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Preferential Elimination of Dieldrin by Some Diatoms Compared to Chlamydomonas and Scenedesmus Species

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Dieldrin named after Otto Diels (famous codiscoverer of the Diels-Alder reaction) (1876-1954) is one of the most intensively investigated pesticides in environmental studies, due to its persistence and wide use.

Like other insecticides, we need more information on the adsorption and uptake of this compound by single species of microorganisms from low concentrations as well as more data on toxic effects of higher concentrations in ecosystems. 1 $\mu g \cdot kg^{-1}$ is considered as a trace concentration (PETROCELLI and ANDERSON, 1975) susceptible to biomagnification in food chains, compared to a LD_{50} of 65 mg . kg^{-1} body weight. No degradation products could be found in various soil microorganisms (VOCKEL and KORTE 1974), but by adsorption e.g. to silica gel, the UV absorption maximum is shifted significantly from 193 nm to 264 nm (GÄB et al., 1974). To find the most effective species or strain of several fresh water algae in eliminating dieldrin by adsorption, four strains of diatoms were compared with ten different species from the genera Scenedesmus and Chlamydomonas.

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MATERIALS AND METHODS

1. Cultivation

The following strains of Chlamydomonas and Scenedesmus from the algal collection of the Institute for Plant Physiology at the University of Göttingen (West Germany) were used:

Chlamydomonas reinhardii	11-329;
Chlamydomonas nostigama	11-35.72
Chlamydomonas aggregate	11-2.72
Chlamydomonas frankii	11-19.72
Chlamydomonas eugametos	11-519
Scenedesmus acuminatus	276-12
Scenedesmus acutiformis	276-11
Scenedesmus dispar	276-13
Scenedesmus obliquus	276-3b
Scenedesmus quadricauda	276-4b

The following diatom strains were taken from our laboratory collection:

Cyclotella cryptica
Nitzschia spec. strain 15
Nitzschia spec. strain 16
Nitzschia spec. strain 23

The algae were cultivated in media as described elsewhere (WERNER and PAWLITZ, in preparation) at 20°C in a light-dark regime of 16 : 8. Light intensity was 10.000 lux for the green algae and 4.000 lux for the diatoms.

The cultures of 300 ml were continuously aerated with 2% CO_2 in air and cultivated in light-thermostats as described by WERNER (1966).

2. Uptake studies

^{14}C-dieldrin (Amersham-Buchler, Braunschweig) was received with a specific activity of 85 $mCi \cdot mMol^{-1}$, equi-

valent to 222 µCi . mg^{-1}. The ^{14}C-dieldrin sample, dissolved in benzene was dried in a rotary evaporator and redissolved in acetone. For uptake experiments, 14 to 18 ml of logarithmically growing algal cultures were brought to an optical density (1.ooo nm) of o.5oo, transferred to 3o ml test tubes and dieldrin added to a final concentration of o.o3 µCi . ml^{-1} corresponding to o.135 µg . ml^{-1}. During this incubation the temperature for all strains was 25 ± 1°C, continuous light intensity 3ooo lux and the tubes aerated through a pasteur pipette. At the times indicated in Fig. 1 to 3, aliquots of the suspensions were centrifuged at 25oo x g for 1o min (Scenedesmus strains and diatom strains), at 45ooo x g for 1o min for Chlamydomonas strains. 1 ml of the supernatant was mixed with 5 ml dioxane scintillator (WERNER et al. 1975), cooled and counted in a Packard TriCarb Liquid Scintillation Spectrometer Model 338o for 5-1o min. The average of three parallels for each strain and each time was determined. As blanks, nutrient solutions without algae were used and referred to as a 1oo% value. By adsorption to the glass surfaces, a significant part of the dieldrin was eliminated also in the blanks during the incubation period of 6 or 27 h. Therefore, the 1oo% value of the blanks had to be determined for each time.

For use as dead cells (in Fig. 1) of Cyclotella cryptica, frozen samples (-20°C) were thawed, resuspended and set to an optical density of o.5ooo. It was demonstrated by cultivation that these diatoms do not survive this procedure. As a shell preparation the same amount of frozen cells was heated in a muffle oven for 1 h at 7oo°C, the remaining shells resuspended and homogenized by ultra sonic treatment.

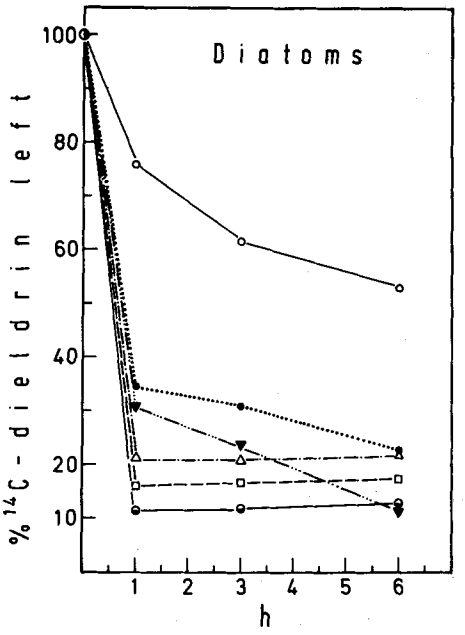
RESULTS AND DISCUSSION

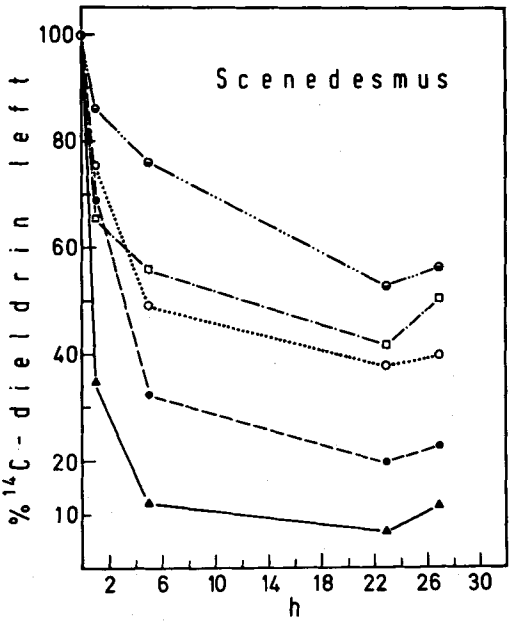
Within 1 h Nitzschia str. 16 and Cyclotella cryptica eliminated between 85 and 9o% of the dieldrin from the liquid with no further effect during the next five h (Fig. 1). The two other Nitzschia strains (15 and 23) were significantly less effective during the first hour, but continued to adsorb the pesticide in the following time. The concentration left behind after 6 h by the two most effective Nitzschia strains is 3-5 x $1o^{-8}$ M. Dead cells of Cyclotella cryptica leave only slightly more of the insecticide behind than living cells, indicating, that viability of the cells is not a prerequisite of adsorption to the cell surface. The shells of the diatoms are also able to adsorb some dieldrin, but much less than living or dead cells. This means, that the organic material, associated with the silica shells (e.g. binding proteins, pectic substances, chitan fibres) (WERNER 1970, WERNER 1977) may be involved in the adsorptive properties of the diatom strains.

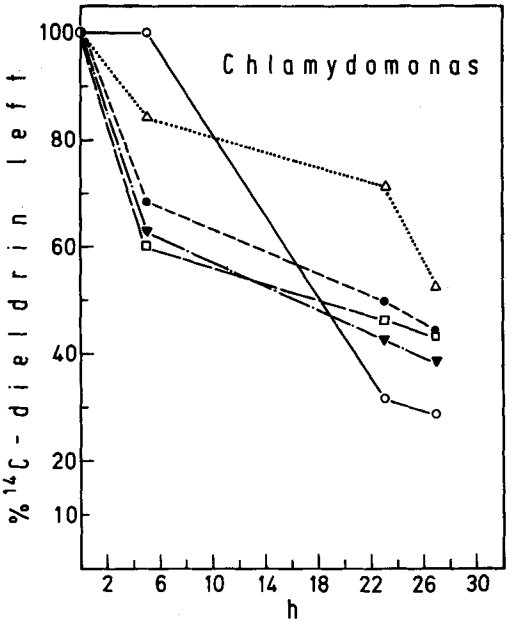
With Scenedesmus species (Fig. 2) we find considerable differences in adsorption of dieldrin. In general, all five species are less effective than the diatoms (Fig. 1). After 1 h only one out of five strains had eliminated more than 35% of the pesticides. Three out of five species even leave about 5o% of the dieldrin in the liquid phase after 24 h. Only Scenedesmus acutiformis eliminates after that time as well as the diatom strains.

All five species of the genus Chlamydomonas tested (Fig. 3) eliminate less than 4o% of the dieldrin within a 5 h period and only about 6o% after 27 h.

As expected, the concentration of dieldrin supplied was not toxic for the cells, since cell numbers increased during the 27 h period in Fig. 1 and 3, e.g. from o.5 x $1o^6$ cells . ml^{-1} to 1.75 x $1o^6$ cells . ml^{-1} with Chlamydomonas eugametos, from 1.65 x $1o^6$ cells . ml^{-1} to 4.o x $1o^6$ cells . ml^{-1} with Chlamydomonas frankii and from 1.75 x $1o^6$ cells .







Elimination of dieldrin from a 3.5×10^{-7} M concentration by:

Figure 1 (diatoms):

- ●——● Nitzschia spec. strain 16
- ▼-...-▼ Nitzschia spec. strain 23
- ●.....● Nitzschia spec. strain 15
- □-----□ Cyclotella cryptica
- △- . -△ Cyclotella cryptica dead cells
- ○——○ Cyclotella cryptica shells

Figure 2 (Scenedesmus)

- ▲——▲ Scenedesmus acuminatus
- ●-----● Scenedesmus dispar
- ○.....○ Scenedesmus acutiformis
- □-.-.-□ Scenedesmus quadricauda
- ●-...-● Scenedesmus obliquus

Figure 3 (Chlamydomonas)

- ○——○ Chlamydomonas reinhardii
- ▼- . -▼ Chlamydomonas aggregate
- □——□ Chlamydomonas nostigama
- ● - - ● Chlamydomonas eugametos
- △.....△ Chlamydomonas frankii

ml^{-1} to $4.2 \cdot 10^6$ cells with Nitzschia spec. strain 16.

Our data with Cyclotella cryptica and dieldrin confirm the results with Chlorella and DDT, that living and killed cells adsorb very fast and to similar extents (SÖDERGREN 1968). Using even lower concentrations of dieldrin (0.05 to 7 $\mu g \cdot l^{-1}$) but for periods of several months ROSE and MCINTIRE (1970) found an accumulation of 1:30.000 in benthic algae. Calculated for our suspensions, already after 1 h an accumulation of more than 1:10.000 in a certain volume of diatom cells (Nitzschia spec. strain 16) compared to an equal volume of nutrient solution must have occurred. This is considerably higher than those accumulation values (up to 1:270) reported for two species of green algae and 2 species of cyanophytes (VANCE and DRUMMOND 1969), or the accumulation of 1:190 found by KEIL and PRIESTER (1969) with Cylindrotheca closterium and DDT.

From our experiments, we could arrange the microalgae used in eliminating dieldrin in the order:

Nitzschia(better than) Scenedesmus(better than) Chlamydomonas,

but the results do not allow us to generalize these findings to the level of algal classes (diatoms-green algae-flagellates) as also emphasized by BAUER (1972).

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