Bioconcentration of Dibrom by *Stigeoclonium pachydermum*

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As the development and use of new pesticides continues, research must be done to assess the effects of these poisons on non-target organisms which are exposed to the poison in equal and sometimes even greater concentrations than that intended for the target pest. When pesticides are applied to an environment, some does reach the target but a great amount often falls on non-target organisms existing in the same habitat. Due to the extremely stable nature of many of these pesticides, they can exist and be concentrated by the subjected plants and animals for periods in excess of 15 - 20 years. This phenomenon of biological uptake and sub-lethal concentration in aquatic food webs has been investigated by several workers (BARKER 1958, CROCKER and WILSON 1965, KEITH 1966, VANCE and DRUMMOND 1969).

The first level in an aquatic food chain is the primary producers, the phytoplankton, which are generally eaten by the lower level herbivores which in turn provide food for the upper level carnivores, generally the fishes. The fact that algae are extremely efficient concentrators of pesticides in the environment has been well documented. MEEKS and PETERLE (1967) found that the alga, Cladophora, in a Lake Erie marsh treated with DDT at the rate of 0.2 lb/acre, concentrated DDT far more than other plants and animals. WARE et al. (1968) suggested that algae may be used as indicators of water contamination by DDT due to their ability to concentrate this pesticide. VANCE and DRUMMOND (1969) found several species of algae concentrated 4 chlorinated hydrocarbon pesticides at levels of 100 to 200-fold.

The effect of this concentration phenomenon is greatly magnified on zooplankton and algal-feeding fish because they are much less resistant to low concentrations, resulting in massive fish kills and related problems. ANDERSON (1945) investigated the toxicity of DDT

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to Daphnia and reported that 1 pg of DDT immobilized them in 32 hrs. WOLLERMAN and PUTNAM (1955) also used Daphnia pulex as a test animal for the rapid screening of systemic pesticides by exposure to prepared extracts and plant filtrates and found several of these systemics to be lethal in concentrations as low as 1 pg. MAKI et al. (1973) showed that low sublethal concentrations of Dibrom significantly reduced the tolerance to low oxygen tension in 3 aquatic animals.

There is a considerable literature concerning the effects of organic insecticides on fish. Much of it involves the lethal concentrations for acute exposure of fish to insecticides. KATZ (1961), and PICKERING et al. (1962) have determined the effects of short term exposure of fish to insecticides, mainly organophosphorus pesticides, and found concentrations less than 1 pg to cause a 96 hr TLm in many species tested. The obvious impact of bioaccumulation of pesticides by algae can be seen from these studies demonstrating the susceptability of upper level carnivores and herbivores to extremely low concentrations of pesticides. With seemingly little or no harmful physiological effect on their own metabolism, the primary producers in an aquatic food web have become lethal food for those herbivores that chance to feed on them.

Our study examined the effects of Dibrom on Stigeoclonium pachydermum, a common green alga. We were especially interested in the uptake and degradation of the pesticide by the alga.

MATERIALS AND METHODS

Unialgal cultures of Stigeoclonium pachydermum were grown throughout the study in BOLD'S Modified Bristol's solution (1949) on a reciprocal shaker at 80 rpm. The cultures were grown at 25°C under continuous illumination of 350 ft-c provided by 40 watt cool white fluorescent tubes. Cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of medium. All experiments were done in triplicate.

Since Dibrom (1,2-dibromo-2,2-dichloroethyl dimethly phosphate) has very low solubility in water, it was necessary to dissolve the pesticide in acetone before it would be inoculated into the algal cultures. To examine the physiological effects of the pesticide alone, the effect of the acetone carrier had to be isolated. An LD_{100}

curve was obtained by inoculating cultures at an O.D. of 0.025 (660nm) with 0, 1, 2, 3, 4, 5, and 6 ml of acetone and measuring growth for 12 days. Growth was measured in a Bausch and Lomb Spectronic 20 at 660 nm.

An LD_{100} curve for Dibrom was obtained by inoculating algal cultures with Dibrom to a final concentration of 0, 0.1, 1, 10, 20, 30, and 50 µg/ml. The cultures were grown for 6 days before inoculation and growth was measured daily for 6 days following inoculation.

Analysis for Dibrom residues was complicated by its extremely rapid rate of decomposition in alkaline media. To circumvent this, cultures were acidified to pH 5.5 with dilute HCl and inoculated with a heavy concentration of Dibrom (1000 µg/ml). Extraction and clean-up were begun 45 min after inoculation. Several extraction procedures were tested (BOSTWICK,AND GIUFFRIDA 1967, STORHERR and GETZ 1964), the most successful being that developed by CHEVRON CHEMICAL CO. (1966). First, the cells were concentrated and separated from the medium with a Foerst Plankton Centrifuge. The cells were ruptured by alternated freezing and thawing and grinding with ground glass. Microscopic examination showed the cells to be successfully ruptured. The cell wall material was separated from the remaining material by centrifugation at 1500 g in an International Centrifuge, Model HN. Microscopic examination confirmed the presence of cell wall material. Extraction of the pesticide was done separately with the cell wall fractions, the remaining cell material, and the culture medium. To each sample was added 1 ml of concentrated HCl, a quantity of anhydrous Na_2SO_4 equal to ½ the wet weight of the sample, and a quantity of saturated Na_2SO_4 solution equal to 3/4 the wet weight of the sample. After thorough mixing, 50 ml of ether were added, the mixture was placed in a separatory funnel and tumbled for 30 min. The ether phase was collected and dried with anhydrous $MgSO_4$. This extract was filtered through a 0.45µ filter, rinsed with fresh ether, and stored in the freezer. This procedure also extracted some of the pigments from the alga so the ether extracts were run through a 12-inch Florisil column to remove the pigments.

Residue analysis was completed on an Aerograph Model 205-B Gas Chromatograph. The instrument parameters were: column temperature: 175°C; detector temperature: 190°C; injector temperature: 190°C; attenuation X4;

column: Dow 10% QF 1 on 60/80 mesh Chromasorb; carrier gas: nitrogen at 40 ml per min.

Standard curves were obtained by injection of 1 ml of acetone containing various Dibrom concentrations. Typical retention time was 2 min. The area of the Dibrom curve was measured in planimeter units and compared with the standard curves to obtain relative concentrations.

Extraction after the pesticide had been in solution 4 hrs was done also. Although care was taken to exactly duplicate the above conditions, no trace of Dibrom or its metabolites could be found.

RESULTS

Acetone at a 1:50 concentration of acetone:medium had no measurable effect on the alga's growth after 12 days.

Concentrations of Dibrom in excess of 20 µg/ml were required to inhibit the growth of S. pachydermum (Fig. 1). Microscopic observation revealed the treatments caused the formation of a thick amorphous coating on the filaments. Also, the characteristic setae at the branch tips were absent.

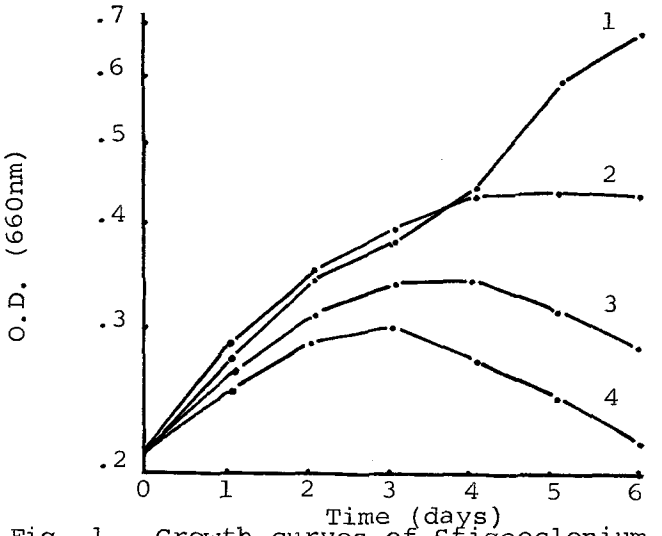

Fig. 1. Growth curves of Stigeoclonium pachydermum grown in Dibrom (1=0-20 µg/ml; 2=30 µg/ml; 3=50 µg/ml; 4=100 µg/ml).

Biocencentration of Dibrom by S. pachydermum appeared to be by absorption of the insecticide to the cell wall rather than by absorption into the cells (Table 1).

TABLE 1

Recovery of Dibrom residues from cell fractions of six-day-old cultures of Stigeoclonium pachydermum incubated in 1000 µg/ml Dibrom for 45 minutes.

Cell Fraction	Planimeter Units	Recovery (% of 1000 µg/ml)
1000 µg/ml in acetone	0.1880	100.00
Cell walls	0.1370	72.80
Remainder of cellular material	0.0042	0.22
Culture medium	0.0012	0.06
Hydrolyzed (assumed)	—	26.90

DISCUSSION

The entomological evidence (BENTON et al. 1964, TAHORI and STERNBERG 1966) developed on Dibrom indicates a very rapid rate of decay of this chemical in metabolic pathways. Also, our attempts to recover the pesticide from an algal culture confirmed that a very rapid interaction between plant tissues and Dibrom took place. When our cultures were acidified by the addition of small amounts of dilute HCl, excellent recoveries of Dibrom were achieved. A study was made (CHEVRON CHEMICAL CO. 1966) in which Dibrom was deposited on lima bean leaves and at various time intervals the leaves were ground, extracted and the Dibrom estimated by gas chromatography. The very rapid rate of disappearance of Dibrom was confirmed when it was found that after 1 hr only 7% of the initial concentrations (60 µg/ml) remained. In the same study, it was found that better quantitative recovery of Dibrom could be obtained from aqueous alfalfa macerate which had been acidified and then fortified and allowed to stand for 1 hr, whereas less than 10% recovery was obtained if the Dibrom and alfalfa were allowed to stand for 1 hr and then acidified and extracted. This agrees

with our findings that Dibrom is effectively destroyed at a very high rate under *in vivo* conditions. The small percentage of residue we found in the cell contents (0.22%) and culture medium (0.06%) were probably due to less than total separation of the cell walls from the cell contents in the centrifugation step.

A rapid breakdown of Dibrom also occurs in the filtered aqueous extract of crop tissue (CHEVRON CHEMICAL CO. 1965). Studies were also made in which the aqueous crop extract was boiled prior to fortification with Dibrom. This boiling had no effect on the rate of breakdown of Dibrom and, therefore, this result indicated that the breakdown reaction was not enzymatic in nature (CHEVRON CHEMICAL CO. 1966). This fact coupled with the observations made on filaments of *S. pachydermum* showing the thick mucilage coat, lack of apical setae, and 72.8% bound residue occurring in the cell wall fractions leads to speculating that there is a chemical reaction between Dibrom and the cell wall during which the pesticide is rapidly degraded.

Our study leaves many questions unanswered (What is the nature of the chemical reaction with the cell wall? Why do the setae disappear?) but it does reinforce the fact that non-target organisms, in addition to being affected themselves, may biologically concentrate large quantities of a pesticide and, thereby, be a source of potential danger to higher members of the food chain.

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