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The effects of selected pesticides on ten isolates of terrestrial algae.

Martha Scheer Salk 1945-
University of Louisville

THE EFFECTS OF SELECTED PESTICIDES ON TEN ISOLATES

OF TERRESTRIAL ALGAE

By

Martha Scheer Salk
B.A., Albion College, 1967
M.S., University of Iowa, 1969

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Date

by the Following Reading Committee:

—————————————————————————
Dissertation Director

—————————————————————————

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—————————————————————————
Chairman

ABSTRACT

Ten algal isolates were obtained from soil into axenic culture and placed into defined media containing from 0 to 50 ppm of DDT, 2,4-D, 2,4,5-T, rotenone, or malathion (in two purities, 95 and 99%). For nine of the isolates, growth was measured photometrically every two weeks for a period of two months. Because of the twisted growth form of the tenth isolate, a visual estimation was used to measure its growth over the same period of time.

The results of the experiments in the light were as follows:

1) 2,4-D and 2,4,5-T were generally neutral in their growth effects. However, both stimulated growth of two isolates, and 2,4,5-T inhibited the growth of two other isolates while 2,4-D inhibited only one.

2) Rotenone and DDT had mixed effects; rotenone inhibited five, stimulated three, and did not effect two isolates, while DDT inhibited four, stimulated one, and have no effect on five isolates.

3) Malathion was neutral toward one isolate and reduced the growth of all others.

4) In several cases, the isolates appeared to use the pesticides to supplement their growth. Two of the isolates were better able to withstand the effects of the pesticides and tended to be stimulated more often than the others.

None of the algae were able to use any of the pesticides as a sole carbon source when grown in defined media in the dark for two

months. Several isolates did not survive under those conditions, and none of the isolates survived in a 10 ppm DDT solution.

The effect that pesticide use may have on natural populations of soil algae is discussed.

ACKNOWLEDGMENTS

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INTRODUCTION

The number of different compounds used as pesticides and the
total magnitude of their use has increased dramatically since the end of
World War II. Each year new pesticides are developed and registered for
use, often with little knowledge of their effects on non-target organisms
or their behavior in the environment. By many different pathways, some
direct, many indirect, most, if not all, of these compounds reach the
soil where they may effect any of the multitude of soil organisms,
ranging from animals such as earthworms to microorganisms such as
bacteria, fungi, and algae. Those studies which are done before
registration of new pesticides usually concentrate on their effects on
man and other large non-target organisms; e. g., mammals, birds, trees,
etc., and exclude the microorganisms which often play a vital role in
soil fertility and dynamics. Therefore, recognizing the existence of
that void, this study was undertaken to determine the effects of a few
selected pesticides on the growth of some soil algae. The pesticides
used were selected from widely varying groups in the hope of discovering
if there were differences between these groups in their effects on the
soil algae. Some of the questions which this study attempted to answer
were: Do the pesticides under consideration have any effect on the
growth of axenic cultures of soil algae? Is there a relationship between
persistence of the pesticide in the soil and its effect on the algae
there? Can any of the algae use any of the pesticides as a sole carbon
source, thereby, degrading it? It is hoped that the information detailed

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in this dissertation will contribute to our understanding of the inter-actions of pesticides and soil algae.

REVIEW OF THE LITERATURE

Soil Algae

Species of diatoms, other chrysophytes, blue-green, and green

algae (Waksman, 1952), as many as 1,000,000 in a gram of well-fertilized

soil (Shields and Durrell, 1964), are an often overlooked part of the

soil microflora. Most works on soil biology ignore them completely or

mention them only briefly, and their importance is probably underestimated

(Lund, 1967). Recent studies on the taxonomy of soil algae have indicated

that some species are the same ones found in fresh water, but many others

are unique to the soil habitat (Deason and Bold, 1960; Mattox and

Bold, 1962; Chantanachat and Bold, 1962; Bischoff and Bold, 1963; Shields

and Durrell, 1964; Groover and Bold, 1969; Kantz and Bold, 1969; Bold,

1970). Other recent studies have dealt with the distribution of soil

algae and annual cyclic changes (Willson and Forest, 1957; Forest, et

al., 1959; Potul'nitskii, 1962; Lund, 1962, 1967; Forest, 1962; Shields

and Durrell, 1964; MacEntee, et al., 1972). Most of these studies

emphasized the importance of combining field and laboratory culture

work for the complete identification and characterization of soil algae.

Factors which determine the growth and distribution of soil

algae are pH, water requirement and availability, depth in soil, and

substrate (Waksman, 1952; Lund, 1962; Shields and Durrell, 1964). They

are most commonly found in soil of pH 5.5 to 8.5 (Lund, 1967), but many

can tolerate a wider pH range and only at extremes of pH is there any

correlation between pH and flora (Lund, 1962); e. g., the green algae

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being particularly abundant in acid soil (Waksman, 1952). Many of the species can survive air drying for periods of up to 10 years (Lund, 1962, 1967; Shields and Durrell, 1964; Trainor, 1970) or shorter periods at extreme temperatures (0 C for 6 days, 60 or 100 C for 1 hour, MacEntee, et al., 1972; Lund, 1962, 1967). While algae are found to a considerable depth, they usually are most abundant near the surface (Willson and Forest, 1957; Waksman, 1952; Shields and Durrell, 1964; Lund, 1967); and if buried by rain or earthworms, they can often return to the surface (Peterson, 1935, cited in Lund, 1967). If furnished with an appropriate carbon source, algae can live heterotrophically in the lab in the dark so that those found at great depths in the soil may actually be growing and multiplying there, although probably at a slower rate than in the light (Waksman, 1952; Parker, et al., 1960; Parker, 1961; Lund, 1967). (However, care must be taken in interpreting these studies since organic substrates are often less easily used in soil than in free solution [Alexander, 1964]). Little information is available on the specific nutrient needs of soil algae, but adding phosphorous and nitrogen (Lund, 1962) or manure (Waksman, 1952) will usually increase their growth while the findings on the effects of other elements are contradictory and may be more closely correlated with species. Drewes, in 1928, was the first to definitely establish the ability of *Anabaena* and *Nostoc* species to fix nitrogen (Waksman, 1952). It is now known that about one-third of the algae which can fix nitrogen are found in the soil (Lund, 1962) and are able to fix nitrogen there (Tiffany, 1951; Waksman, 1952; Shields and Durrell, 1964; Berger, 1965; Lund, 1967).

The interaction of soil algae with other organisms may vary from neutral to protocooperation to competition (Alexander, 1964; Lund, 1967),

but only in a very few cases has a detailed analysis of the specific relationship been attempted (Parker and Bold, 1961; Parker and Turner, 1961). Some algae live more or less symbiotically with higher plants (Lund, 1962; Shields and Durrell, 1964), but they, as a group, flourish most where higher plants are sparse or absent (Lund, 1962).

Algae often serve in the initial stage in plant succession helping to form and adding energy substrates to the soil (Tiffany, 1951; Potul'nitskii, 1962; Alexander, 1964; Shields and Durrell, 1964; Berger, 1965), but there do not seem to be any specific "pioneer" algae, the most abundant adjacent ones simply moving in first (Forest, et al., 1959; Shields and Durrell, 1964). Blue-green algae are among the first organisms which grow on volcanic rock where they form an interwoven mat which stabilizes and forms a non-erodible surface which breaks the force of falling water, improves infiltration, and acts as a substrate for the germination of seeds and spores (Shields and Durrell, 1964). The algal layer, especially in arid and saline soils, is a source of humus and nitrogen, exerts a solvent action on certain soil minerals, and may change the balance of inorganic factors (Lund, 1962; Shields and Durrell, 1964). But in normal soils they do not significantly alter the structure or texture, mainly adding organic matter since either dead or alive they may act as fertilizers (Lund, 1962, 1967). While in climax communities, the algae are mainly heterotrophs using the energy fixed by the higher plants (Alexander, 1964).

Pesticide Persistence

The quantity of pesticides produced and used in the United States each year is staggering. In 1969, 1,333,377,000 pounds (514,099,800 kilograms) were produced here and it is estimated that

over 10^{10} pounds (4.536 x 10^9 kilograms) have been used since 1945,
although there seems to be a slow decline in the use of some pesticides
in the last few years (Matsumura, 1972). The over 900 compounds used
here as pesticides (Crosby, 1973) may reach the soil by many different
routes including direct application, drifting of spray, rainfall, wind
blown dust, accidental spills, manufacturing and agricultural by-products
and wastes, erosion, and residues from both living and dead plants and
animals (Edwards, 1966; Westlake and Gunther, 1966; Crosby, 1973); and
it is probable that pesticides are now present in at least small amounts
in all parts of the world, even where never applied directly (Westlake
and Gunther, 1966). Dr. Howard Reiquam of the Battelle Institute ranks
pesticides as the most hazardous of 19 environmental problems due to their
world wide range, extreme persistence, and complexity in terms of
biological effects, social and political ramifications, air, land, and
water involvement, and effects on nutrition sources, but he expects
them to drop to number 15 in the future as the most persistent are
eliminated (Anon., 1972).

The ultimate environmental fate of any pesticide is difficult to
determine because of dilution and dispersal, chemical and biological
changes, and inadequate means of detection and identification (Crosby,
1973). The main causes of deactivation are photodecomposition and
volatilization from the soil surface, adsorption, leaching, and chemical
and biological decomposition by oxidation, reduction, hydrolysis, or
other transformations, although the complete mechanisms are not usually
known (Horowitz, 1969; Crosby, 1973). Disappearance curves are generally
sigmoidal, the more persistent compounds have a long period of slow
disappearance followed by a period of rapid reduction (Audus, 1950;

Horowitz, 1969; Kearney, et al., 1970). The most important factor determining the stability of a pesticide in the soil is the structure of the chemical itself (Edwards, 1966; Kearney, et al., 1970; Horowitz, 1969; Alexander, 1965a; Lichtenstein, 1972). Of the soil characteristics which effect persistence, the organic matter content is the most important as it is the major adsorptive site for the chemicals (Edwards, 1966) and, therefore, determines how readily available the chemical will be to attack by microorganisms (Kearney, et al., 1967; Wolcott, 1970). Other factors influencing persistence are moisture, soil temperature, cover crops, cultivation, mode of application, formulation used, wind or air movement, and presence of microorganisms which can attack and biodegrade the pesticide (Lichtenstein, 1965, 1972; Horowitz, 1969). Due to the large number of varying effects, no specific "half-life" can be assigned to one particular pesticide (Lichtenstein, 1972).

Persistence of a pesticide in the soil may be due to its basic non-biodegradibility or to its combining with another compound to become unavailable for attack (Alexander, 1965a, 1965b). Many pesticides are recalcitrant since conditions are not always proper for their biological degradation; i.e., organisms may not be present which can degrade them, the compounds may not be in a form suitable for degradation or may not be available to the microorganisms, they may not be capable of inducing formation of the appropriate enzymes for decomposition, or environmental conditions may be toxic or a growth factor missing so the degradation organisms cannot grow or the enzyme cannot work (Kearney, et al., 1967; Audus, 1964; Alexander, 1965a, 1967). Soil type, pH, cation exchange capacity, temperature, organic matter, and moisture content of the soil not only affect the availability and persistence of pesticides in the

soil directly, but also indirectly through effects on microorganisms (Kearney, et al., 1967; Wolcott, 1970; Crosby, 1973). Since different organisms have different metabolic abilities to degrade specific pesticides (Fletcher, 1960; Crosby, 1973), uncommon organisms may become prominent when pesticides are the primary carbon source as the compounds are often unusual and only broken down by a few specific species. Once a suitable population has developed after an initial dose, second and later applications of the pesticide will disappear more rapidly (Alexander, 1971).

Since there is no clear relationship between the structure of a chemical and its sensitivity to degradation, each new compound must be tested individually to find out how it will react (Moore, 1967; Horowitz, 1969). Complicating factors in predicting persistence include a difference in disappearance rates between the lab and the field, the effect of interactions of organisms since many compounds are broken down by combinations of several microbes, and presence of a second carbon compound (e. g. glucose) which can result in degradation of normally resistant compounds (Alexander, 1965b; Cripps, 1971). The latter factor called "cometabolism" or "cooxidation" appears to be widespread and may result when a foreign molecule is acted on by an enzyme which normally has a similar function, but in this case the product is not used for growth because it cannot be further metabolized or because it is toxic (Alexander, 1967, 1971).

Therefore, because of the extensive use of pesticides, their common persistence, and the inadequate knowledge of the ecology of soil algae, this study was undertaken in an attempt to determine some of the interactions between soil algae and pesticides. Since the

problem is very complex, this study is only a bare beginning which included isolating organisms from soil into axenic culture, growing them on artificial media in the lab, and testing the effects of certain pesticides on their growth under carefully controlled conditions.

MATERIALS AND METHODS

Soil samples were collected from several sites in Oldham and Jefferson Counties, Kentucky. Approximately ten grams of soil from each sample were placed in 125 ml Ehrlenmeyer flasks and covered with 50 ml of Tris-buffered inorganic medium (TBIM) (Smith and Wiedeman, 1964), which is described below.

TBIM

Chemical compound	Amount	Final concentration mg/liter	µM/liter
KNO_3	20 ml of 0.1 M solution	202	2000
Na_2HPO_4	10 ml of 0.1 M solution	142	1000
$MgSO_4 \cdot 7H_2O$	3 ml of 0.1 M solution	64	300
$CaCl_2 \cdot 2H_2O$	1 ml of 0.1 M solution	14	100
Tris(hydroxymethyl)-aminomethane (TRIS)	25 ml of 0.2 M solution	606	5000

Each of the above was added to approximately 800 ml of deionized or glass-distilled water. One ml of each of the micronutrient stock solutions was then added and a final dilution to one liter made. The pH of the medium was adjusted to 7.5 with 1.0 N HCl.

Micronutrient Stock Solutions:

		Amount	Final concentration mg/liter	µM/liter
I.	EDTA	50 g	per liter deionized or glass-distilled water	50	170
	KOH, 85%	31 g		31	470
II.	H_3BO_3	11.42 g/liter deionized or glass-distilled water	11.42	185
III.	$FeSO_4 \cdot 7H_2O$	4.98 g/liter acidified water*	4.98	17.8

		Amount		Final concentration mg/liter	μM/liter
IV.	$ZnSO_4 \cdot 7H_2O$	8.82 g		8.82	30
	$MnCl_2 \cdot 4H_2O$	1.44 g		1.44	7.3
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.87 g	per liter	0.87	4.9
	$CuSO_4 \cdot 5H_2O$	1.57 g	acidified water*	1.57	6.3
	$Co(NO_3)_2 \cdot 6H_2O$	0.49 g		0.49	1.7

*Acidified water: 999 ml deionized or glass-distilled water
1 ml concentrated H_2SO_4

The flasks were placed in a plant growth chamber at a temperature of 22 C with a 12 hour cycle of alternating light and dark periods provided by Natur-escent[1] lights, henceforth termed standard conditions. In addition, ten-gram samples of soil were placed in the bottom halves of steril petri plates, moistened daily with deionized water, and left covered in front of a south facing window.

The algae which grew under these conditions were streaked on TBIM solidified with 1.5% agar. Unialgal isolations were made using the methods of Bold (1942), Pringsheim (1946), and Lewin (1959). The algae were isolated into axenic culture using a modification of the atomizing technique of Wiedeman, et al. (1964). Instead of ultrasonication, the algal cells were soaked for two hours in 10 ml of the following solution:

50 ml TBIM

45 ml deionized water

5 ml full strength Walgreen Justrite clear lotion detergent.
The cells were then centrifuged, washed with TBIM 10 times, and sprayed onto a sterile plate of proteose-peptone-TBIM agar. Cells

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1) Duro-Lite Lamps, Inc., Fair Lawn, N.J.

which appeared to be bacteria-free after having grown for several days were inoculated into five ml of TBIM in 10 x 100 mm Pyrex, rimless test tubes and plugged with non-absorbent cotton. Approximately two months later, these cultures were streaked onto proteose peptone, nutrient, yeast extract, and malt extract agar plates, and into fluid thioglycolate to determine if they were axenic. The plates and tubes were incubated for two weeks at 37 C, and any contaminated cultures were disposed of at the end of that time. Stock cultures were grown in 10 ml of TBIM in 18 x 130 mm Pyrex, rimless test tubes plugged with non-absorbent cotton, wrapped with transparent plastic film, and secured with rubber bands. Reserve stocks were kept on TBIM and TBIM plus proteose-peptone agar slants (the latter as a check for possible bacterial contamination). All stocks were grown under standard conditions. After the isolates had grown on the slants, liquid TBIM was added asceptically to the tubes to cover about two-thirds of the exposed agar. The liquid TBIM stocks were used for all experimental inocula.

Five pesticides were selected for use covering a broad range of pesticide types. Each chemical was used at six concentrations: 0.1, 0.5, 1.0, 5.0, 10.0, and 50.0 parts per million. The pesticides used and the molar concentrations used for each were as follows:

2,4-D (2,4-dichlorophenoxy acetic acid); 0.45, 2.26, 4.5, 22.4, 45, and 226 μM;

2,4,5-T (2,4,5-trichlorophenoxy acetic acid); 0.39, 1.96, 3.9, 19.6, 39, and 196 μM;

rotenone ($C_{23}H_{22}O_6$); 0.25, 1.27, 2.5, 12.7, 25, and 127 μM;

malathion (O,O-dimethyl S-bis (carboethoxy) ethyl phosphorodithioate); in two formulations, 95% and 99% purity; 0.30, 1.51, 3.0, 15.1, 30, and 151 μM; and

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane); 0.28, 1.41, 2.8, 14.1, 28, and 141 µM.

A dosage of one pound per acre (1.121 kg/hectare) results in a concentration in the upper soil layer of approximately 2 to 2.5 ppm (Fletcher, 1960). Therefore, concentrations were selected which ranged proportionately around the average recommended dose of 2 to 2.5 pounds per acre. The sodium salts of 2,4-D and 2,4,5-T were used since they are more water soluble than their acid equivalents. Rotenone, DDT, and malathion were dissolved in ethanol before being added asceptically to the sterilized growth medium. Ethanol was added at a rate of 20 ml per liter of growth medium. Since DDT should not be used in an alkaline medium or with iron salts (Stecher, 1968), Bold's Basal Medium II (BBM2) minus iron as described below was used instead of TBIM for the experiments with DDT.

Bold's Basal Medium Modified (BBM2) (Cain, 1965)

Ten milliliters of each of the following stock solutions was added to approximately 800 ml of deionized or glass distilled water;

$NaNO_3$	2.5%	12.5 g/500 ml
KH_2PO_4	1.5%	7.5 g/500 ml
K_2HPO_4	1.0%	5.0 g/500 ml
$MgSO_4 \cdot 7H_2O$	0.75%	3.75 g/500 ml
$CaCl_2 \cdot 2H_2O$	0.25%	1.25 g/500 ml
NaCl	0.25%	1.25 g/500 ml

To this was added one ml of each of the micronutrient stock solutions (as described for TBIM) and a final dilution to one liter made. The pH of the resultant medium was 6.6 after autoclaving and cooling.

Each alga was grown under all the following conditions:

Controls: TBIM

                             TBIM + 2% ethanol,

                             BBM2

                             BBM2 - Fe,

                             BBM2 - Fe + 2% ethanol.

Experimental groups: DDT in 2% ethanol in BBM2 - Fe (6 concen-

                                  trations);

                             malathion, 95% purity in TBIM + 2% ethanol

                                  (6 concentrations);

                             malathion, 99% purity in TBIM + 2% ethanol

                                  (6 concentrations);

                             2,4-D in TBIM (6 concentrations);

                             2,4,5-T in TBIM (6 concentrations); and

                             rotenone in TBIM + 2% ethanol (6

                                  concentrations).

Each experiment was run in six replications under standard conditions.
Growth was measured at two week intervals for a period of two months at
a wavelength of 425 nm with a Spectronic 20, and also at 12 weeks for
those isolates which showed slow growth in one or more pesticides.

A further experiment was conducted in the dark. All controls
and the following concentrations of pesticides were used:

     rotenone, 50 and 10 ppm,

     malathion, 99% purity, 50 ppm,

     malathion, 95% purity, 50 ppm,

     DDT, 10 ppm,

     2,4-D, 50 ppm, and

     2,4,5-T, 50 ppm.

Each experiment was run in six replications at 22 C in the dark for a
period of two months. Growth was determined visually at the end of that
period as none, trace minus minus minus, trace minus minus, or trace
minus.

Photomicrographs were made of each isolate at the end of eight
weeks with a Zeiss RA microscope with Nikon camera and shutter components
on Kodak Panatomic X black and white film.

RESULTS

Description of the Algal Isolates

Ten isolates from soil collected in Jefferson and Oldham Counties, Kentucky were obtained in axenic culture for use in this study. Nine of the isolates are members of the Division Chlorophycophyta. The tenth, isolate 8, could not be readily identified and is, therefore, listed as unknown. This isolate gave a very weak or negative reaction when tested for the presence of starch with potassium iodine and is, therefore, possibly a member of the Division Chrysophycophyta. Since the primary purpose of this investigation was ecological rather than taxonomic, the complete identification of this alga has been reserved for those with taxonomic inclinations. It is possible that this alga is a new species or even a new genus, as the taxonomic study of soil algae is still very much in its infancy and most investigations describe one or more new species (Bold, 1970).

The other nine isolates have been identified to genus, and if the organism were particularly distinct, to species. The isolates are listed in Table 1 following the classification of Smith (1950) with a description of their source. Identifications which are tentative are so indicated by a question mark. Figures 1 - 10 are photomicrographs of each of the isolates.

As can be seen by an inspection of Figure 10, isolate 10, *Hormidium flaccidium*, has an unusual growth habit. This organism forms twisted ropes when growing in culture. Because of this, the colony develops as a tightly intertwined mat. This mat is so interwoven

TABLE I

CLASSIFICATION AND SOURCE OF ALGAL ISOLATES USED IN THIS STUDY

Classification	Isolation number	Isolation source
Division Chlorophycophyta		
Class Chlorophyceae		
Order: Ulotrichales		
Family: Ulotrichaceae		
Hormidium sp. ?	1	Rose bed, Southwest Jefferson County
Hormidium flaccidium A. Br.	10	Rose bed, Southwest Jefferson County
Stichococcus chodati (Bial.) Heering	2	Ant Hill, Horner Wildlife Refuge, Oldham County
Stichococcus chodati (Bial.) Heering	6	Rose bed, Southwest Jefferson County
Order: Chlorococcales		
Family: Chlorococcaceae		
Chlorococcum sp.	3	Woods, Horner Wildlife Refuge, Oldham County
Chlorococcum sp.	5	Sod Field, Horner Wildlife Refuge, Oldham County
Neochloris sp. ?	7	Tobacco Field, Horner Wildlife Refuge, Oldham County
Bracteacoccus sp. ?	4	Cedar Knoll, Horner Wildlife Refuge, Oldham County

TABLE I (Cont'd)

Classification	Isolation number	Isolation source
Family: Oocystaceae		
Chlorella sp.	9	Abandoned field, Horner Wildlife Refuge, Oldham County
Alga of unknown classification	8	Cedar Knoll, Horner Wildlife Refuge, Oldham County

FIGURE 1.   Isolate 1:   *Hormidium* sp. ?

FIGURE 2.   Isolate 2:   *Stichococcus chodati* (Bial.) Heering

FIGURE 3.   Isolate 3:   *Chlorococcum* sp.

FIGURE 4.   Isolate 4:   *Bracteacoccus* sp. ?

FIGURE 5.   Isolate 5:   *Chlorococcum* sp.

FIGURE 6.   Isolate 6:   *Stichococcus chodati* (Bial.) Heering
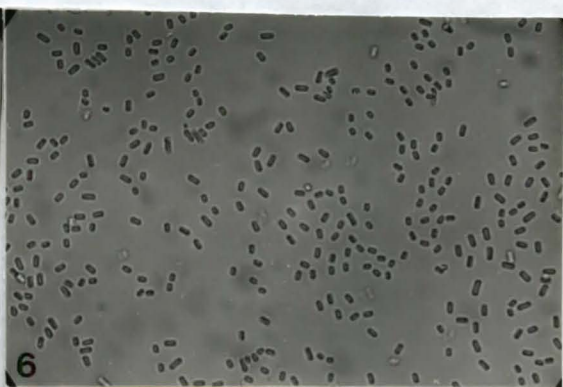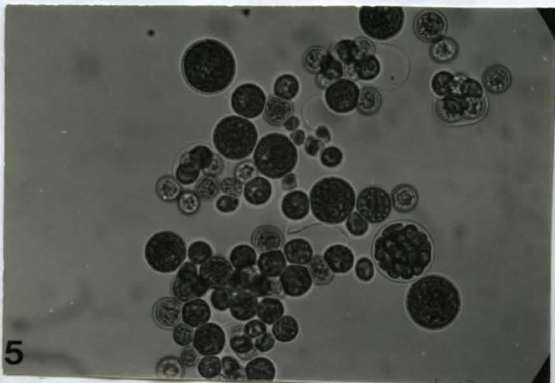
All figures are x 430.
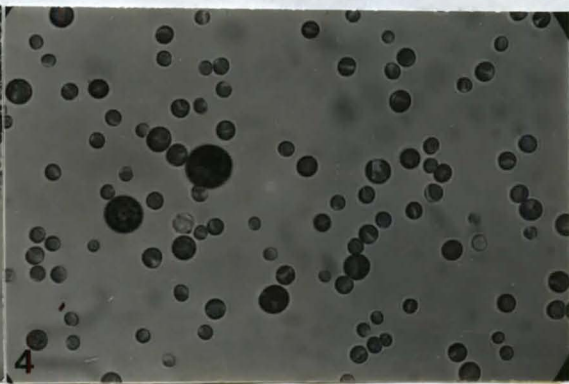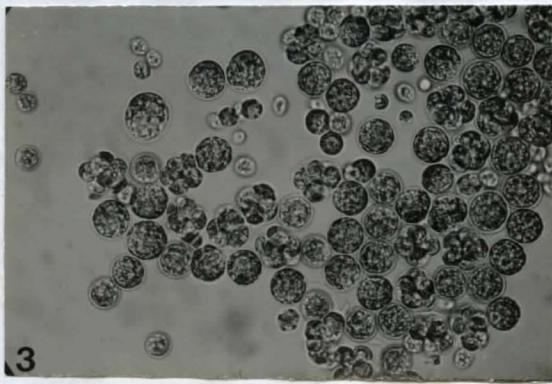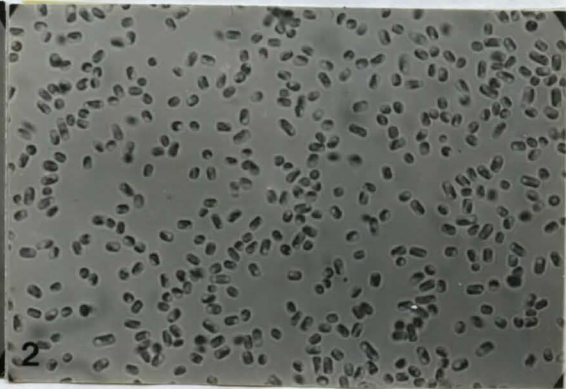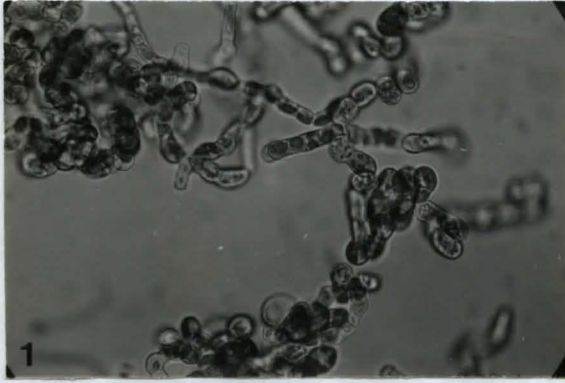
FIGURE 7.   Isolate 7:   *Neochloris* sp. ?

FIGURE 8.   Isolate 8:   Unknown

FIGURE 9.   Isolate 9:   *Chlorella* sp.

FIGURE 10.   Isolate 10:   *Hormidium flaccidium* A. Br.

All figures are x 430.
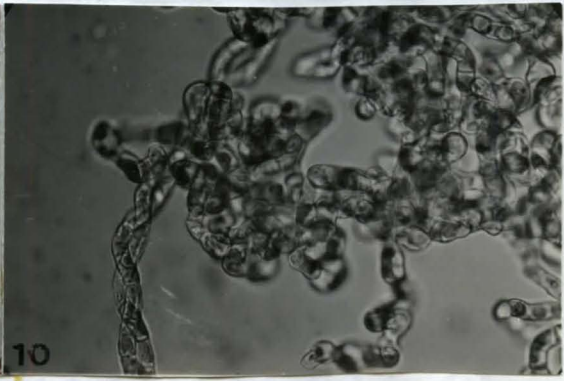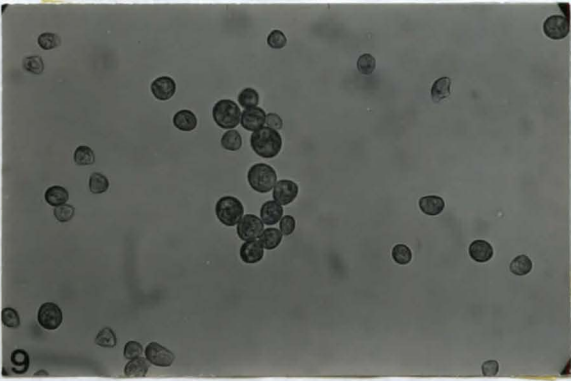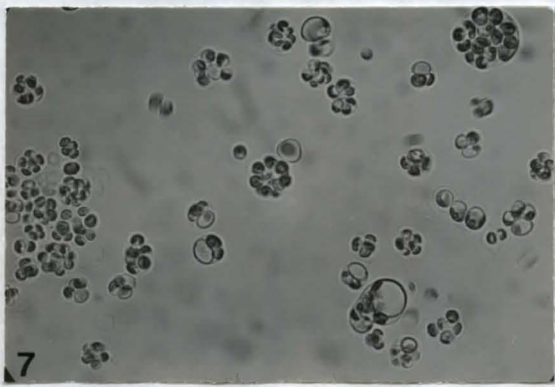
that it is not possible to disperse the colony adequately to get an accurate reading with a Spectronic 20. Therefore, for isolate 10, only visual estimations of growth were possible. The 15 grades used were None, Trace minus minus minus, Trace minus minus, Trace minus, Trace, Trace plus, Fair minus, Fair, Fair plus, Good minus, Good, Good plus, Excellent minus, Excellent, and Excellent plus. Only 2 or more degrees of difference, except in T---, T--, and T-, were regarded as significant (Wiedeman, 1964).

Algal Growth in Control Media

As noted previously, five different media were used for controls. Which medium served as the control for a particular pesticide depended on whether or not the pesticide was water soluble and whether or not it could be used in an alkaline medium. No attempt was made to determine optimal pH for the growth of the isolates or to determine if there were changes in the pH of the media during the course of the experiment as can happen in culture (Wiedeman, 1964). Figures 11-13 show the growth of isolates 1-9 in all five control media, and Table II gives the visual growth measure for isolate 10 in each of them.

There was a very distinct difference in most of the isolates between growth in TBIM media (alkaline) and that in BBM2 media (acid). Only *Hormidium* sp (1) and *Hormidium flaccidium* (10) grew generally as well or better in BBM2 than in TBIM based media, with the difference being more pronounced for isolate 1 than isolate 10. These two species of *Hormidium* had limited growth in all defined media. Growth was so slow that observations were extended to 12 weeks instead of the 8 week period which was adequate for most other isolates under most conditions. Even after 12 weeks, growth was better than fair only for isolate 1 in

FIGURE 11

Effect of control media on growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for the first graph are applicable to the following two graphs.

1. *Hormidium* sp. (1)

2. *Stichococcus chodati* (2)

3. *Chlorococcum* sp. (3)

FIGURE 11 Controls

1

O.D.

0.3
0.2
0.1

2   4   6   8   12
Weeks

2

3

————————— TBIM   ————————— BBM2

— — — — — — TBIM + EtOH   ·········—·····— BBM2 – Fe

······················ BBM2 – Fe + EtOH

FIGURE 12

Effect of control media on growth of isolates 4 - 6. Abscissa - Time
in weeks; Ordinate - Optical density as measured on the Spectronic 20
at 425 nm. The numbers for graph 4 are applicable to the other two
graphs.

4. *Bracteacoccus* sp. (4)

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

FIGURE 12 Controls

4

O. D.

0.7
0.6
0.5
0.4
0.3
0.2
0.1

0 | 2 | 4 | 6 | 8 | 12
Weeks

5

6
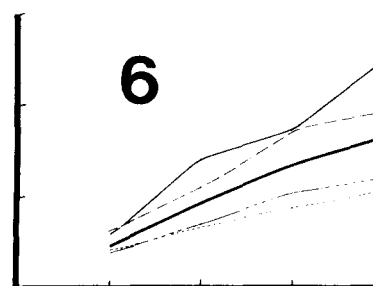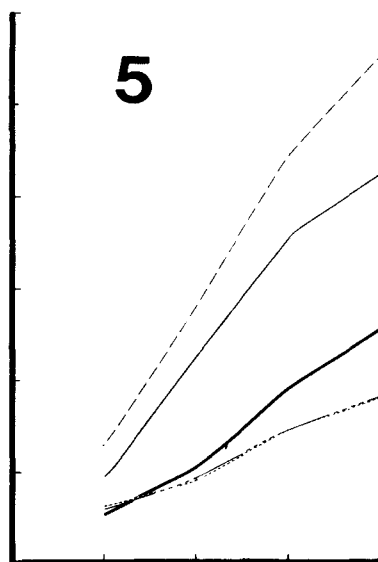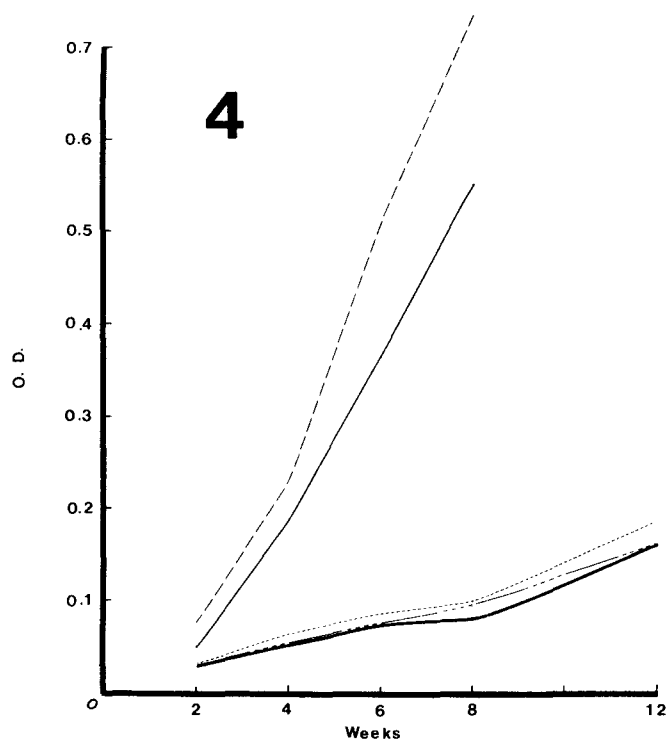
——————— TBIM

——————— TBIM + EtOH

——————— BBM2

——————— BBM2 – Fe

·············· BBM2 – Fe + EtOH

FIGURE 13

Effect of control media on growth of isolates 7 - 9. Abscissa - Time
in weeks; Ordinate - Optical density as measured on the Spectronic 20
at 425 nm. The numbers for graph 7 are applicable to the other two
graphs.

7. *Neochloris* sp. (7)

8. Unknown (8)

9. *Chlorella* sp. (9)

FIGURE 13 Controls

TABLE II

VISUAL ESTIMATIONS OF THE GROWTH OF *HORMIDIUM FLACCIDIUM* (10)
IN CONTROL MEDIA

	Time (weeks)				
Medium	2	4	6	8	12
TBIM	T---	T-	T+	F-	F
TBIM + EtOH	T---	T---	T--	T--	T
BBM2	T---	T--	T-	F-	F
BBM2 - Fe	T---	T--	T-	F-	F
BBM2 - Fe + EtOH	T---	T--	T-	F-	F

BBM2 - Fe, while that same isolate only had a trace of growth at 12 weeks in both TBIM media. Lower growth for these isolates could be due to several factors. Mattox and Bold (1962) reported that only one of their isolates of *Hormidium* sp. grew better than fair in a completely inorganic Bristol's solution which was very similar to the BBM2 used in this study. The one isolate which grew very well in Bristol's solution was from an aquatic environment, the rest being terrestrial in origin (except those from the Culture Collection of Algae at Indiana University whose origins were unknown to Mattox and Bold). The addition of a vitamin mixture to the Bristol's solution increased the growth of many of the cultures to excellent. No attempt was made to determine which specific vitamin (or vitamins) was required. Therefore, the low growth in this study could be due to a vitamin deficiency. Also, tris in some cases is toxic to algae (Mc Lachlan, 1963) which could be the reason for the lower growth of isolate 1 in TBIM than in BBM2. Also, this *Hormidium* sp. might prefer a slightly acid rather than slightly alkaline pH for optimum growth as has been reported for some algal species (Wiedeman, 1964).

For the other eight isolates, growth was significantly depressed in BBM2 as compared to TBIM media. Three isolates, *Bracteacoccus* (4), *Neochloris* (7), and *Chlorella* (9), had such a reduced growth that observations were extended on those isolates in all BBM2 media, both control and experimental, to 12 weeks. Even after 12 weeks, growth in all but one case (*Neochloris* in BBM2 - Fe) was less than in the TBIM control media at 8 weeks. Isolate 8 (unidentified) had a difference in growth only at the 12 week observation point, with significantly more growth at that time in the TBIM media that in the BBM2. Since TBIM was

used for isolation of the algae, their better growth in that medium could simply be a result of selection for organisms which grow well under those conditions.

In most cases, the growth curves for the isolates were very similar in media with and without ethanol. In TBIM, five isolates, *Stichococcus chodati* (2), *Chloroccum* (3) and (5), *Bracteacoccus* (4) and unidentified (8), had slightly better growth in the presence of ethanol at three or more of the sampling points. One isolate, *Neochloris* (7) had better growth in TBIM + ethanol at two sampling points and less growth at the other two. The remaining four isolates, *Hormidium* (1), *Stichococcus chodati* (6), *Chlorella* (9), and *Hormidium flaccidium* (10) had better growth in the absence of ethanol at three or more of the sampling points. This may indicate that the first groups of organisms can use ethanol to supplement their growth in the light. Even for those organisms with slightly depressed growth in ethanol, the reduction was insignificant compared to the differences between growth in TBIM and BBM2 media.

For the control media based on BBM2, there was also little difference in growth rates in most cases with and without ethanol. For *Stichococcus chodati* (2), *Chlorococcum* (3), *Bracteacoccus* (4) and *Hormidium flaccidium* (10), the growth curves in the three media were almost identical. In three other cases (*Chlorococcum* (5), *Stichococcus chodati* (6), and *Chlorella* (1)) there was a slight reduction in growth with the elimination of Fe and the addition of ethanol (BBM2 – Fe + EtOH). *Chlorococcum* (5) had nearly identical curves for growth in BBM2 – Fe with and without ethanol. For the other two isolates, there was less growth in BBM2 – Fe + EtOH than in BBM2 – Fe which was in turn

slightly less than in BBM2. Isolate 1, *Hormidium* sp., had almost identical growth in all three BBM2 media for the first eight weeks. However, at the 12 week sampling point, growth was much higher in BBM2 - Fe and slightly higher in BBM2 - Fe + EtOH than in BBM2. Isolate 8, unidentified, had a reduction in growth from BBM2 to BBM2 - Fe, but the addition of ethanol resulted in a growth curve nearly identical to that of BBM2. Finally, isolate 7, *Neochloris* sp., had better growth in BBM2 - Fe than in BBM2 which was in turn slightly better than BBM2 - Fe + EtOH. None of the differences within the BBM2 control media were as great as the growth differences between the BBM2 and the TBIM media. Therefore, it is concluded that the addition of 2% ethanol or the absence of iron salts from the media did not adversely effect the experimental organisms during the course of the experiment.

Statistical Analysis

A 9 by 6 by 7 factorial analysis of variance was made of the experimental data with the variables being respectively algae, pesticides, and concentrations. Tables III - VI are the summary tables for the analysis of variance for weeks 2, 4, 6, and 8 respectively. Examination of the F values of each table indicates that all variables are significant at least at the $p < 0.01$ level for all main effects and all interactions for each data collection period point. However, as Winer (1971) states,

> In many cases which arise in practice, tests on main effects may
> be relatively meaningless when interactions are significantly
> different from zero.

Therefore, it is difficult to analyze the significance of the two way interactions and the main effects since the three way interaction is significant. This indicates a high level of statictical complexity. Thus, it cannot be said what simple effect the different algae had

TABLE III

ANALYSIS OF VARIANCE TABLE FOR WEEK TWO

	Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1	Mean			3.265985	1	3.265985
2	I	R(IJK)	328.87	.9531845	8	.1191480
3	J	IJ	19.606	.3472589	5	.06945175
4	K	IK	11.456	.1437431	6	.02395718
5	IJ	IJK	6.4375	.1416953	40	.003542381
6	IK	IJK	3.8004	.1003799	48	.002091249
7	JK	IJK	11.5544	.1907437	30	.006358124
8	IJK	R(IJK)	1.5233	.1320663	240	.0005502761
9	R(IJK)			.6827394	1890	.0003612377

I = Algae

J = Pesticides

K = Concentrations

TABLE IV

ANALYSIS OF VARIANCE TABLE FOR WEEK FOUR

	Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1	Mean			22.10565	1	22.10565
2	I	R(IJK)	575.8733	6.632678	8	.8290848
3	J	IJ	14.501162	1.983252	5	.3966503
4	K	IK	5.341488	.3328799	6	.05547998
5	IJ	IJK	12.0705	1.094124	40	.02735309
6	IK	IJK	4.5835	.4985601	48	.01038667
7	JK	IJK	9.8473	.6694537	30	.02231512
8	IJK	R(IJK)	1.5739	.5438679	240	.002266116
9	R(IJK)			2.721203	1890	.001439790

I = Algae

J = Pesticides

K = Concentrations

TABLE V

ANALYSIS OF VARIANCE TABLE FOR WEEK SIX

	Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1	Mean			68.31825	1	68.31825
2	I	R(IJK)	851.18254	21.31157	8	2.663946
3	J	IJ	11.983752	5.199875	5	1.039974
4	K	IK	5.8064516	.8983440	6	.1497240
5	IJ	IJK	15.7500	3.471283	40	.08678204
6	IK	IJK	4.6799	1.237719	48	.02578580
7	JK	IJK	8.3078	1.373275	30	.04577582
8	IJK	R(IJK)	1.7605	1.322389	240	.005509950
9	R(IJK)			5.915161	1890	.003129715

I = Algae

J = Pesticides

K = Concentrations

TABLE VI

ANALYSIS OF VARIANCE TABLE FOR WEEK EIGHT

	Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1	Mean			144.4903	1	144.4903
2	I	R(IJK)	980.61466	44.46577	8	5.558222
3	J	IJ	9.3267721	10.12167	5	2.024333
4	K	IK	2.9131615	.8347748	6	.1391291
5	IJ	IJK	16.0231	8.681816	40	.2170454
6	IK	IJK	3.5257	2.292422	48	.04775880
7	JK	IJK	5.3635	2.179599	30	.07265329
8	IJK	R(IJK)	2.3898	3.250994	240	.01354580
9	R(IJK)			10.71276	1890	.005668126

I = Algae

J = Pesticides

K = Concentrations

without also taking into consideration the effects of pesticides and concentrations on algae. Likewise, there is no simply interpretable effect for either pesticides or concentrations. The statistical analysis further indicates that the joint effects of algae by pesticides, algae by concentrations, or pesticides by concentrations cannot be considered without also considering the third variable. Thus, care must be taken in the interpretation of the independent effects due to algae, pesticides, or concentrations and the two way interactions of these variables. Further, since the interactions are significant, these data cannot be clearly extrapolated to other algae, pesticides, or concentrations. Therefore, the analysis in the following sections will concentrate on the interaction effects.

2,4-D Introduction

The selective herbicidal properties of the chlorinated phenoxy acetic acids were discovered in 1942, but were not publicized until the end of World War II (Martin, 1964). In spite of the numerous studies done since then, the reason for their herbicidal behavior is still not understood at the cellular and molecular level (Moreland, 1967). The recommended agricultural dose is less than 5 lb/A (5.604 kg/H) (Edwards, 1964). In 1968, 142,248 pounds (64,523.69 kg) of all forms of technical material of 2,4-dichlorophenoxy acetic acid (2,4-D) were sold in Kentucky (Moore, 1973), while in 1969, 68,624,000 pounds (31,127,846 kg) of 2,4-D and 2,4,5-T were produced in the United States (Matsumura, 1972). A national survey of pesticide use on croplands in 1969 found that 15.14% of 1684 sites had been treated with 2,4-D at an average level of 0.54 lb/A (0.605 kg/H). Of 188 sites tested for residues, 3 (1.6%) had detectable residues at a mean level of less than 0.01 ppm and a range

of 0.01 to 0.03 ppm (Wiersma, Tai, & Sand, 1972b). In Kentucky, 3.2% of 31 cropland sites surveyed had had 2,4-D application at an average level of 0.50 lb/A (0.56 kg/H) (Wiersma, Tai, & Sand, 1972b).

Degradation of 2,4-D is rapid and can be due to the molecule combining with other cell molecules, to cleavage of the side chain, or to ring hydroxylation or ring cleavage (Hilton, et al., 1963; Alexander, 1965b; Wright, 1971). Reported detoxification times in the lab or greenhouse range from six to seven days to 18 months and in the field from one week to over 160 days (Appendices I & II). Environmental factors which influence the persistence of 2,4-D in soil include depth, organic matter, cation exchange capacity, exchangeable calcium, moisture, free drainage value, total exchangeable bases, soil type, temperature, pH, leaching, type of forest litter, chemical formulation, presence of DDT, and presence of lime (Newman, et al., 1952; Hanks, 1946; Nutman, et al., 1945; Kries, 1947; Martin, 1946; Weaver, 1948; Brown and Mitchell, 1948; DeRose and Newman, 1948; Jorgensen and Hamner, 1948; Crafts, 1949; Norman, et al., 1950; Blackman, et al., 1951; Ogle and Warren, 1954; Bell, 1960; Burger, et al., 1962; Upchurch and Mason, 1962; Upchurch, 1966; Norris and Greiner, 1967; Boyce Thompson Institute, 1971; Montgomery, et al., 1972).

There are three stages in decomposition: immediate initial adsorption on soil colloids, a lag phase, and rapid and complete detoxification (Audus, 1951; Montgomery, et al., 1972). The actual decomposition is due to the action of microorganisms in most cases (Audus, 1949, cited in Bollen, 1961; Audus, 1950; Norman, et al., 1950), as conditions which destroy or inhibit microbes increase persistence (Audus, 1951; DeRose and Newman, 1948; Brown and Mitchell, 1948; Hernandez

and Warren, 1950, cited in Audus, 1964), and over 10 different species
of bacteria, actinomycetes, and fungi have been isolated which can use
2,4-D as a sole carbon source (Appendix III). After initial decomposition,
additional applications are detoxified without the long lag phase (Audus,
1951; Newman, et al., 1952; Rogoff and Reid, 1956). Also, CO_2 is evolved
from soil in which 2,4-D is the only carbon source (Martin, 1946; Jensen
and Peterson, 1952). Finally, adding organisms capable of detoxification
to the soil causes breakdown typical of subsequent rather than initial
application (Audus, 1950).

Determinations of the breakdown pathway suggest that it is not
the same in all species. One frequent intermediate is 2,4-dichlorophenol
(Audus, 1952; Evans and Smith, 1954; Steenson and Walker, 1957; Loos,
et al., 1967), which may be further metabolized to 4-chloro, 2-hydroxy-
phenol (Steenson and Walker, 1957). Other reported intermediates are
6-hydroxy-2,4-dichlorophenoxyacetic acid (Evans and Smith, 1954),
2,4-dichloro-5-hydroxyphenoxyacetic acid (Woodcock, 1964; Faulkner
and Woodcock, 1964, 1965), 2,5-dichloro-4-hydroxyphenoxy-acetic acid
(Faulkner and Woodcock, 1964, 1965), and α-chloromuconic acid (Fernley
and Evans, 1959).

Although a few cases of inhibition of microorganisms by 2,4-D
at normal weed control doses (about 10 ppm or less) are known, most
reports of negative effects are at concentrations much higher than
ordinarily used. In some cases stimulation has been noted either at
normal or even high concentrations (Smith, et al., 1945; Stevenson
and Mitchell, 1945; Martin, 1946, 1963; Lewis and Hamner, 1946; Dubos,
1946; Newman, 1947; Payne and Fults, 1947; Fults and Payne, 1947;

Carlyle and Thorpe, 1947; Worth and McCabe, 1948; Norman, et al., 1950;

Kratochvil, 1951; Gould and Hamstead, 1951; Warren, et al., 1951; Aldrich,

1953; Colmer, 1953; Roberts and Bollen, 1955; Johnson and Colmer, 1955a

and b; Magee and Colmer, 1955; Rogoff and Reid, 1956; Walker and Newman,

1956; Bell, 1957; Frans, et al., 1957; Newman and Downing, 1958; Treater,

et al., 1958; Woodford, et al., 1958; Fletcher, 1960; Whiteside and

Alexander, 1960; Il'in, 1962; Mashtatov, et al., 1962; Petruk, 1964;

Audus, 1964; Dean and Law, 1964; Goodman, 1965; Farm Chemicals Handbook,

1965; Upchurch, 1966; Mickovski, et al., 1968; Montgomery, et al., 1972;

and the following all cited in Pimentel, 1971: Slepecky and Beck,

1950; Anderson and Baker, 1950; Hoover and Colmer, 1953; Rapoport and

Cangioli, 1963; Bounds and Colmer, 1964; Arnold, et al., 1966).

In most cases, studies of 2,4-D and algae also find negative

effects only at high concentrations. In sea water, 1.0 ppm had little

effect on productivity of phytoplankton (Butler and Springer, 1964,

cited in Loosanoff, 1965); while four hours exposure to 1.0 ppm did not

decrease phytoplankton growth (Butler, 1963, cited in Pimentel, 1971).

Growth of *Nitzschia palea* was inhibited by 2 ppm 2,4-D at three days,

but from 7-21 days was the same as the control; *Cylindrospermum*

licheniforme had reduced growth at three days, but not at 7 - 21 days;

and four other algae tested in unialgal cultures (*Microcystis aeruginosa,*

Scenedesmus obliquus, Gomphonema parvulum, and *Chlorella varigata*) were

not affected at any time up to three weeks (Palmer and Maloney, 1955).

At 20 ppm and up, *Anabaena* was significantly inhibited (inhibition

increasing with concentration); *Chlorella* was inhibited by 5 - 100 ppm

(inhibition increasing with concentration), with 1 ppm reducing dry

weight but having no other significant influence; 1 - 50 ppm caused

marked growth inhibition of *Oedogonium*; and 10 ppm reduced growth of

Vaucheria to 74% of controls; while, in some cases, 1 ppm of 2,4-D

promoted growth (Kim, 1961). *Chlorella pyrenoidosa* respiration was

stimulated at moderate concentrations (about 35 ppm) (Erickson, et al.,

1955; Wedding, et al., 1954) and inhibited at high concentrations

(Wedding, et al., 1954). At 2×10^{-3}M, photosynthesis was almost completely

inhibited at pH 3.10 and 4.10, but was stimulated at pH 7.0 and above,

while cells in 9.35×10^{-5}M and higher were progressively decolorized

(Wedding, et al., 1954). At pH 4.49, a 3.02×10^{-3}M concentration of

2,4-D was needed to produce complete photosynthesis inhibition (Erickson,

et al., 1955). Exposure to high concentrations (10^{-2}M) at 10 C for

4 hours did not kill this alga (Wedding, et al., 1959). No toxic effects

of up to 200 ppm technical grade 2,4-D were found on axenic cultures of

Scenedesmus quadricauda, *Chlamydomonas*, and *Chlorella pyrenoidosa* (Vance

and Smith, 1969), while 250 ppm had no effect on *Microcystis aeruginosa*

(Fitzgerald and Skoog, 1952). For a period of 40 hours, 1000 ppm of 2,4-D

had no inhibition at pH 5.1, 6.0, or 7.4 to axenic cultures of *Chlorella*

pyrenoidosa (Tomisek, et al., 1957). Finally, eight algae isolated from

waste stabilization ponds had a maximum tolerance to 2,4-D of 1.0 mM,

with the other 31 isolates having a maximum tolerance at 2.0 mM. None

of the 39 isolates could grow at 10 or 20 mM, and one isolate (*Chlorella*

23) did not survive at 2.0 mM. Two isolates (*Dictyosphaerium* 380 and

Scenedesmus 410) grew better at 2.0 mM than in the controls (Wiedeman,

1964).

2,4-D Results

At the concentrations used in this study, the basic effect of

2,4-D was neutral or stimulatory. A careful examination of Figures 14

to 16 and Table VII reveals that in most cases the growth curves are not greatly different at high or low concentrations of 2,4-D than those for the control. For example, *Stichococcus chodati* (2) shows a typical neutral effect. The growth curve for the control is situated in the midst of the curves for the 2,4-D concentrations, and there is no systematic arrangement of the curves for the experimental concentrations. This same pattern is evident for *Bracteacoccus* (4), *Chlorococcum* (3) and (5), *Stichococcus chodati* (6), unidentified (8), and *Hormidium flaccidium* (10). *Chlorella* (9) had an apparent reduction in growth at the higher concentrations (5 ppm and above) at both the six and eight week sampling points with little or no reduction at lower concentrations. *Hormidium* (1) and *Neochloris* (7) have definite stimulation of growth by 2,4-D, especially at the later sampling points. In both cases, growth at the final sampling point is greatly increased in one of the lower concentrations, with the rest of the concentrations clustered at a point midway between that and the control. It is possible that these two isolates are able to use 2,4-D to some extent to increase their growth in the light. Graph 10 in Figure 16 shows the overall interaction of isolates 1-9 with 2,4-D and confirms the original observation that the basic effect is neutral.

These results agree quite well with the general finding of past experiments that 2,4-D has negative effects on algae only at high concentrations, usually much higher than those used in this study. The only genera used in this experiment which had been reported on previously were *Chlorella* and *Chlorococcum*. The one reference to *Chlorococcum* (Wiedeman, 1964) reported that five isolates of that species grew as well in 2.0 mM 2,4-D (442 ppm) as they did in the control, but they could not grow in 10.0 mM (2210 ppm). Of the five isolates, two had the same growth

FIGURE 14

Effect of 2,4-D on the growth of isolates 1 - 3.   Abscissa - Time in
weeks; Ordinate - Optical density as measured on the Spectronic 20 at
425 nm.   The numbers for the first graph are applicable to the following
two graphs.

1.   *Hormidium* sp. (1)

2.   *Stichococcus chodati* (2)

3.   *Chlorococcum* sp. (3)

FIGURE 14   2,4-D
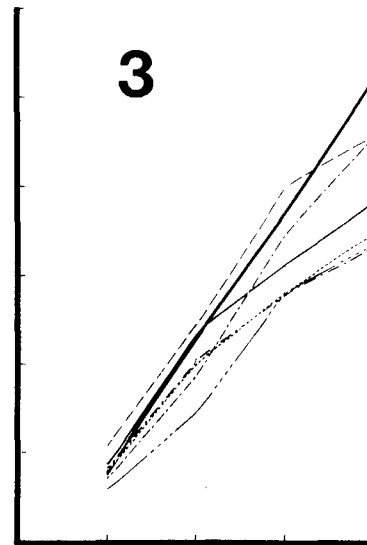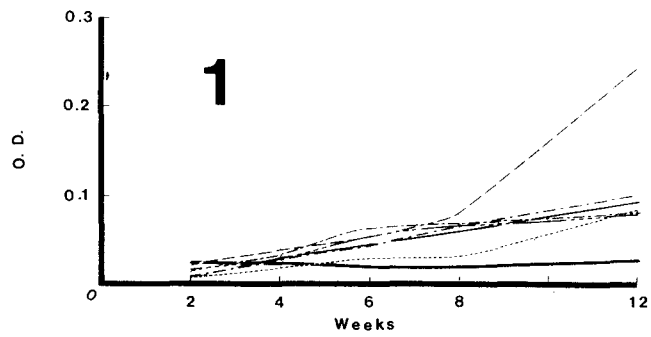
———————— 50 ppm	—·——·——·— 0.5 ppm
—·—·—·—·—·— 10 ppm	———————— 0.1 ppm
···················· 5 ppm	———————— 0 ppm (Control)
—————————— 1 ppm	

FIGURE 15

Effect of 2,4-D on the growth of isolates 4 - 7. Abscissa - Time in
weeks; Ordinate - Optical density as measured on the Spectronic 20 at
425 nm. The numbers for graph 4 are applicable to the following three
graphs.

4. *Bracteacoccus* sp. (4)

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

7. *Neochloris* sp. (7)

FIGURE 15   2,4-D

O.D.

Weeks

50 ppm
10 ppm
5 ppm
1 ppm
0.5 ppm
0.1 ppm
0 ppm (Control)
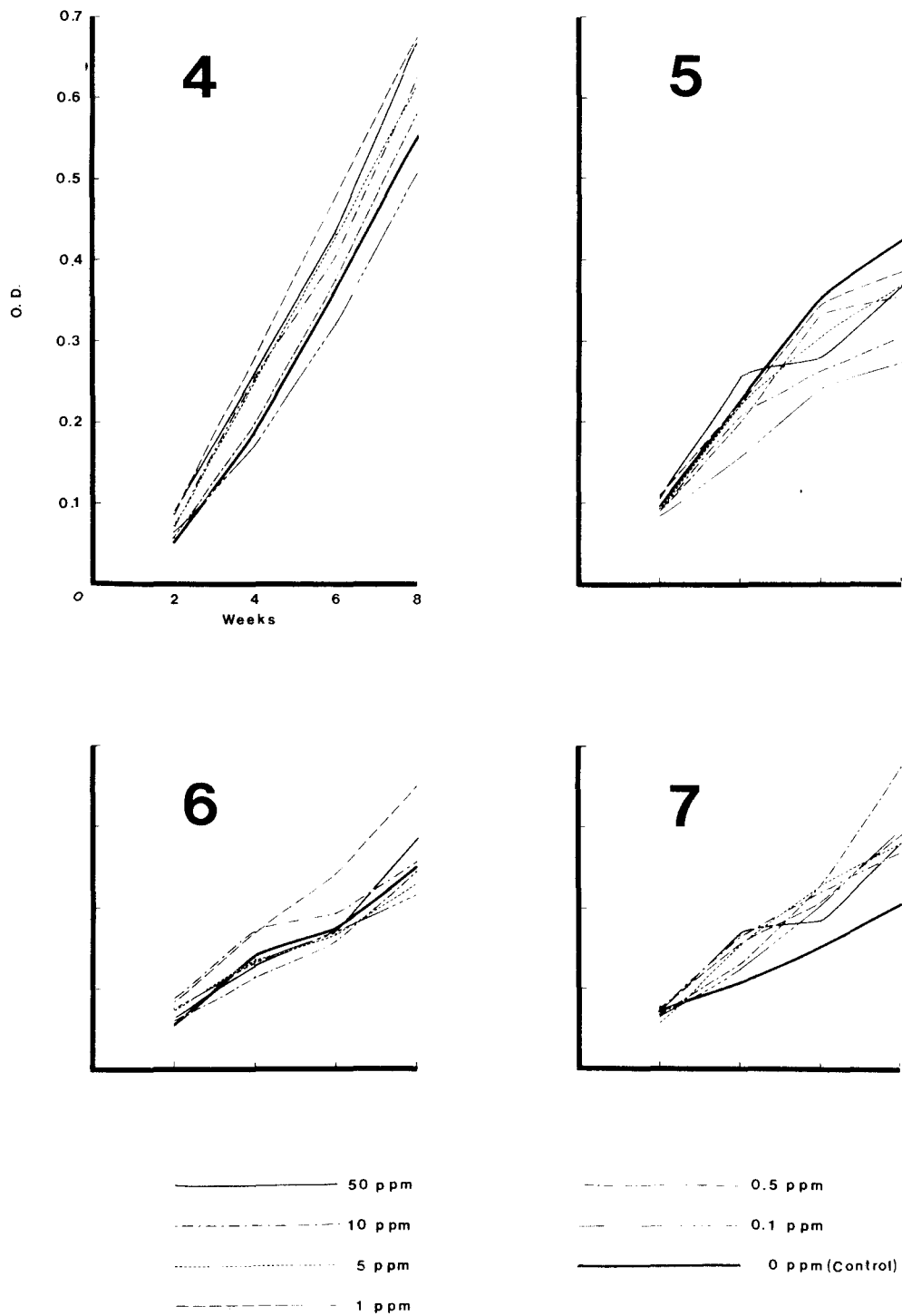
FIGURE 16

Effect of 2,4-D on the growth of isolates 8 and 9 and on a composite
of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical
density as measured on the Spectronic 20 at 425 nm. The numbers for
graph 8 are applicable to the following two graphs.

8. Unknown (8)

9. *Chlorella* sp. (9)

10. Composite of isolates 1 - 9

FIGURE 16 2,4-D
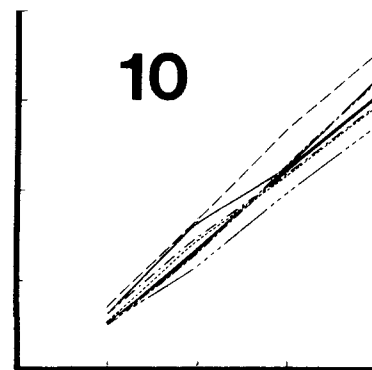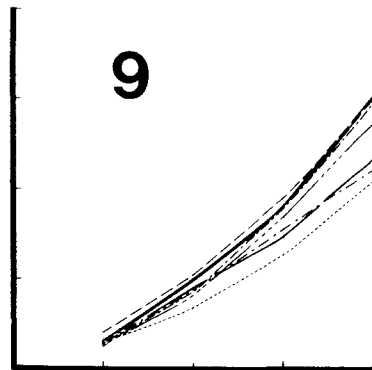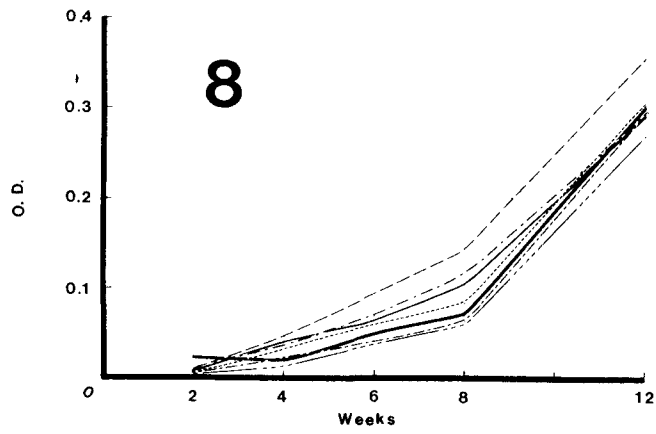
Legend:
- 50 ppm
- 10 ppm
- 5 ppm
- 1 ppm
- 0.5 ppm
- 0.1 ppm
- 0 ppm (Control)

TABLE VII

VISUAL ESTIMATIONS OF THE GROWTH OF *HORMIDIUM FLACCIDIUM* (10)
IN 2,4-D

		Weeks			
Concentration	2	4	6	8	12
50 ppm	N	T--	T-	F	F+
10 ppm	N	T--	T	F	F+
5 ppm	T---	T-	T	F	F+
1 ppm	T---	T--	T	F-	F
0.5 ppm	N	T--	T	F	F+
0.1 ppm	T---	T--	T-	F	F+
0 ppm	T---	T-	T	T-	F

at all concentrations up to 2.0 mM while the other three were stimulated

slightly by all concentrations up to 2.0 mM. The present study found

no stimulation of growth even at concentrations comparable to those used

by Wiedeman which is probably the result of using isolates belonging to

different species or different physiological strains of similar species.

There have been a number of reports of the effects of 2,4-D

on *Chlorella*, both on unspecified species and in a few cases on identified

species. These reports vary from no toxic effects at moderate to high

concentrations, to growth inhibitions in some cases at low concentrations.

Studies which measured photosynthesis and respiration found stimulation

of *Chlorella* at moderate and inhibition at high concentrations, while

other studies reported no negative effects at concentrations close to

those used in this study (Tomisek, et al., 1957; Wedding, et al., 1954;

Vance and Smith, 1969; Wiedeman, 1964). This study found a growth

inhibition at concentrations of 5 ppm and higher, but only after a period

of six weeks. This agrees with the work of Kim (1961) who found that

Chlorella had growth inhibited by 5-100 ppm, with 1 ppm causing only a

reduction in dry weight (which was not measured in this study). The

differences between some of the previous studies and the present report

may be due to different algae used, to different media used, or to

different experimental conditions, with the different organisms being

the most likely reason.

2,4,5-T Introduction

The auxin type herbicide most effective against woody plants

is 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) which has a recommended

usage level of 6 lb/A (6.725 kg/H) or less (Blackman, et al., 1951;

Fletcher, 1960; Martin, 1964; Farm Chemicals Handbook, 1965; Moore,

1967). In 1968, 38,356 pounds (17,398.3 kg) of technical material of all forms of 2,4,5-T were sold in Kentucky (Moore, 1973). A national survey of pesticides used on croplands in 1969 (1684 sites) found that 0.18% of the sites had been treated with 2,4,5-T at an average application of 0.83 lb/A (0.93 kg/H) (Wiersma, Tai, & Sand, 1972b).

Soil decomposition is much slower than for 2,4-D (DeRose, 1946; Audus, 1951; Alexander, 1965b; Walker, 1967), but follows the same basic pattern of adsorption, a long lag phase, and then rapid detoxification (Audus, 1951). The disappearance rate of a second application after detoxification of the first has been reported to be both without a long lag (Audus, 1951) and as long as the initial detoxification (Newman, et al., 1952; Newman and Downing, 1958). There is also a controversy over whether the decomposition is due to microorganisms. Whiteside and Alexander (1960) reported that 2,4,5-T was not broken down by microbes, and no organism which can decompose it has been isolated into pure culture (Newman and Downing, 1958; Walker, 1967). However, those conditions which favor the growth of microbes decrease the persistence of 2,4,5-T in the soil (including soil type, moisture, dose, temperature, and organic matter) (DeRose and Newman, 1948; Norman, et al., 1950; Blackman, et al., 1951; Audus, 1951; Boyce Thompson Institute, 1971). In addition, a bacterial poison, 0.01% sodium azide, destroyed the detoxifying ability of soil which had been able to breakdown 2,4,5-T (Audus, 1951). However, if microorganisms are responsible for the decomposition, they are not the same ones which detoxify 2,4-D (Audus, 1951; MacRae and Alexander, 1965).

Persistence varies greatly both under lab or greenhouse conditions and in the field, but is generally in terms of several

months. Some of the times cited for disappearance in the lab were: over three months (Whiteside and Alexander, 1960); more than 47, 124, and 205 days (different soil types) (Alexander and Aleem, 1961); 270 days (Audus, 1951); 147 days and 11 months (different concentrations) (DeRose and Newman, 1948); and over 15 months (stored in dry soil) (Weaver, 1948). Persistence in the field was reported to be two to five weeks (Klingman, 1961, cited in Pimentel, 1971); over 93 days (DeRose and Newman, 1948); 19 weeks (Newman, et al., 1952); five months (75-100% loss) (Matsumura, 1972); six to seven months (Alexander, 1965b); and 12 months (95% disappearance) (Edwards, 1964).

At concentrations normally used for weed control, reports of negative effects on microorganisms are uncommon (Kratochvil, 1951; Magee and Colmer, 1955; Roberts and Bollen, 1955; Newman and Downing, 1958; Fletcher, 1960; Whiteside and Alexander, 1960; Bounds and Colmer, 1964, cited in Pimentel, 1971). Only at very high concentrations are inhibitions noted (Newman, 1947; Magee and Colmer, 1955; Roberts and Bollen, 1955; Johnson, et al., 1956; Fletcher, 1960; Whiteside and Alexander, 1960), with 2,4,5-T, in general, appearing to be more toxic than 2,4-D (Magee and Colmer, 1955; Johnson, et al., 1956). In some cases, stimulation of soil organisms has even been reported (Roberts and Bollen, 1955; Newman and Downing, 1958).

Studies on the effect of 2,4,5-T on algae generally agree with those on other microorganisms: i. e., adverse effects only at concentrations far exceeding the normal dose for weed control (about 10 ppm). Exposing phytoplankton to 1 ppm of 2,4,5-T for 4 hours did not decrease productivity (Butler, 1963, cited in Pimentel, 1971). A commercial preparation at 2 ppm was toxic to *Microcystis aeruginosa* at three and

seven days (but not at 14 and 21 days), stimulated *Cylindrospermum licheniforme* at three days, and was non-toxic to four other algae (*Scenedesmus obliquus, Chlorella variegata, Gomphonema parvulum,* and *Nitzschia palea*) at three and seven days and to all six unialgal cultures at 14 and 21 days (Palmer & Malovey, 1955). Concentrations up to 200 μg/ml (200 ppm) of technical grade material had no toxic effects on axenic cultures of *Scenedesmus quadricauda, Chlamydomonas eugametos,* and *Chlorella pyrenoidosa* for a period of four days (Vance and Smith, 1969). Finally, the effect of 2,4,5-T at concentrations of 0.02 to 20 mM was tested on 39 axenic algal cultures isolated from waste-stabilization ponds (Wiedeman, 1964). None of the isolates survived in concentrations of 10 or 20 mM; two isolates (*Chlorella,* 23 and *Ankistrodesmus,* 340) did not tolerate 2.0 mM (511 ppm); and two could only tolerate a maximum of 0.1 mM (25.5 ppm) (*Chlorella,* 293 and 370). Two other isolates were stimulated by 2.0 mM (*Dictyosphaerium,* 380 and *Scenedesmus,* 410).

2,4,5-T Results

The effect of 2,4,5-T depended on the isolate under consideration, ranging from stimulation to neutrality to inhibition. *Stichococcus chodati* (2) and (6), *Bracteacoccus* (4), unidentified (8), *Chlorella* (9), and *Hormidium flaccidium* (10) were all basically uneffected by 2,4,5-T at the concentrations used as can be seen by an examination of Figures 17-19 and Table VIII. Two isolates, *Hormidium* (1) and *Neochloris* (7), were stimulated by 2,4,5-T, especially at the later sampling points. Both *Chlorococcum* isolates (3) and (5) were inhibited by the higher concentrations (10 and 50 ppm), but were only slightly effected at lower concentrations. In no case was there total inhibition of growth of any isolate. Figure 19-10 showing the combined effect of 2,4,5-T across isolates 1-9

FIGURE 17

Effect of 2,4,5-T on the growth of isolates 1 - 3. Abscissa - Time
in weeks; Ordinate - Optical density as measured on the Spectronic
20 at 425 nm. The numbers for graph 1 are applicable to the other
two graphs.

1.   *Hormidium* sp. (1)

2.   *Stichococcus chodati* (2)

3.   *Chlorococcum* sp. (3)

FIGURE 17 2,4,5-T

O.D.

0.3
0.2
0.1

1

0   2   4   6   8   12

Weeks

2

3
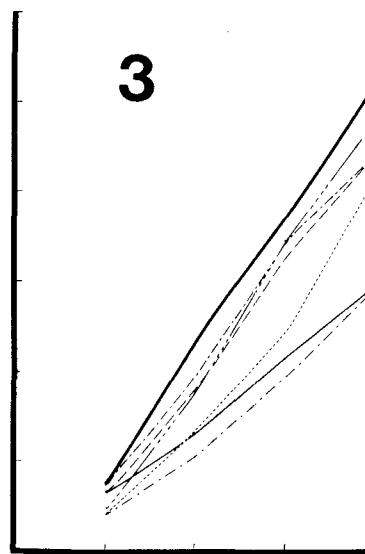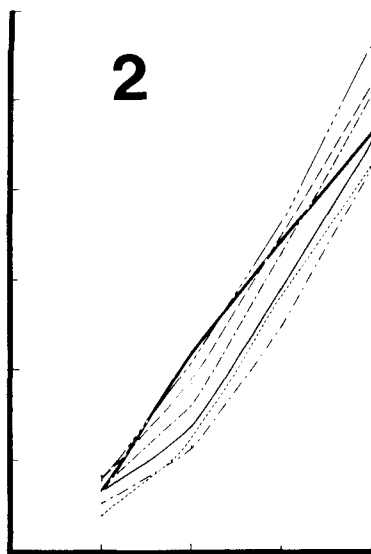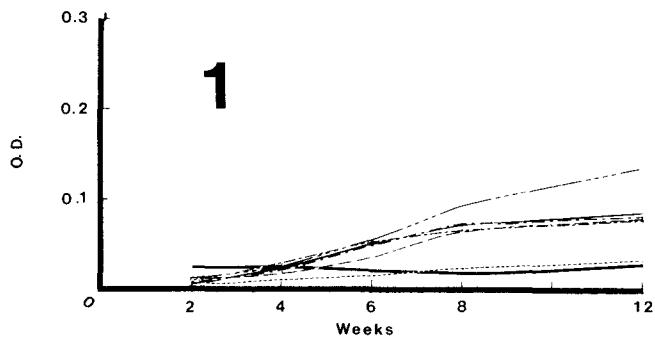
——————— 50 ppm

—·—·—·— 10 ppm

············· 5 ppm

— — — — — 1 ppm

—··—··—··—0.5 ppm

—·—·—·— 0.1 ppm

——————— 0 ppm   (Control)

FIGURE 18

Effect of 2,4,5-T on the growth of isolates 4 - 7. Abscissa - Time
in weeks; Ordinate - Optical density as measured on the Spectronic
20 at 425 nm. The numbers for graph 4 are applicable to the other
three graphs.

4. *Bracteacoccus* sp. (4)

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

7. *Neochloris* sp. (7)

FIGURE 18 2,4,5-T

O.D.

0.7
0.6
0.5
0.4
0.3
0.2
0.1

4

5

O

2 4 6 8

Weeks

6

7
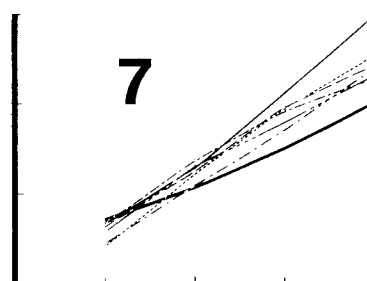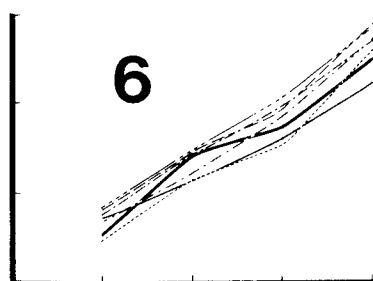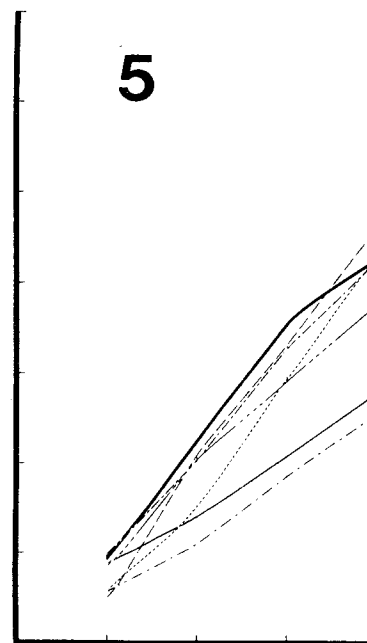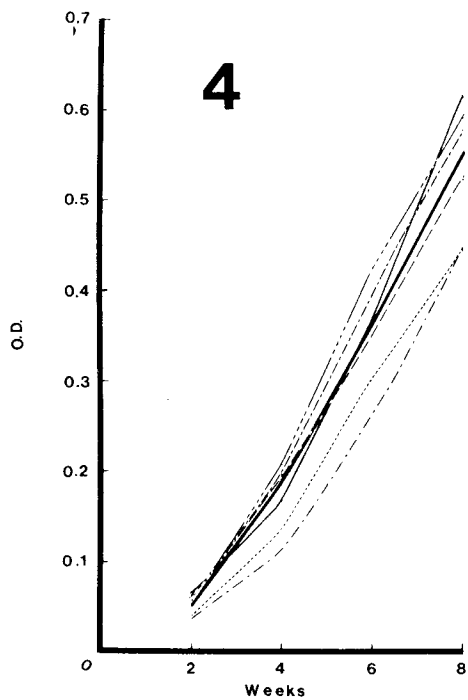
———————— 50 ppm

—·—·—·—·— 10 ppm

················· 5 ppm

——————— 1 ppm

—··—··—··— 0.5 ppm

———·····——— 0.1 ppm

———————— 0 ppm (Control)

FIGURE 19

Effect of 2,4,5-T on the growth of isolates 8 - 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

8.   Unknown (8)

9.   *Chlorella* sp. (9)

10.   Composite of isolates 1 - 9

FIGURE 19 2,4,5-T

Graph 8: O.D. vs Weeks (2, 4, 6, 8, 12)

Graph 9

Graph 10
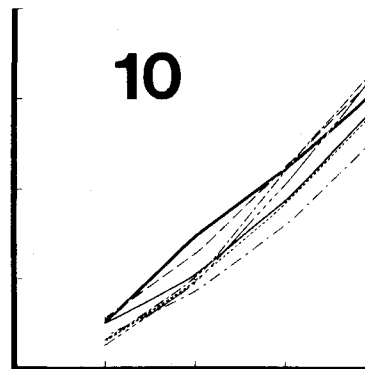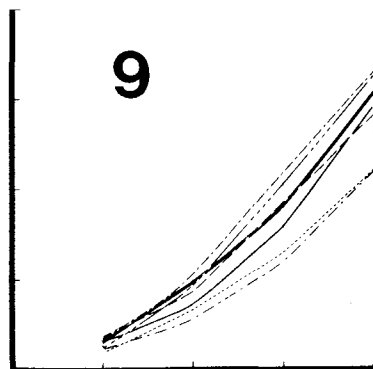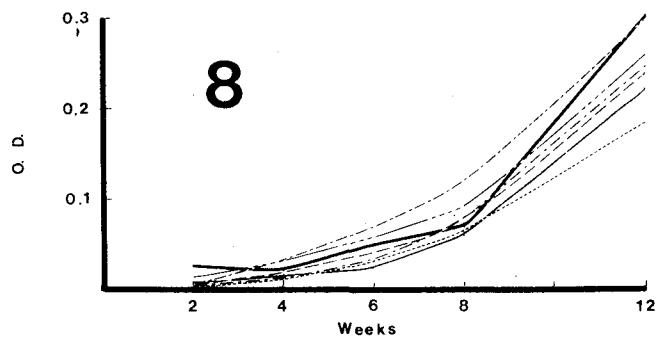
Legend:
———————— 50 ppm
—·—·—·—·— 10 ppm
·················· 5 ppm
— — — — — 1 ppm
—··—··—··— 0.5 ppm
—·····—·····— 0.1 ppm
———————— 0 ppm (Control)

TABLE VIII

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN 2,4,5-T

Weeks

Concentrations	2	4	6	8	12
50 ppm	N	T---	T--	T	T+
10 ppm	N	T--	T-	T	F
5 ppm	T---	T--	T-	T+	F
1 ppm	T---	T--	T	F-	G-
0.5 ppm	N	T--	T-	T+	F-
0.1 ppm	T---	T-	T-	F-	F+
0 ppm	T---	T-	T	F-	F

verifies the basic neutrality of 2,4,5-T to soil algae at the concentrations tested.

This study generally agrees with past work, finding negative effects rarely and mainly at higher concentrations. An earlier report on five isolates of *Chlorococcum* sp. (Wiedeman, 1964) found that they could tolerate concentrations up to 2.0 mM (511 ppm), but could not survive at 10.0 mM. Only one of those five isolates was stimulated and two were slightly inhibited (but not significantly) by concentrations comparable to those used in this study. A number of studies reported on effects of 2,4,5-T on *Chlorella* sp. Palmer and Maloney (1955) found no toxic effects of 2 ppm over a period of 21 days, while Vance and Smith (1969) reported no toxic effects of technical grade chemical at concentrations up to 200 ppm. Wiedeman (1964) worked with six isolates of *Chlorella* sp. and found varying tolerances to different concentrations of 2,4,5-T. None of the isolates could tolerate a concentration of 10.0 mM (about 2554 ppm), one grew as well in 2.0 mM (511 ppm) as it did in the control, three others could grow as well as in the control at concentrations only up to 0.2 mM (51 ppm), while the other two isolates had growth equal to the control at a maximum concentration of 0.1 mM (25 ppm). It is possible that more inhibiting effects might have been found in this study if concentrations higher than 50 ppm had been used, but it was desired to keep this study within the limits that would most commonly be encountered by the organisms in nature.

A comparison of the graphs for 2,4-D and 2,4,5-T for isolates *Chlorococcum* (3) [Figures 14-3 & 17-3] and (5) [Figures 15-5 & 18-5] shows that they are more susceptible to 2,4,5-T than to 2,4-D. Close inspection of the graphs for those isolates in 2,4-D shows that both

are inhibited by 2,4-D to some extent at the final sampling point with inhibition much less evident at the earlier periods. This may indicate that the *Chlorococcum* isolates are sensitive to both 2,4-D and 2,4,5-T, but more sensitive to the latter. This agrees with Wiedeman (1964) who also found that the *Chlorococcum* isolates he tested were more sensitive to 2,4,5-T than to 2,4-D. *Chlorella* isolate (9) shows the opposite reaction, being more sensitive to 2,4-D than to 2,4,5-T. But even the inhibition due to 2,4-D is not as prominent as that due to the other pesticides tested. In the case of 2,4,5-T there appears to be a slight inhibition at the higher concentrations, but this is not as obvious as that with 2,4-D. It appears likely that the addition of one chlorine atom, changing 2,4-D to 2,4,5-T, can have a large influence on the effects on soil algae.

DDT Introduction

In 1874, Othmar Zeidler first described 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane, commonly called DDT; but it was not until 1939, that Paul Müller recognized its value as an insecticide (Farm Chemicals Handbook, 1965). Since that time the total amount used may be as high as 10^6 short tons (9,078 x 10^5 metric tons) (Moore, 1967), and residues are often found in the soil of over 100 ppm (Wiersma, Mitchell, and Stanford, 1972). In 1972, the use of DDT in the U. S. was halted for crops (with three minor exceptions), for nonhealth applications, and for health purposes without a prescription (Ruckelshaus, 1972). The governmental findings leading to that decision stated that the risk from continued use outweighed the benefits since suitable, but less persistent, substitutes were available. The specificity of DDT may be due to its high penetrability through insect cuticle but not through vertebrate skin

(Martin, 1964), or it may be due to differences in rates of degradation (O'Brien, 1966). Its precise mode of action is unknown, but it appears to interfer with nerve transmission (O'Brien, 1966, 1967). DDT is nonselective and often kills the natural enemies of the pest it is applied to control, thereby causing an increase in the undesired pest (Wurster, 1973).

Shortly after DDT use began on a large scale, it became apparent that it persisted in soils for many years, even decades. Since the late 1940's, reports have shown that even a single application will persist for many years, that repeated applications accumulate to amounts much higher than amounts applied in any one year, that it is seldom found in quantity in the soil below plow depth, that it is concentrated by organisms in the food chain, and that DDT and its residues are found in soils where they have never been directly applied (Smith, 1948; Chisholm, et al., 1950 and 1955; Foster, 1951; Fleming and Maines, 1953; Allen, et al., 1954; Ginsburg and Reed, 1954; Ginsburg, 1955; Lichtenstein, 1957; Lichtenstein and Schulz, 1959; Lichtenstein, et al., 1960; Taschenberg et al., 1961; Clore, et al., 1961, cited in Alexander, 1965b; Roberts, et al., 1962; Wheatley, et al., 1962; Bridges, et al., 1963; Edwards, 1963, 1964; Woodwell and Martin, 1964; Harris, et al., 1966; Nash and Woolson, 1967; Woodwell, et al., 1967; Woodwell, 1967; Cole, et al., 1967; Chacko and Lockwood, 1967; Ko and Lockwood, 1968a; Dimond, et al., 1970; Kearney, et al., 1970; Cox, 1970a, 1970b; Lichtenstein, et al., 1971; Matsumura, 1972; Brown, 1972; Tarrant, et al., 1972; Wiersma, Tai and Sand, 1972a; Wiersma, Mitchell, and Stanford, 1972; Menzie, 1972; Yule, 1973, and the following, all cited in March, 1965: Randolph, et al., 1960; MacPhee, et al., 1960; Gallaher and Evans, 1961).

Factors which influence DDT persistence in the soil include soil type, temperature, ultraviolet light, moisture, amount of mixing into soil, plowing, and organic matter (Foster, 1951; Fleming and Maines, 1953; Eno, 1958; Lichtenstein and Schulz, 1959 and 1961; Lichtenstein, et al., 1960).

Although first speculated on almost 20 years ago (Jones, 1956, cited in Walker, 1967), evidence has only accumulated in the last 10 years to show that DDT can be degraded by microorganisms. No single microbe is known which can break it down all the way to CO_2, H_2O, and chlorine, but it appears that several can break it down by acting in sequence. None can use DDT or any of its reduction products as a sole carbon source, so breakdown must be a form of cometabolism (Chacko, et al., 1966; Wedemeyer, 1967b; Alexander, 1971; Focht, 1972; Pfaender and Alexander, 1972). Organisms which have been shown to be involved in at least one step of the breakdown are listed in Appendix IV. The main metabolite is TDE (DDD), but other breakdown products have been identified and are listed in Appendix V. Some of the breakdown steps require the absence of air, others its presence (Pfaender and Alexander, 1971; Focht, 1972; Alexander, 1972; Focht and Alexander, 1971; Wedemeyer, 1966, 1967a, and 1967b). If microorganisms can break it down, why does DDT persist and accumulate in the soil? This may be because of the need for alternating anaerobic and aerobic conditions, or more likely because organisms which break it down are not abundant and they have no selective advantage in decomposing it since they cannot use it as a carbon source (Pfaender and Alexander, 1972; Alexander, 1972; Chacko, et al., 1966; Focht, 1972).

DDT has been reported to have effects on higher plants varying from negative to neutral to stimulation (Chapman and Allen, 1948;

Appleman and Sears, 1946; Gould and Hamstead, 1951; Thurston, 1953;
Chisholm, et al., 1955; Harcourt and Cass, 1955; Clower and Matthysse,
1954; Eno and Everett, 1958; Eder, 1963; Bridges, et al., 1963;
Lichtenstein, et al., 1962; Lawler and Rogers, 1967; National Academy
of Sciences, 1968).

The effects of DDT on microorganisms and their processes are
usually reported to be neutral. Negative effects are normally only
found at very high concentrations, while a few cases of stimulation
have even been reported (Appleman and Sears, 1946; Wilson and Choudhri,
1946; Smith and Wenzel, 1947; Fults and Payne, 1947; Gould and Hamstead,
1951; Bollen, et al., 1954a and 1954b; Eno, 1968; Eno and Everett, 1958;
Pathak, et al., 1961, cited in Marth, 1965; Bartha, et al., 1967; Ko
and Lockwood, 1968b; Ledford and Chen, 1969; Tarrant, et al., 1972; Payne
and Fults, 1947; Eder, 1963; MacRae and Vinchx, 1973; Roberts and Bollen,
1955; the following all cited in Bollen, 1961; Martin, et al., 1959;
Jones, 1956; and Braithwaite, et al., 1958).

Since DDT has a very low water solubility (about 1.2 ppb.
[Bowman, et al., 1960]), studies on its effects on phytoplankton are
often at concentrations which might be found in water, but which are
far below amounts normally used for insect control. Algae which have
been reported to be uneffected or only mildly effected by DDT under such
conditions are listed in Table IX. However, algae can accumulate DDT
passively and rapidly to a level several hundred times higher than the
concentration in which they are growing, and surveys of algae isolated
from nature have found many with high DDT concentrations in their cells
(Rice and Sikka, 1973; Keil and Priester, 1969; Gregory, et al., 1969;
Woodwell, et al., 1967; Cox, 1970a and 1970b; Vance and Drummond, 1969).

TABLE IX

ALGAE UNAFFECTED BY DDT AT LOW CONCENTRATIONS

Algal species	Conditions	Reference
Chlorella sp.		Wilson and Choudhri, 1946
Agmenullum quadruplicatum	100 ppb DDT	Boush and Batterton, 1972
Anacystis nidulans	100 ppb DDT	Boush and Batterton, 1972
Dunaliella tertiolecta	80 ppb	Bowes, 1972
Cyclotella nana	80 ppb, short lag before division started	Bowes, 1972
Thalassiosira fluviatilis	80 ppb, short lag before division started	Bowes, 1972
Amphidinium carteri	80 ppb, short lag before division started	Bowes, 1972
Coccolithus huxleyi	80 ppb, short lag before division started	Bowes, 1972
Porphyridium sp.	80 ppb, short lag before division started	Bowes, 1972
Phytoplankton culture	1.0, 0.1, 0.05, 0.01 ppm	Loosanoff, et al., 1957

(mainly *Chlorella*, *Chlamydomonas*, other common species, and zooplankton)

Dunaliella tertiolecta	100 ppb	Menzel, et al., 1970
Coccolithus huxleyi	100 ppb	Menzel, et al., 1970
Chlamydomonas reinharatii	0.2-20 ppm	Morgan, 1972
Cylindrospermum licheniforme	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955
Scenedesmus obliquus	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955
Chlorella variegata	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955

TABLE IX (Cont'd)

Algal species	Conditions	Reference
Nitzschia palea	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955
Microcystis aeruginosa	2 ppm (toxic at first)	Palmer and Maloney, 1955
Gomphonema parvulum	2 ppm (reduced growth slightly at first)	Palmer and Maloney, 1955
Anacystis nidulans	1 ppm	Gregory, et al., 1969
Scenedesmus obliquus	1 ppm	Gregory, et al., 1969
Euglena gracilis	1 ppm	Gregory, et al., 1969
Microcystis aeriginosa, *Anabaena cylindrica*, *Scenedesmus quadricauda*, and *Oedogonium* sp. in unialgal cultures	up to 1.0 ppm	Vance and Drummond, 1969

There are several reports that DDT might upset a natural balance. In some cases, the changes were concluded to be no greater than those caused by climate or other ecological factors (Bishop, 1947; DeKoning and Mortimer, 1971). Others reported that the effects might influence the algal population composition in nature (Lazaroff and Moore, 1966; Woodwell, et al., 1967; Mosser, et al., 1972; Batterson, et al., 1972). Shane (1948) described a bloom of *Synedra* sp. in a reservoir due possibly to stimulation by a DDT spray or to a reduction in the number of zooplankton grazers because of the DDT spray. A pond treated with 0.02 ppm DDT had a disappearance of *Chara* sp. and *Spirogyra* sp. within two months with the regrowth the following summer less than 20% of the previous year's high (Bridges, et al., 1963).

Negative effects on algae have been reported at low, normal, and high concentrations of DDT. Butler (1963, cited in Pimentel, 1971) found that phytoplankton communities exposed to DDT at 1 ppm in the lab for four hours had a reduction in productivity of 77.2%. Stadynk (1967, cited in Vance and Drummond, 1969) reported a significant reduction in ^{14}C assimilation by *Scenedesmus quadricauda*. Wurster (1968) found that DDT as low as a few ppb reduced photosynthesis in lab cultures of four species of marine algae in axenic culture and also a natural mixed phytoplankton community. *Chlorella* grown in less than 0.3 ppb DDT for three days had great morphological changes which disappeared when the DDT was removed (Södergren, 1968). The LD_{100} for *Anabaena cylindrica* is between 15 and 20 ppm DDT in unialgal culture, while for *Microcystis aeruginosa*, *Scenedesmus quadricauda,* and *Oedogonium* sp. it is over 20 ppm (Vance and Drummond, 1969). At the end of four days, DDT at 100 ppm reduced the growth of *Scenedesmus* and *Euglena,* but not *Chlorella* and *Ankistrodesmus* and varying pH did not change the effect of DDT on

Chlorella (Christie, 1969). Growth of *Monochrysis lutheri* was reduced

75% by 0.60 ppm DDT, while *Protococcus* sp. growth was reduced 50%,

Dunaliella euchlora growth was reduced 25%, and *Chlorella* sp. and

Phaeodactylum tricornutum growth was reduced very little by concentrations

up to 1.0 ppm DDT (Ukeles, 1962). Cell division of *Skeletonema costatum*

was blocked after two to three divisions by 100 ppb DDT, while division

of *Cyclotella nana* was slowed (Menzel, et al., 1970). Another report

found that 80 ppb DDT delayed cell division of *Skeletonema costatum* for

nine days, after which it continued at the normal rate (Bowes, 1972). At

250 ppm, *Coccolithus huxleyi* and *S. costatum* had lag phases of two and

ten days respectively before normal cell division began. The only morpho-

logical differences cited were that cells of *S. costatum* in lag phase

looked "unhealthy". Finally, a wide range in tolerance of waste stabili-

zation pond algae to DDT was reported (Wiedeman, 1964). Four isolates

(*Pediastrum*, 161 and 265, and *Chlorella*, 185e and 370) had no tolerance

for even 0.02 mM, the lowest concentration used. One isolate (*Chloro-

coccum*, 155) grew quite well at the highest concentration (20 mM), while

11 others showed at least some growth at that concentration. Four other

isolates (*Chlamydomonas*, 29, *Stigeoclonium*, 96, and *Chlorococcum*, 78 and

292) grew as well in 10.0 mM as they did in the controls.

DDT Results

DDT is a most perplexing compound to work with. Its solubility

in water is very low, so it must be dissolved in a solvent before being

added to the growth medium. Unfortunately, at the highest concentration

used in this study, 50 ppm, the DDT precipitated when added to the medium.

This precipitate scattered light and made it impossible to obtain accurate

readings with the Spectronic 20 at that concentration. Therefore, only

visual readings are given for the growth of the isolates in 50 ppm DDT (Table X), but these follow the pattern for other concentrations for each isolate. For isolates 1 through 9, graphs of the effect of DDT on the growth curves, Figures 20-22, are given as usual for concentrations of 10 ppm and below where there was not the problem of interference from the DDT precipitate. Table XI gives visual estimations of growth for isolate 10. Because of the slow growth of several of the isolates in BBM2, observations were made additionally at 12 weeks for algae 1, 4, 7, 8, 9, and 10. The general effect of DDT was neutral, although in several cases there was a wide spread in the growth levels, especially at the final sampling point. The following isolates were either uneffected or only mildly effected: *Hormidium* (1), *Chlorococcum* (3) and (5), *Stichococcus chodati* (6) and unidentified (8). *Bracteacoccus* (4) and *Neochloris* (7) show a growth pattern with inhibition at the higher concentrations and either no effect or a slight stimulation at the lowest DDT concentrations. *Chlorella* (9) showed a neutral effect at the 2, 4, and 6 week sampling points, but a stimulation by DDT at the 8 and 12 week points. Finally, *Stichococcus chodati* (2) and *Hormidium flaccidium* (10) had depressed growth at all concentrations of DDT. Figure 22-10 shows the effect of DDT on the total of isolates 1-9. This indicates a rather mixed reaction over all with little systematic effect.

These results are comparible to those in the literature which found widely varying effects of DDT depending upon the organism being examined. Only *Chlorella* and *Chlorococcum* of the algae used in this study have been previously reported. Wiedeman (1964) described the effects of concentrations of DDT up to 20 mM (7090 ppm) on five isolates of *Chlorococcum*. One of these isolates grew as well in the highest concentration as in the controls (although the maximum growth was found

FIGURE 20

Effect of DDT on the growth of isolates 1 - 3. Abscissa - Time in
weeks; Ordinate - Optical density as measured on the Spectronic 20
at 425 nm. The numbers for graph 1 are applicable to the other two
graphs.

1.   *Hormidium* sp. (1)

2.   *Stichococcus chodati* (2)

3.   *Chlorococcum* sp. (3)

FIGURE 20 DDT

1

O.D.

0.5
0.4
0.3
0.2
0.1
0

2   4   6   8   12
Weeks

2

3
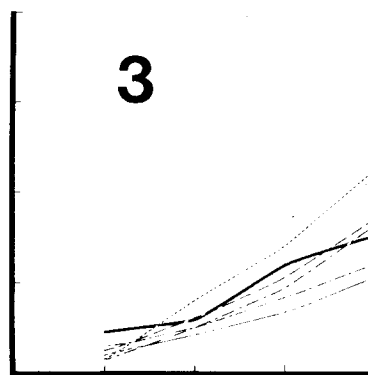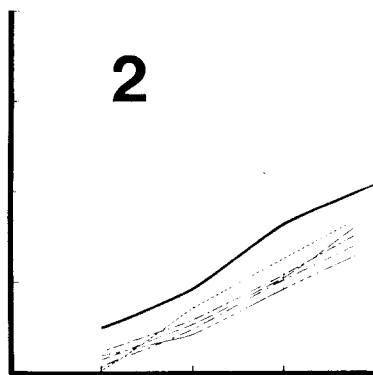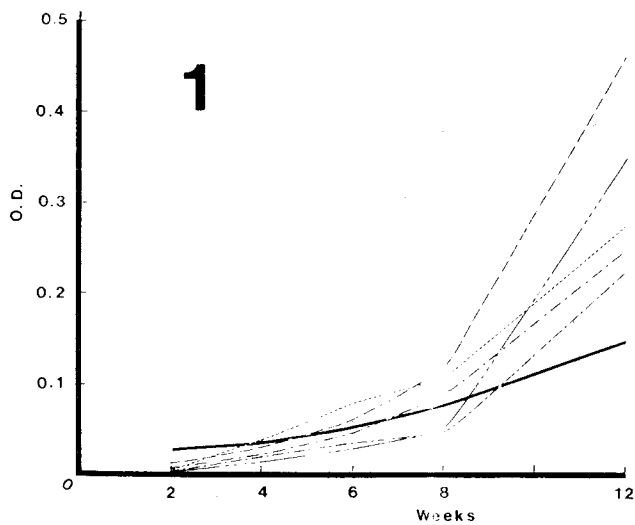
——————— 10 ppm

··················· 5 ppm

— ·· — ·· — 1 ppm

— ·· —— ·· 0.5 ppm

— ·· —— ·· 0.1 ppm

—————— 0 ppm (Control)

FIGURE 21

Effect of DDT on the growth of isolates 4 - 7.   Abscissa - Time in
weeks; Ordinate - Optical density as measured on the Spectronic 20
at 425 nm.   The numbers for graph 4 are applicable to graphs 5 and 7.

4.   *Bracteacoccus* sp. (4)

5.   *Chlorococcum* sp. (5)

6.   *Stichococcus chodati* (6)

7.   *Neochloris* sp. (7)

FIGURE 21 DDT
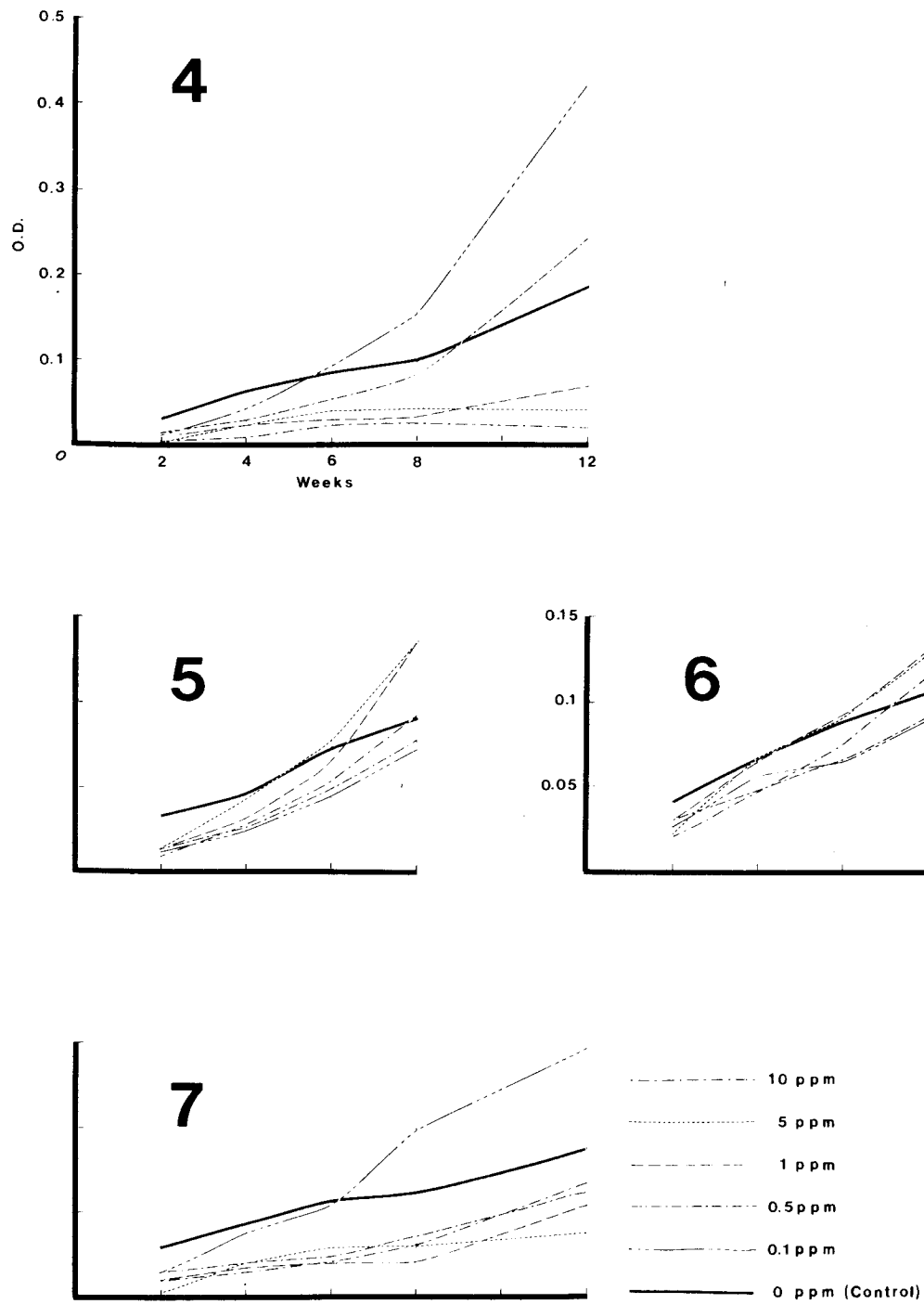
FIGURE 22

Effect of DDT on the growth of isolates 8 and 9 and a composite of
isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density
as measured on the Spectronic 20 at 425 nm. The numbers for graph 8
are applicable to the other two graphs.

8.   Unknown (8)

9.   *Chlorella* sp. (9)

10. Composite of isolates 1 - 9

FIGURE 22 DDT

Figure 8, 9, 10

Legend:
- 10 ppm
- 5 ppm
- 1 ppm
- 0.5 ppm
- 0.1 ppm
- 0 ppm (Control)
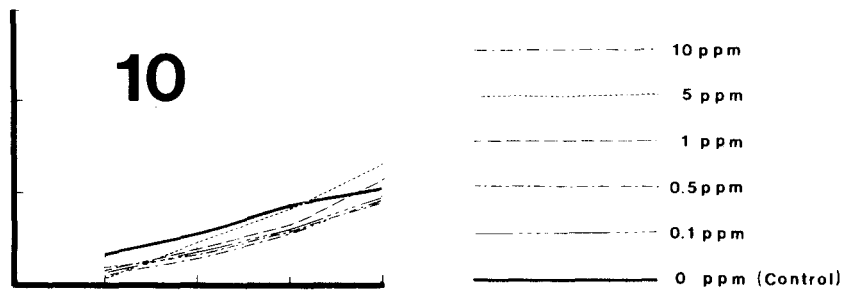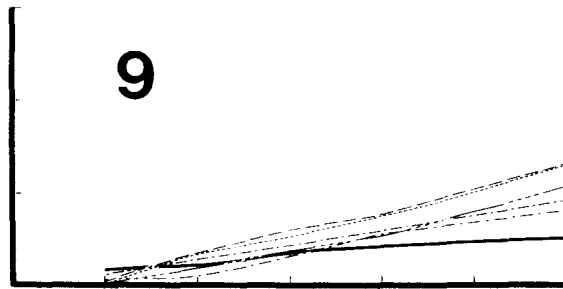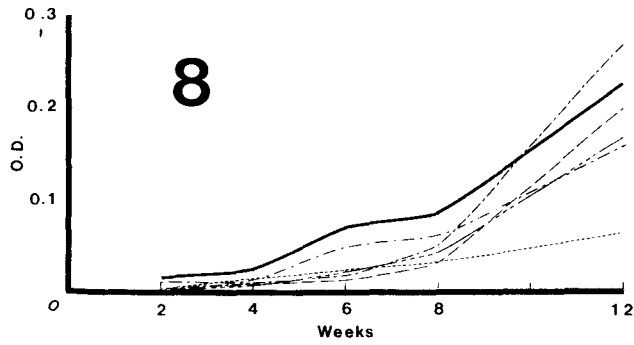
TABLE X

VISUAL ESTIMATIONS OF THE GROWTH OF ISOLATES 1 - 9
IN 50 PPM DDT

Weeks

Isolate #	2	4	6	8	12
1	T---	T---	T--	T-	T+
2	T---	T--	T-	T	
3	T--	T--	T-	T+	
4	N	T---	T---	T---	T---
5	T---	T--	T-	T	
6	T---	T--	T-	T	
7	N	N	N	N	T-
8	N	N	N	T--	F-
9	N	T---	T---	T--	T

TABLE XI

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN DDT

	Weeks				
Concentration	2	4	6	8	12
50 ppm	N	N	T---	T-	T
10 ppm	N	N	T---	T-	T+
5 ppm	N	N	T--	T	T
1 ppm	N	T---	T--	T	F
0.5 ppm	N	T---	T--	T	F-
0.1 ppm	T---	T--	T--	T+	F
0 ppm	T---	T--	T-	T+	F

in concentrations of 1.0 and 2.0 mM). Three other isolates could survive in the highest concentration, with greatly reduced growth, and two of those isolates could grow as well in 10.0 mM (3545 ppm) as in the control. The final isolate had a growth equal to the control at a maximum concentration of 2.0 mM (709 ppm). In four of the five cases, growth was stimulated by at least one of the concentrations, most commonly 1.0 and 2.0 mM. The results at the concentrations used in this study are comparable to those from Wiedeman (1964).

A number of studies have mentioned the effects of DDT on *Chlorella* sp. The reported results vary greatly. Less than 0.3 ppb DDT caused great morphological changes, but no growth effects (Södergren, 1968); while from 1.0 ppm to 100 ppm did not reduce growth even with varying pH's (Ukeles, 1962; Christie, 1969). Finally, Wiedeman (1964) reported that two of the six isolates of *Chlorella* which he investigated did not grow as well in the lowest concentration used (0.02 mM or 7.1 ppm) as they did in the control. The other four isolates were inhibited at concentrations of either 0.2 or 1.0 mM (70.9 or 354.5 ppm), and none of the isolates was stimulated by any concentration used. Two other studies have also reported little or no effect of DDT on *Chlorella* at low concentrations (Wilson and Choudhri, 1946; Falmer and Maloney, 1955). The present study found neutral effects for the first six weeks of observation and a stimulation for weeks eight and 12. This stimulation may not have been noticed in earlier studies since most of the studies reported in the literature were of much shorter duration (or of unspecified duration). However, the neutral effects for the first six weeks agree with most previous studies.

Rotenone Introduction

Rotenone is a botanical or natural compound with a biosynthesis similar

to that of the isoflavonoids (Fukami and Makajima, 1971) and the following

structure:
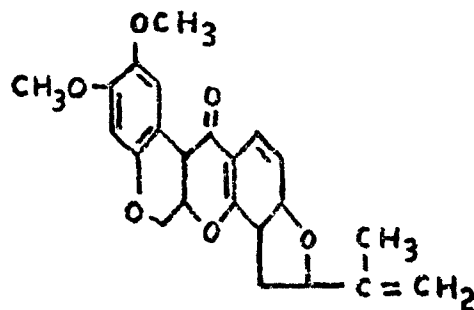
It is extracted from the dried roots of *Derris elliptica* in southeast

Asia and Indonesia and *Lonchocarpus* spp. in South America, Peru being

the chief source (Farm Chemicals Handbook, 1965). The crude extract

from the "tubaroot" includes a number of other derivatives, some of

which are not insecticidal (O'Brien, 1967). This extract has long been

used as a fish poison by Malayan natives; and in 1848, T. Oxley suggested

that it might be useful as an insecticide against leaf-eating caterpillars

(Fukami and Nakajima, 1971). In 1912, Nagai isolated the active ingre-

dient from tubaroot naming it rotenone; and in 1961, Miyano, Kobayashi,

and Matsue first synthesized it. In addition to rotenone, 10 related

compounds are known from plants of the legume family, some of which also

have insecticidal properties.

Rotenone is short lived as an insecticide (O'Brien, 1967), break-

ing down on exposure to heat or light to at least 20 different, mainly

noninsecticidal compounds, the major toxic one being $6\alpha\beta$, $12\alpha\beta$-rotenolone

(Cheng, et al., 1972). Toxicity is almost completely lost in as short a

period as 10 days due to decomposition or other chemical changes and not

to absorption and translocation in plants (Pagan and Morris, 1953).

Rotenone becomes firmly bound in the mitochondria (Yamamoto, 1969)

and acts by inhibiting the coupled oxidation of $NADH_2$ and the reduction

of cytochrome b, thereby blocking oxidation of all substrates oxidized by the NAD system (glutamate, α-ketoglutarate, pyruvate, etc.), but not interfering with the oxidation of succinate (O'Brien, 1967). The reason for its high toxicity to insects, fish, and pigs, but low toxicity to most mammals is still unknown, but differences in degradation rates have been proposed as a possible explanation.

Little research has been done on the effects of rotenone on nontarget species as its use has been greatly curtailed since the introduction of many synthetic insecticides in the last 30 years. Only 2,254 pounds (1022.4 kg) of the technical material were sold in Kentucky in 1968 (Moore, 1973). Rotenone does not leave harmful residues on vegetable crops (Farm Chemicals Handbook, 1965) and has no effect on cucumbers (Harcourt and Cass, 1955).

Several studies have noted effects on aquatic microorganisms. Hooper (1948) found a decrease in the number of protozoans after treating a lake with derris root (cited in Cope, 1965). Hoffman and Olive (1961) noted that rotenone at a concentration of 1.0 ppm applied to a lake reduced the number of entomostraca, rotatoria, and protozoa. Kiser, Donaldson, and Olson (1963) treated a shallow lake with 0.5 ppm rotenone and reported a complete removal of the open water zooplankton species for three months, a more gradual and shorter disappearance of the shore edge species, and only a reduction in numbers of those species in the dense weed patches. Of 42 cladoceran and copepod species in that lake none was permanently eliminated. A second lake was treated with 1 ppm of rotenone which penetrated to the thermocline at 30 feet (9.144 m) in six hours killing Cladocera and Copepoda as it sank. The zooplankton did not return to their preapplication level for several months after the

lakes had become non-toxic to fish and been restocked. None of these studies made any mention of the effects of rotenone on phytoplankton or other plants.

Rotenone Results

The most common effect of rotenone on algal growth was inhibition, but it was also neutral in some cases and stimulatory in others (Figures 23-25 and Table XII). These isolates which were definitely inhibited include *Stichococcus chodati* (2), *Chlorococcum* (3) and (5), *Bracteacoccus* (4), and unidentified (8). In several cases the pattern of inhibition was quite marked, with a gradual increase in growth from the highest concentrations to the control. That pattern is particularly apparent for isolates 3, 4, and 5. *Chlorella* (9) and *Hormidium flaccidium* (10) (Figure 25-9 and Table XII) were uneffected by rotenone as growth was almost identical at all concentrations and in the control. For the remaining three isolates, there was some stimulatory effect. *Stichococcus chodati* (6) was stimulated only at 10 and 50 ppm with the other concentrations being very close to the control. *Neochloris* (7) was stimulated by all concentrations considerably at eight weeks, slightly at six weeks, and hardly at all at weeks two and four. *Hormidium* (1) was stimulated by all concentrations slightly at four weeks and considerably at the six, eight, and 12 week periods. Since rotenone is known to break down in the light to several other compounds, it is possible that those organisms which are stimulated by its presence are actually not responding to the rotenone but to one of its breakdown products since the stimulation occurs only at the later observation times when breakdown is likely to have occurred. Figure 25-10 indicates the overall effect of rotenone across all algae studied. It confirms the above discussion indicating that rotenone

FIGURE 23

Effect of rotenone on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 1 are applicable to the other two graphs.

1. *Hormidium* sp. (1)

2. *Stichococcus chodati* (2)

3. *Chlorococcum* sp. (3)

FIGURE 23 Rotenone

Legend:
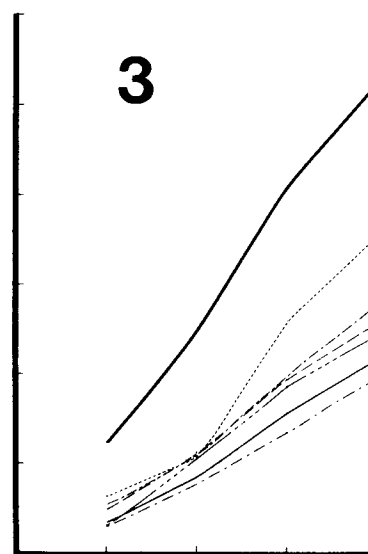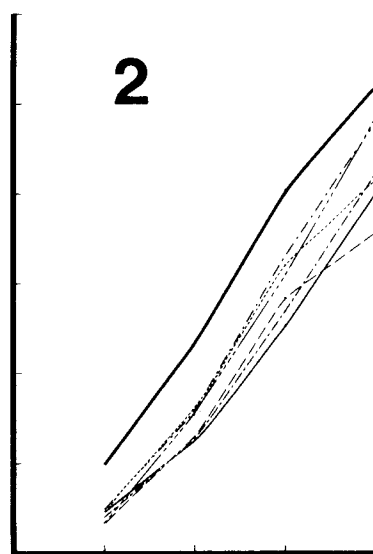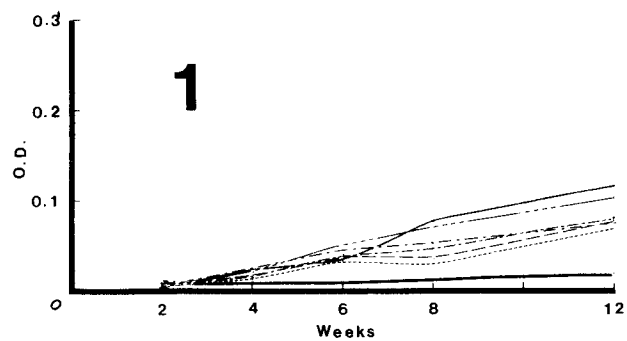
50 ppm

10 ppm

5 ppm

1 ppm

0.5 ppm

0.1 ppm

0 ppm (Control)

FIGURE 24

Effect of rotenone on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other three graphs.

4. *Bracteacoccus* sp. (4)

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

7. *Neochloris* sp. (7)

FIGURE 24 Rotenone

50 ppm

10 ppm

5 ppm

1 ppm

0.5 ppm

0.1 ppm

0 ppm (Control)
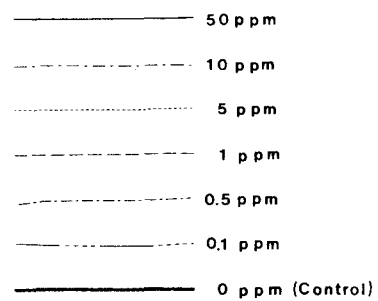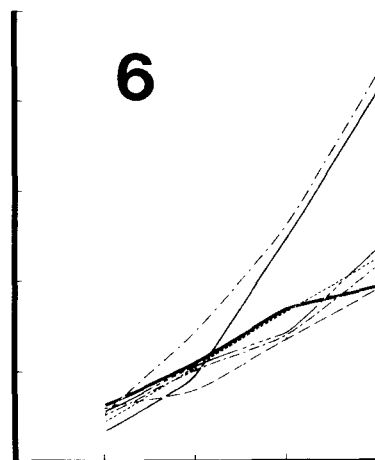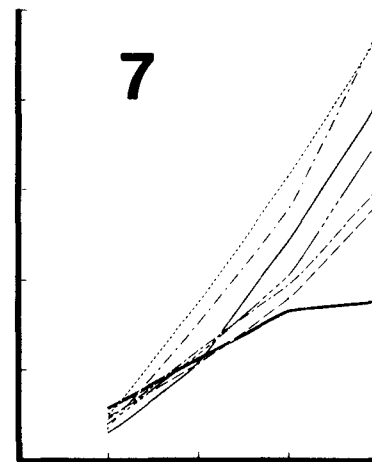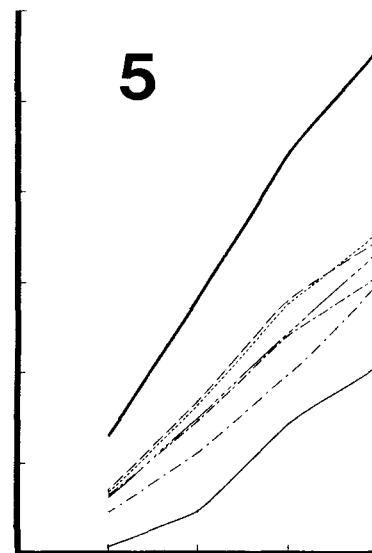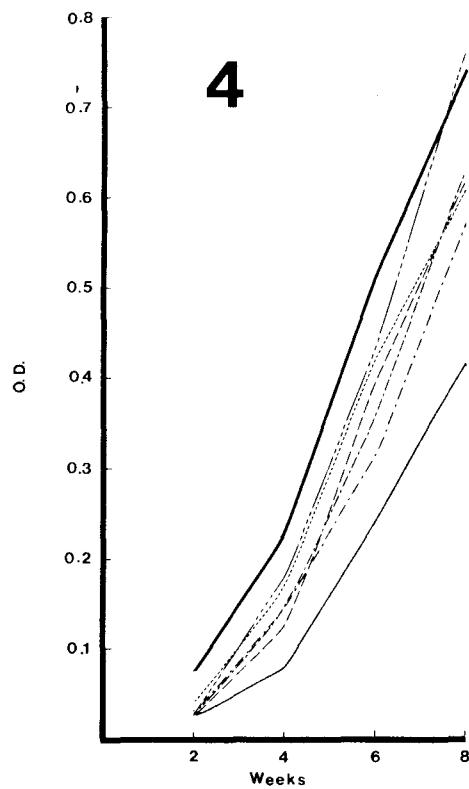
FIGURE 25

Effect of rotenone on the growth of isolates 8 and 9 and a composite
of isolates 1 – 9.   Abscissa – Time in weeks; Ordinate – Optical
density as measured on the Spectronic 20 at 425 nm.   The numbers
for graph 8 are applicable to the other two graphs.

8.   Unknown (8)

9.   *Chlorella* sp. (9)

10.   Composite of isolates 1 – 9

FIGURE 25 Rotenone

O.D. vs Weeks

8

9

10
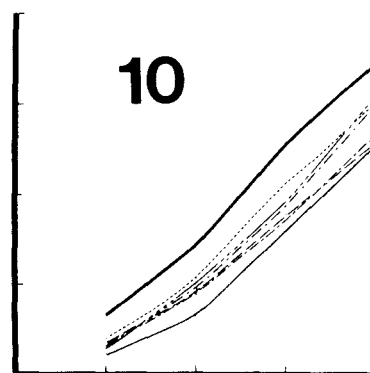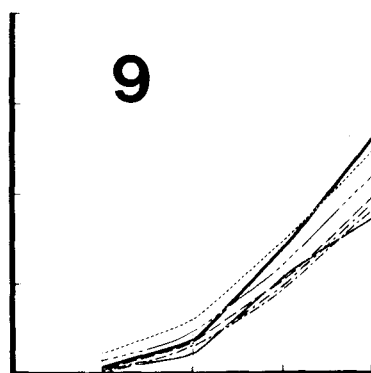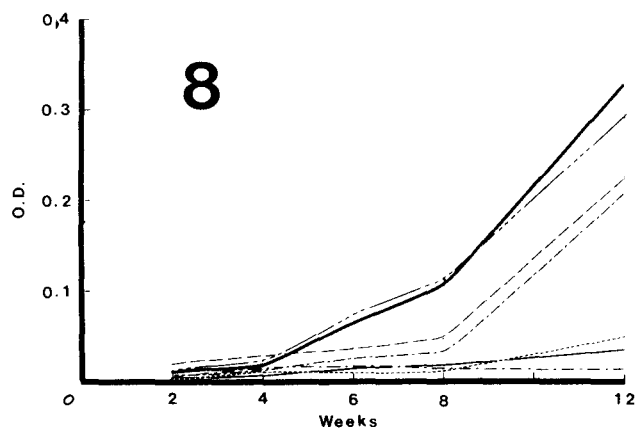
50 ppm
10 ppm
5 ppm
1 ppm

0.5 ppm
0.1 ppm
0 ppm (Control)

TABLE XII

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN ROTENONE

	Weeks				
Concentration	2	4	6	8	12
50 ppm	N	N	N	T---	T+
10 ppm	N	N	T---	T--	T
5 ppm	N	T--	T-	T+	F-
1 ppm	N	T---	T--	T-	T
0.5 ppm	N	T---	T--	T-	T
0.1 ppm	N	T---	T--	T-	T+
0 ppm	N	T---	T----	T-	T+

is more inhibitory than stimulatory to the isolates. The control is basically at a higher level than the experimental conditions, with the highest concentration being lower than the others.

Since no previous work has been reported on rotenone and algae, no comparison of the present study and past studies can be made. The general finding of an inhibition of zooplankton appears to be similar to the prevalent negative effect on growth which was found in this study.

Malathion Introduction

O,O-dimethyl S-bis [(carboethoxy)ethyl] phosphorodithioate, commonly called malathion, is a short-lived synthetic organophosphate pesticide (O'Brien, 1966). It was introduced in the early 1950's (Haller, 1952) and has had increased use in recent years as a replacement for many of the persistent organochloride pesticides, 51,467 pounds (23,345.4 kg) being sold in Kentucky in 1968 (Moore, 1973). The organophosphate insecticides as a group act by inhibiting acetylcholinesterase and, thereby, disrupting nerve function (Casida, 1974). Early studies (Metcalf and March, 1953) indicated that they were transformed *in vivo* by oxygen and enzymes to their active forms, as the highly purified insecticides *in vitro* did not greatly inhibit insect cholinesterase. Malathion has a low toxicity for mammals, 1375 mg/kg being the acute oral LD_{50} for rats (Farm Chemicals Handbook, 1965), while 100 ppm in the diet is considered safe for man (Spiller, 1961); and it is, therefore, used in preference to others of this group which are more toxic to humans.

In soil outdoors, malathion is very short-lived, but it may be more persistent in soil indoors (Spiller, 1961). There is no evidence of its volitilization from soils (Harris and Lichtenstein, 1961), but degradation there apparently by chemical hydrylosis is directly related to

adsorption and pH (Menzie, 1972). A national survey of croplands in 43 states (1684 sites) reported that 7.54% of the sites had been treated with malathion at an average rate of 0.17 lb/A (0.19 kg/H) (Wiersma, Tai, and Sand, 1972b). Of 66 samples tested for residues, only 2 (3%) had traces of malathion at rates of 0.04 and 0.36 ppm. Other reports on persistence included a 75-100% loss in bioactivity in one week (Kearney, et al., 1969); a persistence of only two days (Laygo and Schulz, 1963, cited in Pimental, 1971); an 85% reduction in three days and a 96.6% loss in eight days when applied at five lb/A (about 3.2 ppm) (Lichtenstein and Schulz, 1964); and even with very heavy applications (76.6 lb/A [85.86 kg/H] the first year and 16 lb/A [17.9 kg/H] the second and third years) complete disappearance at the end of one year (Roberts, et al., 1962). Wiersma, Mitchell, and Standford (1972) sampling onion fields for malathion residues reported that 13.6% had been treated with an average of 2.86 lb/A (3.21 kg/H), but found no residues either in the soil or in the onions grown in those fields.

Phytotoxicity to higher plants varies with the species from none (Haller, 1952; Roberts, et al., 1962; Spiller, 1961) to moderate (Haller, 1952; Clower and Matthysse, 1952; Stafford, 1954; Haviland and Highland, 1955; and Lichtenstein, et al., 1962) to outright killing (Clower and Matthysse, 1954); and the effects include lower yields (Gojmerac, 1957) and reduced germination (Starks and Lilly, 1955; Strong, et al., 1959, and Gojmerac, 1956 and 1957).

There appears to be little negative effect of malathion on soil fungi and bacteria, possibly because most spraying is done when the fungi are not active (Spiller, 1961). *Trichoderma viride*, a soil fungus, and *Pseudomonas* sp., a soil bacterium isolated from heavily sprayed northern Ohio soils, could metabolize malathion (Matsumura and Boush, 1966) while autoclaved soil did not break it down. Certain colonies of *T. viride*

from a culture collection had a very marked ability to cause the breakdown of malathion through the action of carboxylesterase(s). Degradation of malathion added to soil at 150 and 1500 ppm was indicated by a high level of CO_2 production and was increased by the addition of glucose (Bartha, et al., 1967). An initial inhibition of nitrification decreased with time as the chemical was either detoxified or a resistant nitrifying population developed.

Reports of the effects of organophosphates on algae are few. Methy parathion at 3 ppb applied to a lake three times between June 19 and August 7 caused a reduction in zooplankton followed by an *Anabaena* bloom which had not occurred the previous year when there was no treatment (Cook and Conners, 1963). Hurlbert, et al., (1972) reported a rapid increase in phytoplankton and a reduction in herbivores after treating several ponds with Dursban at 0.025 and 0.25 lb/A (0.028 and 0.28 kg/H). They found blooms of blue-green algae in four ponds and a diatom in a fifth pond and concluded that the changes in species were likely due to a reduction in grazing. Malathion at 7.25 ppm inhibited growth of *Euglena gracilis* by 48.9% in the light and 19.2% in the dark (Moore, 1970). Inhibition was less at lower concentrations and 0.15 ppm stimulated growth in the dark by 11.4%. Christie (1969) reported on the effects of malathion on several species of algae from waste stabilization ponds. After four days 100 mg/l caused growth effects ranging from complete inhibition of *Ankistrodesmus* to a 2/3 reduction for *Scenedesmus* and a very slight reduction for *Chlorella* to an increase of 2-1/2 times for *Euglena*. After seven days at the same concentration growth of *Chlorella* was greatly stimulated while *Scenedesmus*, *Euglena*, and *Schroderia* had growth reductions of 1/6, 1/3 and 1/2 respectively. Further experiments were carried out with axenic cultures of *Chlorella pyrenoidosa* in a defined medium. The pH of that

medium influenced the effect of 100 mg/l over a period of seven days; at
pH 9.0, growth was reduced for days one to five, but only slightly depres-
sed by day seven; while at pH 6.0, growth reduction was very slight for
all seven days. Lower concentrations, 0-10 mg/l, at pH 6.0 resulted in
less than a 10% growth reduction for *Chlorella*. The general conclusion
was that malathion was capable of altering the composition of a mixed algal
community from waste stabilization ponds, but would not display a persis-
tent inhibitory effect.

Malathion Results

Two different purities of malathion were used in this investiga-
tion. The first was 95% purity, the technical grade. This is the form
that is sold for public use, either diluted or mixed with an inert chemical.
The other, 99% purity, was laboratory grade. Figures 26-31 and Table XIII
show that the majority of the isolates were adversely effected by both
forms of malathion with some very dramatic reductions in the growth curves,
but no complete inhibition of growth at the concentrations used. *Stich-
ococcus chodati* (2), *Chlorococcum* (3) and (5), *Bracteacoccus* (4), and
unidentified (8) show a definite reduction in growth in both malathion 95
and 99. In some cases, isolates 2 and 4 in particular, there is a systema-
tic drop in growth as the concentration of malathion is increased, while in
other cases the presence of even a small amount of pesticide reduces growth
and additional pesticide does not decrease growth further. In both puri-
ties, *Stichococcus chodati* (6) has reduced growth at the first three
sampling points, but at the last time period grows as well in at least
one of the experimental conditions as in the control. This may indicate
an initial suppression which is overcome as the pesticide is either de-
graded or tied up in the cells. *Hormidium* (1), *Neochloris* (7), and

FIGURE 26

Effect of malathion - 95 on the growth of isolates 1 - 3. Abscissa -
Time in weeks; Ordinate - Optical density as measured on the Spectronic
20 at 425 nm. The numbers for graph 2 are applicable to graph 3.

1.   *Hormidium* sp. (1)

2.   *Stichococcus chodati* (2)

3.   *Chlorococcum* sp. (3)

FIGURE 26 Malathion-95

Legend:
- 50 ppm
- 10 ppm
- 5 ppm
- 1 ppm
- 0.5 ppm
- 0.1 ppm
- 0 ppm (Control)
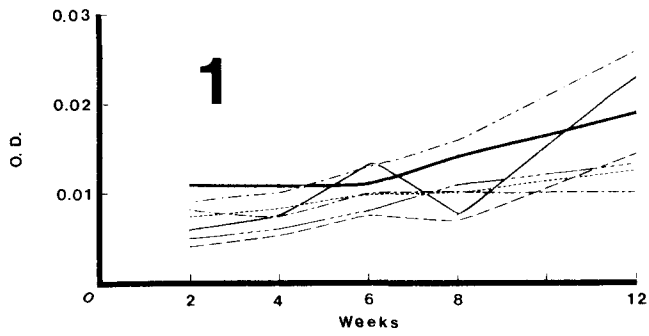
FIGURE 27

Effect of malathion – 95 on the growth of isolates 4 – 7. Abscissa – Time in weeks; Ordinate – Opitcal density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other three graphs.

4. *Bracteacoccus* sp. (4)

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

7. *Neochloris* sp. (7)

FIGURE 27   Malathion-95
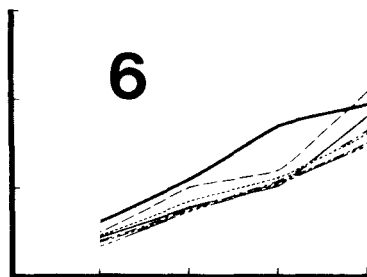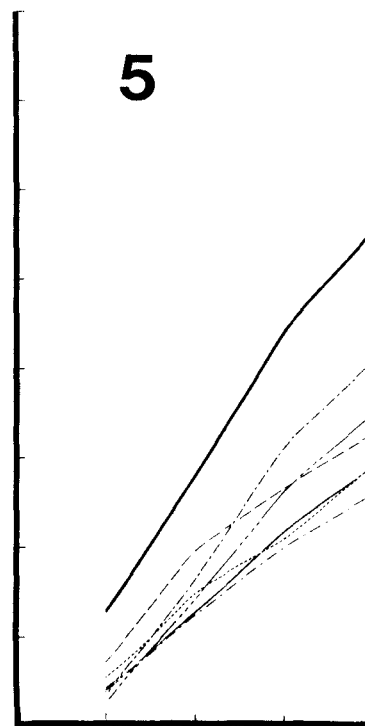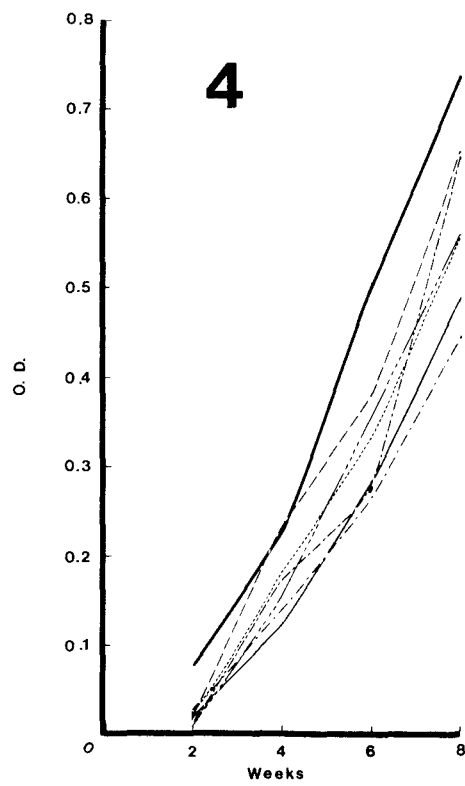
	50 ppm
	10 ppm
	5 ppm
	1 ppm

	0.5 ppm
	0.1 ppm
	0 Control

FIGURE 28

Effect of malathion-95 on the growth of isolates 8 and 9 and a composite of isolates 1 – 9. Abscissa – Time in weeks; Ordinate – Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

8. Unknown (8)

9. *Chlorella* sp. (9)

10. Composite of isolates 1 – 9

FIGURE 28 Malathion-95

8

9

10
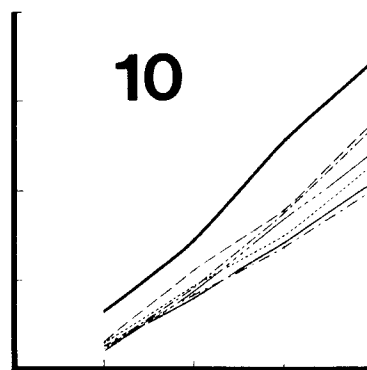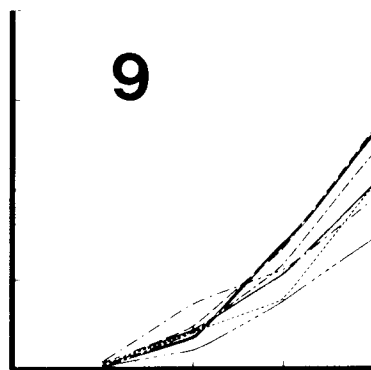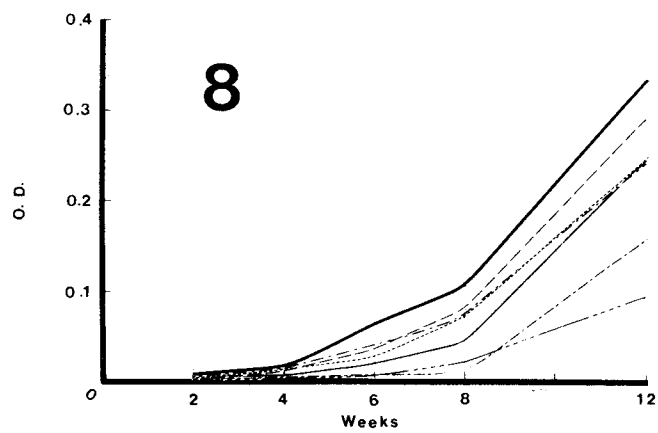
———————— 50 ppm	—·——·——·—— 0.5 ppm
—·——·——·——·— 10 ppm	—··——··——··— 0.1 ppm
······················ 5 ppm	———————— 0 ppm (Control)
—— —— —— ——· 1 ppm	

FIGURE 29

Effect of malathion-99 on the growth of isolates 1 - 3. Abscissa -
Time in weeks; Ordinate - Optical density as measured on the Spectronic
20 at 425 nm. The numbers for graph 2 are applicable to graph 3.

1. *Hormidium* sp. (1)

2. *Stichococcus chodati* (2)

3. *Chlorococcum* sp. (3)

FIGURE 29   Malathion-99

Legend:
- 50 ppm
- 10 ppm
- 5 ppm
- 1 ppm
- 0.5 ppm
- 0.1 ppm
- 0 ppm (Control)

103
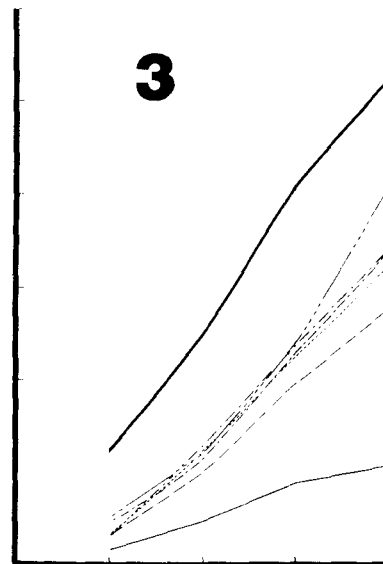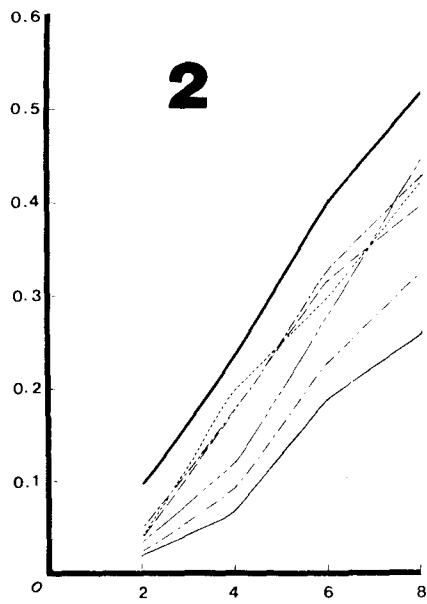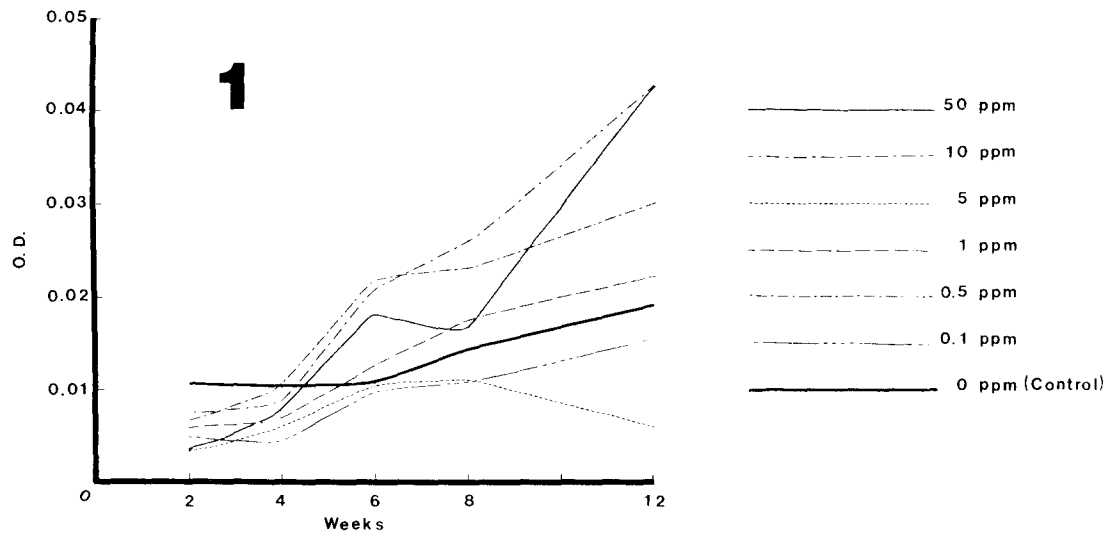
FIGURE 30

Effect of malathion-99 on the growth of isolates 4 - 7. Abscissa -
Time in weeks; Ordinate - Optical density as measured on the Spectronic
20 at 425 nm. The numbers for graph 4 are applicable to the other three
graphs.

4. *Bracteacoccus* sp. (4)

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

7. *Neochloris* sp. (7)

FIGURE 30 Malathion-99
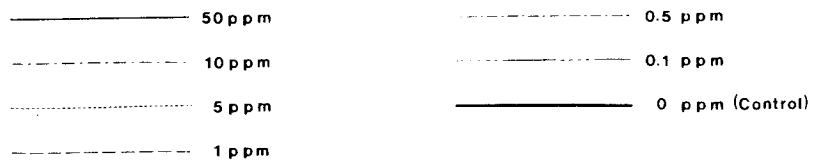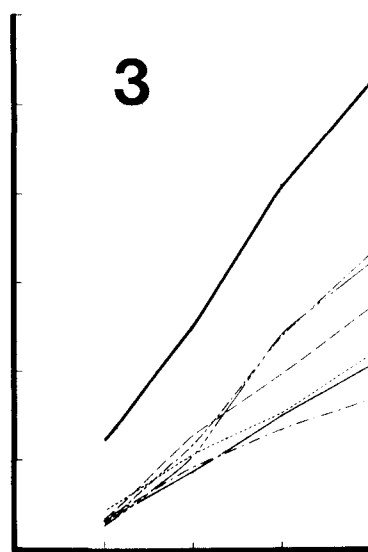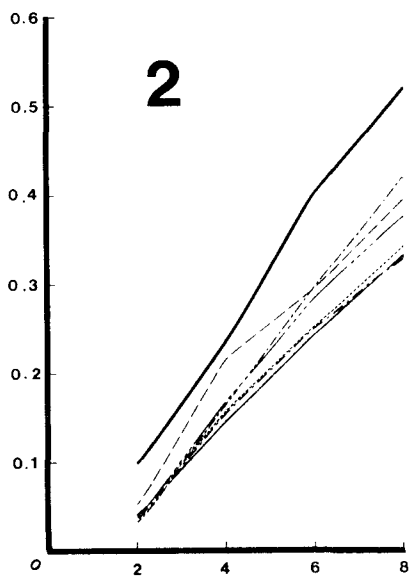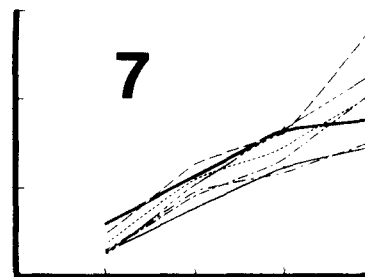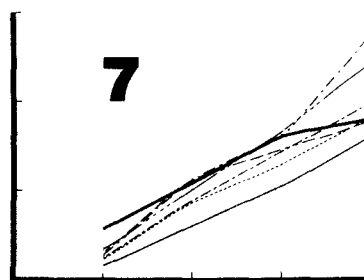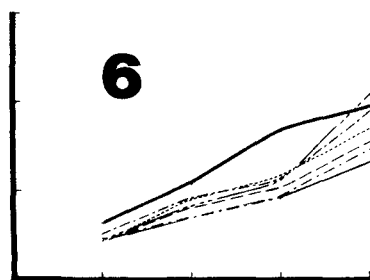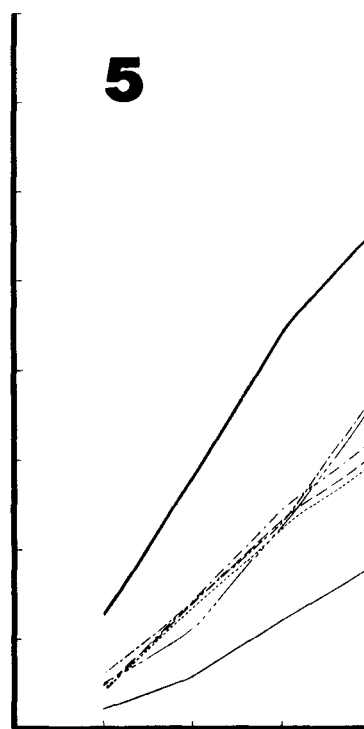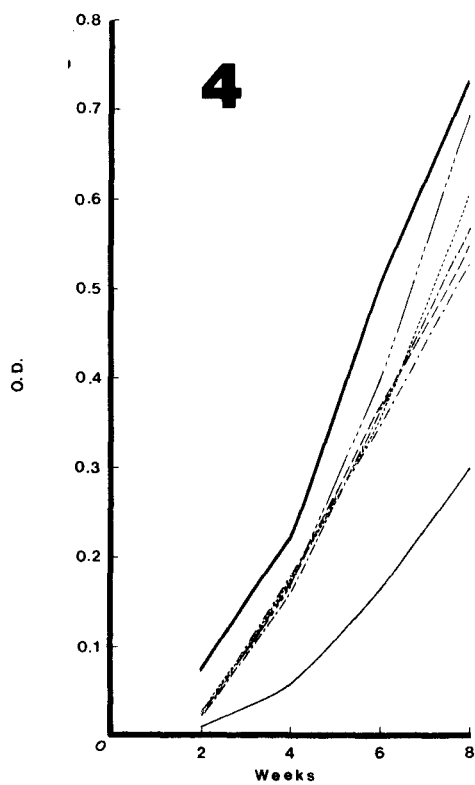
FIGURE 31

Effect of malathion-99 on the growth of isolates 8 and 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

8.   Unknown (8)

9.   *Chlorella* sp. (9)

10.   Composite of isolates 1 - 9.

FIGURE 31 Malathion-99
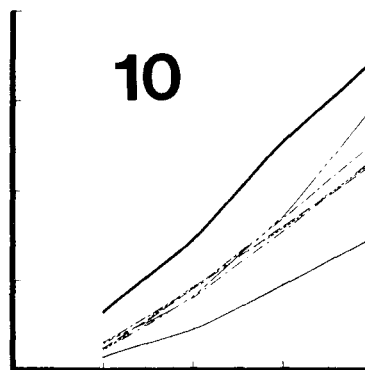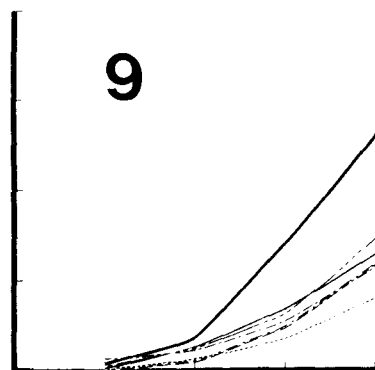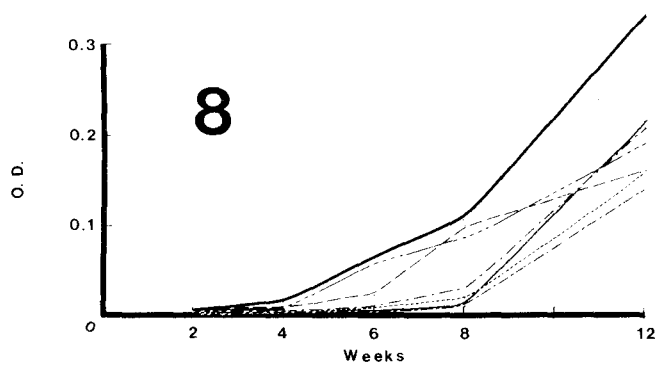
TABLE XIII

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN MALATHION-95 AND 99

Malathion-95 Concentration	Weeks 2	4	6	8	12
50 ppm	N	T----	T--	T--	T--
10 ppm	N	T----	T---	T--	T-
5 ppm	N	T----	T--	T-	T-
1 ppm	N	T----	T--	T--	T-
0.5 ppm	N	T----	T--	T--	T-
0.1 ppm	N	T----	T--	T-	T-
0 ppm	N	T----	T---	T-	T+

Malathion-99 Concentration	Weeks 2	4	6	8	12
50 ppm	N	T----	T--	T	T+
10 ppm	N	T--	T-	T+	F
5 ppm	N	T----	T-	T	T
1 ppm	N	T--	T--	T-	T
0.5 ppm	N	T--	T-	F	F+
0.1 ppm	N	T--	T-	T+	F
0 ppm	N	T----	T---	T-	T+

Hormidium flaccidium (10) are in general uneffected by malathion. All
have a slight depression in growth at the first sampling period, but by
the second data point growth in at least one concentration is equal to the
control and by six or eight weeks, there are no significant differences.
The only case of a significant difference in the response of an isolate to
the different purities is for *Chlorella* (9). In that case, malathion 95
does not cause a reduction in the growth, but the malathion 99 does. The
growth reduction in the malathion 99 is particularly striking at the six
and eight week sampling points.

These results appear to agree with the literature reports which
found varying effects depending on species. The only genus used in this
study which had been reported on before was *Chlorella*. In that case,
Christie (1969) reported that *Chlorella pyrenoidosa* was inhibited more by
100 ppm of malathion at pH of 9.0 than at a pH of 6.0, but that by day
seven, the difference was no longer significant. It is possible that the
differences found in this study between the effects of malathion 95 and
99 may be due to a slightly different pH of the medium or to changes in
the pH of the medium during growth. But since this study was over a much
longer period than that of Christie, a definite comparison cannot be made.
Further research is needed to clarify this point and investigate probable
causes.

Figures 28-10 and 31-10 indicate the overall effect of malathion
on all isolates tested. It confirms the negative effects described above,
with the control having a curve much above that of any of the experimental
concentrations, and the highest concentrations being lower than the
intermediary concentrations. One striking difference between the two
figures is that there is a significant gap between the 10 and 50 ppm
concentrations for malathion 99, but an overlap for these curves for

malathion 95. This may indicate a difference between the two purities at higher concentrations, with manufacturing impurities masking the effect of the 95% purity.

Dark Experiment-Results

The purpose of this experiment was to determine if any of the isolates could use any of the pesticides as a sole carbon source. Since the amount of plant mass in any isolate was very small, growth was measured only visually. The results are shown in TABLE XIV. In the control groups, the presence of ethanol did not result in any systematic growth increase. It is difficult to determine if the results indicated in TABLE XIV are true growth or simply the ability of the isolates to survive, but not divide, in those conditions in the dark, the latter being the more likely. Parker (1961) reported heterotrophic growth of several algae isolated from soil both in defined media with added sugars and in Texas soil-water flasks when a bacterium also isolated from the same soil was present. In the latter case, growth was much slower than in the glucose solution. At the end of 14 months, there was a 16-fold increase in algal cells over the original, indicating an average of only four divisions of each of the original cells. Therefore, it is possible that even if the algae used in this study could grow at a very slow rate in the pesticides in the dark, the two month incubation time would not have been long enough to show that. The concentrations of sugars used in Parker and in other studies on heterotrophic growth (Wiedeman, 1964; Wiedeman and Bold, 1965) are always much higher than that of the pesticides used in this study so that might also be a factor in the present lack of heterotrophic growth. One result of this experiment which is readily evident upon inspecting TABLE XIV is that none of the algae could survive in 10 ppm of DDT in

TABLE XIV

GROWTH OF ALGAE IN VARIOUS PESTICIDES IN THE DARK

Isolate #

Pesticide	1	2	3	4	5	6	7	8	9	10
Rotenone, 10 ppm	N	T--	N	T--	N	N	T---	N	N	N
Rotenone, 50 ppm	N	T--	N	T--	N	T---	T---	N	N	N
Malathion-99, 50 ppm	N	T--	T---	T--	N	N	T--	N	T---	T---
Malathion-95, 50 ppm	N	T--	T---	T--	N	T--	T--	N	T---	T---
DDT, 10 ppm	N	N	N	N	N	N	N	N	N	N
2,4,5-T, 50 ppm	N	T--	T---	T--	T---	T--	T---	T---	T---	T---
2,4-D, 50 ppm	N	T--	T---	T--	T---	T--	T--	N	T---	T---
Controls:										
TBIM	T---	T--	T---	T--	T---	T--	T---	N	N	N
TBIM-EtOH	N	T--	T---	T--	N	T--	T--	N	T---	T---
BBM$_2$	N	T--	N	T--	T---	T--	T--	N	T---	N
BBM$_2$ - Fe	N	T--	T---	T--	T---	T--	T---	N	T---	T---
BBM$_2$ - Fe + EtOH	T---	T--	T---	T--	T---	T--	T--	N	T---	N

the dark, even though they all could grow in this concentration in the light. This could not be due to the BBM2 medium or any of its variants as most of the isolates could survive in one or more of them.

No algae were found in this study which were able to use any of the pesticides as a sole carbon source and grow in the dark. It is, however, possible that an investigation geared primarily at isolating algae that could so use them would be successful. This is particularly likely for the herbicide 2,4-D which has been found to be readily broken down and used as a carbon source by many bacteria, fungi, and actinomycetes (Appendix III). Because of the similarity between blue-green algae and bacteria (Stanier, et al., 1971), a search for an alga capable of breaking down 2,4-D or other pesticides would probably most profitably start with representatives of that group isolated from soil which had been sprayed at some time with the pesticide in question. Another group which might provide an alga which can utilize pesticides as a sole carbon source is the chlorococcalean algae (Division Chlorophycophyta, Order Chlorococcales, Family Chlorococcaceae) since that group exhibited a great degree of heterotrophy in past studies (Parker, 1961).

Although none of the isolates grew heterotrophically in the dark with the tested pesticides, there were some cases of stimulation of growth in the light. Wiedeman (1964) and Wiedeman and Bold (1965) described five ways in which organisms responded to supplemental carbon sources. The organisms in this study would fall in either their category 2 (obligate autotrophs needing both light and air for growth) or category 3 (obligate phototrophs which can grow in light either aerobically or with air excluded), a distinction which cannot be made on the basis of the present study. It is possible that the organisms in this study which

were stimulated by one or more of the pesticides fall into category 3.
That is, they can use the pesticide as a carbon source to supplement the
natural carbon supply from carbon dioxide or can perhaps use it as a sole
carbon source if air is excluded, as long as light is present, but cannot
grow in the absence of light even in the presence of the stimulatory pes-
ticide. A second possibility is that the isolates which are enhanced by
the pesticides in the light are able to use them as a supplemental source
of carbon in a form of cometabolism, but cannot use them as a sole carbon
source in the dark. Further study will be needed to determine if either
of these hypotheses is correct.

Results - Overall Effects of All Pesticides on Each Isolate

Figures 32 and 33 show the average effects for each isolate for
all six pesticides and give a good indication of the general reaction of
each alga to pesticides. *Stichococcus chodati* (2), *Chlorococcum* (3) and
(5), *Bracteacoccus* (4), and *Chlorella* (9) are all definitely inhibited by
the addition of pesticides to the growth medium. Although the degree of
inhibition varies, these organisms all show a definite pattern with lower
growth in all concentrations of pesticides. In the case of isolates 3, 4
and 5, the inhibition is particularly orderly with the growth curves for
10 and 50 ppm falling below those for the other pesticide concentrations
which in turn are lower than that for the controls. *Stichococcus chodati*
(6) and unidentified (8) have patterns which are indefinite, not having
any obvious order to the different concentrations and the controls. This
is a particularly interesting difference between isolates 2 and 6 which
have been tentatively identified as the same species. Finally, *Hormidium*
(1) and *Neochloris* (7) have a pattern which indicates an overall stimula-
tion in growth due to the presence of pesticides. In both cases this is

FIGURE 32

Interactions of all pesticides on the growth of isolates 1 - 4.
Abscissa - Time in weeks; Ordinate - Optical density as measured on
the Spectronic 20 at 425 nm. The numbers for graph 2 are applicable
to graphs 3 and 4.

1. *Hormidium* sp. (1)

2. *Stichococcus chodati* (2)

3. *Chlorococcum* sp. (3)

4. *Bracteacoccus* sp. (4)

FIGURE 32 Interactions

Figure 1: O.D. vs Weeks
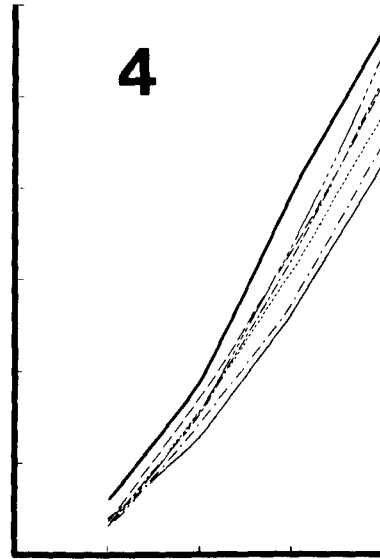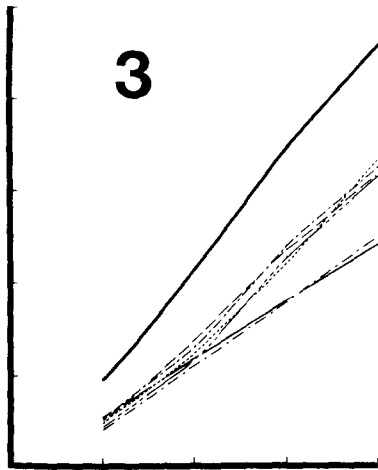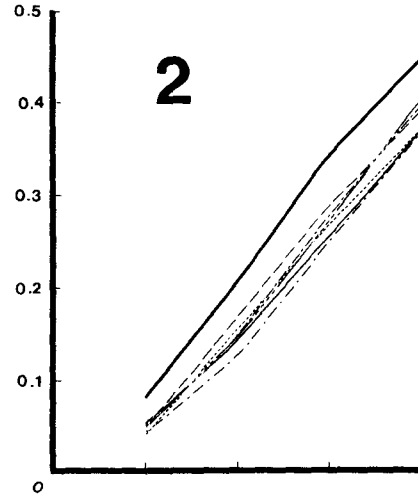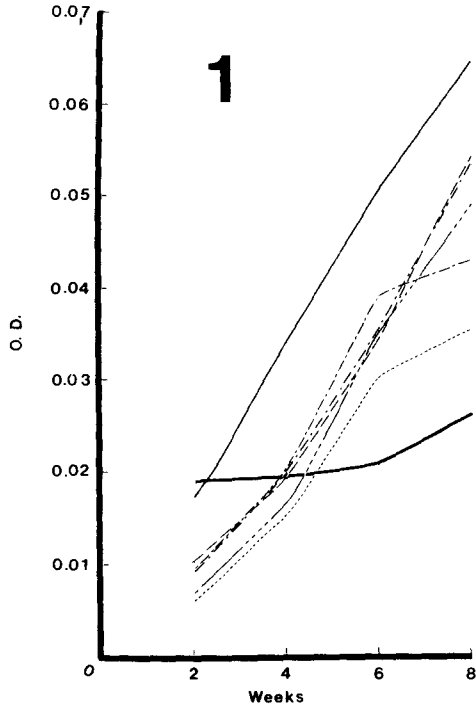
Legend:
- 50 ppm
- 10 ppm
- 5 ppm
- 1 ppm
- 0.5 ppm
- 0.1 ppm
- 0 ppm (Control)

FIGURE 33

Interactions of all pesticides on the growth of isolates 5 - 9.
Abscissa - Time in weeks; Ordinate - Optical density as measured
on the Spectronic 20 at 425 nm. The numbers for graph 5 are
applicable to graphs 6, 7, and 9.

5. *Chlorococcum* sp. (5)

6. *Stichococcus chodati* (6)

7. *Neochloris* sp. (7)

8. Unknown (8)

9. *Chlorella* sp. (9)

FIGURE 33 Interactions

5

6

7

8

9
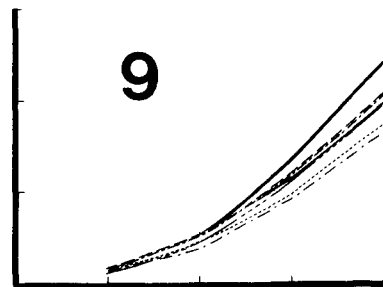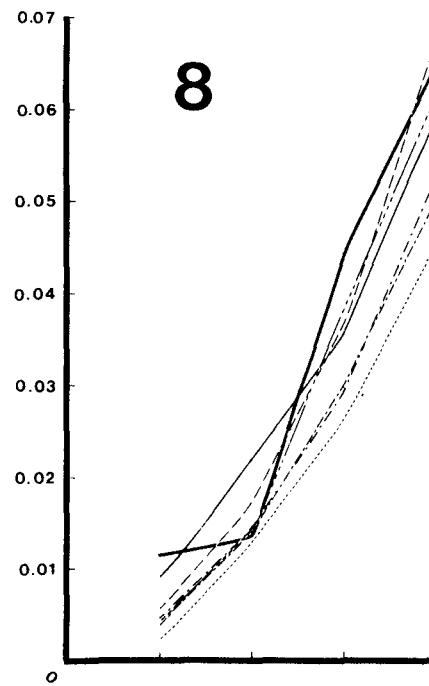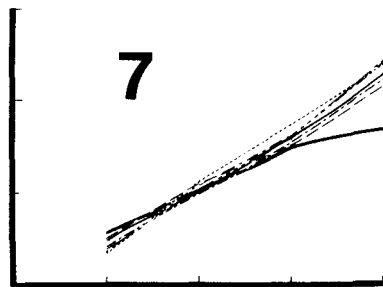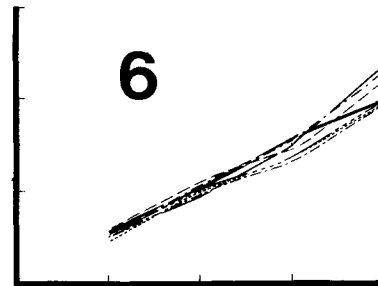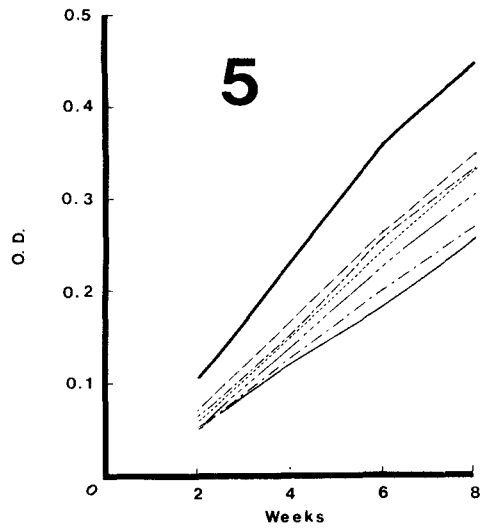
50 ppm

10 ppm

5 ppm

1 ppm

0.5 ppm

0.1 ppm

0 ppm (Control)

more marked at the later data points which might indicate an ability of these organisms to overcome any initial inhibition due to the pesticides and then use them to supplement their growth.

DISCUSSION

This section will be divided into four parts covering: 1) stimulation due to certain pesticides; 2) analysis of growth effects of each pesticide; 3) the possible natural interactions of these pesticides and soil algae; and 4) suggestions for further research.

Stimulation Effects

The stimulation of growth of some isolates by four of the five pesticides tested (only malathion did not increase the growth of any isolates) is interesting since it was shown that none of the algae can use the pesticides as a sole carbon source in the dark. Other workers have also reported increased growth in some cases with the pesticides used. Since the tubes in which the isolate were growing were not shaken, it is probable that the amount of CO_2 in the media was the limiting factor in growth. It is known that suboptimal photosynthesis rates in higher plants are often the result of a lack of CO_2 (Salisbury and Ross, 1969), and it is possible that this situation occurred in the tubes in the present experiment toward the end of the daily 12 hour light period. Since the stimulation of growth is most pronounced at the later sampling periods, there might be a greater deficiency of CO_2 as the number of photosynthesizing cells increased. Wiedeman (1964) and Wiedeman and Bold (1965) have shown that some algae can utilize supplemental carbon sources under varying conditions of light and air. Organisms which could grow either aerobically or with air excluded but only in the light were labeled obligate phototrophs. Wiedeman (1964) reported a photometabolic process for many of the algae he studied when they were grown in glucose, mannose,

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fructose, sodium acetate, and casein hydrolysate with exogenous carbon dioxide excluded. It is possible that the organisms in this study which were stimulated by the pesticides are obligate phototrophs, being able to take advantage of the supplemental carbon source (pesticide or ethanol) to increase their growth when the available CO_2 has been depleted or reduced to a very low level, but only in the presence of light. Further studies with CO_2 excluded or studies following the breakdown of the pesticides in the culture medium could clarify this point.

It is interesting to note that isolates 1, *Hormidium* sp., and 7, *Neochloris* sp., were both stimulated by 2,4-D, 2,4,5-T and rotenone, and were uneffected by malathion. The only pesticide toward which they reacted differently was DDT, with 1 being uneffected and 7 being inhibited by the highest concentrations used. These 2 isolates appear to be the most resistant to pesticides and would be the best species to test for possible pesticide degradation. Further studies of the means by which they are able to use a variety of pesticides to enhance their growth and to avoid the negative effects often found with other isolates could provide further insights into the pesticide-soil algae interaction.

Growth Effects

The five different pesticides used in this study fall into the following general sequence from least inhibitory to most inhibitory toward the growth of the isolates used in this study: 2,4-D, 2,4,5-T, DDT, rotenone, and malathion. Discussion of the possible mechanisms for these effects will follow in that order.

2,4-D

As early as 1950, 2,4-D was known to effect many plant processes (Norman, et al., 1950; Freeland, 1950; Van Overbeek, et al., 1951; Wort

and Cowie, 1953). However, in spite of 30 years of work, the mode of action of 2,4-D and the reason for its specificity for dicots over monocots is still not completely understood (Moreland, 1967; Salisbury and Ross, 1969). The current theory is that 2,4-D and other auxin type herbicides act at the nuclear level by enhancing synthesis of RNA and protein and, therefore, tissue proliferation. The extra synthesis induced by these herbicides might preclude normal cell development and function, thereby, being the basis for herbicidal action by upsetting the natural metabolic balances resulting in uncontrolled metabolism and death of the plant (Hilton, et al., 1963; Moreland, 1967; Key, 1963; Key, et al., 1960; Salisbury and Ross, 1969; Chrispeels and Hanson, 1962). However, many responses are observable within a few minutes of application, a period too short for RNA synthesis. Therefore, it may be that auxins and auxin-like herbicides act in more than one way (Moreland, 1967; Salisbury and Ross, 1969). Even if this hypothesis is correct for the mode of action of 2,4-D and other auxin-type herbicides, it does not account for 2,4-D's specificity for broad leaf plants. It is possible that auxin selectivity is associated with cyclic AMP which is known to be involved in the physiological responses of animal hormones which are often exceedingly specific (Salisbury and Ross, 1969). It is also possible that differential selectivity of 2,4-D is due to differential penetration, translocation, and inactivation rates in susceptible and resistant plants.

This study found that nine of the ten algae tested were either unaffected or stimulated by 2,4-D. From the preceeding discussion, it would appear than 2,4-D either does not penetrate into the algal cell or if it does, is rapidly detoxified there. Since 2,4-D is an auxin-mimicing herbicide, and auxins in general have little effect on algae, even though at least some algae are known to contain IAA (Salisbury and Ross, 1969),

it is not too suprising that 2,4-D is basically neutral toward algae.
The one isolate which was inhibited, *Chlorella* (9), may be more sensitive
to auxins and may not be able to breakdown those compounds readily when
they enter the cell or to keep them from entering.

The stimulation of two isolates, *Hormidium* (1) and *Neochloris* (7),
is interesting since it was shown that neither can use 2,4-D as a sole
carbon source in the dark. Other workers have also reported stimulation
of algal growth in some cases in the presence of 2,4-D. Respiration of
Chlorella pyrenoidosa was stimulated at concentrations comparable to those
used in this study (about 35 ppm, Erickson, et al., 1955), while Wedding,
et al., (1954) reported a stimulation of photosynthesis in the same algae
at pH of 7.0 and above (comparable to the pH of the TBIM medium in this
study) at 2,4-D concentrations of 2×10^{-3} M (442 ppm). Wiedeman (1964)
reported that two isolates, *Dictyosphaerium* 380 and *Scenedesmus* 410, grew
better at 2.0 mM 2,4-D than they did in the control. Therefore, it is
possible that the stimulation in the present study was due to similar
mechanisms with the increase in growth due to increased phytosynthesis
and respiration.

2,4,5-T

The general effects of 2,4,5-T in this study were found to be only
slightly different that those of 2,4-D, two isolates being inhibited by
2,4,5-T (*Chlorococcum* (3) and (5)) while only one was inhibited by 2,4-D.
2,4,5-T, like 2,4-D, is one of the chlorophenoxy herbicides and is,
therefore, similar to naturally occurring auxins. Structurally, the only
difference between 2,4-D and 2,4,5-T is that the latter has an additional
chlorine atom at the number 5 position of the benzene ring in place of an
hydrogen. This small structural difference, however, has a large

physiological difference. 2,4,5-T is very highly toxic toward woody plant species and is commonly used to remove undesired shrub growth. Because of a dioxin which results as a manufacturing impurity, 2,4,5-T was suspended from use around the home, near water, on food crops, and in South Vietnam in April, 1970. This dioxin is supposed to be very toxic to humans and other organisms. Since the 2,4,5-T used in this study was a purified form and not that generally sold in the market place, these dioxins should not have had any effect on the results reported here.

A comparison of Figures 14-16 and 17-19 shows that two isolates, *Chlorococcum* (3) and (5) are more susceptible to 2,4,5-T than to 2,4-D. The inhibition at concentrations of 10 and 50 ppm is quite obvious in both isolates in 2,4,5-T. The probable mechanism which results in inhibition due to 2,4-D in the algae is also probably acting in the case of 2,4,5-T so the analysis described above will not be repeated.

The isolates which were stimulated by 2,4-D, *Hormidium* (1) and *Neochloris* (7), were effected in the same way by 2,4,5-T. Isolate 1 has a greater stimulation due to 2,4,5-T while 7 is more effected by 2,4-D. The stimulation may be a result of photometabolism of 2,4,5-T under conditions of low CO_2 as was discussed in the first section of the discussion. The only reports on the effects of 2,4,5-T on physiological processes is that 1 ppm did not decrease productivity of a phytoplankton sample over a period of four hours (Butler, 1963, cited in Pimentel, 1971). Therefore, the effect on photosynthesis or other physiological processes is uncertain.

DDT

Although the insecticidal properties of DDT have been known for over 35 years, an understanding of its mechanism and selectivity at a

cellular level is still far from complete. Its primary effect is on the central nervous system (Moore, 1967), apparently in increasing the potassium permeability of the axons of the sensory nerves (O'Brien, 1967). The effects it has on plants may be related, but cannot be exactly the same since plants have no nerves! Reports on the effects of DDT at the cellular level with higher plants have shown a significant respiration reduction (Lichtenstein, et al., 1962) and a reduction in the light reaction of photosynthesis in susceptible varieties of barley (Lawler and Rogers, 1967). The latter reaction to DDT was controlled by a single major gene, susceptibility being dominant to resistance. With algae, DDT has been reported to reduce productivity of phytoplankton communities (Butler, 1963, cited in Pimentel, 1971); reduce C^{14} assimilation (Stadynk, 1967); reduce photosynthesis (Wurster, 1968); and block or delay cell division (Menel, et al., 1970; Bowes, 1972). Other studies have reported morphological changes and both growth stimulations and growth reductions from varying concentrations of DDT.

The present study found varying effects due to DDT depending upon the isolate under consideration. The inhibition found in four of the isolates tested here could be due to any of the mechanisms discussed above. However, the actual basis for this negative effect cannot be determined from the present study. The difference in susceptibility between the isolates, especially striking between the two isolates tentatively identified as *Stichococcus chodati* (2 and 6), is probably due to either differential breakdown rates or to differential effects within the cell. Resistance is probably not due to ability to keep the molecule from entering the cell since it has been shown that uptake is passive and very rapid even with dead cells (Södergren, 1968).

Rotenone

Rotenone acts by inhibiting the coupled oxidation of $NADH_2$ and the reduction of cytochrome b, a pathway common to all aerobic cells. It is not surprising then that it had negative effects on the growth of half of the isolates tested in this study. What is most surprising is that it was neutral toward two isolates and stimulated the growth of three others. Rotenone could have lost its effectiveness due to breakdown from exposure to light within the early part of the experiment. Some of the breakdown products are also toxic, and it may be that the isolates most affected are those that are inhibited both by rotenone and its toxic breakdown products. Those algae which were not effected may have a resistance to the breakdown products or may be able to modify them to other nontoxic chemicals. Two of the three isolates which were stimulated by rotenone were the same ones which were stimulated by 2,4-D and 2,4,5-T (*Hormidium* (1) and *Neochloris* (7)). These two isolates appear to be particularly well adapted to using supplemental carbon sources to increase their growth when light is present. The third isolate which was stimulated by rotenone was *Stichococcus chodati* (6) while the other isolate tentatively identified as the same species, (2), had a definite inhibition in growth due to rotenone. This difference may be due to difference in the physiological responses of the two strains. Finally, the pattern of stimulation of isolate 6 is interesting since it is stimulated only by the two higher concentrations and not by the lower ones indicating a possible threshold level for stimulation. Further studies will be necessary to determine the reason for the patterns of stimulation and the difference in response of two similar isolates.

Malathion

Malathion, an organophosphate, had the strongest effect of any of the pesticides tested, inhibiting seven isolates, being neutral to three and stimulating none. The effect of inhibition is quite systematic, with the highest concentrations inhibiting most and successively lower concentrations being less inhibitory. The organophosphates exert their inhibitory action in animals by reacting with cholinesterase (O'Brien, 1967). Specifically, the OH^- of a serine at the active site of cholinesterase combines with the phosphorous of the organophosphate, forming a covalent bond between the enzyme and the pesticide. Since esterases are common in algal cells, malathion likely exerts a negative effect on algae by tying up and inactivating essential esterase enzymes. This would explain the systematic effect regularly seen in this study. At higher concentrations more enzymes would be tied up with a greater disruption of the intermediary metabolism of the cells as a consequence. Organophosphates are activated *in vivo* when S-alkyl isomerization occurs (a CH_3 - group moves from an oxygen atom to the sulfur atom. This makes the phosphorous more positive than it is in the original form, and it is able to react more rapidly with esterases.) Another change which can take place *in vivo* is the complete replacement of the sulfur atom by oxygen. (For malathion, the resulting sulfurless compound is called malaoxon and is a more potent cholinesterase inhibitor than malathion). Christie (1969) found that at least some of the C^{14} malathion added to an axenic culture of *Chlorella pyrenoidosa* was converted to malaoxon. Therefore, a similar conversion may have occurred in the algae in this study which were inhibited by malathion.

Carboxyesterases and phosphatases are known in mammals and insects which degrade malathion to malathion acid (which can be further cleaved to

malathion diacid) or to dimethyl phosphorothioate. Degradations of this type may also be possible in algae and may account for the few isolates which were found to be uneffected by malathion. Certain compounds are known which can, however, tie up the enzymes which are responsible for this hydrolysis and, therefore, increase the toxicity.

Therefore, it is probable that the inhibitory effect of malation is due to its combining with essential esterases in the algal cells. Those organisms which are not effected are probably better able to convert malathion to nontoxic compounds or to decouple the enzyme-insecticide bond. That no cases of stimulation were found indicates that none of the isolates studied were able under any conditions to profit from the presence of malathion, even if it were degraded.

Natural Interactions

As S. J. L. Wright has stated (1971):

> The microbiologist usually aims to study herbicide degradation
> under defined conditions, using pure microbial cultures and pure
> chemicals rather than commercial formulations. Such conditions
> are often so far removed from those in the soil environment
> as to render laboratory studies quite artificial. This is an
> unfortunate admission for physiological-ecological investiga-
> tions and indicates that information obtained from *in vitro*
> experiments cannot necessarily be projected with certainty to
> the field where the microbial population and environment are
> infinitely more complex.

The factors which must be considered for a full study of the effects of a given pesticide on a given species include; 1) the properties of the pesticide; 2) the manner of its application; 3) the extent to which the species contacts the pesticide; 4) the response of the species (acute and chronic toxicity); 5) information on the factors which control the population size of the species in the absence of the pesticide; and 6) toxicity information on the pesticide's effects on any species which control the given species in the absence of the pesticide. If all this

information were known, then a hypothesis could be made on the effect the pesticide may have on the species in nature (Moore, 1967). Since the present study does not include all these factors, the caution of Wright must be kept in mind during the speculation which follows.

Of the five pesticides tested in this study, the two with the most harmful effects, rotenone and malathion, are also the ones which are least persistent. (However, DDT, the most persistent pesticide, is nearly as inhibitory). Therefore, the effects of these two pesticides, although they may be temporarily drastic, may not be long lasting. But it must be remembered that even sublethal effects may have a potentially great ecological importance. In addition, there are indirect effects which a pesticide may have on any one species; delayed toxic effects, reduction of habitat, and removal of symbionts, competitors, and grazers (Moore, 1967). Because none of these pesticides had a uniform effect across all the algae tested, they could alter the species composition of a mixed soil population and have some of the indirect effects noted above. As early as 1953, Aldrich suggested that 2,4-D might change the composition of microorganism populations with repeated applications by encouraging the growth of organisms which can decompose 2,4-D and use it as a carbon source while at the same time reducing the populations of other organisms which are susceptible to its effects. Cook and Conners (1963) and Hurlbert, et al. (1972) reported changes in natural species composition following application of different organophosphates. Christie (1969) found that malathion altered the species composition of a mixed algal community associated with waste stabilization ponds. Similarly the presence of DDT caused changes in species proportions in a mixed culture of two algal species (Mosser, et al., 1972) and in natural algal populations (Lazaroff and Moore, 1966; Woodwell, et al., 1967; Batterson, et

al., 1972; Shane, 1948; and Bridges, et al., 1963). Kiser, et al. (1963) and Hooper (1948, reported in Cope, 1965) found reductions in zooplankton in lake environments due to the presence of rotenone. So the present pesticides, even when there are no direct effects, may change the species composition of the soil algae community.

It appears from this study that none of the algal isolates by themselves would be able to break down the pesticides in the soil. It is, however, possible that the algae could in concert with other micro-organisms cause a change in structure of the chemicals which would lead to their gradual breakdown and disappearance. Alexander (1965b) indicates that microorganisms cannot always detoxify and decontaminate soils at a sufficient rate to keep the level of even readily biodegradable pesticides low. And since some of these chemicals are modified in various ways by different microbes, they may not have the same toxicities in every soil. Studies, therefore, must also focus on all potential detoxification products and how they effect soil microorganisms.

Therefore, although it is difficult to say with great certainty from this study alone, it is probable that the pesticides tested would have an effect in nature on the species tested and would alter the ecological balance of the soil microflora.

Suggestions for Further Research

This study, like most research, while answering a few questions has raised many new ones. Efforts should be made to expand the present study to include organisms of different algal groups, especially the blue-greens and diatoms which are common members of the soil microflora. Also, work should be done using mixed populations to determine what effects the pesticides have on diversity of the soil flora and the ef-

fects that interactions of more than one organism isolated from the same habitat can have on the pesticides. Further work could be done using technical grades of the pesticides studied here to determine if there are differences due to the presence of manufacturing impurities. A concerted effort should be made, particularly among the blue-green algae, to find isolates which can use pesticides, especially 2,4-D as a sole carbon source. Along this line, a dark experiment of 12-14 month duration could be performed to determine if any of the isolates could use the compounds as a sole carbon source in the dark, but at a very slow rate. Tests could be undertaken with the organisms which were stimulated by the pesticides to determine if they are actually breaking down any of the pesticides to any extent. Other studies could evaluate the ecological effects of pesticide interaction (potentiation). Also, a study to determine the mechanisms of resistance of certain isolates would be informative. Finally, detailed morphological and physiological studies may find effects due to the presence of pesticides which were not apparent from the present study.

While this study was only a beginning, it has shown that there are definite interactions between soil algae and pesticides which have not been considered before. These interactions have been found even at concentrations normally used as pesticidal levels and not only at the very high levels often used in studies of this type. Future studies could involve soil scientists, biochemists, bacteriologists, mycologists, and phycologists working together to determine the ultimate fate of pesticides in the environment and their effect on the soil microflora.

SUMMARY AND CONCLUSIONS

A study of the effects of five different pesticides on soil algae
was made. This project involved isolating into axenic culture ten algae
from soil collected in Oldham and Jefferson Counties, Kentucky, and then
determining the effects of different concentrations of each pesticide on
the growth curve of each isolate over an eight or 12 week period, and also
determining if any of the isolates could use any of the pesticides as a
sole carbon source.

The algae isolated into unialgal, bacteria-free (axenic) culture
were, with one possible exception, members of the Division Chlorophycophy-
ta. Isolate 8, still unidentified, gave a very weak or negative response
for starch when tested with potassium iodine. It is, therefore, possible
that isolate is a member of the Xanthophycophyta. Two basic growth media
were used, TBIM for most of the experiments and BBM2 for the experiments
with DDT. All organisms, except for #1, grew better in TBIM than in BBM2,
possibly because that was the medium used for isolation purposes or
because the algae were better able to grow in an alkaline than an acid
medium.

Statistical analysis revealed that the three way interaction of
pesticide-algae-concentrations was significant at all data collection
points. Therefore, the two way interactions of pesticide-algae, pesti-
cide-concentration, and algae-concentrations and the main effects must be
interpreted with caution. Due to the significant interactions, the re-
sults of this study cannot be generalized to other algae, pesticides, or
concentrations.

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The results of each pesticide in the light may be summarized as follows:

1) 2,4-D was basically neutral, inhibiting one isolate, stimulating two others, and showing neutral effects toward the other seven.

2) 2,4,5-T was also basically neutral: it inhibited the growth of two algae, stimulated two others, and had no effect on the growth of the other six.

3) DDT had mixed effects: negative effects were found with four isolates, stimulation with one, and neutrality with the other five.

4) Rotenone also had mixed effects: inhibiting five isolates, stimulating three, and having no effect on the other two.

5) Malathion was used in two purities, 95% and 99%, the first being technical and the second laboratory grade. Except for isolate 9, the results were the same with both purities. There were six cases of inhibition, three cases of neutrality, and none of stimulation. Isolate 9 was inhibited by one of the purities but not the other.

The results of the dark experiment showed that none of the isolates could use any of the pesticides as a sole carbon source to sustain growth in the dark at a level comparable to that in the light. At least some of the isolates could, however, survive for a period of eight weeks in all the pesticides except DDT. At the end of eight weeks there were no visible traces of algae in any of the tubes containing DDT.

Other observations and results can be summarized as follows:

1) In at least some cases, the isolates could use the pesticides to supplement their growth. This is probably a result of phototrophy since they could not use any of the pesticides to support their growth in the dark.

2) Isolates 1, *Hormidium* sp., and 7, *Neochoris* sp., are best able to withstand the presence of pesticides and to use them to supplement their growth.

3) Although four of the pesticides in this study (all but DDT) are rapidly broken down in the soil, repeated applications could still have an effect on the soil microflora. Changes in the proportions of different algal species could occur as a result of stimulation of certain organisms and inhibition of others.

4) Finally, suggestions for further studies were made.

APPENDICES

APPENDIX I

DECOMPOSITION TIME FOR 2,4-D UNDER LAB CONDITIONS

Time for Decomposition	Conditions	Reference
3-8 weeks	wet and dry soil	Jorgensen and Hamner, 1948
2 weeks - 18 months	wet and dry storage	Mitchell and Marth, 1946
6 weeks		Hans, 1946
120 days	lake water	Aly and Faust, 1964
3 to over 7 months (95% disappearance)	different soils	Montgomery, et al., 1972
24 hours (81-85%)	lake muds previously adapted	Aly and Faust, 1964
2-3 weeks		Jensen and Peterson, 1952
10 days		Whiteside and Alexander, 1960
6-7 days		Bell, 1957
less than 13 weeks		Kries, 1947
8, 11, or 21 days; 6-7 days	varying concentrations; retreatment	Newman and Thomas, 1950
23, 26, or 94 days	different soils	Alexander and Aleem, 1961
less than 8 weeks	greenhouse, unleached	DeRose, 1946
14 days		Audus, 1951
11 or 15 months	dry storage, different concentrations	Weaver, 1948
67 days	in greenhouse	DeRose and Newman, 1948
40, 80, or 160+ days	different dosages and soil pHs	Martin, 1946
over 7 weeks		Taylor, 1947
over 6 months	organisms from activated sludge	Schwartz, 1967

APPENDIX II

DECOMPOSITION TIMES FOR 2,4-D UNDER FIELD CONDITIONS

Time for decomposition	Conditions	Reference
less than 93 days		DeRose and Newman, 1948
over 45 days		Weaver, 1948
less than 80 days		DeRose, 1946
1 month		Matsumura, 1972
4-6 weeks	95% disappearance	Edwards, 1964
1 or 4 weeks or longer	different soils and doses	Ogle and Warren, 1954
6 weeks; 5 weeks	untreated soil; soil treated previous year	Newman, et al., 1952
1-4 weeks		Klingman, 1961 (cited in Pimentel, 1971)
4-18 weeks		Hernandez and Warren, 1950 (cited in Pimentel, 1971)
2 or 6 weeks	different concentrations	Cope, et al., 1970

APPENDIX III

ORGANISMS WHICH CAN USE 2,4-D AS A SOLE CARBON SOURCE

Organism	Reference
Bacterium globiforme type	Audus, 1950, 1951; Audus and Symonds, 1955
Rhizobium meliloti	Nilsson, 1957 (cited in Audus, 1964)
Flavobacterium aquatile	Jensen and Peterson, 1952; Jensen, 1960 (cited in Audus, 1964)
F. peregrinum	Steenson and Walker, 1957, 1958
Achromobacter sp.	Bell, 1957, 1960; Steenson and Walker, 1957, 1958
Pseudomonas sp.	Fernley and Evans, 1959
Aspergillus niger	Woodcock, 1964
Corynebacterium sp.	Rogoff and Reid, 1956
Mycoplana sp.	Walker and Newman, 1956
Penicillium sp.	Mickovski, et al., 1968
Scopulariopsis brevicaulis	Mickovski, et al., 1968
Fusarium neoceras	Mickovski, et al., 1968

Unidentified:

Gram-negative bacterium, looked like *Corynebacterium*	Jensen and Peterson, 1952
Gram negative, motile rod	Evans and Smith, 1954
Bacterium	Loos, et al., 1967

APPENDIX IV

MICROORGANISMS INVOLVED IN AT LEAST ONE STEP OF DDT BREAKDOWN

Microorganism	Reference
Yeast	Kallmand and Andrews, 1963; and Ledford and Chen, 1969
Lake plankton	Miskus, et al., 1965
Proteus vulgaris	Barker, et al., 1965
Nocardia sp.	Chacko, et al., 1966
Streptomyces aureofaciens	Chacko, et al., 1966
S. cinnamoneus	Chacko, et al., 1966
S. viridochromogenes	Chacko, et al., 1966
S. albus	Chacko, et al., 1966
S. antibioticus	Chacko, et al., 1966
Escherichia coli	Wedemeyer, 1966
Klebsiella pneumonial	Wedemeyer, 1966
Aerobacter aerogenes	Wedemeyer, and Kearney, et al., 1969
23 plant pathogenic and saprophytic bacteria	Johnson, et al., 1967
Trichoderma viride	Matsumura and Boush, 1968; and Patil, et al., 1970
Gastrointestinal microflora of the rat	Braunberg and Beck, 1968
Geotrichum sp.	Ledford and Chen, 1969
Rumen microorganisms	Fries, et al., 1969
Hydrogenomonas sp.	Focht and Alexander, 1970, 1971; Focht, 1972; Alexander, 1972; Pfaender and Alexander, 1972
Pseudomonas sp.	Patil, et al., 1970
P. aeruginosa	Cope, 1965
P. fluroscens	Cope, 1965

APPENDIX IV (Cont'd)
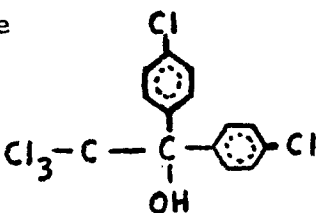
Microorganism	Reference
Bacillus sp.	Patil, et al., 1970
Micrococcus sp.	Patil et al., 1970
Mucor alternans	Anderson, et al., 1970
Monilaceae sp.	Focht, 1972
Arthrobacter sp.	Pfaender and Alexander, 1972
Unidentified soil microbe	Patil, et al., 1970
Gram positive rod	Ledford and Chen, 1969

APPENDIX V

DDT METABOLITES

Metabolites	Reference
Dicofol	Matsumura and Boush, 1968; Anderson, et al., 1970 Patil, et al., 1970
TDE (DDD) (2,2-bis(p-chlorophenyl)-1,1-di- chloroethane)	Ledford and Chen, 1969 Ko and Lockwood, 1968b Kallman and Andrews, 1963 Miskus, et al., 1965 Barker, et al., 1965 Wedemeyer, 1966, 1967b Chacko, et al., 1966 Johnson, et al., 1967 Guenzi and Beard, 1967 Braunberg and Beck, 1968 Fries, et al., 1969 Johnson, et al., 1972 Anderson, et al., 1970 Patil, et al., 1970 Matsumura and Boush, 1968 Ott and Gunther, 1965 Matsumura, et al., 1971 Menzie, 1972 Pfaender and Alexander, 1972
DDE (1,1-dichloro-2,2-bis(p- chlorophenyl)ethylene)	Anderson, et al., 1970 Matsumura and Boush, 1968 Wedemeyer, 1967b Ott and Gunther, 1965 Matsumura, et al., 1971 Menzie, 1972 Pfaender and Alexander, 1972
DDA (2,2-bis-(p-chlorophenyl)acetate)	Menzie, 1972 Wedemeyer, 1967b Patil, et al., 1970 Anderson, et al., 1970
DBP (4,4'-dichlorobenzophenone)	Menzie, 1972 Anderson, et al., 1970 Pfaender and Alexander, 1972 Wedemeyer, 1967b, 1967a
DDDE	Ott and Gunther, 1965
DDNS (1-bis(p-chlorophenyl)-ethane)	Matsumura et al., 1971
p-chlorobenzoic acid	Menzie, 1972

APPENDIX V (Cont'd)

Metabolite	Reference

Kelthane

$Cl_3-C-\underset{\underset{OH}{|}}{C}$ (with two chlorophenyl groups, one para-Cl above and one para-Cl to the right)

Menzie, 1972

1,1-bis(p-chlorophenyl)ethane — Anderson, et al., 1970

p-chlorophenylacetic acid — Alexander, 1972
Focht and Alexander, 1970, 1971
Pfaender and Alexander, 1972

DDMS (1-chloro-2,2-bis(p-chloro-phenyl)ethane) — Pfaender and Alexander, 1972
Wedemeyer, 1967b

DDMU (1-chloro-2,2-bis(p-chloro-phenyl)ethane) — Wedemeyer, 1967b

DDNU (unsym-bis(p-chlorophenyl)ethylene) — Wedemeyer, 1967b

DPM (Dichlorophenylmethane) — Wedemeyer, 1967a

DBH (Dichlorobenzhydrol) — Wedemeyer, 1967a

Phenylacetic acid — Focht and Alexander, 1971

2-phenyl-3,3,3-trichloroproprionic acid — Focht and Alexander, 1971

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VITA

Martha Scheer Salk, first child of Frederick August Scheer, Jr. and Alice Lepay Scheer, was born in Detroit, Michigan on April 16, 1945. She attended public elementary and secondary schools in Dearborn, Michigan, and graduated from Edsel Ford High School in June, 1963. She matriculated at Albion College, Albion, Michigan, the following September and graduated in June, 1967, with highest honors and departmental honors in biology. While at Albion, she received many academic honors including membership in the Albion College Fellows, the highest academic honorary on the Albion campus, and was elected to the Beta of Michigan chapter of Phi Beta Kappa. In February, 1967, she was married to Gary Clive Salk. She received her Master of Science degree in botany from the University of Iowa in February, 1969 where she was the recipient of an NDEA Title IV Fellowship for the period from September, 1967 to August, 1968. From September, 1969 to May, 1970 she taught general biology parttime at Dutchess Community College in Poughkeepsie, New York. She enrolled at the University of Louisville in January, 1971 and received her Ph.D. from that institution in May, 1975.

Permanent address: Gilbert Associates, Inc.

Reading, Pennsylvania