University of Montana

## ScholarWorks at University of Montana

Graduate Student Theses, Dissertations, & Professional Papers

Graduate School

1976

# Toxicity of Zectran on microalgae subjected to natural environmental stress

William Reid Molina
*The University of Montana*

## Recommended Citation

TOXICITY OF ZECTRAN ON MICROALGAE

SUBJECTED TO NATURAL ENVIRONMENTAL STRESS


By

William R. Molina

B.S., University of Oregon, 1973


Presented in partial fulfillment of the requirements
for the degree of
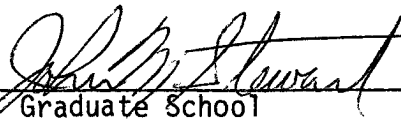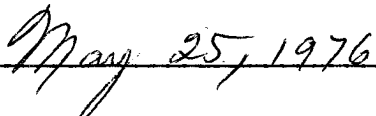
Master of Science

University of Montana


1976


Approved by:

_R. Sheridan_
Chairman, Board of Examiners

_John M. Stewart_
Dean, Graduate School

_May 25, 1976_
Date

UMI Number: EP34534

# UMI

### Dissertation Publishing

# ProQuest

# ABSTRACT

Molina, William R., 1976                    Environmental Studies

Toxicity of Zectran on Microalgae Subjected to Natural Environmental Stress

Director:  Dr. Richard Sheridan  $\mathcal{R} \cdot P \mathcal{S}$

Toxic effects of the carbamate insecticide, Zectran, on photosynthetic rates of unicellular green algae, Chlorococcum sp. were assayed over a range of light intensities, temperatures and pH values to observe changes in tolerance under conditions of physiological stress.  At optimum conditions of 25 C, 1500 ft-c. and pH 7.5, Chlorococcum exhibited a tolerance threshold for photosynthesis and respiration between 1 and 10 parts per million (ppm) Zectran.  One ppm Zectran showed no toxic effects at the stress temperatures 7 and 38 C.  Toxicity greatly increased, however, with increasing time and temperature at 10 and 15 ppm.

Light intensities of 250, 1500, and 10,000 foot candles (ft-c.) were used as suboptimal, optimal and supraoptimal conditions respectively.  Again, 1 ppm showed no toxic effects over 24 hours at sub-, supra- or optimal light intensities.  At 10,000 ft-c., the higher concentrations 10 and 15 ppm showed 13 - 20% greater inhibition than at lower intensities.

Exposure to 10 and 15 ppm for 1 hour at pH 10 reduced photosynthesis by 33% and 50% respectively over the control rates.

Permutations of stress conditions were tested at the three Zectran concentrations.  Photosynthesis was inhibited 27% by 1 ppm after 24 hours at conditions of 38 C and 10,000 ft-c.  At all concentrations, combinations of high light and high temperature had similar detrimental effects.  Temperature extremes proved to have the most influence governing toxicity.  Low light reduced the toxic response of Chlorococcum and low temperature provided the greatest degree of protection for the organism.  Exposure at pH 10 was protective at low light and temperature and detrimental at high light and temperature.  In most cases, as the period of exposure increased, toxicity also increased.

The results indicate that environmental conditions at the time of pesticide application can have an important effect on nontarget organism response.

## ACKNOWLEDGMENTS

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

# LIST OF FIGURES

CHAPTER I

INTRODUCTION

Increased variety and use of chemical pesticides over the past
four decades has stimulated research concerning the role of these chem-
icals in the environment. The persistency and world-wide distribution
of DDT (1,1,1- Trichloro-2,2- bis(p-chlorophenyl) ethane) were primary
factors involved in directing the emphasis of pesticide development
towards chemicals which are toxic to only a narrow range of organisms
and which chemically degrade into non-toxic substances. Research in
the development of such pesticides is a complex task involving monitor-
ing of the chemical breakdown of the pesticide in air, soil, water,
and biological tissues and testing the toxicity of the pesticide and
its breakdown products on target and non-target organisms. While the
number of organisms employed in toxicological studies has expanded,
the methodology of tests has never been varied to include the possible
effects that environmental conditions might have on an organism's
ability to tolerate poisons. Pesticide tolerance is generally investi-
gated under conditions favorable to the organism's growth and well-
being. In nature, however, an organism is not always favored with
optimum conditions and consequently may not respond in the same manner
to pesticide concentrations deemed acceptable in the laboratory.

Although usually neglected in pesticide studies, algae are of
primary importance in aquatic ecosystems. Detritus and algae are the

1

major basic foodstuffs of aquatic animals (Hynes, 1963). Algae also play an important role in the oxygenation of water and in nutrient, mineral, and carbon cycling. During the course of the annual solar cycle in temperate lakes, attached algae and phytoplankton are subjected to temperature fluctuations, nutrient, oxygen and carbon dioxide concentration extremes and hydrogen ion concentration variations. Certain minimum and maximum levels of these physical parameters constitute the limits of favorable conditions for the physiological processes of different algae. An alga exposed to conditions approaching its threshold of minimum requirement or maximum tolerance of these physical conditions, is under stress, i.e., its physiological processes are not operating at optimum, and its tolerance to biological or chemical disturbances may be narrowed.

This study was thus undertaken to test the hypothesis that environmental stresses do affect an organism's tolerance to a pesticide. The carbamate insecticide, Zectran$^{(R)}$ (4-dimethylamino-3, 4-xylylmethyl-carbamate), has been used as an effective substitute for DDT in control of the western spruce budworm (Choristoneura occidentalis) and was the pesticide employed in this study. Furthermore, this investigation was initiated to achieve the following specific objectives: 1. To determine light intensities, temperatures and hydrogen ion concentrations which constitute stress conditions to the green alga Chlorococcum sp.. 2. To test the hypothesis that natural stresses as suboptimal or supraoptimal light intensity, temperature and hydrogen ion concentration act to alter the toxic threshold of the pesticide Zectran on freshwater

algae.  3.  Test combinations of stress factors at various Zectran concentrations to evaluate their effects on Zectran toxicity.

CHAPTER II

REVIEW OF THE LITERATURE

Research has shown that Zectran (Figure 1) degrades in ultraviolet
or sunlight (Abdel-Wahab and Casida, 1967) and that under field condi-
tions, its residue levels decrease within 24 hours (Pieper and Miskus,
1967). The $LD_{50}$ (amount of toxicant required to kill 50% of the test
population) of Zectran for spruce budworm is 1 µg/g body weight (Rob-
erts et al., 1969). Survey studies conducted to determine Zectran's
potency on mule deer, avians, bullfrogs (Tucker and Crabtree, 1969),
and aquatic insects have shown these organisms to be relatively toler-
ant to the pesticide (Gibson and Chapman, 1972). Zectran is a choli-
nesterase inhibitor, and therefore interferes with normal transmission
of nerve impulses. Zectran's action is of short duration and does not
produce cumulative effects (Lang and Miskus, 1967).
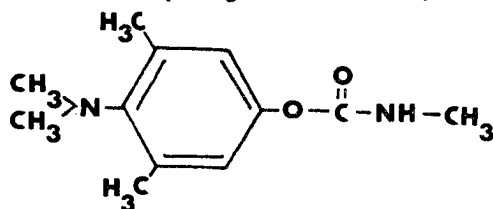


Figure 1. Structural formula for Zectran.

Work done by members of the Forest Service's Insecticide Evaluation
Project in Berkeley, California, shows that Zectran is absorbed in iso-
lated plant cells by diffusion and is not actively pumped into the cell
cytoplasm. Work by this group also shows that the diffusion rate of

4

Zectran through the cell membrane was less than that of the fat-soluble insecticides such as DDT (Anon., 1970).

Zectran is especially safe to fish. It is one of the least toxic chemicals tested on game fish (U.S. Fish and Wildlife Service, Anon., 1970).

Two recent reports (Snyder and Sheridan, 1974; Sheridan and Simms, 1975) have shown either growth or photosynthetic and respiratory rates in eleven species of algae to be inhibited by Zectran concentrations between 1 and 10 parts per million (ppm).

Lazaroff (1966) screened several hundred algal isolates for sensitivity to the pesticides Lindane, Dieldrin and DDT. The results indicated that chlorinated insecticides, at levels as low as 1 ppm, might select for resistant cells within populations of unialgal cultures. Lazaroff concluded that these selective effects could be expected to influence the composition of natural algal populations.

Wurster (1968) found that DDT concentrations as low as a few parts per billion (ppb) in water reduced photosynthesis in laboratory cultures of four coastal marine species and in a natural phytoplankton community. He warned that phytoplankton population changes caused by DDT could have insidious effects of more ecological importance than the obvious, direct mortality of larger organisms.

Menzel (1970) conducted a study on the effects of DDT, Dieldrin and Endrin on the photosynthesis and growth rates of four marine phytoplankton. He found that tolerance to these insecticides by algae ranged from high in Dunaliella to low in Cyclotella. He postulated

that Dunaliella's resistance was due to its adaptation to unstable estuarine conditions.

Ukeles (1962) measured growth of five species of marine phytoplankton in the presence of 17 different toxicants including bactericides, solvents, insecticides, fungicides and herbicides. The carbamate compounds Sevin and Nabam were tested and concentrations of 10 ppm were lethal to all species tested. Members of the class Chlorophyceae were found to be most resistant to the toxicants tested. Ukeles determined that even the most sensitive organisms could withstand exposure to sublethal concentrations of toxicants for long periods of time by maintaining dormancy. Cells resumed growth and reproduction when transferred to basal medium without toxicant.

Relatively little research has as yet been done investigating the interaction of physical influences with toxicants and algae. Most of the work which has been done concerns the influence of temperature and this literature is reviewed by Cairns et al., (1975). They describe the interaction between hydrogen ion concentration and temperature in hot springs. A number of blue-green algae inhabit hot springs at temperatures above 70 C, while the sole photosynthetic organism in habitats with a pH less than 5 and temperatures greater than 50 C is the eucaryotic Cyanidium caldarium. Whether or not this same pH-temperature relationship exists for mesothermal algae is not documented.

In working with metallic elements and the blue-green alga Plectonema boryanum, Harvey (1967) showed that the alga concentrated different elements at different rates depending on the temperature. Similarly, Jacobsen (1971) showed that the rate of $^{144}$Cs uptake by

Chlamydomonas increased 15-fold between 4 and 22 C.

Enhanced toxicity of antibiotics to algae is achieved at elevated temperatures. Cairns et al. note that less antibiotic is needed to inhibit growth of organisms maintained at supraoptimal temperatures.

Experimental work concerning the effect of hydrogen ion concentration on the toxicity of chemicals on algae is minimal. Brooks (1925) showed that the diffusion rate of arsenite into cells of Valonia macrophysa is lowest at pH 7 and increases under alkaline conditions. Wedding and Erickson (1957) showed that the concentration of 2,4-dichlorophenoxyacetic acid needed to achieve 50% inhibition of growth in Chlorella pyrenoidosa was lowest at pH 4 and increased with increasing pH. These results support the theory of Simon and Beevers (1952) that weak organic acids penetrate cell walls chiefly in their undissociated form.

The range of bioassay techniques used in toxicity studies of algae includes: motility, gamete production, cytoplasmic streaming, protein synthesis, $CO_2$ fixation, growth, $O_2$ uptake or production (Cairns et al., 1975), pigment extraction (Stepanek et al., 1961), fluorescent microscopy (Hannemann, 1975), cell lysis and detachment in the case of attached algae (Braginsky, 1965).

In an attempt to characterize environmental stress, the biological effects of light and temperature have been reported by a number of investigators. McCombie (1960) found that growth rates in Chlamydomonas reinhardi were steadily reduced with incremental decreases in light intensity down to 75 foot candles (ft-c.). Sorokin and Krauss (1962) studied the combined effects of temperature and illuminance on Chlor-

ella growth rates of synchronous cultures. The authors found that at
light intensities below 100 ft-c. (less than 1/29 the saturation illu-
minance) cell growth was temperature dependent. They observed that the
lower the temperature, the greater the effect increased light intensi-
ties had on growth inhibition. At the optimum growth temperature, in-
creasing light intensity was not inhibitory to 3,000 ft-c. Temperatures
only 3 C above those optimal for growth caused a 45% reduction in growth
rate and a reduced light saturation value. Cells growing under sub-
and supraoptimal temperatures could not maintain their maximum growth
rates for as long as cells at optimum temperatures. Cells used in this
experiment were not acclimated to experimental conditions. The authors
postulated that the destructive effects caused by high light and low
temperature may be due to the inadequate rate of chlorophyll synthesis
at low temperatures.

Jitts et al. (1964) reported that algae cell division showed a
stress response with greater inhibition of the growth rate occurring at
high light intensities when the cells were at sub- and supraoptimal
temperatures. This effect has been cited to explain phytoplankton suc-
cession (Jitts et al., 1964). Succession is favored when the water
temperature rises above the optimum range of one species into the opti-
mum range of another, and the physiological optimum of the algae at
these temperatures depends on the mean prevailing light intensity.

That plants can adapt physiologically to a wide range of light
intensities is well known. Rabinowitch (1952) has pointed out that
shade-adapted plants often contain a greater concentration of photo-

synthetic pigment per unit area or volume than the corresponding sun-adapted species. Shade-adapted plants have a steeper initial photosynthesis / light curve resulting from more efficient light absorption by the higher concentration of pigments. With increasing light intensities the photosynthetic enzyme / pigment ratio changes and cells develop mechanisms to dispose of the excess photosynthetic products above those used in the usual dissimilatory processes (Myers, 1946). Extremes in light intensity to which algae cannot successfully adapt do occur. Very low intensities can remain below the compensation point, and very high light intensities photo-oxidize chlorophyll resulting in inhibition of photosynthesis.

The rate of photosynthetic inhibition increases very rapidly as temperature approaches the tolerable limit. Ouellet (1951) has examined this phenomenon and suggests that a key step is blocked at that temperature, possibly by the heat denaturation of an enzyme or by the alteration of some structure in the cell. Chloroplasts are known to become rapidly inactivated near 45 C (Holt and French, 1946).

Hutchinson (1967) points out that the nature of the action caused by sub- and supraoptimal conditions tends to be asymmetrical. At conditions less than optimum, retardation of growth is characteristic, while at supraoptimal conditions, active inhibition, injury and death intervene.

Hydrogen ion concentration can cause stress to algal cells directly by altering intercellular pH or permeability of the cell membrane to nutrients. Indirect effects occur by induced changes in the

ionic form of nutrients in the medium (Ouellet and Benson, 1952).
Hydrogen ion concentration regulates the distribution of carbon dio-
xide among the chemical species $H_2CO_3$, $HCO_3^-$, and $CO_3^{--}$. High pH
values shift the bicarbonate equilibrium towards carbonate, but the
growth and survival of many algae seem to be endangered only at pH
values exceeding 10 (Steemann Nielsen, 1955).

CHAPTER III

MATERIALS AND METHODS

## Source of Experimental Cells

Chlorococcum sp., growing in a gelatinous mass, was collected in Rattlesnake Creek, Rattlesnake Canyon, Missoula, Montana.

Cells were plated on sterile nutrient agar disks of the following composition: $H_2O$, 1,000 ml; $KNO_3$, 0.25 g; $K_2HPO_4$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; Fe EDTA (13% Fe), 0.008 g; $Na_2SiO_3 \cdot 9H_2O$, 0.05 g; soil water, 1 part soil to 2 parts $H_2O$, filtered after autoclaving, 50 ml; and 10 g Difco-Bacto agar. Colonies appearing on the disk were replated four times. A single colony was transferred into liquid medium and cultured at 20 C, 1500 foot candles (ft-c.). The culture was uni-algal, but it was not axenic.

## Stock Cultures

Cells were cultured in 1,000 ml Roux flasks equipped with bubblers fed from cotton filtered line air after having passed through a water column. Bubbling supplied $CO_2$ and maintained agitation sufficient to keep cells evenly dispersed. The Roux flasks were clamped upright in 20 gallon (U.S.) aquaria maintained at a constant 20 $\pm$ 1 C with heater-circulators and uniform illumination of 1500 ft-c. provided by two Westinghouse "Cool White" SHO Lamps. Culture density was maintained by daily dilutions to an optical density (O.D.) of approximately 0.1 at 710 nm against distilled water. This density represents ca. 1/3 the

maximum density attained by the cells, and it was well within the log phase of growth.

## Growth Medium

Medium A (Table 1), (Sheridan, 1972), contains sufficient concentrations of macro- and trace elements to sustain logarithmic growth to high cell densities. Stock cultures and all reported experiments employed Medium A.

TABLE 1.  The Composition of Medium A.

| | |
|---|---|
| Distilled water | 1000 ml |
| NTA | 0.1 g |
| Sheridan's Trace Elements | 0.5 ml |
| $FeCl_3 \cdot 6H_2O$ | 2.0 ml (0.9368 g/200 ml) |
| $CaSO_4 \cdot 2H_2O$ | 0.06 g |
| $MgSO_4 \cdot 7H_2O$ | 0.0988 g |
| NaCl | 0.0077 g |
| $KNO_3$ | 0.1032 g |
| $NaNO_3$ | 0.6888 g |
| $Na_2HPO_4$ | 0.1111 g |

pH 8.2.  Autoclaving 40 min. changes pH to 7.5

Sheridan's Trace Elements-$H_2O$, 1000 ml; HCL, 3.0 ml; $MnCl_2 \cdot 4H_2O$, 2.0 g; $ZnCl_2$, 0.5 g; $H_3BO_3$, 0.5 g; $CuCl_2 \cdot 2H_2O$, 0.025 g; $CoCl_2 \cdot 6H_2O$, 0.025 g; $V_2O_4(SO_4)_3 \cdot 16H_2O$, 0.25 g.

## Zectran Dilutions

As formulated by Dow Chemical Company, Zectran is dissolved in a petroleum carrier and designated FS-15.  FS-15 was homogenized in medium A using a Waring blender to make an emulsified stock solution containing 50 ppm Zectran.  Each experimental concentration for 1, 10 and 15 ppm Zectran was made up from stock solutions which were prepared

within a few hours of experiments. To give perspective to the experimental concentrations, it is useful to calculate the concentration resulting from the direct spraying of a lake at the concentration approved for forest application. Spraying a 4046 $m^2$ (1 acre) x 1 m deep lake with the recommended 68 grams Zectran per acre yields a concentration of 0.166 ppm which is approximately 10 times less than the lowest experimental concentration.

For experiments, 90 ml of 1.1, 11, and 16.5 ppm Zectran solutions were placed in 120 ml culture tubes, and 9 ml of suspended cells were added resulting in the experimental concentrations of 1, 10 and 15 ppm.

## Stress Conditions

Stress conditions in this study were defined as sub-lethal environmental conditions resulting in reduced growth or photosynthesis.

### Temperature

Experiments to determine optimum and stress temperatures for growth were conducted by measuring growth rates versus temperature over a 34 C range. Experiments were performed using cells in 800 ml of medium A in the 1000 ml Roux flasks at 1500 ft-c., pH 7.5. Twenty ml of stock cells were introduced into flasks at temperatures ranging from 7 to 41 C. Five ml aliquots of cells were aseptically sampled at the first hour and every 24 hours for up to 11 days. The optical density at 710 nm (Shimadzu MPS 50-L spectrophotometer) against distilled water was considered proportional to cell number during exponential growth.

### Light

Light intensity curves were determined using total $^{14}C$ incorporated

per chlorophyll content at varying light intensities during a 15-minute period. Low light intensities were achieved by shading the cultures from the fluorescent light bank with wire screens and high light intensities were provided by two quartz-iodine "Quartzlite" 500 watt, 120 volt outdoor flood lamps. All experiments involving light stress were performed at 25 C, pH 7.5.

One set of experiments utilized cells acclimated to the tested light intensity for 48 hours before the experiment. A second set of experiments exposed stock culture cells growing at 1500 ft-c. to the experimental light intensities with no acclimation period.

### Hydrogen Ion Concentration

Carbon-14 uptake by cells in medium A adjusted to pH values of 2, 3.5, 4.5, 7.5, 10, 11, 11.5 and 12 was measured to ascertain pH values which constitute stress conditions. Cell growth resulted in detectable pH increases in 2 hours. These experiments were, therefore, limited to 1 hour during which time there was no measurable pH change. For this same reason, it was not possible to precondition cells to experimental pH values. The use of buffers was avoided because of possible growth-enhancing or toxic effects introduced with their use. Only the higher pesticide concentrations of 10 and 15 ppm were assayed. All hydrogen ion stress experiments were performed at 1500 ft-c. and 25 C. Measurement of pH was made using a Beckman expanded scale pH meter.

## Zectran Toxicity Under Stress Conditions

Cells used in toxicity experiments were exposed to experimental conditions of light and temperature extremes without Zectran for 48 hours prior to experiments. Cells were then centrifuged (4,000 x g) and resuspended in culture media to an optical density of 0.2 at 710 nm against distilled water. The cells were then distributed to culture tubes containing Zectran and subjected to various experimental conditions.

## Permutations of Physical Stress

Combinations of light and temperature at pH 10 and 7.5 were investigated to determine the effect of combinations of physical stress on pesticide toxicity. A pH value of 10 was the only one likely to occur naturally which elicited a stress response, and it was the only extreme pH value used in these experiments. Samples at this pH value were tested only after 1 hour exposures while permutations involving pH 7.5 were sampled at 1 and 24 hours.

## Carbon-14 Uptake Measurements

Procedure:

1)  Two, 4 ml aliquots of cells from experimental cultures were placed in 8 ml screw cap glass vials.

2)  0.1 ml of 0.2 $\underline{M}$ carbonate-bicarbonate buffer Warburg mixture #11 (Umbreit $\underline{et}$ $\underline{al}$., 1964) added to each vial and shaken.

3)  Vials placed in rack in water bath at experimental temperature.

4)  0.2 ml $NaH^{14}CO_3$ (2.5 $\mu c/ml$) injected into vials and shaken.

5) 1500 ft-c. illumination provided by 2 incadescent lamps for 15 minutes.

6) 1 ml 6$\underline{M}$ acetic acid injected into vials to halt photosynthesis and drive off unincorporated $^{14}CO_2$.

7) Vial contents and one distilled water rinse emptied into 20 ml scintillation vials.

8) Solutions evaporated to dryness at 50 C.

9) 15 ml scintillation mixture (6g PPO, 0.2g Popop, 2000 ml toluene) added to vials.

10) $^{14}C$ uptake measured as cpm in a Searle Isocap 300 liquid scintillation system set for 10 minute count time.

Carbon-14 incorporation against time curves were determined for all experimental conditions to insure that the $^{14}CO_2$ supply was constant and not limiting during the 15 minute period of photosynthesis. In all cases, uptake was linear for at least 30 min. Dark blanks were included in each experiment.

High bacterial growth occurred in the stock solutions of $NaH^{14}CO_3$ stored at room temperature or under refrigeration. This problem was eliminated by storing the stock frozen in individual 3 ml aliquots.

## Chlorophyll Determination Method

Chlorophyll was extracted and the concentration determined in methanol using the formulas of Holden (1965). Procedure:

1) 4 ml of experimental culture sampled.

2) sample centrifuged at 4000 x g for approximately 7 minutes.

3) supernatant decanted, pellet dispersed with agitation.

4)   4 ml absolute methanol added, tube corked and stored in the dark.

5)   24 hours later cells centrifuged (4000 x g, 7 min).  Pellet must be white.

6)   optical density of chlorophyll in methanol determined at chlorophyll peaks of 650 and 665 nm.

7)   total chlorophyll a + b calculated as mg chlorophyll per liter using the formula:

Total chlorophyll = 25.5 (O.D. 650) + 4.0 (O.D. 665).

## Light Intensity Measurements

Light intensities were measured using a Weston model 756 illumination meter.

CHAPTER IV

RESULTS AND DISCUSSION

## Zectran Concentrations vs Growth

Figure 2 presents data demonstrating the influence of 1, 10 and 15 ppm Zectran on the growth rate of Chlorococcum sp. over a 48 hour period, 25 C, and 1500 ft-c. Zectran stimulated growth at 1 ppm and inhibited growth at 10 and 15 ppm.

## Temperature vs Growth

Figure 3 presents the generation times (hours per doubling of optical density) for cells grown at 1500 ft-c. and temperatures ranging from 7 - 41 C. The temperature range for maximum growth is approximately 18 - 34 C. Small changes in temperature below 10 and above 35 C rapidly increase generation times for the cells. Reaction rates of cellular processes gradually decrease as temperatures becomes suboptimal, and this is reflected in the increased time required for cell division. As temperatures increase to supraoptimal levels, constituents of the physiological systems change form and cease to function normally; the rapid increase in generation time at high temperatures reflecting this. Denaturation of enzymes and the alteration of lipids are considered to be of primary importance in the destruction of cellular functions at high temperatures (Heilbrunn, 1943; Ouellet, 1951).

Figure 2.  Growth rates of Chlorococcum at 25 C,

1500 ft-c., and pH 7.5 at 1, 10 and 15 ppm Zectran.

Cell concentrations measured as optical density

at 710 nm.  Graphs present the mean values of
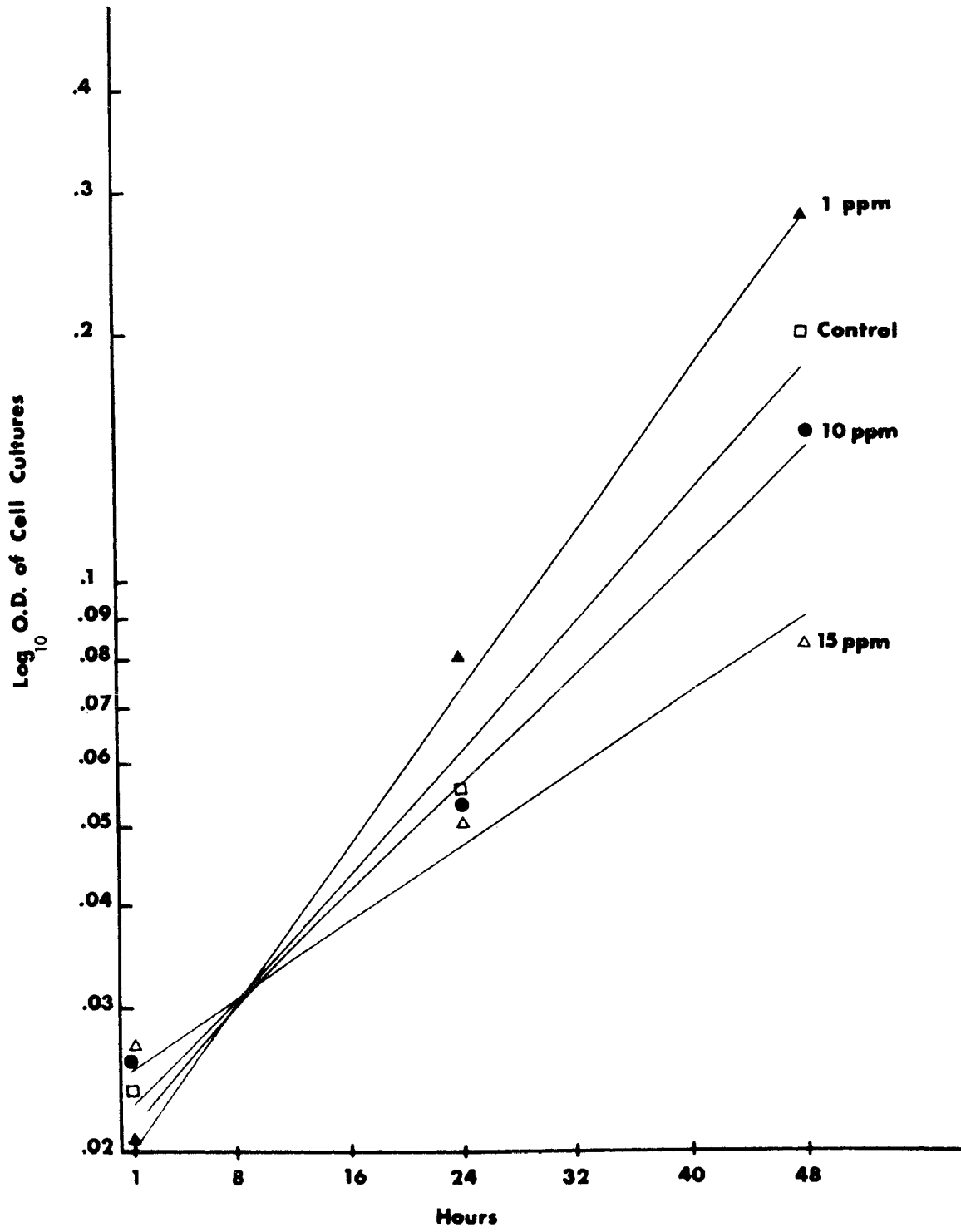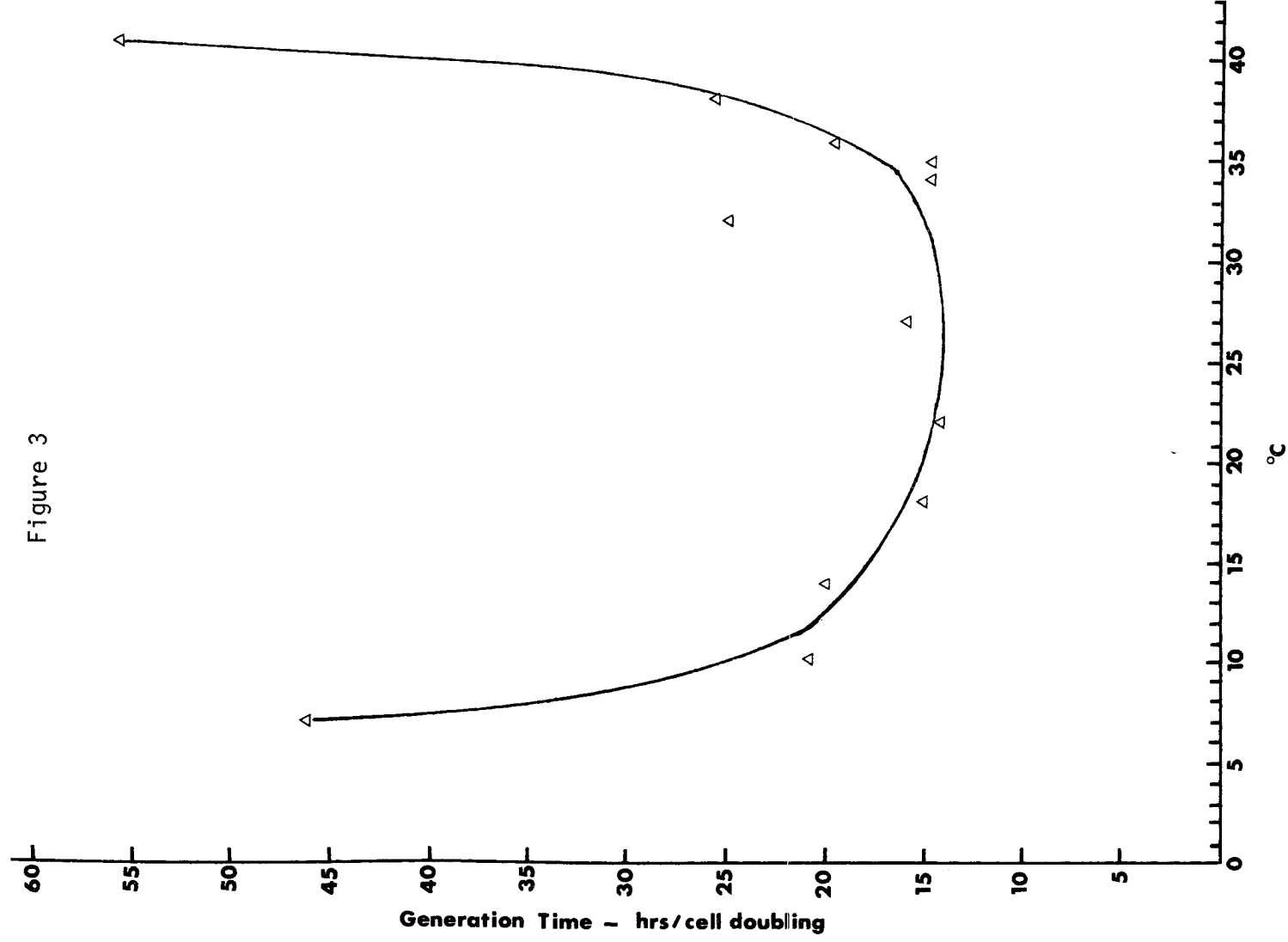
three separate trials.

Figure 2

Figure 3. Generation times (hours per optical density doubling) of <u>Chlorococcum</u> at 1500 ft-c., pH 7.5 over a range of temperatures.

Figure 3

For use in this study, 7 and 38 C were considered sub- and supra-optimal temperatures respectively, and 25 C was optimal.

## Temperature Stress and Zectran Toxicity

Figure 4 presents the result of Chlorococcum exposure to 1, 10 and 15 ppm Zectran at sub-, supra- and optimal temperatures.

One hour exposure to 1 ppm at all three temperatures caused slight inhibition of $^{14}$C uptake with no significant difference in inhibition occurring between 7 and 38 C. Twenty-four hours exposure at 7 C to 1 and 10 ppm resulted in photosynthetic rates greater than in control cultures while at 15 ppm this stimulation did not occur.

After one hour of exposure to 10 ppm, cells at 7 C showed approximately 15% inhibition; at 25 C, 23% inhibition, and at 38 C, 40% inhibition. After a 24-hour exposure, the cells at 7 C showed a 10% stimulation of photosynthesis while inhibition in the 25 and 38 C cells increased to 58% and 75%, respectively.

Exposure to 15 ppm Zectran for one hour resulted in 20% inhibition of photosynthesis by cells at 7 C, 24% at 25 C, and 58% at 38 C. After 24 hours of exposure at 7 C, no stimulation of carbon uptake was exhibited; there was instead an approximate 12% inhibition. The longer exposure of 24 hours of cells at 25 and 38 C to 15 ppm also increased the toxic effect of Zectran resulting in a 52% inhibition for 25 C cells and mortality of the 38 C cells. Zero $^{14}$C uptake and bleached cells were the criteria for death.

Figure 4.  Carbon-14 uptake by Chlorococcum 1 and 24 hours

at suboptimal, optimal and supraoptimal temperatures, 1500

ft-c. pH 7.5 and 1, 10 and 15 ppm Zectran.  Vertical lines

represent confidence limits at the 95% level of significance.
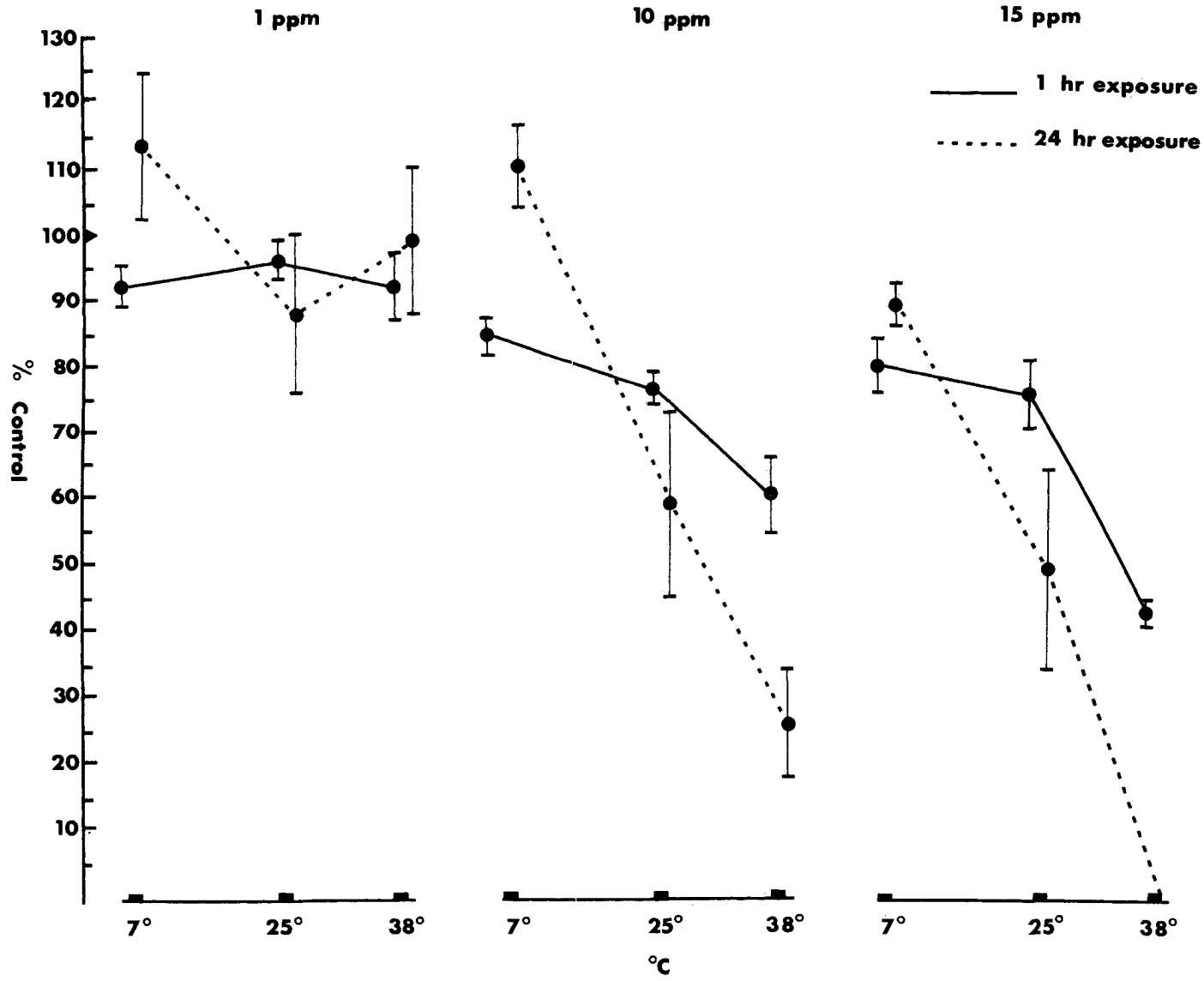
Graphs present the mean results of five trials.

Figure 4

## Light Intensities vs Photosynthetic Rates

Photosynthetic rates of acclimated cells were optimal between 250 - 1500 ft-c. (Figure 5). The uptake of $^{14}C$ by acclimated cells decreased at light intensities greater than 1500 ft-c. Figure 5 also shows how cells acclimated to 1500 ft-c. and then exposed to 250, 600, 1500, 2500, and 5000 ft-c. for 1 hour had much lower photosynthetic rates at the unaccustomed light intensities compared to cells preconditioned by 48 hours growth at those intensities. This effect demonstrated the cell's ability to adapt, especially at the lower intensities, to varying light intensities over time, resulting in the maintenance of optimum photosynthetic rates. Adaptation usually involves changes in the concentration of photosynthetic pigments, photosynthetic enzymes and other enzymes (Jorgensen and Steeman Nielsen, 1965). Figure 5 also demonstrates that cells suddenly exposed to high light intensities show a drastic reduction in photosynthetic rates. While this high light-caused inhibition is somewhat mitigated over time by biochemical adaptation, photosynthetic rates are never as great as those achieved at lower intensities. This "permanent" reduction in photosynthetic capability has been reported to be the result of photooxidation of the photosynthetic apparatus by high light intensities (Steeman Nielsen, 1952). Light intensity stress was achieved at 10,000 ft-c. Adaptation of photosynthesis to low light made light limitation a difficult stress condition to impose. The light intensity of 250 ft-c. was used for low light intensity stress.

Figure 5. Carbon-14 uptake per mg chlorophyll by Chlorococcum after 1 hour exposure to 25 C, pH 7.5 and a range of light intensities. The figure presents data from cells which were both preconditioned to the light intensities tested and cells which were not preconditioned. Data are the mean values of three trials.

Figure 5

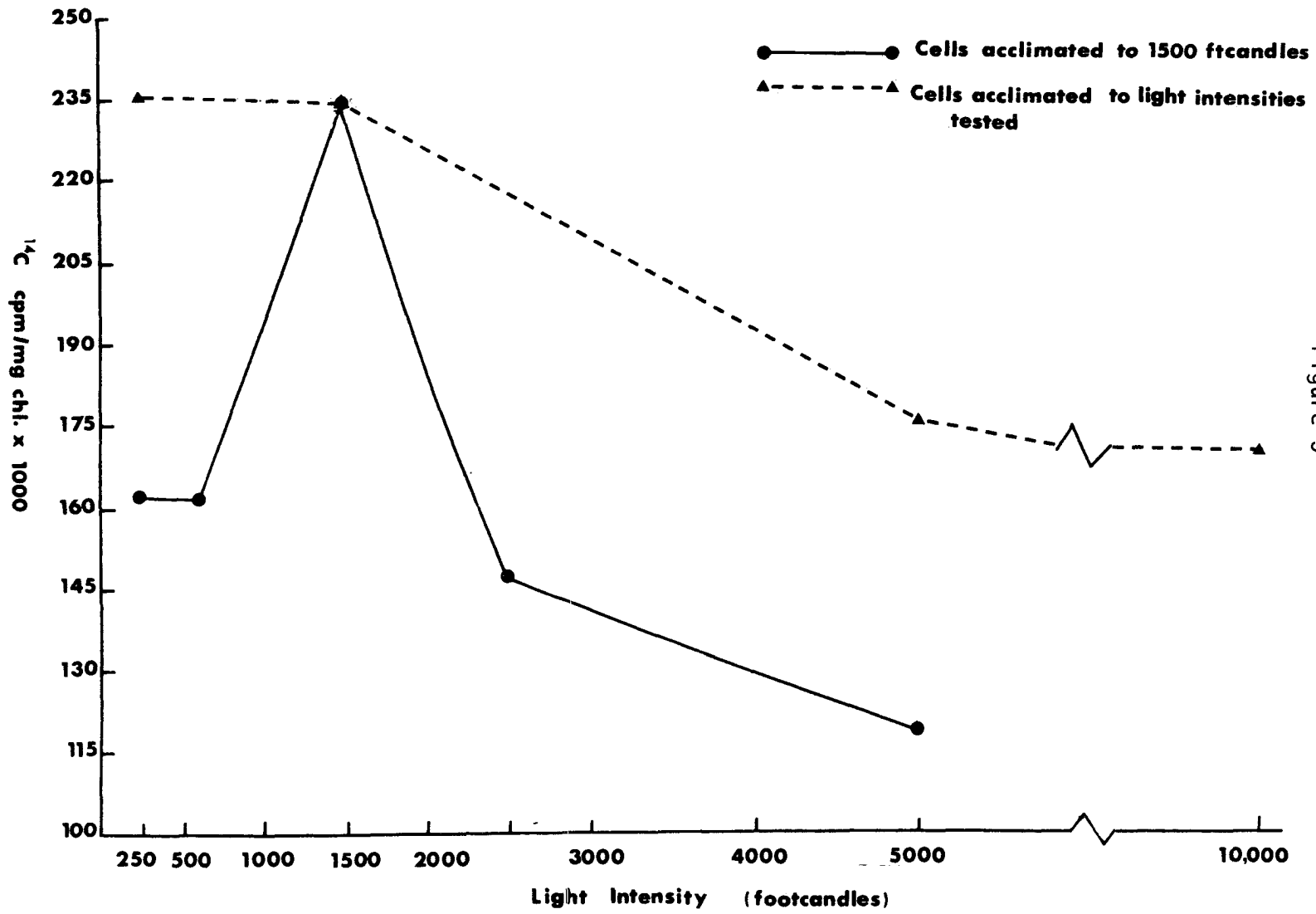Figure 6.  Carbon-14 uptake as percent control at 1 and 24 hours by Chlorococcum at suboptimal, optimal and supra-optimal light intensities at 25 C, pH 7.5 and 1, 10 and 15 ppm Zectran.  Data are the mean values of 5 trials.
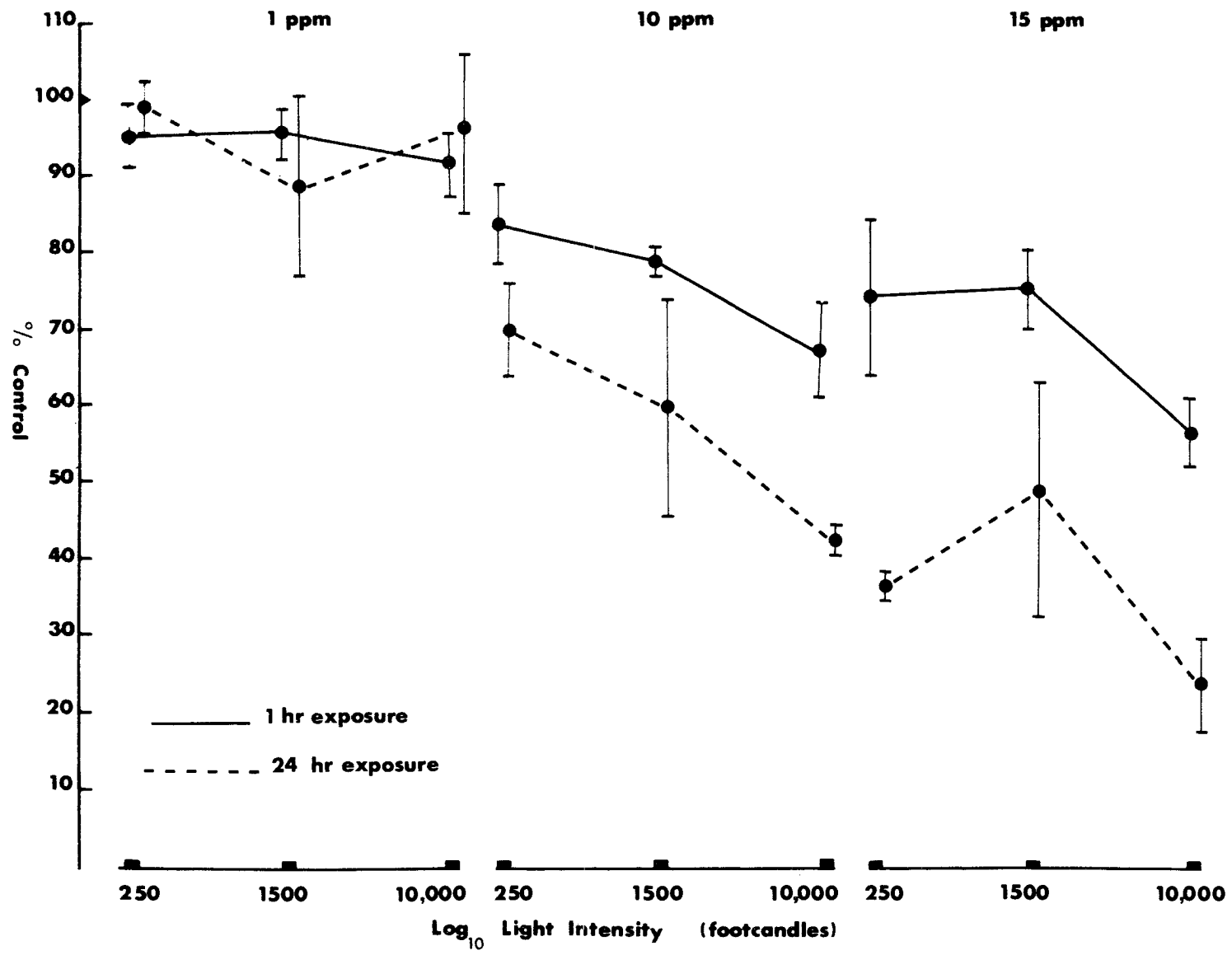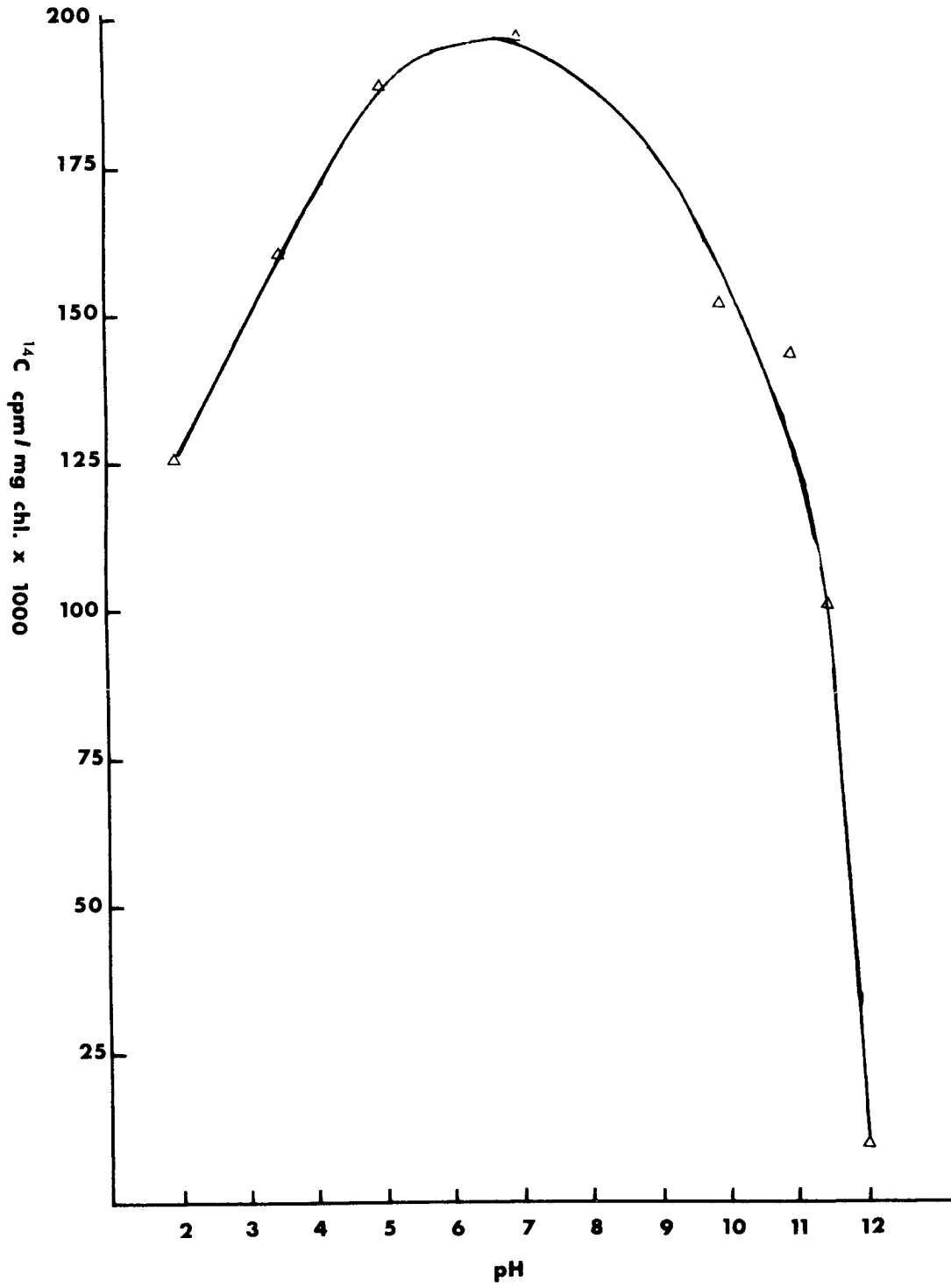
Figure 6

Figure 7. Carbon-14 uptake per mg   chlorophyll by <u>Chlorococcum</u> after 1 hour exposure to various pH values at 25 C, and 1500 ft-c. Data are the mean values of three trials.

Figure 7

high pH is not in the limitation of carbon.

## Hydrogen Ion Stress and Zectran Toxicity

The results of experiments in which Chlorococcum was exposed to 10 and 15 ppm at different pH values are presented in Figure 8. Photosynthesis rates at 10 and 15 ppm were approximately 20 and 27% less than the controls at pH 2, and these rates were not significantly different from those which occurred at pH 5 and 7.5. Less tolerance to Zectran was demonstrated by cells at the higher pH values of 10 and 11.5. Inhibition caused by 10 and 15 ppm after 1 hour exposure was 31 and 48% respectively at pH 10, and 36 and 53% at pH 11.5

## Zectran Toxicity and Combinations of Stress Conditions

The results of experiments in which Chlorococcum was exposed to 1, 10 and 15 ppm Zectran at different combinations of light intensity, temperature and both pH 10 and 7.5 are presented in Figures 9 - 11. These data are ordered so that those physical conditions which increased or decreased the alga's ability to tolerate Zectran are apparent.

Inhibition of photosynthesis at 1 ppm Zectran was generally low under the combinations of test conditions shown in Figure 9. Stimulation of photosynthesis was achieved at low temperature (7 C), optimal light intensity (1500 ft-c.), and pH 10. Photosynthetic rates equal to controls occurred at low temperature, low light and pH 10. Greatest inhibition (27%) resulted after 24 hour exposure to high temperature (38 C), high light (10,000 ft-c.), and pH 7.5. The highest inhibition (22%) after 1 hour exposure to 1 ppm occurred at high temperature, high

Figure 8. Carbon-14 uptake as percent control by Chlorococcum

after 1 hour exposure to 10 and 15 ppm Zectran at 25 C, 1500 ft-c.

and the pH values indicated. Data are the mean values of three

trials. Vertical lines represent confidence limits at the 95%

level of significance.

Figure 8

Figure 9. Carbon-14 uptake as percent control by Chloro-coccum after 1 and 24 hours exposure to 1 ppm Zectran at the conditions indicated. Data are the mean values of three trials. Vertical lines represent confidence limits at the 95% level of significance.

Figure 9

37

Figure 10.  Carbon-14 uptake as percent control by Chlorococcum
after 1 and 24 hours exposure to 10 ppm Zectran at the conditions indicated.  Data are the mean values of three trials.
Dashed lines indicate dead cultures; vertical lines represent
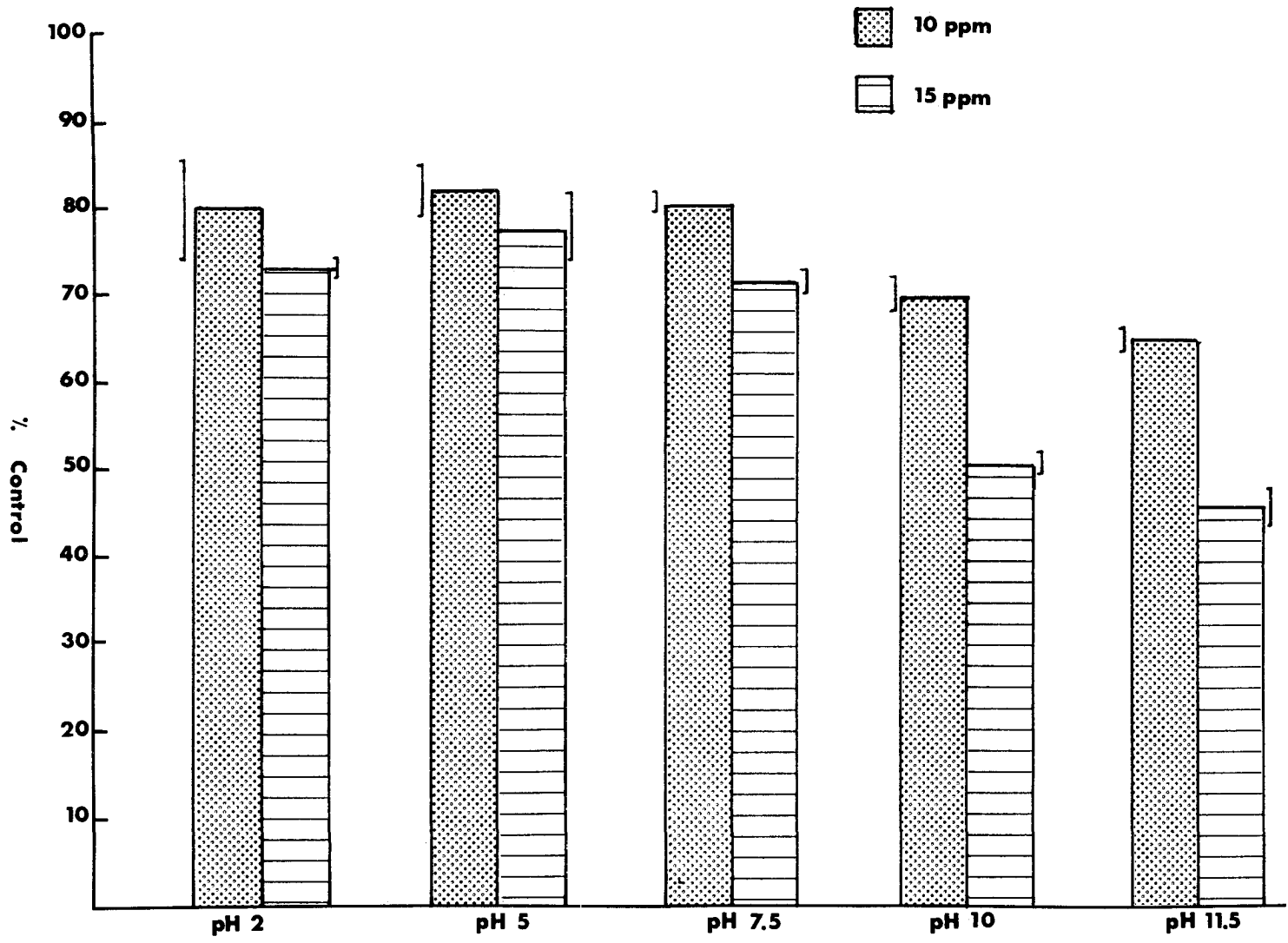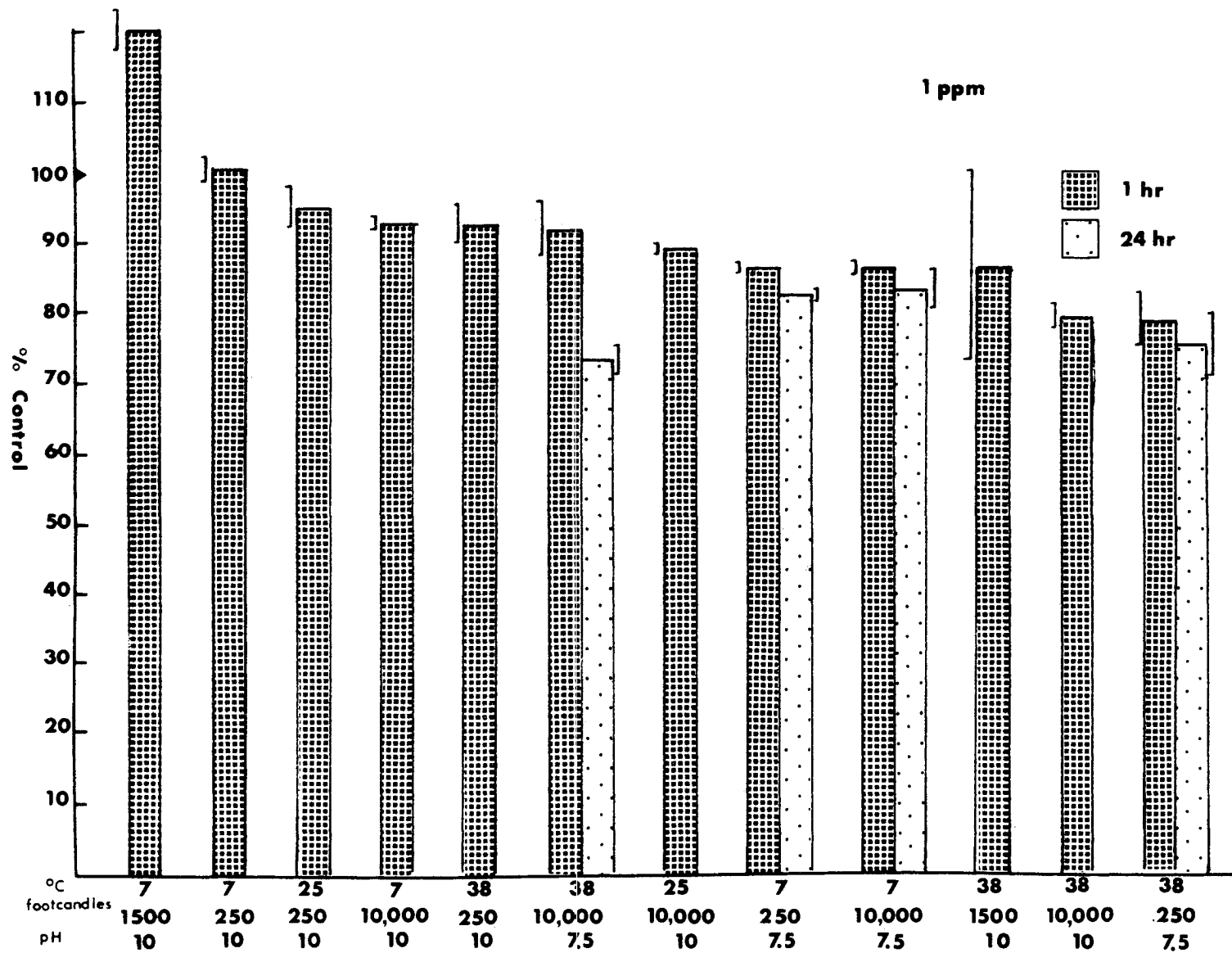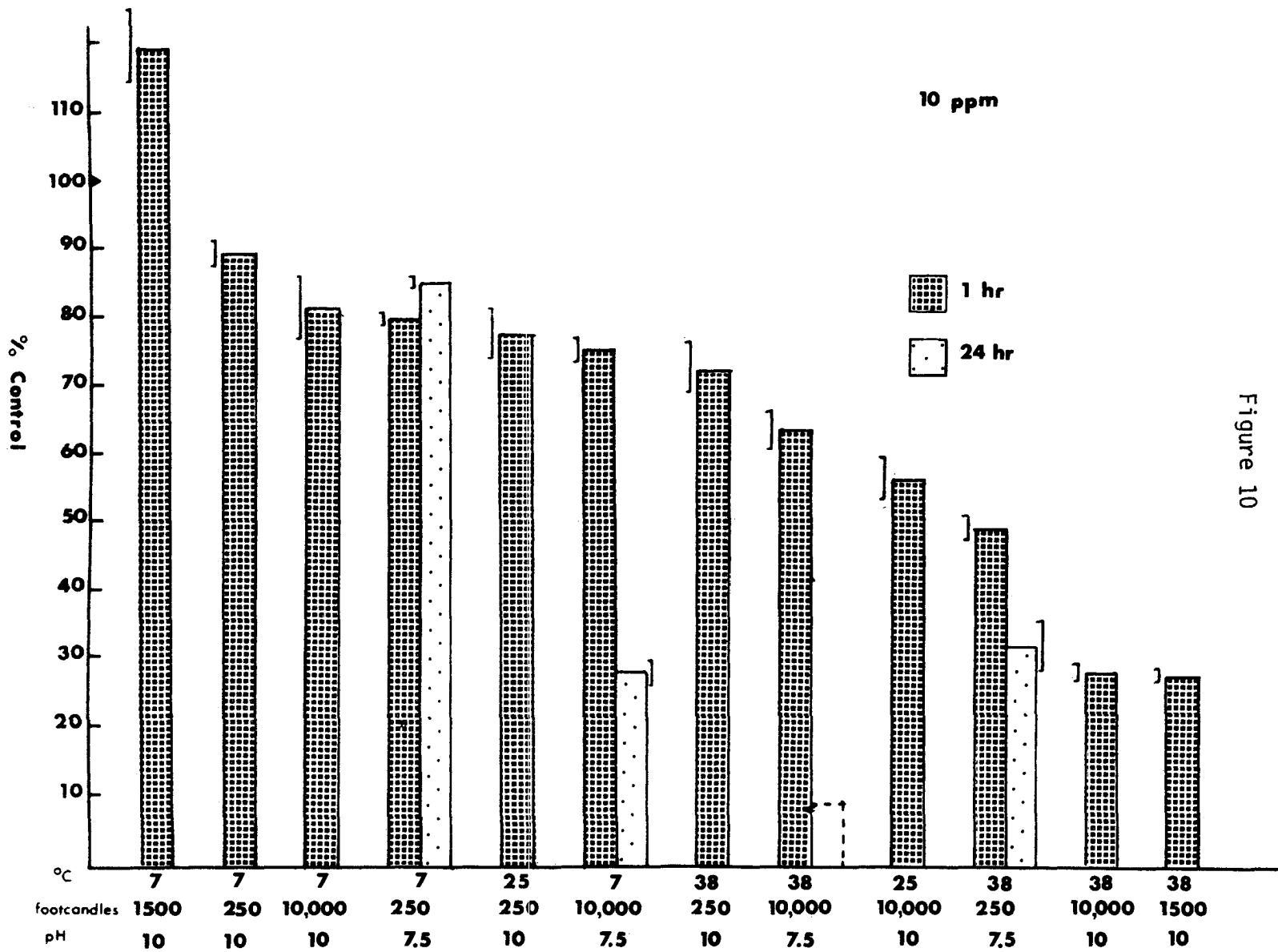confidence limits at the 95% level of significance.

Figure 10

39

Figure 11.  Carbon-14 uptake as percent control by <u>Chlorococcum</u>
after 1 and 24 hours exposure to 15 ppm Zectran at the conditions
indicated.  Data are the mean results of three trails.  Dashed
lines indicate dead cultures; vertical lines represent confidence
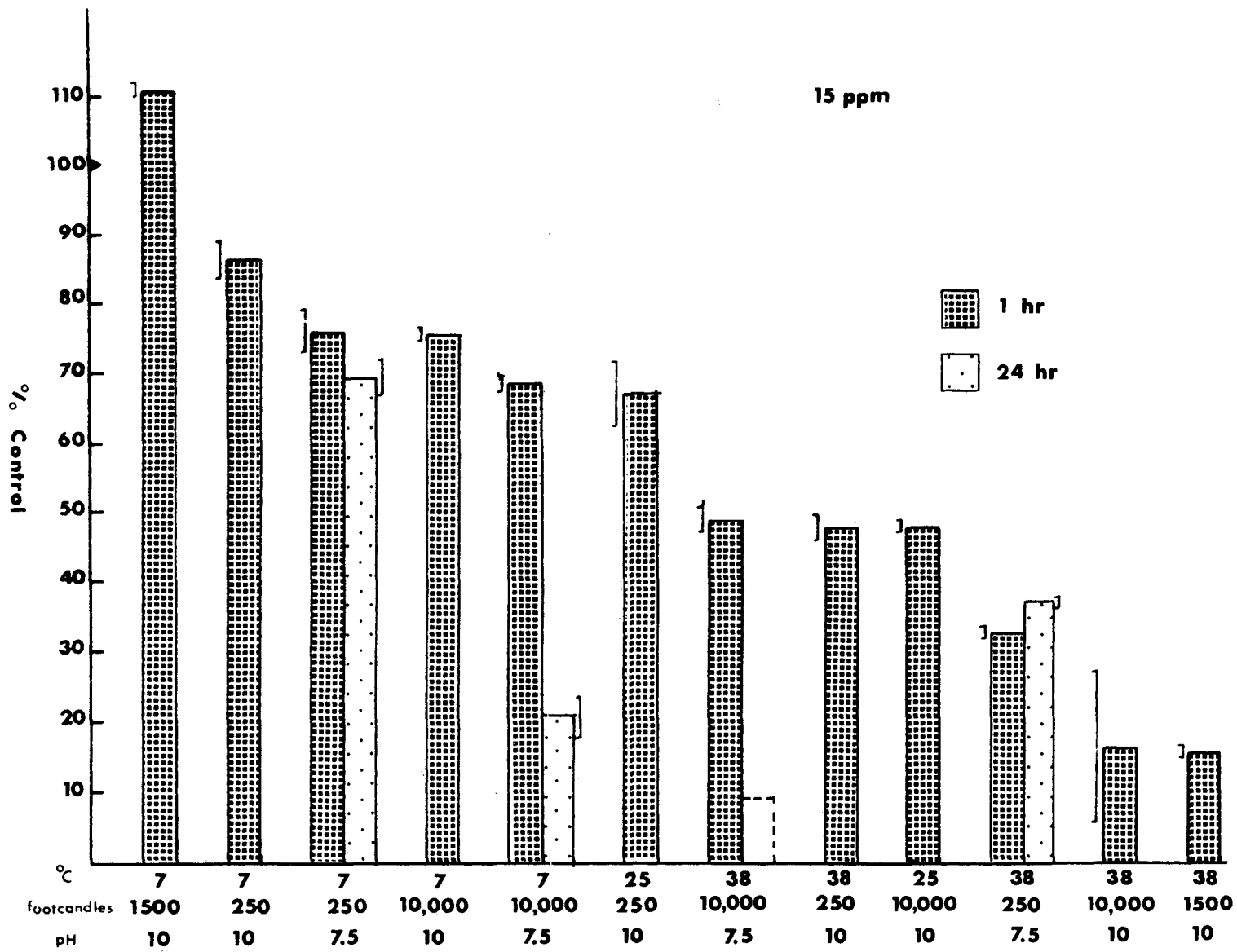limits at the 95% level of significance.

Figure 11

41

and low light and both pH 7.5 and 10.

Photosynthesis at 10 ppm Zectran (Figure 10) was inhibited under most conditions. Stimulation again occurred at 7 C, 1500 ft-c. and pH 10. All other stress conditions involving 7 C inhibited photosynthesis between 11 and 25% while most stresses at the higher temperatures resulted in rates from 38 to 75% below the controls. Two exceptions at the higher temperatures include 25 and 38 C in conjunction with low light and pH 10 which resulted in inhibition to photosynthesis of 23 and 28% respectively. Inhibition of photosynthesis by cells at 7 C occurred between 1 and 24 hours at 10,000 ft-c., pH 7.5 while at the same temperature and pH a slight stimulation occurred after 24 hours in cells at 250 ft-c. After 1 hour exposure to 10 ppm Zectran, cells at 38 C, pH 10 , 10,000 and 1500 ft-c. showed the greatest inhibition while 24 hour exposure to 38 C, pH 7.5 and 10,000 ft-c. resulted in cell death.

The results of experiments performed at 15 ppm Zectran (Figure 11) showed the same general ordering of stress conditions as was found at 10 ppm Zectran.

There is no apparent explanation for the slight stimulation observed at 15 ppm, high temperature, low light intensity and pH 7.5 after 24 hours while under these same conditions, 24 hour exposure to 10 ppm resulted in an inhibition.

Figures 12, 13 and 14 present the same results as are shown in Figure 11. However, in these figures, either light intensity, temperature or pH is held constant, and combinations of the remaining two con-

ditions are plotted in the order of their inhibitory effect. This graphic technique aids in illustrating the effect of each environmental stress condition on Zectran's toxicity.

At 250 ft-c. (Figure 12A) it can be seen that the toxic effect of Zectran was greatest at 38 C. Seven degrees and pH 10 were the most "protective" to the organism, and 38 C and pH 7.5 were the most detrimental. Twenty-four hour exposure at 25 C and pH 7.5 and 1 hour exposure at 25 C and pH 10 both exhibited inhibitions intermediate between 7 and 38 C.

At 10,000 ft-c. (Figure 12B), temperature had similar effects, but high pH became more important in increasing the pesticide's toxicity. That 10,000 ft-c. constitutes a greater stress on the cells than 250 ft-c. is evidenced by the generally greater levels of inhibition and cell death after 24 hours at 10,000 ft-c., pH 7.5.

At 7 C (Figure 13A) and pH 10 Zectran was stimulatory. At 7 C, conditions of 10,000 ft-c., and pH 7.5 showed the greatest inhibitory effects. The effects of high light at low temperature on the alga's tolerance are similar to the findings of Sorokin and Krauss (1961) that this combination of conditions reduced the growth rates of their cells. The authors postulated that chlorophyll synthesis at low temperatures was less than the rate of chlorophyll photo-oxidation.

Chlorococcum's tolerance to 15 ppm Zectran at 38 C was low under all conditions (Figure 13B). Comparison of Figures 13A and B shows that 7 C, 1500 ft-c. and pH 10 had a slightly stimulatory effect while at 38 C these same conditions of light and pH had the most inhibitory

Figure 12.  (A) Carbon-14 uptake as percent control by Chloro-

coccum after 1 and 24 hours exposure to 15 ppm Zectran at

250 ft-c. and the indicated variations of temperature and pH.

Vertical lines represent confidence limits at the 95% level

of significance.  (B) Carbon-14 uptake by Chlorococcum after

1 and 24 hours exposure to 15 ppm Zectran at 10,000 ft-c. and

the indicated variations of temperature and pH.  Dashed lines

refer to dead cultures.
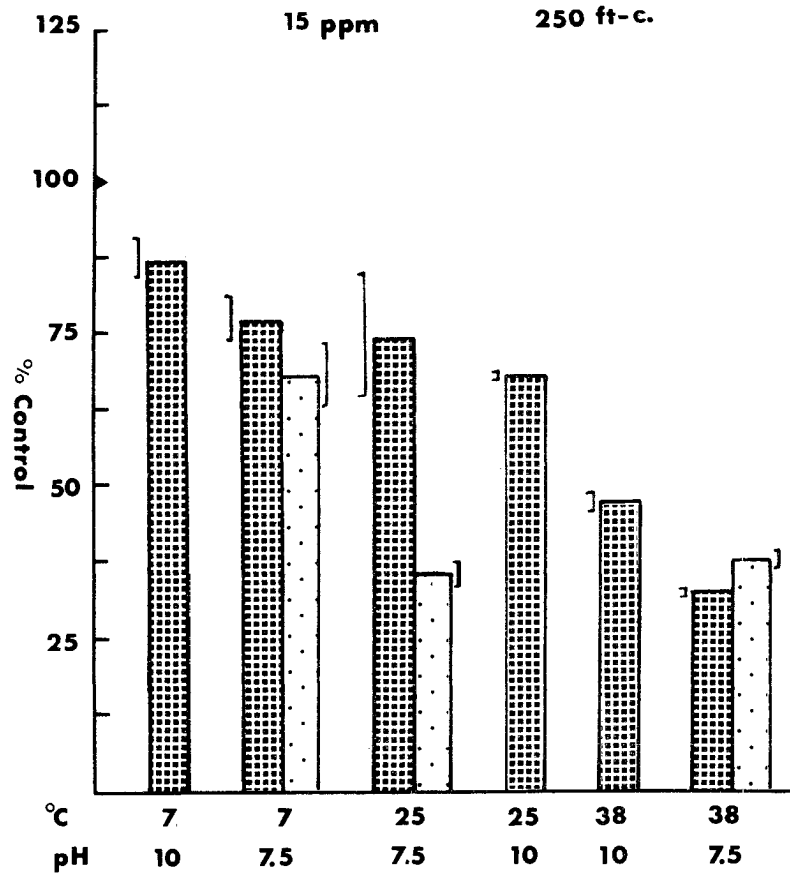
Figure 12

Figure 13.  (A) Carbon-14 uptake by Chlorococcum after 1 and

24 hours exposure to 15 ppm Zectran at 7 C and the indicated

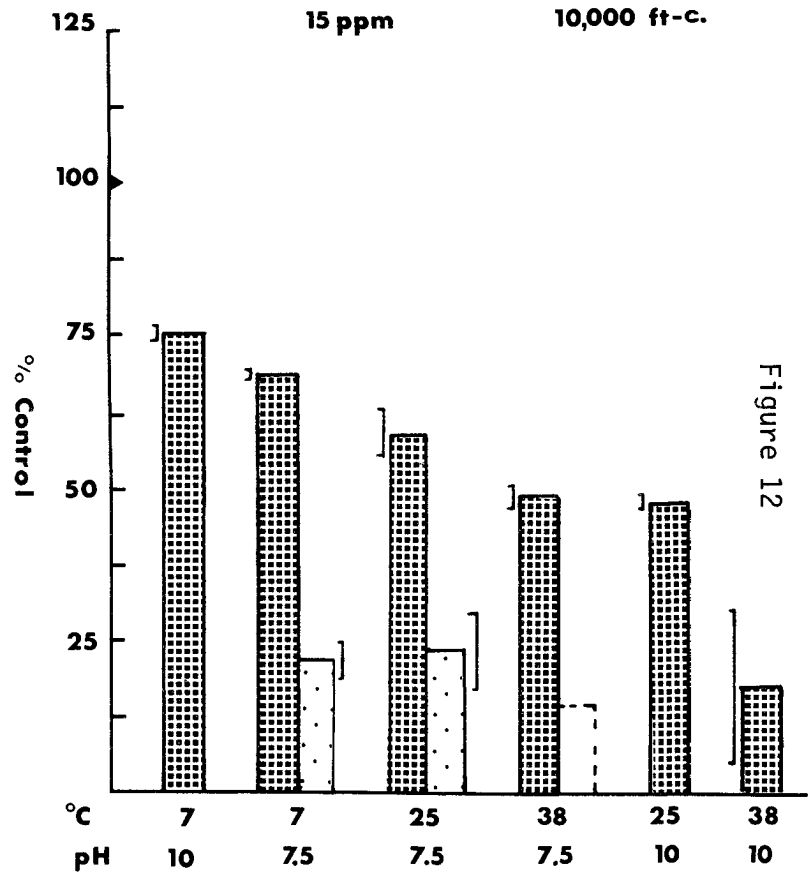variations of light and pH.  Vertical lines represent confi-

dence limits at the 95% level of significance.

(B) Carbon-14 uptake by Chlorococcum after 1 and

24 hours exposure to 15 ppm Zectran at 38 C and the indicated

variations of light and pH.  Broken lines refer to dead cultures.
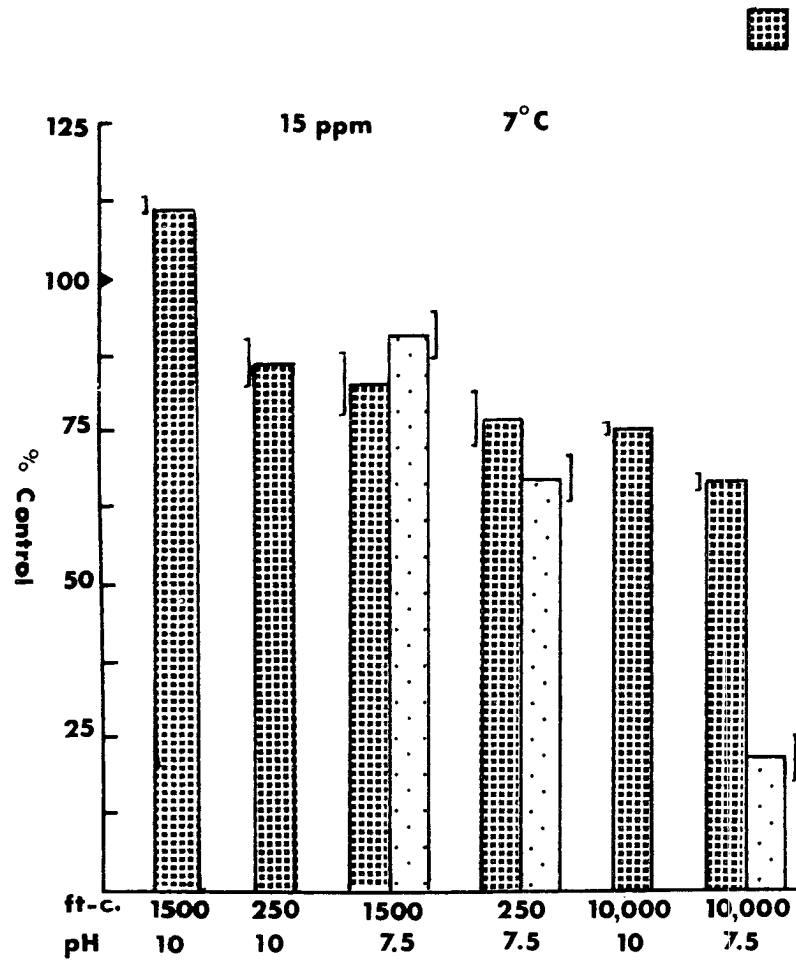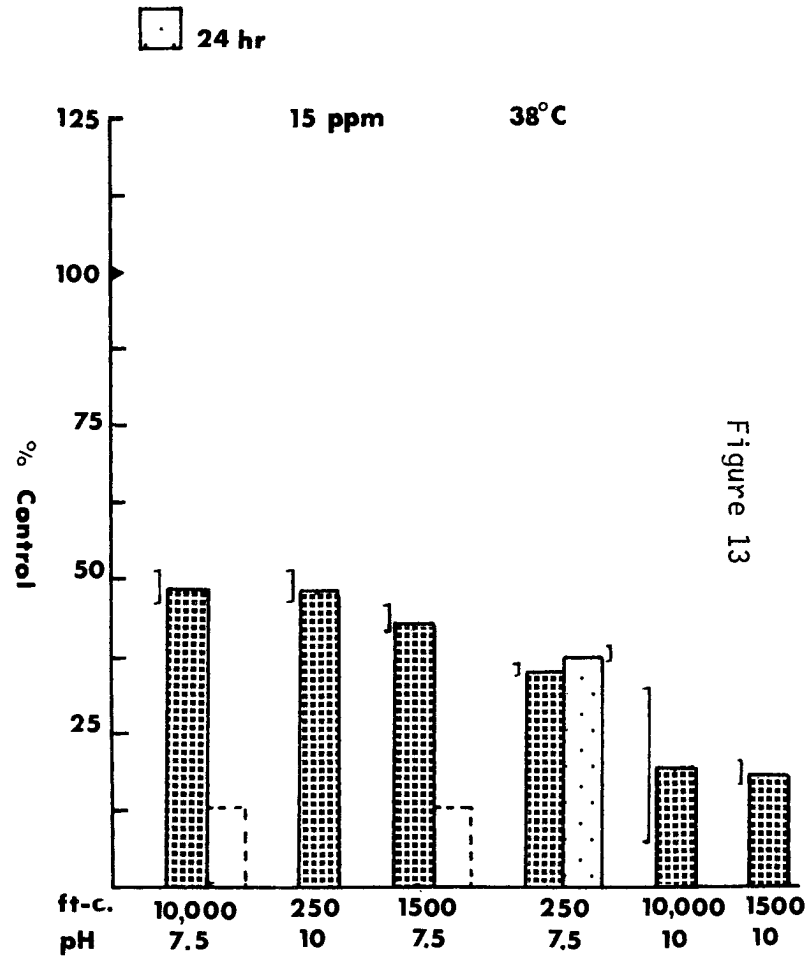
Figure 13

effects. Conversely, at 7 C, 10,000 ft-c. and pH 7.5 resulted in the greatest inhibition while at 38 C, 10,000 ft-c. and pH 7.5 were among the least inhibitory. This reversal of effects indicates that pH 10 enhanced Zectran's toxicity at the higher temperatures and light intensities.

Figure 14 shows the results when pH is held constant at 10 and light and temperature vary. Low and high temperatures were responsible for the extreme effects shown: 7 C caused a 10% stimulation, and 38 C caused 85% inhibition. The effects of low and high light had the same effect as the temperature extremes; 250 ft-c. resulted in a 33% inhibition and 10,000 ft-c. a 53% inhibition. The optimum conditions of 25 C, 1500 ft-c. produced intermediate effects of 50% inhibition.

Figure 14. Carbon-14 uptake by Chlorococcum after 1 hour

exposure to 15 ppm Zectran at pH 10 and the indicated

variations of light and temperature. Vertical lines re-

present confidence limits at the 95% level of significance.

Figure 14

49
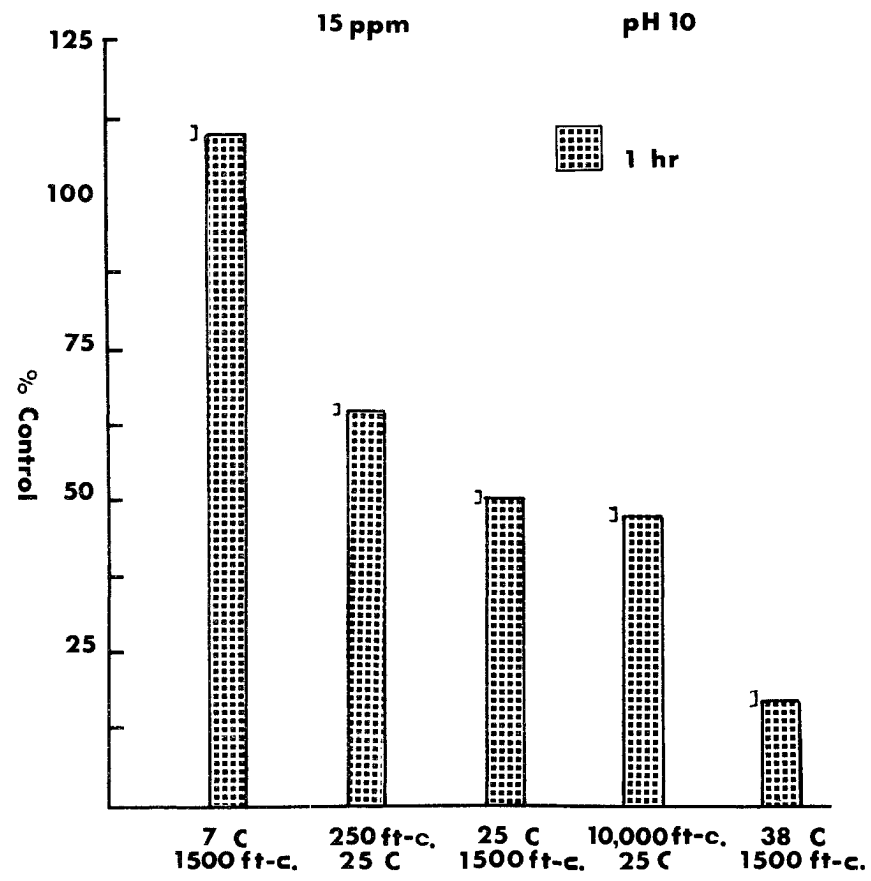
CHAPTER V

CONCLUSIONS

The results showed that environmental conditions did influence Chlorococcum's tolerance to the insecticide Zectran.

Relatively high concentrations of the pesticide caused stimulation of photosynthesis by cells at 7 C and at both pH 7.5 and 10. Sheridan and Simms (1975) also reported stimulation of photosynthesis by cells treated with Zectran.

The five stress conditions employed were insecticide concentration, growth medium pH, light intensity, temperature and duration of exposure to Zectran. Of these, temperature caused the greatest changes in toxicity. Even under conditions of extreme temperature stress, however, 1 ppm Zectran was not toxic.

Increased exposure time increased Zectran's toxic effects under almost all stress light and temperature conditions.

High light intensities slightly increased inhibition by Zectran. This could be due to chlorophyll photo-oxidation and the resultant decrease in photosynthetic activity.

After 24 hours of exposure to 15 ppm at 38 C (Figure 13B), cells at both 10,000 and 1500 ft-c. died while cells at 250 ft-c. exhibited photosynthesis rates approximately equal to their rates after 1 hour of exposure to Zectran. The light saturation level for an organism increases with increasing temperature (Sorokin, 1960). At this high

temperature, illumination at 250 ft-c. may have been sufficiently low to limit cellular respiration thereby allowing the cells to survive 24 hours of exposure to Zectran.

High pH values along with high temperatures and light intensities enhanced Zectran's toxicity. At low temperatures the effect is reversed. Studies involving toxicants which are organic acids or electrolytes should employ pH as a test parameter because the dissociation of these substances has been shown to be important regarding the toxic effect (Erickson et al., 1955; Cairns et al., 1975).

Cell permeability to various solutes has been found to increase with increasing temperatures, light, pH and age of the cell (Stadelmann, 1962). A similar function was noted in this study, and permeability to the Zectran molecule might play some interacting role in this effect.

It is possible to arrange the stress conditions according to their influence on Zectran toxicity to Chlorococcum. In order of increasing toxic effect, they are: low temperature; low light; high light; high temperature. Thus, high temperature and high light maximized and low temperature and low light minimized Zectran's toxic effect. The extent to which combinations of these factors regulate toxicity is shown in the results section (Figures 9, 10, 11). The extremes presented in these graphs are useful in predicting the relative tolerance of Chlorococcum under various field conditions. The greatest tolerance to Zectran was found at conditions of low temperature, low light and high pH. With the exception of high pH, these field conditions can be expected

to occur in early spring, late fall and winter in temperate climates. Conditions enhancing toxicity are high temperature, high light and high pH. These would be found in summer. Therefore, Zectran's effect on non-target organisms could be minimized by avoiding application in the summer months.

Tolerance levels of other algal species in various ecological habitats could be expected to show responses different from those reported here. Toxic stress imposed by combinations of chemicals and physical conditions could cause imbalances of the flora, producing population explosions and dominance of the community by one or a few species. The long-range effects of this process are indeed both complex and significant. It seems prudent, then, that toxicity studies determining "safe" levels of toxicants in nature should include the examination of physical and biological interactions in order to fully characterize the safety of the toxicant.

CHAPTER VI

SUMMARY

## Conclusions and Recommendations

1. Toxic response of Chlorococcum sp. to Zectran concentrations increased with temperature rise and at high light intensities.

2. The effect of hydrogen ion concentration was dependent on light and temperature; high pH increased toxicity at high light and temperature and minimized toxicity at low light intensities and temperatures.

3. Combinations of low light and low temperature at pH 10 minimized Zectran's toxic effects while combined conditions of high light, high temperature at pH 10 greatly increased the toxic effect.

4. Stress conditions in order of their increasing toxic effect are: low temperature; low light; high light; high temperature.

5. In most cases, toxicity increased with exposure time.

6. High water temperatures, light intensities and pH conditions found in summer increased Zectran toxicity to Chlorococcum.

7. In order that toxicological studies adequately define maximum concentrations of toxicants allowable in ecosystems and minimum concentrations necessary for results, the studies should assay toxicity under regimes of stress pertinent to organisms and conditions involved.

LITERATURE CITED

Abdel-Wahab, A.M. and Casida, J.E., 1967. Oxidation of 4-dimethylami-
noaryl methylcarbamate insecticides (Zectran and Matacil) on bean
foliage and of lakylaminophenyl methylcarbamate on silica gel
chromatoplates. J. Agr. Food Chem. 15: 479-487.

Anon. Research on Zectran. A substitute for DDT in the control of
spruce and jackpine budworm. Report revised from July 1970 staff
report on file with The Insecticide Evaluation Project, Pacific
Southwest Forest and Range Experimental Station, Forest Service,
U.S. Dept. Agr., P.O. Box 245, Berkeley, California.

Bollen, W.D., Lu, K.D. and Tarrant, R.F., 1970. Effect of Zectran on
microbial activity in a forest soil. USDA Forest Service District
Note PNW-124.

Branginsky, L.P., 1965. On methods of screening of algicides effective
against blue-green algae. Ecology and Physiology of Blue-green
Algae. Akad. Nauk, U.S.S.R.

Brooks, M.M., 1925. The effects of internal and external pH on the
penetration of arsenic from arsenate and arsenite solutions into
a living cell. Am. J. Physiol. 72: 222 (Abstr.).

Cairns, J., Jr., Heath, A.G., and Parker, B.C., 1975. Temperature in-
fluence on Chemical Toxicity to Aquatic Organisms. J. Water
Pollut. Control. Fed. 47 (2): 267-280.

Erickson, L.C., Wedding, R.T., and Brannaman, B.L., 1955. Influence of
pH on 2.4-D and acetic acid activity in Chlorella. Plant Physiol.
30: 69-74.

Gibson, H.R. and Chapman, D.W., 1972. Effects of Zectran insecticide on aquatic organisms in Bear Valley Creek, Idaho. Trans. Am. Fish Soc. 101: 330-344.

Hannemann, W., 1965. Versuche uber die Anwendung von Algiziden in der Saidenbachtalsperre. Wiss. Zeitschr. d. Karl-Marx-Univ., Leipzig, Math. - Nat. Reiche, 14(2): 315-327.

Harvey, R.S., 1967. Temperature effect on the sorption of radionuclides by a blue-green alga. Rept. DP-MS-66-88, U.S. Atomic Energy Comm., Washington, D.C.

Heilbrumn, L.V., 1943. An Outline of General Physiology. W.B. Saunders Co., Philadelphia, Pa., 274-277.

Holden, M., 1965. Chemistry and Biochemistry of Plant Pigments. Academic Press, New York, 93.

Holt, A.S. and French, C.S., 1946. The phytochemical production of oxygen and hydrogen ion by isolated chloroplasts. Arch. Biochem. 9: 25.

Hood, D.W. and Park, K., 1962. Bicarbonate utilization by marine phytoplankton in photosynthesis. Physiol. Plantarum 15: 273-282.

Hutchinson, G.E., 1967. A Treatise on Limnology. Vol. II. Introduction to Lake Biology and the Limnoplankton. John Wiley and Sons, Inc., New York.

Hynes, H.B.N., 1963. The Biology of Polluted Waters. Liverpool University Press.

Jacobsen, A.F., 1971. Effect of temperature upon the uptake of [144]Cesium by Chlamydomonas. Nucl. Sci. Abs., abst. no. 55162.

Jitts, H.R., McAllister, C.D., Stephens, K. and Strickland, J.D.H., 1964. The cell division rates of some marine phytoplankters as a function of light and temperature. J. Fish. Res. Bd. Canada 21: 139-157.

Jorgensen, E. and Steeman-Nielsen, E., 1965. Adaptation in plankton algae, p. 39-46. In C.R. Goldman (ed.), Primary Productivity in Aquatic Environments. Mem. Ist. Ital. Idrobiol., 18 Suppl., University of California Press, Berkeley.

Lang, J.M., and Miskus, R.P., 1967. Zectran fed orally to mice. . . cholinesterase levels in blood determined. U.S. Forest Serv. Res. Note PSW-140.   Pacific S.W. Forest and Range Exp. Sta., Berkeley, California.

Lazaroff, N. and Moore, R.B., 1966. Selective effects of choorinated insecticides on algal populations. J. Phycology Suppl. 2: 7.

Menzel, D.W., Anderson, J. and Randtke, A., 1970. Marine phytoplankton vary in their response to chlorinated hydrocarbons. Science 167: 1724.

Myers, J., 1946. Culture conditions and the development of the photosynthetic mechanism:  IV Influence of light intensity on photosynthetic characteristics of Chlorella. J. Gen. Physiol., 29: 429-440.

McCombie, A.M., 1960. Actions and interactions of temperature, light intensity and nutrient concentrations on the growth of the green alga, Chlamydomonas. J. Fish. Res. Bd. Canada. 17: 871-894.

Ouellet, C., 1951. The path of carbon in photosynthesis. XII Some

    temperature effects. J. of Exp. Botany 6: 316-320.

_____, and Benson, A.A., 1952. The path of carbon in photo-

    synthesis XIII. pH effects in $C^{14}O_2$ fixation by Scenedesmus.

    J. of Exp. Botany 8: 237-245.

Pieper, G.R. and Miskus, R.P., 1967. Determination of Zectran resi-

    dues in aerial forest spraying. J. Agr. Food Chem. 15: 915-916.

Rabinowitch, E.I., 1951. Photosynthesis and Related Processes. Vol.

    II, pt. 1. Interscience, New York.

Roberts, R.B., Miskus, R.B., Duckels, C.K. and Sakai, T.T., 1969.

    In vivo fate of the insecticide Zectran in spruce budworm, tobacco

    budworm and housefly larvae. J. Agr. Food Chem. 17: 107-111.

Sheridan, R.P., 1972. Kinetics of chlorophyll a and plastoquinone A:

    Changes in response to light intensity. J. Phycology 8: 166-169.

_____, and Simms, M., 1975. Effect of the insecticide Zectran

    (Mexacarbate) on several algae. Bull. of Env. Contam. and Tox.

    13(5): 565-569.

Simon, E.W. and Beevers, H., 1952. The effect of pH on the biological

    activities of weak acids and bases. I. The most usual relation-

    ship between pH and activity. New Phytologist 51: 163-190.

Snyder, C.E. and Sheridan, R.P., 1974. Toxicity of the pesticide Zec-

    tran on photosynthesis, respiration and growth in four algae.

    J. Phycol. 10: 137-139.

Sorokin, C., 1960. Kinetic studies of temperature effects on the cel-

    lular level. Biochim., Biophys. Acta. 38: 197-204.

_____ and Krauss, R., 1962. Effects of temperature and illumination on _Chlorella_ growth uncoupled from cell division. _Plant Physiol._ 37: 37-46.

Stadelmann, E.J., 1962. Permeability, p. 493-528. In R.A. Lewin (ed.) _Physiology and Biochemistry of Algae._ Academic Press.

Steeman Nielsen, E., 1952. On detrimental effects of high light intensities on the photosynthetic mechanism. _Physiol. Plant._ 5: 334-344.

_____, 1955. The production of organic matter by the phytoplankton in a Danish lake receiving extraordinarily great amounts of nutrient salts. _Hydrobiolgia_ 7: 68-74.

Stepanek, M. and Cervenka, R., 1961. A new method for the determination of the vitality of water algae. Measurement of the effect of algicides. Sci. Pop. Inst., Chem. Technol., Prague, _Techn. of Water_ 5(1): 189-226.

Tucker, R.K. and Crabtree, D.G., 1969. Toxicity of Zectran insecticide to several wildlife species. _J. Econ. Entomol._ 62: 1307-1310.

Ukeles, R., 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. _Applied Microbiology_ 10: 532.

Umbriet, W.W., Burris, R.H. and Stauffer, J.F., 1964. _Manometric Techniques._ 4th ed., Burgess, Minneapolis.

Wedding, R.T. and Erickson, L.C., 1957. The role of pH in the permeability of _Chlorella_ to 2,4-D. _Plant Physiol._ 32: 503-512.

Wurster, C., 1968. DDT reduces photosynthesis by marine phytoplankton.

Science 159: 1474-1475.