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# Effects of Two Herbicides on Selected Aquatic Bacteria

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Chemical herbicides are commonly used to control nuisance aquatic vegetation. Since the development of the first effective organic herbicide in 1932 (Brian 1964), over 150 formulations of agricultural and aquatic herbicides have been approved for use (Ashton and Crafts 1981). Concern about the increased usage of herbicides, their fate in the environment, and their effect on nontarget organisms has also increased. Extensive literature exists on the impact of various herbicides on fish, zooplankton, and invertebrates (Serns 1975, 1977). Degradation of selected herbicides in the environment also has been documented (Alexander 1964). Although bacteria are important in the processes of decomposition and nutrient cycling in aquatic ecosystems, the effect of herbicides on aquatic bacteria has received little attention (Alexander 1964).

Herbicide applications in aquatic systems may affect bacterial populations directly by either inhibiting or stimulating the metabolism of specific functional groups or of the entire community. Alternatively, bacteria may be affected by changes in various water quality parameters (Serns 1975), loss of vegetative substrate, loss of food sources, or increases in nonherbicide toxins released by vegetation.

The objectives of this study were to: (1) assess the effects of simazine and endothall on bacterial populations in aquatic ecosystems, and (2) determine the effects of those herbicides on growth and respiration of common bacterial isolates obtained from aquatic ecosystems. Herbicides used were dipotassium endothall (7-oxabicyclo(2.2.1)heptane-2,3-dicarboxylic acid) and simazine (2-chloro-4,6-bis(ethylamino)-s-triazine).

Endothall, a contact herbicide, effectively controls several aquatic macrophytes by a variety of mechanisms (Serns 1977). Inorganic salts of endothall at recommended application rates generally are not harmful to nontarget organisms (Serns 1977), but amine formulations are 10 times more phototoxic and 100 times more toxic to fish than are other formulations (Walker 1963). Endothall dissipates rapidly in the environment (Armstrong 1974), and microbial activities are known to be at least partially

responsible for its degradation (Comes et al. 1961). Studies of the effects of endothall on bacteria (aquatic or terrestrial) have been inconclusive (Anderson 1978).

The triazine simazine effectively controls a variety of submerged aquatic macrophytes and algae (Walker 1964). It is a systemic herbicide with a delay of 2-6 weeks between application and toxic responses, i.e., chlorosis and decay (Walker 1964). Simazine interferes with the Hill reactions, thus preventing photosynthesis (Good 1961). At normal application rates simazine generally has been found nondeleterious to nontarget organisms, including soil bacteria (Burnside et al. 1961).

The data presented here were obtained as part of a study comparing the effects of herbicides and hybrid carp on aquatic ecosystems. This project (Federal Aid Project F-37-R) has been funded by the Illinois Department of Conservation, Illinois Department of Energy and Natural Resources, U.S. Fish and Wildlife Service, and Illinois Natural History Survey.

## MATERIALS AND METHODS

Bacterial populations in treated and untreated ponds were sampled before and after treatment to determine changes in total numbers of and in dominant aerobic, heterotrophic bacteria. In addition, bacterial isolates most abundant in the water column and on submersed macrophytes were exposed to maximum recommended concentrations of the herbicides in in vitro growth tests and in respirometry procedures.

Ponds 1c and 1e are gravel pits with surface areas of 2,885 and 2,400 $m^2$ and mean depths of 1.14 and 1.45 m, respectively. Ponds 2c and 2s are man-made ponds with surface areas of 682 and 689 $m^2$ and mean depths of 1.02 and 0.72 m, respectively. All ponds are located near Champaign, Illinois. Water temperatures of the ponds ranged from 10.0 to 31.0°C during the sampling season (5 May to 1 October 1981). All ponds were stocked with bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus), and largemouth bass (Micropterus salmoides) in comparable numbers. All ponds had a history of excessive macrophyte growth, primarily Potamogeton, Najas, and Chara (Gorden and Waite 1981).

The dipotassium salt of endothall was applied to pond 1e at 0.3 ppm on 5 June 1981. Bacterial samples were obtained from it and pond 1c (control) on two dates prior to endothall application and at 3, 5, 7, and 18 days and monthly following application. On 9 June 1981, pond 2s was treated with 1.0 ppm simazine. Samples were collected from it and pond 2c (control) on two dates prior to treatment and at 3, 7, 10, and 16 days and monthly after treatment.

On each sample date, four samples were collected from the water column, four from sediments, and two from macrophytes. Water and sediment samples were equally divided between areas of high

and low macrophyte concentrations. Water and sediment samples were collected and transferred to sterile containers. A 6-8-cm terminal portion of an apparently healthy macrophyte was collected aseptically and placed in 99 ml of sterile buffer solution.

All samples were serially diluted in sterile buffer, pour plated in duplicate on half-strength nutrient agar, and incubated inverted at 30°C for 7-10 days prior to enumeration of colony generating units (CGU) $ml^{-1}$ water, CGU $g^{-1}$ wet weight sediment, and CGU $g^{-1}$ dry weight macrophytes. The nonparametric Kruskal-Wallis test was used to analyze these data (Sokal and Rohlf 1969). Six of the most abundant bacterial isolates were selected for studies of the effects of simazine and endothall on growth and respiration. These isolates were tentatively identified as Aeromonas hydrophila, Acinetobacter anitratus, Bacillus sp., Micrococcus sp., Pseudomonas vesicularis, and Pseudomonas sp. (Buchanan and Gibbons 1974).

Fresh endothall-nutrient broth media was prepared prior to each test by diluting filter-sterilized endothall in sterile nutrient broth to a final concentration of 5 ppm (highest recommended application rate). Eight 10-ml tubes (four without herbicide and four containing 5 ppm of endothall) were inoculated with homogenized bacterial stock and incubated at 25°C for 24 hours. Initial and final bacterial cell densities were determined by serial dilution. The effects of endothall on each bacterial isolate was determined using a t test of means.

Fresh simazine-nutrient broth solutions were prepared by adding simazine to sterile nutrient broth to a final concentration of 3 ppm (highest recommended application rate). Inoculation, culture, counting, and analysis procedures were identical to those used in the endothall studies. Due to simazine's extremely low solubility in water (Davis et al. 1959) and its inability to withstand autoclaving (Burnside et al. 1961), it was added to sterile nutrient broth without any treatment to ensure its sterility. Plating of the solution prior to the addition of bacteria revealed that simazine contained no bacteria capable of growth on the plating medium.

Using a Gilson differential respirometer (Model #SGRP-14), oxygen consumption of six bacterial isolates was determined. Bacterial isolates (1.0 ml) were transferred to eight sterile reaction vessels, four of which contained 1.0 ml of either endothall-nutrient broth solution (5 ppm) or simazine-nutrient broth solution (3 ppm); four contained only nutrient broth. The final volume in each reaction vessel was 2.0 ml. Procedures followed those of Umbreit et al. (1972) and Gilson Medical Electronics (undated). Dilution of the stock culture was necessary to measure oxygen consumption over an extended time. Readings were taken at least every hour until maximum oxygen uptake was reached. Respirometry data were analyzed using a t test of means.

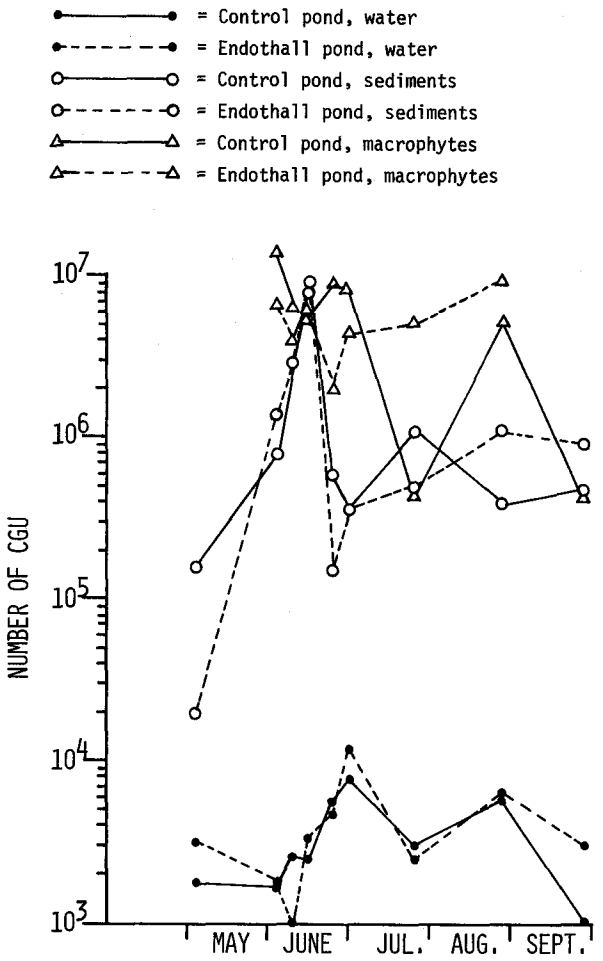


Figure 1. Mean total number of colony generating units (CGU) in water (number $ml^{-1}$), in sediments (number $g^{-1}$ wet weight), and on macrophytes (number $g^{-1}$ dry weight) in the control pond and the pond treated with endothall (0.3 ppm) on 5 June 1981.

## RESULTS AND DISCUSSION

Neither simazine nor endothall had a significant effect on total bacterial numbers present in the water column, present in sediments, or attached to macrophytes. No significant differences (Kruskal-Wallis, $P<0.05$) were found in the numbers of bacteria present in herbicide-treated ponds versus their respective control ponds on any sampling date. In all four ponds, the number of bacteria in water ranged from 3.0 to 330 x $10^2$ CGU $ml^{-1}$, in sediments from 2.0 to 1,100 x $10^4$ CGU $g^{-1}$ weight weight, and on macrophytes from 4.4 to 420 x $10^5$ CGU $g^{-1}$ dry weight (Fig. 1).

Approximately 340 bacteria were isolated, the most commonly identified genera being Pseudomonas, Bacillus, Aeromonas, and Corynebacterium. No alterations in the dominant CGU were noted following herbicide application.

Simazine at 3 ppm and endothall at 5 ppm had no measurable effect on the growth of any bacterial isolate in pure culture.

Endothall at 5 ppm apparently slightly inhibited the respiration rate of Micrococcus sp., slightly stimulated respiration of Pseudomonas vesicularis, and had no obvious effect on the respiration rate of Bacillus sp. (Table 1); none of these differences were significant (t test, $P<0.05$). Significantly higher respiration rates were observed in endothall-treated replicates than in controls of Aeromonas hydrophila (each time period), Pseudomonas sp. (at 1 hour), and Acinetobacter anitratus (at 1.0 and 1.5 hours) (Table 1). Within several hours the rates of treated replicates resembled those of controls.

Simazine at 3 ppm increased the respiratory activity of four isolates; however, only with Micrococcus sp. was the effect significant (t test, $P<0.05$). Respiration rates of controls versus simazine-treated replicates leveled off with time.

"Herbicides [are] usually applied in a particular environment at levels of a few parts per million. At such concentrations the

TABLE 1

Mean oxygen consumption (ul) of Bacillus sp. and Pseudomonas sp. (per $10^7$ cells) and Aeromonas hydrophila and Acinetobacter anitratus per $10^8$ cells) for endothall treatment (E) and controls (C).

| | Bacillus sp. | | Aeromonas hydrophila | | Pseudomonas sp. | | Acinetobacter anitratus | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | C (n=4) | E (n=3) | C (n=4) | E (n=4) | C (n=8) | E (n=8) | C (n=8) | E (n=8) |
| 0.5 | 4.9 | 6.1 | 7.1 | 8.4* | 0.0 | 0.0 | 0.0 | 1.5 |
| 1.0 | 14.8 | 16.3 | 16.3 | 18.1* | 0.4 | 1.8* | 3.7 | 6.1* |
| 1.5 | 30.9 | 31.1 | 27.0 | 29.0* | 2.3 | 4.9 | 12.1 | 15.0* |
| 2.0 | 55.0 | 56.4 | 43.4 | 45.8* | 7.8 | 10.4 | 25.0 | 28.6 |
| 2.5 | 85.0 | 87.8 | 56.7 | 59.2* | 15.4 | 18.8 | 45.2 | 51.1 |
| 3.0 | 121.8 | 123.4 | 69.3 | 71.4* | 26.8 | 30.6 | 81.2 | 85.0 |
| 3.5 | 150.4 | 152.4 | | | 41.0 | 45.2 | | |
| 4.0 | 182.2 | 184.6 | | | 62.6 | 67.8 | | |
| 4.5 | 213.4 | 213.6 | | | | | | |
| 5.0 | 247.8 | 246.5 | | | | | | |
| 5.5 | 282.0 | 279.8 | | | | | | |

*t test, $P<0.05$

chemicals have a profound effect upon susceptible plants, but microorganisms or microbial transformations seem little affected at the low levels recommended for weed control" (Alexander 1971). Our results generally support that summation, since simazine and endothall were found to have no significant effect on bacterial populations in situ or in growth experiments. However, these herbicides did affect the respiratory activity of the dominant bacteria. Generally, these effects were not statistically significant or became insignificant with time, indicating that initial effects of the herbicides on respiratory activity of bacteria were short lived. Also, these effects may be masked or buffered by complex interactions in the aquatic environment.

Field applications of herbicides have profound and measurable effects on aquatic ecosystems in general (Serns 1975; Walker 1963). Similar effects might be expected on lower-trophic-level populations and their functional rates. Certain bacteria in treated ponds were affected directly by the herbicides and were either inhibited or stimulated, but their numbers were either too low or the effects were too subtle to be observed (Gorden et al. 1982). Also, herbicide was assimilated by the aquatic vegetation and adsorbed on sediments and was not readily available (West et al. 1979).

Indirect effects on bacterial populations undoubtedly occurred due to the death and degradation of plant biomass (Gorden et al. 1982). A more critical study of the bacteria associated with plant senescence and decomposition may reveal changes in dominant populations and functional processes. Our observations of bacterial populations at 1, 3, and 5 days may have been too infrequent to document subtle or short term changes in bacterial numbers; however, little change in bacterial populations occurred following recommended applications of simazine (Roslychy 1977) and endothall (Anderson 1978) to terrestrial systems.

Chemicals will continue to enter aquatic ecosystems. Terrestrial herbicides routinely enter aquatic systems as a result of soil erosion and point and nonpoint sources of pollution. In some instances, these herbicides may be concentrated in aquatic habitats at levels which are detrimental to microbial communities. The use of chemical herbicides to control aquatic macrophytes is likely to continue and may increase until biological or mechanical methods of control are greatly improved. However, at recommended rates, annual applications of simazine and endothall do not appear to be detrimental to the aerobic, heterotrophic bacterial populations of pond ecosystems.

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