg^{-1} atrazine concentrations on algal productivity and species composition in a pond study, and significant effects of 1 to 5 μg kg^{-1} concentrations in concurrent laboratory work. Bryfogle and McDiffett (1979) observed changes in productivity patterns, species dominance and chlorophyll pigment ratios at 0.05 to 0.40 mg kg^{-1} concentrations of simazine (a closely related s-triazine) in laboratory cultures. Both studies employed planktonic algae rather than the attached forms responsible for most photosynthesis in lotic systems, but lotic communities may respond in a similar manner to atrazine input. Kosinski and Merkle (1984) noted significant inhibition of community productivities in laboratory streams treated with atrazine concentrations of 1.0 to 10.0 mg kg^{-1}. Kosinski (1984) also reported that 1.0 and 10.0 mg kg^{-1} atrazine caused biomass reduction and changes in the species composition of stream periphyton. We are aware of no other studies concerning response of lotic algae to herbicides.

The objective of this study was to monitor short term effects of atrazine on the algal communities of artificial streams to determine if concentrations of atrazine, that have been measured in runoff and stream waters, could significantly affect community productivity and species composition.

MATERIALS AND METHODS

Sixteen recirculating artificial streams were placed on the flat roof of a three story building in central Texas. Each stream was 2.43 m long, 12.5 cm wide and 6 cm deep, constructed of plywood, painted with a water-resistant white enamel paint and lined with polyethylene plastic sheeting 0.1 mm thick. Each stream held 12 L of water at an average depth of 4 cm. A single layer of stream-worn pebbles (diameters 4-8 mm) was placed in the center 1.8 m of each stream. The water was recirculated by means of a water pump placed at the foot of each stream. Water was pumped to the upper end through a plastic hose, maintaining a water flow rate of about 5 cm sec^{-1} in the stream. Additional water was added only to replace evaporative losses.

Minter Spring, Brazos County, Texas, was chosen as the source of water and algae for this study since it provides a supply of constant quality. This water is nearly anoxic and has a constant temperature of 21°C. It is highly mineralized with specific conductance of 54.6 mS m^{-1} at 25°C, alkalinity of 74 mg kg^{-1} $CaCO_3$, nitrate concentration of 404 µ g kg^{-1}, and soluble reactive phosphorus concentration of 21 µg kg^{-1} (Kosinski and Merkle 1984). The principal algae genera present are Anabaena, Nitzschia, Rhopalodia and Navicula. Algal spores and cells present in the water were permitted to colonize the streams. Evaporative water losses were initially replaced by Minter Spring water, permitting nutrient accumulation to facilitate colonization. Deionized tap water was later used to avoid excessive evaporative concentration of nutrients and minerals.

Community productivities were estimated with open water oxygen methods which estimate net community productivity (NCP) by correcting the rate of change of the dissolved oxygen concentration for exchange with the atmosphere. Dissolved oxygen concentrations were monitored (September 22, 1980 through October 30, 1980) on selected days at half-hour intervals, from sunrise to sunset, with a dissolved oxygen meter. The meter was calibrated with azide modified standard Winkler titrations (APHA 1975) at each half-hour reading. Net community productivity was calculated for each stream and daily cumulative net productivity was summed for the period from 7:00 AM to 7:00 PM, in order to standardize values throughout the study period. Additional readings were taken at night to obtain an indication of night-time respiration since the rate of change of dissolved oxygen levels at night reflect only the interaction of respiration and diffusion. Equilibrium values of dissolved oxygen concentrations at given temperatures were calculated (Benson and Krause 1980).

The oxygen exchange coefficient was estimated in order to correct for atmospheric exchange. An empirical determination was done by placing water partially deoxygenated with sodium sulfite and cobalt chloride in an uncolonized stream. Oxygen concentrations were then monitored at one minute intervals. The rates of change in oxygen levels during these intervals were regressed on the mean

saturation deficits during the intervals, the slope estimating the oxygen exchange coefficient (Owens 1974). All estimates were corrected for temperature differences and standardized to 20°C (Bansal 1973). A mean oxygen exchange coefficient of 0.0568 m hr^{-1} was obtained from four separate determinations and used for productivity and respiration calculations.
Colonization was permitted for 5 weeks, beginning September 12, 1980, since initial studies indicated that about 4 weeks were necessary to develop adequate algal growth. Four horizontally positioned glass microscope slides were placed in the upper and lower portions of all streams at the beginning of the study. Slides were randomly selected at weekly intervals during the study, the algal community on each was scraped into a vial and preserved with buffered formalin solution. The preserved algae were suspended in 10 mL of distilled water and two 1 mL aliquots were taken from each sample, placed in Sedgewick-Rafter cells and five random fields of view examined. All algae cells present in these fields were identified to genus and counted. The six most numerous genera, comprising 95% of all cells, were statistically analyzed for variations in densities. The sampling scheme used was determined by the optimal allocation method limited by time constraints (Snedecor 1967) for the most numerous genus of algae, Chroococcus.

Conductivities were monitored with a electro-mho meter every few days and alkalinities were determined at weekly intervals by sulfuric acid titration (APHA 1975). Both the diurnal temperature and pH fluctuations were recorded at weekly intervals on the same days that the dissolved oxygen concentrations were monitored. A pH meter was used to monitor hourly values from sunrise to sunset. Temperature readings were taken with the oxygen meter for all streams at half-hour intervals, and were used to calculate equilibrium oxygen concentrations. Phosphate analyses were performed on water samples from all streams taken two days before treatment, the day of treatment, three days and one week after treatment. The samples were frozen until later examination. Soluble reactive phosphate concentrations were determined by the ascorbic acid spectrophotometric technique with a lower detection limit of 5 $\mu g\ kg^{-1}$ (APHA 1975).

The brand of atrazine used in this study was Aatrex 80 WP manufactured by the CIBA-Geigy Corporation. Four concentrations of the herbicide in the artificial streams were initiated on October 23, 1980, after the colonization period. Concentrations of 0.0, 0.1, 1.0 and 10.0 mg kg^{-1} active ingredient were randomly assigned to sixteen streams with four streams per treatment. The loss of one of the 10 mg kg^{-1} streams, due to leaks, at the beginning of the study unbalanced the experimental design and the results were analyzed accordingly. Atrazine has an approximate half-life of 3.2 days in such laboratory streams (Kosinski and Merkle 1984) and no additional herbicide was added after the initial treatment.

RESULTS AND DISCUSSION

Analyses for treatment effects were performed by date (Table 1) since there was a significant decline in the control streams' dawn-to-dusk net community productivities (NCP) through time ($P < 0.0001$). There were no significant differences between stream productivities before herbicide application, however, differences became highly significant upon treatment ($P < 0.0001$). Duncan's multiple range test indicated significant differences ($P < 0.05$) between the control, the 0.1 mg kg^{-1} treatment and a third group consisting of both the 1.0 and 10.0 mg kg^{-1} treatments. The results were essentially identical three days later, but after a week, the 0.1 mg kg^{-1} treatment was no longer different from the control. These results are similar to those of other studies which report significant inhibition of algal oxygen production at atrazine concentrations of 1.0 to 10.0 mg kg^{-1} (Butler 1977). Even the 0.1 mg kg^{-1} concentration significantly depressed productivity, which is also consistent with the literature (de Noyelles et al. 1982; Kosinski and Merkle 1984). All NCP values were fairly low, indicating high respiration relative to oxygen production.

Table 1. Daily cumulative NCP values by date and treatment, atrazine applied October 23. Sample size of four.

	Treatment (atrazine mg kg^{-1})			
Date	0.0	0.1	1.0	10.0
Sept 22	0.335[1]	0.093	0.189	0.147
	(0.064)	(0.098)	(0.049)	(0.144)
	A[2,3]	A	A	A[3]
Oct 01	-0.028	-0.088	-0.100	-0.004
	(0.103)	(0.070)	(0.081)	(0.063)
	A	A	A	A[3]
Oct 21	-0.042	-0.065	-0.109	-0.030
	(0.064)	(0.063)	(0.048)	(0.030)
	A	A	A	A[3]
Oct 23	-0.049	-0.410	-0.719	-0.890
	(0.066)	(0.038)	(0.063)	(0.046)
	A	B	C	C[3]
Oct 26	-0.182	-0.395	-0.596	-0.663
	(0.059)	(0.017)	(0.014)	(0.034)
	A	B	C	C[3]
Oct 30	-0.214	-0.286	-0.468	-0.502
	(0.032)	(0.286)	(0.011)	(0.034)
	A[3]	A	B	B[3]

[1]Treatment means (oxygen g m^{-2} day^{-1}) with standard errors (in parentheses).
[2]Different letters indicate significant differences between treatment means within dates (Duncan multiple range test).
[3]Sample size of three.

Respiration values were determined solely from night-time observations made on all streams before and after treatment. There were no significant differences in respiration rates between treatments. However, rates did decrease through the study period, possibly due to decreasing ambient temperatures (Table 2). The lack of any detectable effect on respiration rate was not surprising since atrazine's action as an inhibitor of the Hill reaction of photosynthesis (Gardner 1981) should not directly affect respiration. Correcting for temperature differences was unnecessary for comparisons between treatments since all streams exhibited nearly identical temperature regimes.

Table 2. Respiration rate by date and treatment, atrazine applied October 23. Sample size of four unless denoted otherwise.

	Treatment (atrazine mg kg^{-1})			
Date	0.0	0.1	1.0	10.0
Sept 22	0.085[1] (0.004)	0.083 (0.003)	0.092 (0.005)	0.083[2] (0.002)
Oct 01	0.076 (0.004)	0.092 (0.016)	0.084 (0.002)	0.079[2] (0.004)
Oct 21	0.061 (0.002)	0.061 (0.003)	0.059 (0.002)	0.066[2] (0.001)
Oct 23	0.067 (0.002)	0.062 (0.002)	0.065 (0.002)	0.072[2] (0.003)
Oct 30	0.050 (0.002)	0.052 (0.003)	0.045 (0.002)	0.052[2] (0.002)

[1]Treatment means (oxygen g m^{-2} hr^{-1}) with standard errors (in parentheses).
[2]Sample size of three.

Initial examination showed that algae cell distributions in the Sedgewick-Rafter cells were slightly contagious. Therefore, the data were transformed according to Taylor's Power Law (Taylor 1961) and then analyzed for significant treatment effects using an ANOVA model. Analyses indicated that only Nitzschia showed significant differences between treatments ($P < 0.0210$). This occurred October 30, 1980, (one week after treatment) when Duncan's multiple range test showed significant differences ($P < 0.05$) between the 0.1 mg kg^{-1} treatment, the 10.0 mg kg^{-1} treatment and a third group consisting of both the control and the 1.0 mg kg^{-1} treatments. The control had approximately 2.7×10^4 cells cm^{-2} on the colonization slides, the 0.1 mg kg^{-1} treatment had about twice this density (4.5×10^4 cells cm^{-2}) and the 10.0 mg kg^{-1} treatment had about half the control value (1.0×10^4 cells cm^{-2}). Other studies have reported reductions in algal growth at lower atrazine concentrations (Boger and Schlue 1976; Rohwer and Fluckiger 1979). Unfortunately, not enough species specific information is available for comparison. De Noyelles et al. (1982) reported selective effects of atrazine on phytoplankton species at 0.02 and 0.50 mg kg^{-1} concentrations, in

which patterns of species abundances changed with herbicide application due to differential algal species resistances, interspecific competition and changes in predator/prey relationships. Kosinski (1984) noted extreme inhibition of Rhopalodia, Phormidium and Cladophora by 1.0 and 10.0 mg kg^{-1} atrazine in artificial streams accompanied by large biomass reductions in the algal communities. Bryfogel and McDiffett (1979) noted selective effects of 0.05 to 0.40 mg kg^{-1} simazine on algae in mixed cultures which caused shifts in species dominance patterns. The present study did not show any major shifts in numerical importances of the other dominant algal groups so the increase in Nitzschia cell numbers for the 0.1 mg kg^{-1} treatment cannot be explained.

There were no significant differences in conductivity or alkalinity between treatments. Conductivity varied from 61.0 mS m^{-1} (at 25°C), initially, to 41.2 mS m^{-1} by the end of the study. Alkalinity steadily increased from an initial value of 62.25 mg kg^{-1} (as calcium carbonate) to 77.00 mg kg^{-1}, possibly due to abundant limestone in the substrate. Soluble reactive phosphate concentrations were very low, with most of the samples showing zero or trace amounts. Therefore, insufficient information was available for statistical analysis. The changes in pH indicated agreement with the patterns of NCP rates throughout the day. Average values were relatively high, slightly over 8.0, probably due to the calcareous substrate and the removal of carbon dioxide by photosynthesis.

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