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Toxicity of Certain Insecticides to Protozoa

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In a recent study of the effects of insecticides on mixed microbial populations, WEBER & ROSENBERG (1981) noted the absence of protozoans in laboratory ecosystems which had been treated with carbaryl. In addition, of those insecticides tested, carbaryl demonstrated the most pronounced inhibition of the culture's ability to decompose cellulose.

Since grazing by protozoa is an important selection factor for some bacterial populations (GUDE 1971), and protozoal predation on bacteria has been shown to stimulate decomposition of cellulosic plant materials (FENCHEL & HARRISON 1976; HARRISON & MANN 1975), the observed correlation between the absence of protozoans and inhibition of cellulose decomposition in the presence of carbaryl (WEBER & ROSENBERG 1981) suggests that the toxicity of insecticides to protozoans may exert a profound effect on cellulose decomposition processes.

Through the widespread use of insecticides, many ecosystems can become contaminated as a result of runoff as well as direct application. The toxicity to protozoans of carbaryl, malathion, toxaphene, and 1-naphthol, the major hydrolysis product of carbaryl, was therefore examined.

MATERIALS AND METHODS

Rice infusion broth (RIB) was prepared by adding 4 grains of brown rice to 150 mL of half-strength artificial seawater (LYMAN & FLEMING 1940) and allowing the mixture to stand overnight. Protozoa were isolated from a salt marsh by placing a small amount of decaying plant material and sediment in 150 mL of RIB and incubating for one week at room temperature under the ambient light/dark regime.

To separate protozoa from particles of detritus, a number of large ciliates were collected from the culture with a capillary pipette and placed in fresh RIB which was incubated for several days. This resulted in a detritus-free culture dominated by the ciliate Euplotes sp. and a number of unidentified microflagellates. By removing some of the culture fluid and replacing it with fresh RIB every few days, this culture was maintained in the laboratory for 3 months.

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The number of ciliates present in the culture was determined by taking ten 0.1 mL samples and placing them in separate wells of a 96-well microtiter plate (FALCON, Oxnard, CA). The number of ciliates in each well were counted using a dissecting microscope, and the average number per well calculated. For toxicity tests, the culture density was adjusted to two organisms per well at the start of each experiment to standardize the inoculum.

For concentrations of each compound to be tested, 5.0 mL of adjusted protozoan culture was placed in each of 2 small glass petri dishes (5 x 3 cm). Five mL of test solution at twice the desired final concentration in seawater was added to each dish, and the cultures incubated for 24 h. Control dishes received seawater with carrier (acetone). Twenty-four 0.1 mL samples were taken from each culture dish, placed in separate wells of a 96-well microtiter plate, and the number of ciliates in each well was counted. Percent mortality in each dish was calculated using the number of ciliates per 24 wells from control dishes as 0%. This experiment was performed in duplicate for each substance, with a total of 4 replications for each concentration as well as controls.

RESULTS AND DISCUSSION

Dose-response curves for the compounds tested can be seen in fig. 1. The term "% Mortality" in this case indicates the number of protozoans present expressed as percent of controls after 24 h exposure to toxicants. The differences between controls and treated cultures are due to differences in the growth rates of the organisms as well as to mortality.

The concentrations of carbaryl, 1-naphthol, and malathion used in this experiment are well within the range of the solubilities of these compounds in water. Carbaryl and 1-naphthol are both soluble in water at approximately 30 ug/mL (KARINEN et al. 1967; LAMBERTON & CLAEYS 1970). The solubility of malathion has been reported as 145 ug/mL (BURCHFIELD et al. 1965).

The water solubility of toxaphene is approximately 0.5 ug/mL, (GUYER et al. 1972) a value which is exceeded by the concentrations of this compound used in the present study. These data were included, however, to indicate that toxaphene may exert toxic effects on protozoa under laboratory conditions. Toxaphene has been reported in surface runnoff at concentrations as high as 0.5 ug/mL (WAUCHOPE 1978).

The lowest concentrations of toxaphene, carbaryl, and 1-naphthol exhibiting toxicity presented in Fig. 1 are comparable to concentrations observed in the environment. It should be noted that although carbaryl is degraded by many types of microorganisms under a wide variety of soil conditions, the major end product of this degradation is 1-naphthol (KARINEN et al. 1967, OSMAN & BELAL 1980), which is as toxic as the parent compound, carbaryl (Fig. 1). Studies on the concentration of carbaryl in the environment may therefore not reflect the total concentration of toxic materials. Surface runoff from areas that have been treated with carbaryl may contain enough carbaryl plus

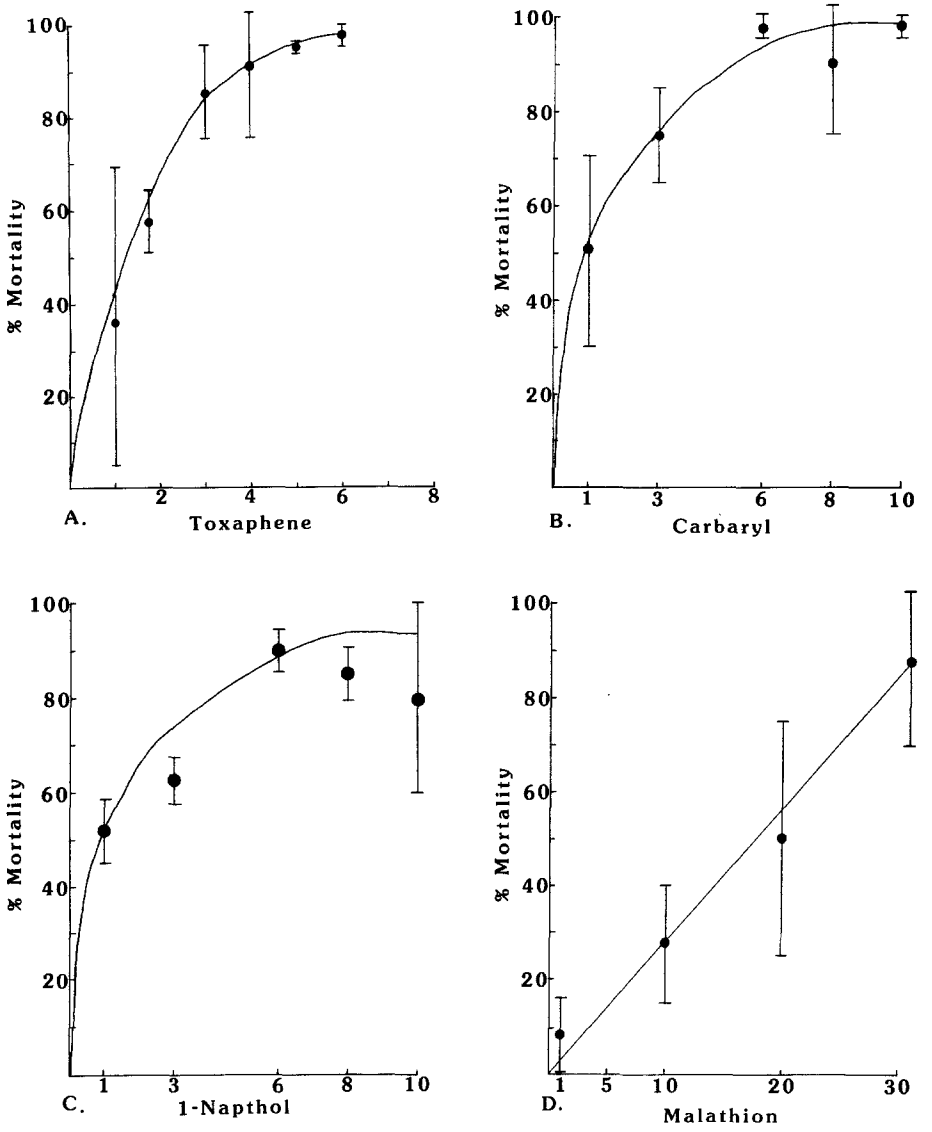

Fig. 1: Dose-Response Curves of Protozoans to Various Insecticides. Insecticide Concentrations are Measured in µg/mL.

1-naphthol to cause significant mortality among protozoans.

Malthion has been reported in surface water at concentrations ranging from 0.8 to 3.2 ug/mL $^{-1}$ (MURRAY & GUTHRIE 1980). The low toxicity of malthion for protozoans (Fig. 1) and evidence indicating it is rapidly degraded by soil bacteria (MOSTAFA et al. 1972, CONGREDO et al. 1978) indicates runoff levels of this compound would not cause significant mortality among protozoan populations.

Protozoans have been used as an assay system for the toxicity of aquatic pollutants, however these assays involved the use of sophisticated equipment (HONIG et al. 1980). The method described here, although strictly qualitative, is inexpensive and convenient, and can be performed with mixed as well as pure cultures.

The results reported here indicate that runoff levels of carbaryl (1-naphthol) may be sufficient to cause significant mortality in populations of protozoans. In light of the role that these organisms play in decomposition processes, the effects of agricultural chemicals as well as other pollutants on protozoa should be further investigated. The method presented here can be used to facilitate such investigation.

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