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Effects of Pesticides on Decomposition of Organic Matter and Nitrification in Sewage

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Much is known about the possible toxicity of pesticides to microorganisms growing in laboratory media and to microbial populations and communities in soils. For example, the effects and fate of propoxur (KUSESKE et al. 1974, GUPTA et al. 1975), dichlorvos (BALLINGTON et al. 1978), chlorpyrifos (MILES et al. 1979), and carbaryl (RODRIGUEZ & DOROUGH 1977) in soil have been investigated. However, the toxicity and degradability of fewer chemicals have been assessed in aquatic ecosystems. BOURQUIN (1977) found malathion to be readily degradable by salt-marsh bacteria, and BUTCHER et al. (1977) suggested that chlorpyrifos enhanced algal blooms in pond water. LICHTENSTEIN et al. (1966) showed the disappearance of carbaryl in lake water in 1-3 days, and ALY & EL-DIB (1973) reported that propoxur disappeared from river water in 8 days. MURRAY & GUTHRIE (1980) studied the toxicity of pesticides to lake microorganisms. Studies of the effects of a number of pesticides on nitrification in soil have shown some inhibition at high concentrations (usually greater than 50 ppm) of the test compounds (KUSESKE et al. 1974, GUPTA et al. 1975). GARRETSON & SAN CLEMENTE (1968) and WINELY & SAN CLEMENTE (1970) reported that aldrin, lindane, and TDE at concentrations below 10 µg/mL inhibited Nitrobacter agilis and Nitrosomonas europaea in culture media. Propoxur was toxic but at levels greater than 10 µg/mL, and malathion had no effect at 1000 µg/mL.

Although plants treating domestic sewage rarely receive high concentrations of pesticides, such high levels can arise from discharges of chemical manufacturing plants or from shop wastes from military installations at which pesticides are being used (MEIER et al. 1976). The toxicity of such an influx to the sewage microflora must be evaluated, as detrimental effects on sewage processes such as respiration and nitrification may lead to serious environmental consequences. Hence, a study was undertaken to determine the toxicity of eight pesticides to respiration and nitrifying activity of sewage microorganisms.

MATERIAL AND METHODS

Respiratory Activity and Degradation. Insecticides (greater than 95% purity) in anhydrous diethyl ether were added to 300-mL BOD (biochemical oxygen demand) bottles. The ether was allowed to evaporate, and 300 mL of BOD diluent was added. The insec-

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ticides used were carbaryl, propoxur, bendiocarb, diazinon, chlorpyrifos, malathion, and dichlorvos. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was dissolved in 0.2 M K_2HPO_4, and dilutions were added to 300 mL of diluent. The pesticide solutions were freshly prepared for each experiment.

A standard BOD diluent was used (AMERICAN PUBLIC HEALTH ASSOCIATION 1975). The final pH was approximately 7.2. The solution was aerated for 4-6 h at room temperature and then mixed with fresh municipal sewage added at a rate of 0.33% (v/v). BOD bottles containing pesticide were filled with this inoculated diluent and stoppered. The incubation was at 25°C. Initial dissolved oxygen (D.O.) was determined using an azide modified titration method (AMERICAN PUBLIC HEALTH ASSOCIATION 1975), and subsequent D.O. determinations were made using a polarographic oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH). The values were compared with an air-saturated control that was preserved by adding 1.0 g of KCN to BOD bottles at zero time. Five replicates of the no-pesticide treatment as well as of each pesticide concentration were used. Toxicity was considered as the percent D.O. in the presence compared to that observed in the absence of the pesticide at the end of the 160-190 h test period.

The assay for pesticide degradability was performed using the same conditions except that the inoculum was either 0.033% (v/v) carbon-depleted liquid from the toxicity tests that had been incubated at least 190 h or sewage (0.33%, v/v) stored for 160 h or more at 4°C. This storage procedure led to marked decline in BOD. Whereas the oxidation of carbonaceous material in the sewage inoculum plus the pesticide was measured in the toxicity tests, in these experiments the pesticide was the chief carbon source available for microbial metabolism. Degradation was measured by comparing the final percent D.O. at the end of 160-190 h with the amount expected based on complete oxidation of the pesticide to CO_2. Aerobic microbial degradation of the pesticides at a concentration of 10 µg/mL would consume all the available O_2 in the BOD bottles.

Nitrification. An enrichment of nitrifying microorganisms was obtained by incubating raw municipal sewage with 100 µg NH_4-N/mL on a rotary shaker at 29°C for 15 days. The enrichments were then filtered through Whatman No. 1 filter paper and a 5-µm Millipore filter, and the filtrate was centrifuged at 11,700 X g for 15 min. The resulting pellet was used to inoculate 3 L of a salts solution containing 8.5 mg K_2HPO_4, 21.8 mg KH_2PO_4, 17.7 mg Na_2HPO_4, 22.5 mg $MgSO_4 \cdot 7H_2O$, 36.4 mg $CaCl_2.2H_2O$, 25 µg $FeCl_3 \cdot 6H_2O$, 1.7 mg NH_4Cl, and 471 mg $(NH_4)_2SO_4$ (pH 7.2) per liter of distilled water. The flasks were incubated on a rotary shaker at 29°C for 21 days, during which time the pH was periodically readjusted to 7.2 with K_2CO_3. The microbial cells were collected as above and used to inoculate a more highly buffered solution containing 4.88 g K_2HPO_4, 3.81 g KH_2PO_4, 0.20 g

$MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2 \cdot 2H_2O$, 10 mg $FeCl_3 \cdot 6H_2O$, 2 mg $MnCl_2 \cdot 4H_2O$, 1 mg $NaMoO_4 \cdot 2H_2O$, and 0.385 g NH_4Cl per liter of distilled water. The pH was adjusted to 7.2. The enrichment procedure was repeated once more, by which time complete oxidation of the ammonium to nitrate occurred within 7 days.

For tests of pesticide effects, the 2,4-D dissolved in 0.2 M K_2HPO_4 and the other pesticides in anhydrous diethyl ether were added to 14 X 125 mm test tubes, and the ether was allowed to evaporate, leaving a pesticide residue in each tube. An equivalent amount of ether was added to the appropriate pesticide-free controls. The cells in 3 L of the nitrifying enrichment were collected as above, suspended in 75 mL sterile distilled water, and used to provide a 2% inoculum (v/v) for sterile nitrification medium with or without NH_4 (100 µg N/mL). Tubes receiving 7.2 mL of this inoculated medium were incubated at 29°C on a rotary shaker operating at 70 rpm. At each sampling time, 3 replicate tubes were removed for duplicate analysis for nitrate by the chromotropic acid method (AMERICAN PUBLIC HEALTH ASSOCIATION 1975).

RESULTS AND DISCUSSION

Carbaryl, malathion, diazinon, chlorpyrifos, dichlorvos, bendiocarb, or 2,4-D in concentrations ranging from 0.1 to 100 µg/mL had little or no toxicity to the O_2 depletion caused by microorganisms degrading organic matter in sewage (Table 1). In the solutions with no pesticide, 0-5% of the D.O. remained at the end of the test period.

Table 1. Toxicity of pesticides to microorganisms oxidizing organic matter in sewage in a 160-190 h period

Pesticide	Pesticide concentration (µg/mL)		
	0.1	1.0	100
	% D.O. remaining		
Carbaryl	8[a]	13	2
Propoxur	18	11	52
Bendiocarb	ND[b]	32	8
Diazinon	ND	ND	5
Chlorpyrifos	2	9	7
Malathion	1	1	7
Dichlorvos	2	8	7
2,4-D	ND	2	3

[a]Value given is highest % D.O. remaining in repeated trials.

[b]Not determined.

These data indicate that all but one of the pesticides pose little or no threat to the activity of microorganisms responsible for the biodegradation of organic matter in sewage. Although the results varied somewhat among different experiments, 1.0 µg/ml of each of the chemicals had little or no detrimental effect. Even at 100-fold higher concentrations, no suppression of microbial activity was evident, except for propoxur. Propoxur, moreover, was toxic even at 10 µg/mL, as a test with this concentration revealed that 51% of the D.O. remained after 187 h as compared to 2% in a pesticide-free control.

Toxicity tests were conducted at different times and with different samples of sewage. Probably for this reason, some differences in results were obtained when an experiment was repeated with the same pesticide concentration. For example, in a trial with 10 µg of carbaryl/mL, a slight delay occurred before the onset of oxygen utilization as compared to the no-pesticide control. However, complete oxygen consumption was witnessed in the test period, despite the initial lag. Alternatively, in an experiment with 10 µg malathion/mL, the initiation of oxidation was seemingly enhanced slightly as compared to the no-pesticide control. In one instance, moreover, 100 µg 2,4-D/mL was toxic, and 85% of the D.O. remained after 167 h. However, because this herbicide probably would never exist in sewage or other aquatic microbial habitats at such high concentrations, the occasional observation of suppression is not deemed to be ecologically significant.

A study was conducted to determine the susceptibility of the pesticides to biodegradation when provided as sole carbon sources to carbon-depleted inocula. The results were similar for the two types of inocula. Less than 5% of the available O_2 was depleted in the 160-190 h test period when propoxur, dichlorvos, or 2,4-D was present as the sole added carbon source at 10 µg/mL, suggesting little or no degradation. However, carbaryl at 10 µg/mL was degraded, and 74% of the O_2 available was removed. Similarly, 40, 65, and 47% of the available O_2 was depleted when bendiocarb, diazinon, and malathion, respectively, were the sole carbon sources at 20 µg/mL. The results with 10 µg chlorpyrifos/mL were inconsistent. The values for O_2 depletion were corrected for O_2 utilization in pesticide-free controls.

Although no degradation of propoxur and 2,4-D was noted here, propoxur decomposition has been reported in river water and sewage (ALY & EL-DIB 1973) and 2,4-D in lake water (FRANK 1972). Carbaryl is also readily degraded in lake water (LICHTENSTEIN et al. 1966). On the other hand, 8 weeks was required for 95% of propoxur to disappear from river water (EICHELBERGER & LICHTENBERG 1971), and the half-life for malathion disappearance from river water has been reported to be almost one month (PARIS et al. 1975). The failure to observe degradability of some of these compounds may be a result of the use of a short incubation period, the need for a second carbon source if the compounds are cometabolized, or the absence of growth factors.

The effects of the pesticides on nitrification were assessed from plots of the logarithmic increase in nitrate formation, on the assumption that the responsible organisms were autotrophic bacteria. The pattern of nitrification and the effect of one of the pesticides are shown in Fig. 1. It is evident that the rate of

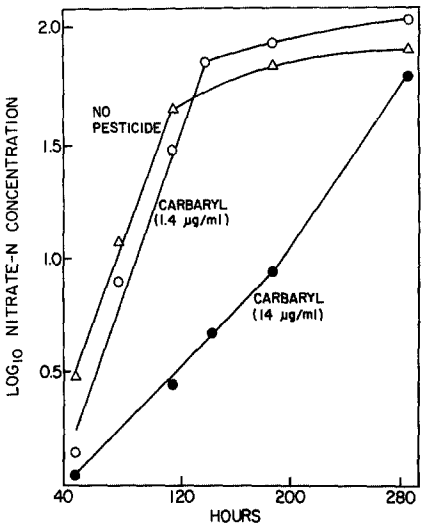

Fig. 1. Effect of carbaryl on nitrification by an enrichment culture derived from sewage.

logarithmic increase is not influenced by 1.4 µg carbaryl/mL, but 14 µg carbaryl/mL was toxic to nitrification. Two studies were conducted using inocula from separate 7-day-old nitrifying enrichments. Because of the use of different inocula, the growth rates of the nitrifiers were different in the absence of any pesticide. Nitrate formation was not detected in solutions not supplemented with ammonium. A summary of the effects of the chemicals is given in Table 2. Carbaryl at the higher concentration was toxic, but propoxur, bendiocarb, 2,4-D at neither concentration and carbaryl at 1.4 µg/mL were not inhibitory as indicated by a comparison at the 5% level of significance of the slopes of the regression lines. Diazinon, chlorpyrifos, malathion, and dichlorvos at 1 and 10 µg/mL also were not toxic. Indeed, the stimulation of nitrification by 10 µg chlorpyrifos and dichlorvos/mL was statistically significant at the 10% level, but the apparent enhancement by 10 µg diazinon and malathion was not statistically significant. Although carbaryl showed some toxicity at the higher concentration, even this inhibition appears to have been relieved somewhat after about 200 h (Figure 1), possibly because of the destruction of the molecule. The shorter generation times in the presence of 10 µg/mL

Table 2. Generation times of nitrifying microorganisms from sewage in the presence of pesticides

Expt.	Chemical	Chemical conc (μg/mL	Generation time (h)
	None	-	19
	Propoxur	1.4	18
		14	15
1	Carbaryl	1.4	18
		14	54
	Bendiocarb	1.4	20
		14	22
	2,4-D	1.4	26
		12	19
	None	-	29
	Diazinon	1.0	39
		10	16
2	Chlorpyrifos	1.0	31
		10	19
	Malathion	1.0	33
		10	14
	Dichlorvos	1.0	22
		10	14

of several pesticides is typical of the oligodynamic effect, the stimulation of a microbial process by low concentrations of a chemical that is toxic at higher levels.

This investigation has demonstrated that only propoxur was toxic to the microbial decomposition of organic matter in sewage and only carbaryl was detrimental to nitrification. However, high levels of even these compounds were needed to have an antimicrobial action. Moreover, several of these pesticides, although not propoxur, were metabolized during the test period so that possible harm that might ensue following their entry into sewage systems would soon be dissipated.

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