Accumulation of Aldrin and Dieldrin by Blue-green Algae and Related Effects on Photosynthetic Pigments

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The chlorinated insecticides aldrin and dieldrin were used extensively in midwestern agricultural practices, particularly for the soil control of corn pests, until recently. Resistant insect strains have reduced their effectiveness, and environmental persistence has further decreased their desirability. These pesticides have been found in most surface, subsurface, and finished water samples in Iowa (JOHNSON and MORRIS, 1971; RICHARD et al., 1975). June and July peaks of dieldrin in surface drainage followed environmental conversion of aldrin which had been applied in May (JOHNSON and MORRIS, 1971). Microorganisms, plants, and photooxidation in light and air convert aldrin to the epoxide, dieldrin, which is extremely inert to biological and chemical degradation. These insecticides are highly water-insoluble but adsorb strongly on soil particles in agricultural runoff. The amounts associated with physical and biological components of aquatic systems have been studied (MORRIS and JOHNSON, 1970; METCALF et al., 1973).

During summer months in the Midwest, dense blooms of blue-green algae occur in waters enriched by nutrients from agricultural runoff. The object of our study was to clarify the potential that these algal populations have for bioconcentration and exchange of persistent pesticides in aquatic environments. Previous work indicated that blue-green algae concentrate chlorinated pesticides introduced at a level of 1 mg/l under laboratory conditions (VANCE and DRUMMOND, 1969). To approximate the much lower levels found in natural waters (JOHNSON and MORRIS, 1971; RICHARD et al., 1975), we introduced aldrin or dieldrin into cultures of Anabaena cylindrica, Anacystis nidulans, and Nostoc muscorum to give concentrations of 1 µg/l and measured bioconcentration. Toxicity levels also were studied to better understand the impact of these persistent pesticides on populations of blue-green algae.

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Materials and Methods

Cultures of Anabaena cylindrica (Culture Collection of Algae at Indiana University, 629), Anacystis nidulans (CCAIU, 625), and Nostoc muscorum (Kaiser Research Foundation M12-4-1) were grown in 1-liter batches of Kratz-Myers medium (KRATZ and MYERS, 1955) or a modified Chu 10 medium (GERLOFF et al., 1950) in 2800-ml Fernbach flasks for 7 days at 23°C under 1350 lux fluorescent and 1000 lux incandescent light on a 12-hour photoperiod.

The test pesticide was introduced into cultures during log-phase growth to give a final concentration of 1 µg/l. Control cultures received no pesticide. Because aldrin and dieldrin have low solubility in water, stock solutions were made by dissolving the pesticide in a minimum of acetone and then diluting to 200 ml with water containing 0.05% Triton X-100. Pesticide grade solvents were used in all steps.

After 7 days, the algae were harvested by centrifugation, lyophilized to ensure uniformity among samples, and extracted 3 times with 2.5 ml acetonitrile. An equal volume of 2% aqueous Na_2SO_4 was added to the collected supernatants. The mixture was extracted 3 times with 2 ml hexane. The extracts were evaporated to 0.3 ml under nitrogen and cleaned up on a 200 mm x 14 mm glass column packed with 40 g Florisil and anhydrous Na_2SO_4 (1:1). After elution with 200 ml 6% ethyl ether/petroleum ether (v/v) and then 200 ml 15% ethyl ether/petroleum ether (v/v), the eluate was evaporated to 1 ml on a steam bath.

Chromatographic analyses were performed on a Varian Aerograph gas chromatograph equipped with a 1.83 m x 0.32 cm glass column packed with 10% DC-200, an electron capture detector, and nitrogen carrier gas. Confirmational analysis was performed by thin-layer chromatography on alumina-coated plates. Heptane was used as the developing solvent and $AgNO_3$ as the chromogenic agent. Plates were viewed under ultraviolet light.

The growth medium, after harvesting of algae, was filtered and analyzed. Preparation included partitioning of the remaining pesticide into hexane, followed by dehydration with Na_2SO_4. Procedures for concentration and GLC analysis were the same as above.

The toxicity of the test pesticides was investigated by inoculating triplicate log-phase cultures of the algae to give final pesticide concentrations of 1, 10, 100, and 1000 µg/l. After 7 days, 5-ml samples of each culture were collected on Gelman Type A glass fiber

filters. The absorbance was read against a blank filter in a Beckman DB-G spectrophotometer at 618 nm (tne absorption maximum for phycocyanin, the major accessory photosynthetic pigment in blue-green algae) and 678 nm (the absorption maximum for chlorophyll a).

Results and Discussion

The ability of selected blue-green algal species to significantly accumulate aldrin and dieldrin from aqueous solution has been demonstrated (Tables 1 and 2).

TABLE 1
Concentration of Aldrin from Aqueous Medium by Three Species of Blue-green Algae

Alga	Initial conc. of aldrin in medium (µg/l)	Aldrin extracted from algæ (ng/g)			
		1	2	3	Mean
Anabaena cylindrica	1	1535	921	1420	1292
Anacystis nidulans	1	835	1192	947	991
Nostoc muscorum	1	trace	trace	trace	trace

TABLE 2
Concentration of Dieldrin from Aqueous Medium by Three Species of Blue-green Algae

Alga	Initial conc. of dieldrin in medium (µg/l)	Dieldrin extracted from algae (ng/g)			
		1	2	3	Mean
Anabaena cylindrica	1	219	226	158	201
Anacystis nidulans	1	650	472	391	504
Nostoc muscorum	1	2080	1578	1887	1848

Table 1 records the concentration of aldrin from solution by cultures of Anabaena cylindrica and Anacystis nidulans after growth in the presence of aldrin for 7 days. Our factor for the concentration of aldrin by Anabaena cylindrica is considerably larger than that reported by VANCE and DRUMMOND (1969) when they started with 1 mg/l aldrin. The initial concentration of pesticide, the density of the culture, and consequently the number of available binding sites may be partially responsible for the difference in the concentration factor (NEUDORF and KHAN, 1975). Another explanation may be the method of sample preparation. We used lyophilization in order to reduce variability in water content and sample weight so that valid comparisons could be made between algal species.

A species variation is noted in the apparent metabolism of aldrin to dieldrin by Nostoc with subsequent excretion of dieldrin. This is our interpretation of the presence of dieldrin in the medium after the 7-day exposure to aldrin, since there was no conversion of aldrin to dieldrin in a control under the same conditions but lacking algae. Anabaena and Anacystis did not provide evidence of this metabolic conversion of aldrin to the epoxide, dieldrin, followed by excretion.

All three species concentrated dieldrin from aqueous solution (Table 2). Anabaena cylindrica showed the lowest capacity for concentration and Nostoc muscorum the highest. Species variation is demonstrated in the differing levels of the pesticides accumulated by the three algae. Although the test species are laboratory cultures, they are representative of genera found in natural waters. Species variation should be expected in native populations as well. Benthic algae have been found to concentrate aqueous concentrations of 0.05 - 7 µg dieldrin/l to 0.1 - 200 mg/l, with filamentous algae accumulating greater amounts than unicellular diatoms (ROSE and MCINTIRE, 1970).

The effect of aldrin and dieldrin on the test species is evidenced in Figures 1-3. Concentrations of primary (chlorophyll a) and accessory (phycocyanin) photosynthetic pigments were used as indicators of physiological health and growth for the purpose of comparing pesticide-treated cultures with control cultures. At 100 µg/l pesticide, decreases in pigment absorption were evident, and at 1000 µg/l, a serious impact occurred. Introduced aldrin was found to be more toxic than dieldrin to all three species. Transmission electron microscopy did not reveal any uniquely characteristic alteration in subcellular structure, although

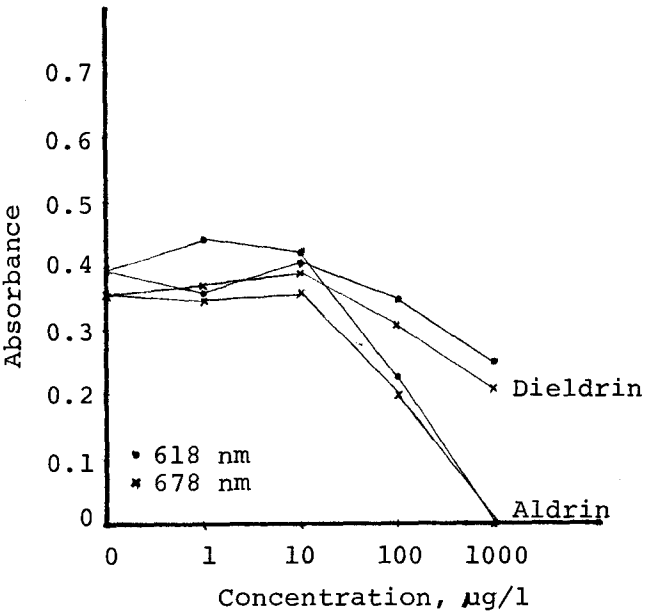

Figure 1. Response of Anabaena cylindrica to aldrin and dieldrin, as measured by photosynthetic pigment absorption after 7 days.

fewer cells with normal ultrastructure were observed in samples which had been exposed to higher concentrations of pesticides.

When BATTERTON et al. (1971) exposed two blue-green algal species, Anacystis nidulans and a marine isolate of Agmenellum quadruplicatum, to 475 or 950 µg/l aldrin or dieldrin, the pesticides were not algicidal but did depress growth rates. Aldrin caused less growth inhibition than dieldrin; dieldrin inhibited Agmenellum less than Anacystis. These exposures lasted only 30-36 hours, and lag periods of up to 12 hours were noted in the presence of pesticide. Aldrin and dieldrin were reported (VANCE and DRUMMOND, 1969) to be algicidal to the blue-green alga Microcystis aeruginosa at < 5 mg/l after 7 days, but the LD_{100} for Anabaena cylindrica was > 15 mg/l; Warburg analysis indicated that concentrations of aldrin and dieldrin up to 1 mg/l had no significant effect on respiration in short-term measurements. O'KELLEY and DEASON (1976) reported inhibition of a majority of 21 strains of Warrior River green algae and diatoms by 0.01, 0.1, and 1.0 mg/l aldrin or dieldrin. Results obtained in these field and laboratory studies

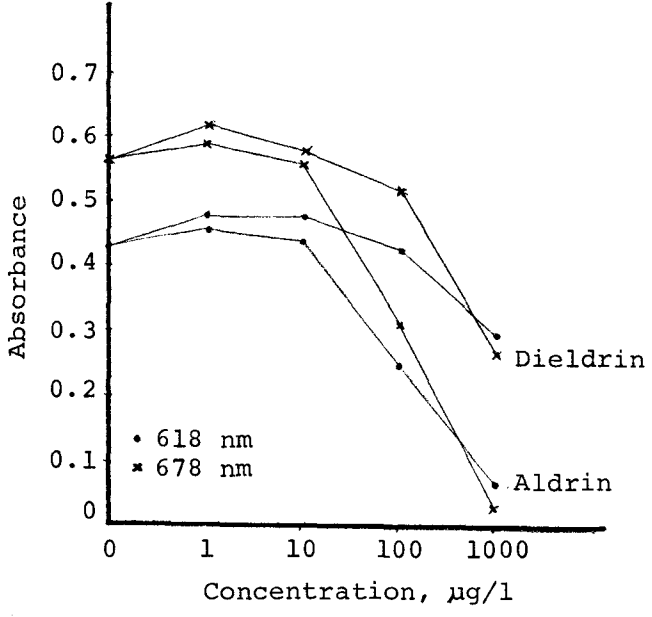

Figure 2. Response of Anacystis nidulans to aldrin and dieldrin, as measured by photosynthetic pigment absorption after 7 days.

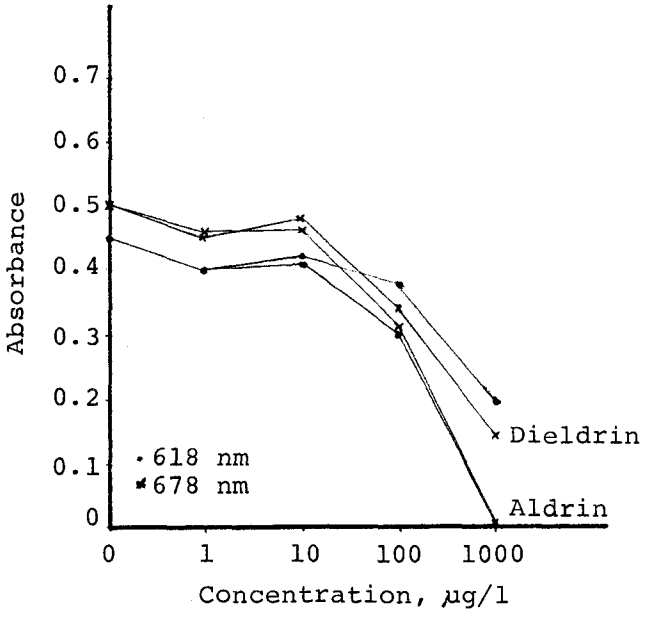

Figure 3. Response of Nostoc muscorum to aldrin and dieldrin, as measured by photosynthetic pigment absorption after 7 days.

as well as in our experimental work indicate that aldrin and dieldrin are not toxic to algal species at 1 µg/l. Since reported levels of aldrin and dieldrin found in aquatic environments in the Midwest (JOHNSON and MORRIS, 1971; RICHARD et al., 1975) are well below 1 µg/l, neither an algicidal nor a growth inhibiting effect on the algae would be anticipated in natural waters.

METCALF (1974), using a laboratory model ecosystem which included seven representative organisms in a food web, found that 86-96% of the total ^{14}C in labeled aldrin was stored as dieldrin in algae, snails, and fish. The highest level of bioconcentration was in the bottom-feeding snails. SANBORN (1974) confirmed the bioconcentration of dieldrin by all organisms in an ecosystem and its inertness to biological and chemical degradation. Although blue-green algae concentrate aldrin and dieldrin from solution, they are not preferentially grazed by predators in natural environments and probably play a minor role in bioconcentration of these pesticides at higher levels in the food web.

However, the accumulation of aldrin and dieldrin by blue-green algae may be important in the exchange of these chlorinated hydrocarbons between agricultural runoff and the aquatic environment. Since dense blue-green blooms frequently are found in lakes and ponds during periods of heavy seasonal runoff, significant concentrations of these persistent insecticides may be removed from the water column via adsorption or uptake by algae. LESHNIOWSKY et al. (1970) demonstrated adsorption of aldrin from colloidal dispersion on floc-forming Lake Erie bacteria with subsequent removal of the pesticide from the water phase as the flocs settled. Aldrin and dieldrin are found in lake sediments and may have been deposited by settling of flocs and organic detritus including dead algae. The role played by blue-green algae in the exchange of pesticides must be considered in aquatic environments characterized by dense algal blooms and large-scale use of agricultural pesticides. These "nuisance bloom" algae may be beneficial in reducing levels of pesticides which are potentially available to bioconcentration through the food web.

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