

AD-A255 364



## REPORT DOCUMENTATION PAGE

TIC

SEP 14 1992

2

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION/AVAILABILITY OF REPORT

Approved for public release;  
distribution unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

AFOSR-TR- 92-0212

6a. NAME OF PERFORMING ORGANIZATION

Alabama A &amp; M University

6b. OFFICE SYMBOL  
(If applicable)

7a. NAME OF MONITORING ORGANIZATION

AFOSR/NL

6c. ADDRESS (City, State and ZIP Code)

P.O. BOX 13, Normal Alabama, 35762

7b. ADDRESS (City, State and ZIP Code)

Building 410  
Bolling AFB DC 203328a. NAME OF FUNDING/SPONSORING  
ORGANIZATIONAir Force Office of Scientific  
Research8b. OFFICE SYMBOL  
(If applicable)

NL

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

AFOSR : F49620-91-C-0063

8c. ADDRESS (City, State and ZIP Code)

Bldg. 410, Bolling AFB, DC 20322- 6448

10. SOURCE OF FUNDING NOS.

PROGRAM  
ELEMENT NO.PROJECT  
NO.TASK  
NO.WORK UNIT  
NO.

611030

3484

RS

11. TITLE (Include Security Classification)

Effects of halogenated hydrocarbonson aquatic  
organisms

12. PERSONAL AUTHOR(S) Mahasin G. Tadros

13a. TYPE OF REPORT  
Annual Technical

13b. TIME COVERED

FROM 8/1/91 TO 8/1/92

14. DATE OF REPORT (Yr., Mo., Day)

1992, August 30

15. PAGE COUNT

36

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

FIELD GROUP SUB. GR.

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

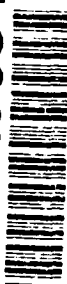
Algae- Halogenated hydrocarbons

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report summarizes progress for the first year of the subcontract, AFOSR F49620-91-C-0063 entitled "Effects of Halogenated Hydrocarbons on aquatic organisms.

This research dealt with several experiments evaluating the reponse of different algal species towards selected halogenated hydrocarbons. Two groups of algal species were assayed. The response of the algal species towards the chemical was evaluated under various growth conditions. Species varied in their response towards the chemicals. The green species were more sensitive than the diatoms, in respect to temperature. Within each group there were tolerant and sensitive species. In conclusion, when bioassaying the halogenated hydrocarbons, various algal species as well as growth parameters should be considered.

92-25094



20. DISTRIBUTION/AVAILABILITY OF ABSTRACT

UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS ☐

21. ABSTRACT SECURITY CLASSIFICATION

22a. NAME OF RESPONSIBLE INDIVIDUAL

DR Walter Kozumbo

22b. TELEPHONE NUMBER  
(Include Area Code)

(202) 767-5021

22c. OFFICE SYMBOL

NL

DD FORM 1473, 83 APR

EDITION OF 1 JAN 73 IS OBSOLETE.

Effects of Halogenated Hydrocarbons on Aquatic Organisms

First Annual Technical Report

to

Air Force Office of Scientific Research

Contract AFOSR: F49620-91-C-0063

August 30, 1992

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| DTIC TAB           | <input type="checkbox"/>                   |
| Unannounced        | <input type="checkbox"/>                   |
| Justification      |  |
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| Distribution/      |  |
| Availability Codes |  |
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| A-1                |  |

## **SUMMARY**

This report summarizes progress for the first year of the subcontract, AFOSR F49620-91-C-0063 entitled "Effects of Halogenated Hydrocarbons on aquatic organisms.

This research dealt with several experiments evaluating the response of different algal species towards selected halogenated hydrocarbons. Two groups of algal species were assayed. The response of the algal species towards the chemical was evaluated under various growth conditions. Species varied in their response towards the chemicals. The green species were more sensitive than the diatoms, in respect to temperature. Within each group there were tolerant and sensitive species. In conclusion, when bioassaying the halogenated hydrocarbons, various algal species as well as growth parameters should be considered.

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## INTRODUCTION

This report summarizes progress for the first year of the subcontract, AFOSR F49620-91-C-0063 entitled "Effects of Halogenated Hydrocarbons on aquatic organisms.

Chlorinated hydrocarbons are natural components of oil deposits and commonly find their way into surface waters as a result of discharges from refineries, waste oil, disposal, and accidental spills. Municipal wastewater discharges have also been recognized as sources of aliphatic and aromatic hydrocarbons (Barrick, 1982). Chlorinated hydrocarbons may enter the environment as a result of their use as solvents, heat transfer fluids, flame retardants or chemical intermediates or as waste products of the elector-industry (Jan and Malnersic, 1980). Among the most common solvents used form halogenated hydrocarbons are: trichloroethylene, tetrachloroethylene and dichloroethylene. These compounds are among the dominant contaminants detected in gound water (Barber et. al., 1988; Love and Eilers, 1982). Organic solvents can make their way into the environment as industrial wastes. Because of their carcinogenic potential, contamination of soil and water by solvents is cause for serious concern.

Relatively few reports have been published on the comparative toxicity of solvents towards tests organisms, and these dealt primarily with fish and aquatic invertebrates (Alexander et. al., 1878; Bouman et. al., 1981; LeBlanc and Suprenant, 1983). However, only few data of toxicity effects of solvents on algae have published (pearson and McConnell, 1975; Lay et. al., 1984; Stratton, 1987).

Algae have been considered to be good indicators of bioactivity of industrial wastes (walsh et. al., 1984). Algae are ubiquitous in aquatic ecosystems, where they incorporate solar energy into biomass, produce oxygen that is dissolved in water and used by aquatic organisms, function in cycling and mineralization of chemical elements, and serve as food for herbivorous and omnivorous animals. When they die, they sink as food for herbivoruous and amnivorous animals. When they die, they sink to the sediment where their chemical constituents are transformed, solubilized, and recycled into the water. These functions are dependent upon phytoplankton population dynamics which, in turn, depend upon seasonal variability in temperature, intensity of solar radiation, nutrient

concentrations in the water, and grazing by animals. Natural and anthropogenic alterations of water, and grazing by animals. Natural and anthropogenic alterations of water quality can upset the balance of these controlling factors and bring about changes in species composition of the algal community, rates of production, biomass, and water chemistry. If water quality is altered by toxicants or growth stimulants from industrial, agricultural or municipal sources, normal algal function may be upset, causing gross changes in structure and function of the receiving aquatic ecosystem.

## **OBJECTIVE:**

During the first year of the project the following studies were performed in order to:

.Compare the response of fresh water and saltwater (estuarine) single algal species, to different concentrations of the halogenated hydrocarbons, under different growth parameters: temperature and light intensity.

## **EXPERIMENTAL DESIGN:**

The response of algal species to chemicals was determined at 20 ° C and 30 ° C, under two light irradiances : 80 and 120  $\mu\text{Em}^{-2} \text{ s}^{-1}$

## **MATERIALS AND METHODS:**

### **Algal species:**

Assays were conducted with freshwater and saltwater algal species:

#### **Fresh water Green :**

*Gleocystis sp.*, *Scenedesmus sp.*,  
*Nannochloris sp.*, *Tetraselmis sp.*,  
*Chlorella sp.*, and *Nitzschia sp.*, (diatom)

#### **Salt water (estuarine) :Diatom :**

*Cylindrotheca*, *Nitzschia pusilla*, *Navicula saprophila*, *Nitzschia dissipata*, *Thalassiosira weissflogii* , and *chlorococcus sp.*

All algal species were obtained from the University of Texas algal collection (UTEX). The algal species were checked for bacterial contamination before use.

**Culture Medium:**

"F/2" Guillard and Ryther (1962)

**Macroelements:** (concentration mM/L medium)

|                        |  |  |
|------------------------|--|--|
| NaNO <sub>3</sub> 0.88 | NaH <sub>2</sub> PO <sub>4</sub> 0.036 | Na <sub>2</sub> SiO <sub>3</sub> 0.107 |
|------------------------|--|--|

**Trace Metals** (concentration uM/L medium):

|            |      |        |       |
|------------|------|--------|-------|
| Zinc       | 0.08 | Copper | 0.04  |
| Manganese  | 0.90 | Iron   | 11.70 |
| Cobalt     | 0.05 | EDTA   | 11.50 |
| Molybdenum | 0.03 |        |       |

**Vitamins:** (concentration ug/L medium)

|               |        |
|---------------|--------|
| Cyanocobalmin | 0.05   |
| Biotin        | 0.05   |
| Thiamine, HCL | 100.00 |

The culture medium was used for all species. For marine species, the medium was enriched with commercial artificial sea salt mix (Instant ocean, aquarium system, Inc. East Lake, OH.) to 20 parts per thousand (ppt) salinity.

Distilled water was used for preparation of media. The pH of media was adjusted to 8.0 with sodium hydroxide.

**Inoculum:**

Inoculations were prepared with cultures in log growth phase, obtained by frequent replenishment of medium. Cultures were acclimated to the growth conditions of the treatment for 72 h prior to the exposure by maintaining the growth rates constant. The initial inoculum was standardized to  $7 \times 10^4$  cells/ml. in all treatments.

**Culturing:**

All cultures were performed in triplicate in sterile optically matched tubes. Cultures were incubated on shakers in incubators at different temperatures (20, 30°C) under two light irradiations (80 and 120  $\mu\text{E m}^{-2} \text{s}^{-1}$ ), in light-dark cycle (16hr. light: 8hr. dark).

**Chemicals for testing:**

The following volatile halocarbons were tested:

Chloroform, Carbon Tetrachloride and Trichloroethylene. Test compounds were ordered from J.T. Baker Chemical Co.

### **Concentrations and Treatments:**

All test organisms were assayed in water-soluble fraction concentrations of 0.05, 0.1, 0.2, 0.3. The 100 % solution was prepared by adding part of chemicals to 100 parts dilution water (volume to volume) and stirring in a covered glass bottles with Teflon-coating-lined screw caps for 2 hours. After allowing the solution to settle for 1h, the water-soluble fraction was siphoned into another container for distribution to the test containers. The assay was carried out in tubes containing 25ml medium. All assays were conducted in triplicate test tubes.

All algal cultures were treated with different concentrations of the halocarbon. The concentration of halocarbons was not measured, because the gas liquid chromatograph was not yet ordered.

### **Growth Monitoring:**

Cultures were incubated for 96 h. The population density was determined by cell counting using a hemacytometer. Ten microscopic fields were counted and averaged. Responses of species were estimated by:

- A. Population density measured by cell counting using Hemacytometer. From population density the growth rate (u) of each species was calculated from the expression:

$$u = \frac{\log_{10} N - \log_{10} N_0}{t - t_0}$$

Where:

N = population density at the end at the test

N<sub>0</sub> = population density at the beginning of the test

t - t<sub>0</sub> = length of time of the test

- B. Toxicity was calculated in percentages of the control

### **QUALITY CONTROL AND STATISTICS :**

Culturing media were sterilized by autoclaving before treatment with hydrocarbons. All glass used for experiments were also sterilized by



autoclaving. The temperatures of autoclaves were monitored on a per-use basis. Spectrophotometers, pH meters, and analytical balances were calibrated on a regular basis. All glassware (pyrex) were cleaned using 1% HCL followed by rinsing thoroughly with deionized water. The triplicate tests analyzed at each parameter (e.g. Temperature, salinity...) each test was performed twice. All errors were expressed as the standard error of the mean (SEM). Occupational safety and Health Administration (OSHA) regulations regarding the safe handling of chemicals and safety of personnel were followed.

## **RESULTS AND DISCUSSION :**

A series of experiments were performed, using the chemicals at different concentrations, at 10° C, 15° C, 20° C, 25° C, and 30° C under two light irradiances: 100 and 200  $\mu\text{Em}^{-2}\text{s}^{-1}$  (as proposed in proposal). The light irradiance was too high, where most of the algal species did not grow. So the light irradiance was lowered to 80 and 120  $\mu\text{Em}^{-2}\text{s}^{-1}$ . The same applied to the temperature, which led to limiting the experiments to two temperatures (20° C and 30° C).

The chemicals were tested, after being dissolved in acetone as we proposed in the proposal. We find that acetone, alone stimulates the growth more than the control. Therefore the chemicals were dissolved in water at very low concentration (see methods).

### **Response of species to chemicals under different growth conditions.**

#### **A Green species**

##### **1. *Gleocystis* sp. (Figure 1.)**

Trichloroethylene enhanced the growth of the organism at all concentrations. However the response was more when the alga was incubated at lower temperature (20°C). Increasing the light intensity, Trichloroethylene enhanced the growth of the alga than the other chemicals.

##### **2. *Scenedesmus* sp. (Figure 2.)**

The percentage of survival of the alga was higher than the control in case of Carbon Tetrachloride and Chloroform than Trichloroethylene, especially in 0.1% and 0.2% concentrations. Also the growth of the alga was higher in 20°C than in 30°C. The experiments show also that low light irradiation ( $80 \text{ uEm}^{-2} \text{ s}^{-1}$ ) enhanced the utilization of the chemicals than high light irradiation ( $120 \text{ uEm}^{-2} \text{ s}^{-1}$ ).

### **3. *Chlorella sp.* (Figure 3.)**

The figure shows that the species was sensitive to all chemicals in both temperatures and both light irradiations.

### **4. *Chlorococcum sp.* (Figure 4.)**

The percentage of survival decreased with increasing the concentration of the chemicals. Lowering the temperature to 20°C and high light irradiation, the species was more sensitive than when was exposed to 30°C.

### **5. *Nannochloris sp.* (Figure 5.)**

Carbon Tetrachloride enhanced the growth of the alga, in low concentration (0.1%) and at 30°C (fig.5:). Trichloroethylene enhanced the growth of Nannochloris (fig.5:). Carbon Tetrachloride and Trichloroethylene enhanced the growth of the alga at high light intensity (fig. 5:). It was noticed that the phenol was toxic to the alga at both temperatures.

### **6. *Tetraselmis sp.* (Figure 6.)**

Trichloroethylene enhanced the growth of the alga at low concentration more than carbon tetrachloride and chloroform. Increasing the concentration, the species started to be sensitive with the exception Trichloroethylene enhanced the growth (fig.6:). In experiments run in high light intensity the chemicals started to lower the growth of the alga compared to the control, specially at high temperature (fig.6:).

### **Comparison of the chemicals in terms of growth conditions:**

Comparison of the chemicals (Figures 7,8,9,10) in terms of the response of the green species to Carbon Tetrachloride, Trichloroethylene, Chloroform and Phenol under growth conditions:

### Temperature 20° C (Figure 7,8)

***Scenedesmus*, *Nannochloris* and *Tetraselmis*** were promoted in Chloroform, Carbon Tetrachloride and Tetrachloroethylene.

***Chlorella* and *Chlorococcum*** were the most sensitive to the chemicals. However, all the species were tolerant to Trichloroethylene at high light intensity (Fig.8)

### Temperature 30° C (Fig.9,10)

All the species tolerated all chemicals, with the exception ***Chlorella*** was sensitive.

Comparison of the green species in terms of their response to the chemicals (Fig 7,8,9,10) the species could be grouped as following:

**Most tolerant: *Gleocystis*, *Scenedesmus*, *Nannochloris*, *Tetrasalmis***

**Medium Tolerant: *Chlorococcum***

**Most sensitive: *Chlorella***

It should be noted that ***Chlorococcum*** is a saltwater species and all other species are fresh water ones

### B. DIATOMS

#### 1. ***Cyclotella sp***: (Figure 10)

This diatom was sensitive to all chemicals specially chloroform and phenol under all growth conditions.

#### 2. ***Cylindrotheca sp***: (Figure 11)

The diatom survived the chemicals at 20° C but lower than the control. The diatom was more sensitive to the chemicals with increasing the temperature to 30° C (figure 11)

#### 3. ***Nitzschia pusilla sp***: (Figure 12)

The diatom was sensitive to chloroform and phenol. Chloroform was more toxic at high temperature (30° C).

**4. *Navicula saprophila* sp:** (Figure 13)

The diatom was tolerant to carbon tetrachloride and trichloroethylene followed by phenol, at low concentrations. The species was more sensitive to high concentrations ( 0.2. 0.3 %). Increasing the temperature (30° C) increased the sensitivity of the diatom (fig. 15: ).

**5. *Nitzschia* sp:** (Figure 14)

The diatom tolerated the chemicals at 20° C temperature more than at 30°C (Figure 14).Carbon tetrachloride and chloroform were enhancing the growth of the diatom.

**6.*Nitzschia dissipate*:** (Figure 15)

The diatom tolerated the different concentrations of carbon Tetrachloride and Tetrachloroethylene at both temperatures ( 20° C, 30° C). Chloroform was inhibitory to some extent, phenol was toxic in all concentrations.

**7.*Thalassiosira weisflagii*:** (Figure 16)

The diatom tolerated carbon tetrachloride, chloroform and Tetrachloroethylen.It was sensitive to phenol in temperatures.

Comparison of the chemicals (Figures: 17, 18, 19, 20) in terms of the response of the diatoms to carbon Tetrachloride, Trichloroethylene, Chloroform and Phenol under growth conditions.

**Temperature 20° C** (Figure 18, 19)

***Cyclotella*, *Cylindrotheca* and *Nitzschia pusilla***, promoted in low concentrations of Tetrachloroethylene, Carbon Tetrachloride and Chloroform but not in high concentrations ( 0.2 %). ***Navicula saprophila*, *Nitzschia*, *Nitzschia dissipita*** were promoted at all concentrations.

***Nitzschia dissipata* and *Thalassiosira weisflagii***, tolerated the chemicals under these conditions.

**Temperature 30° C** ( figures 20. 21)

***Nitzschia dissipata* and *Thalassiosira weissflogii*** tolerated Trichloroethylene, ***Nitzschia saprophila*** tolerated the chemicals to somewhat degree.

In case of Carbon Tetrachloride, most of species tolerated the chemical except ***Cylindrotheca* and *Nitzschia pusilla***, they were sensitive.

In case of Chloroform, all species were sensitive except ***Nitzschia dissipata*** and ***Thalassiosira weissflogii*** were sensitive to some degree.

Comparison of the Diatoms in terms of their response to the chemicals (Figures 18, 19, 20, 21) the species could be grouped as following:

**Most tolerant: *Nitzschis dissipata*, *Thalassiosira***

**Medium Tolerant: *Nitzschia saprophila*,**

**Most Sensitive: *Cyclotella*, *Cylindrotheca*, *Nitzschia pusilla*.**

It is noteworthy that ***Nitzschia sp*** is a freshwater species and all other species are saltwater species.

## **CONCLUSIONS:**

. Algal species varied in their response to the chemicals. As a result the species were grouped according to their sensitivity as following:

### **GREEN:**

Most Tolerant : ***Gleocysits*, *Scenedesmus*, *Nannochloris*, *Tetrasalmis*.**

Medium Tolerant : ***Chlorococcum*** (salt water)

Most sensitive : ***Chlorella***

### **DIATOMS:**

Most Tolerant : ***Nitzschia dissipiatas* *Thalassiosira***

Medium Tolerant : ***Nitzschia saprophila***

Most sensitive : ***Cyclotella*, *Cylindrotheca*,**

***Nitzschis pusilla*,**

***Nitzschia sp*** (fresh water)

. Tolerant species may indicate their ability to accumulate or degrade the chemicals. This question will be answered later in future work during this project.

- . Changes in the light intensity of the growth conditions did not produce changes in the response of the algal species towards the chemicals.
- . Changes of the growth conditions as temperature, produced variations in the response of algal species towards the chemicals. The green species were sensitive to the chemicals in 20° C than in 30° C. Diatoms tolerated chemicals in both temperatures.

#### **FUTURE PLANS:**

We will continue to investigate the effect of halogenated hydrocarbons on the aquatic organisms in the following experiments:

- .The effect of the chemicals will be assayed in growth media complete and deficient in one element (nitrogen as nitrate, phosphorus as phosphate or silicon as silicate).
- .The response of algal species to the chemicals will be determined in the original medium after being enriched with various sea salt concentrations 15, 25 or 35 ppt (parts per thousands).
- .The above experiments will be performed with single species.

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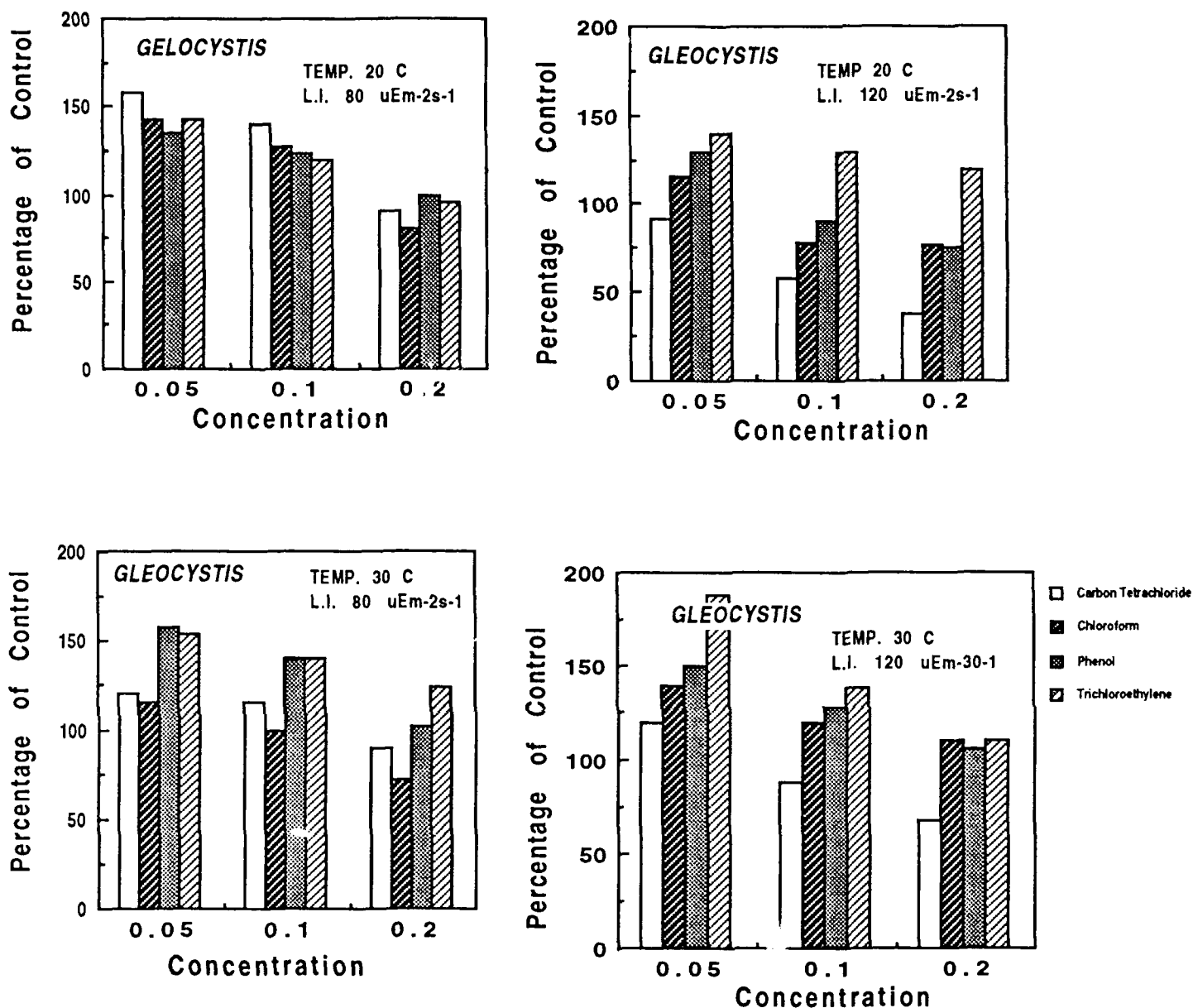


Figure 1: Effect of chemicals on growth of green alga, *Gleocystis* sp. as a percentage of the control. Standard deviation did not exceed 2%.



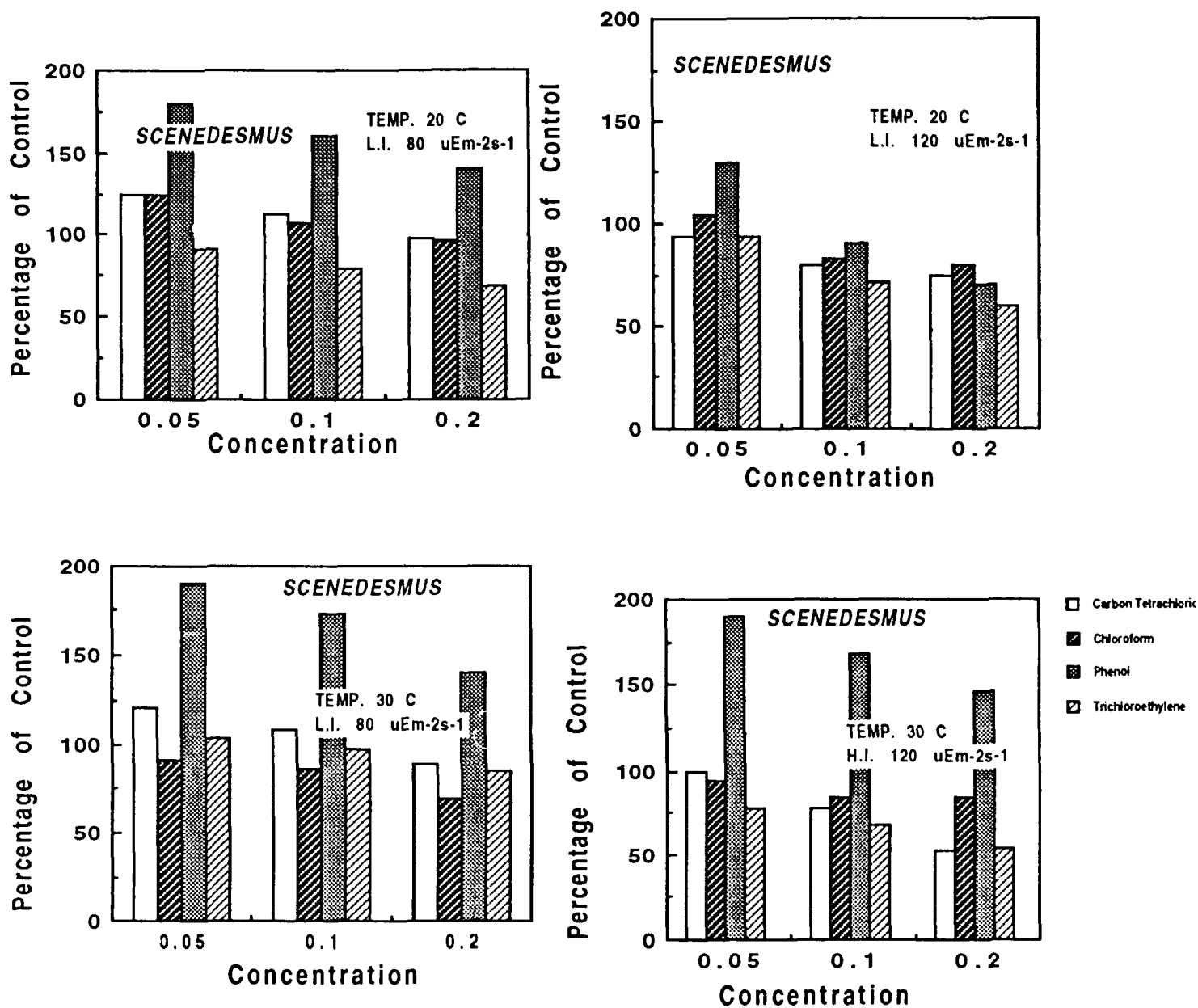


Figure 2: Effect of chemicals on growth of green alga, *Scenedesmus* sp. as a percentage of the control. Standard deviation did not exceed 2%.

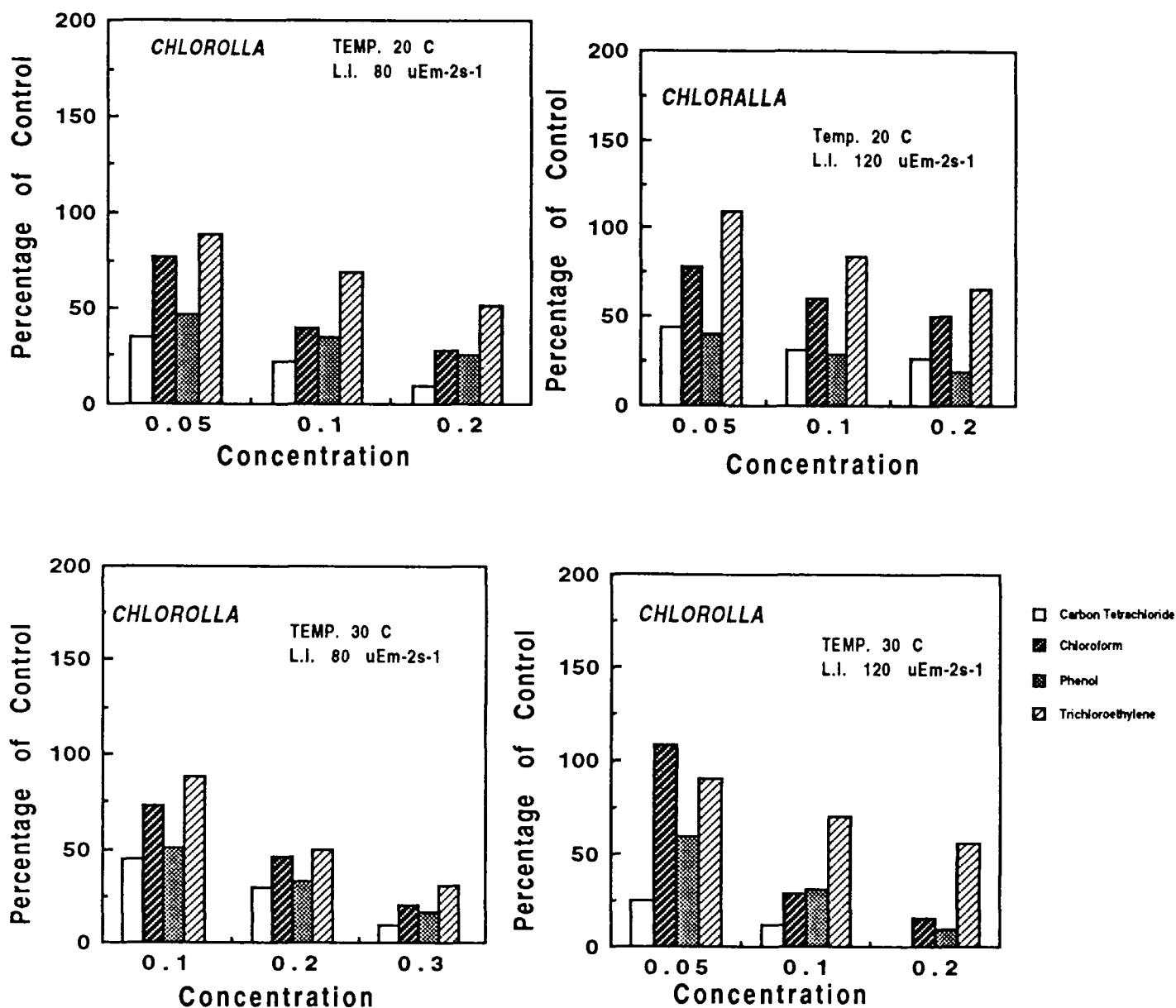


Figure 3: Effect of chemicals on growth of green alga, *Chlorella sp.* as a percentage of the control. Standard deviation did not exceed 2%.

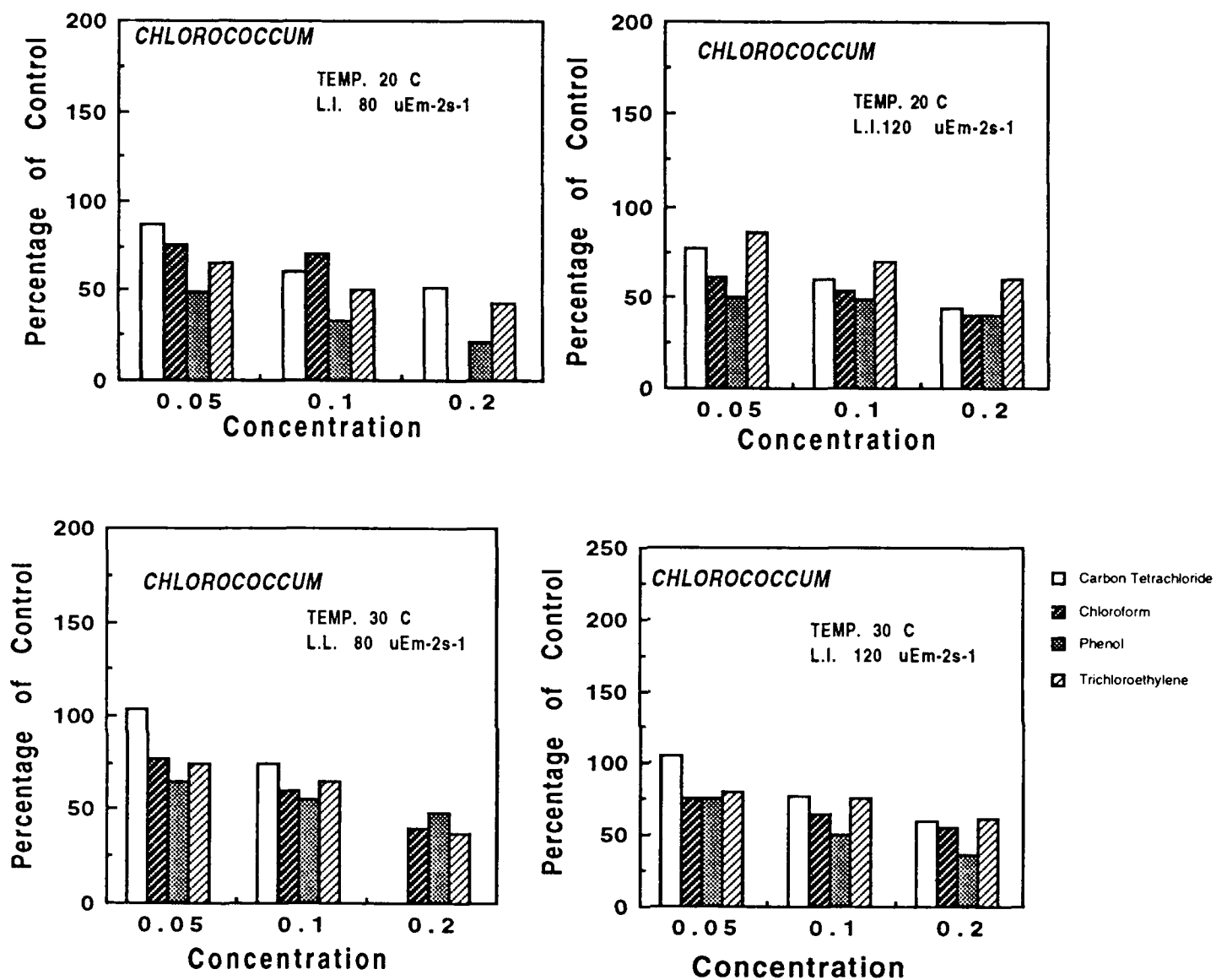


Figure 4: Effect of chemicals on growth of green alga, *Chlorococcum* *sp.* as a percentage of the control. Standard deviation did not exceed 2%.

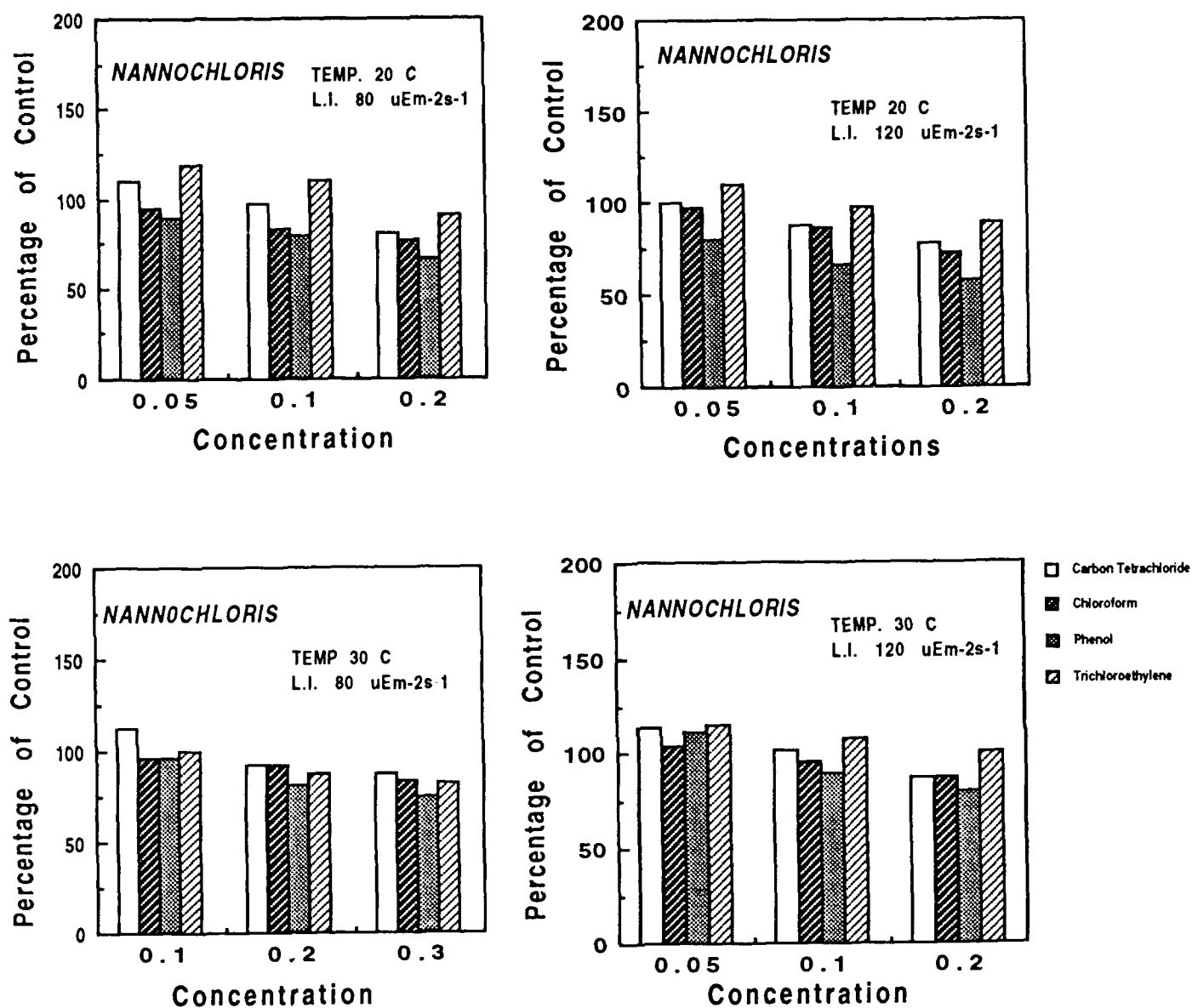


Figure 5: Effect of chemicals on growth of green alga, *Nannochloris* sp. as a percentage of the control. Standard deviation did not exceed 2%.

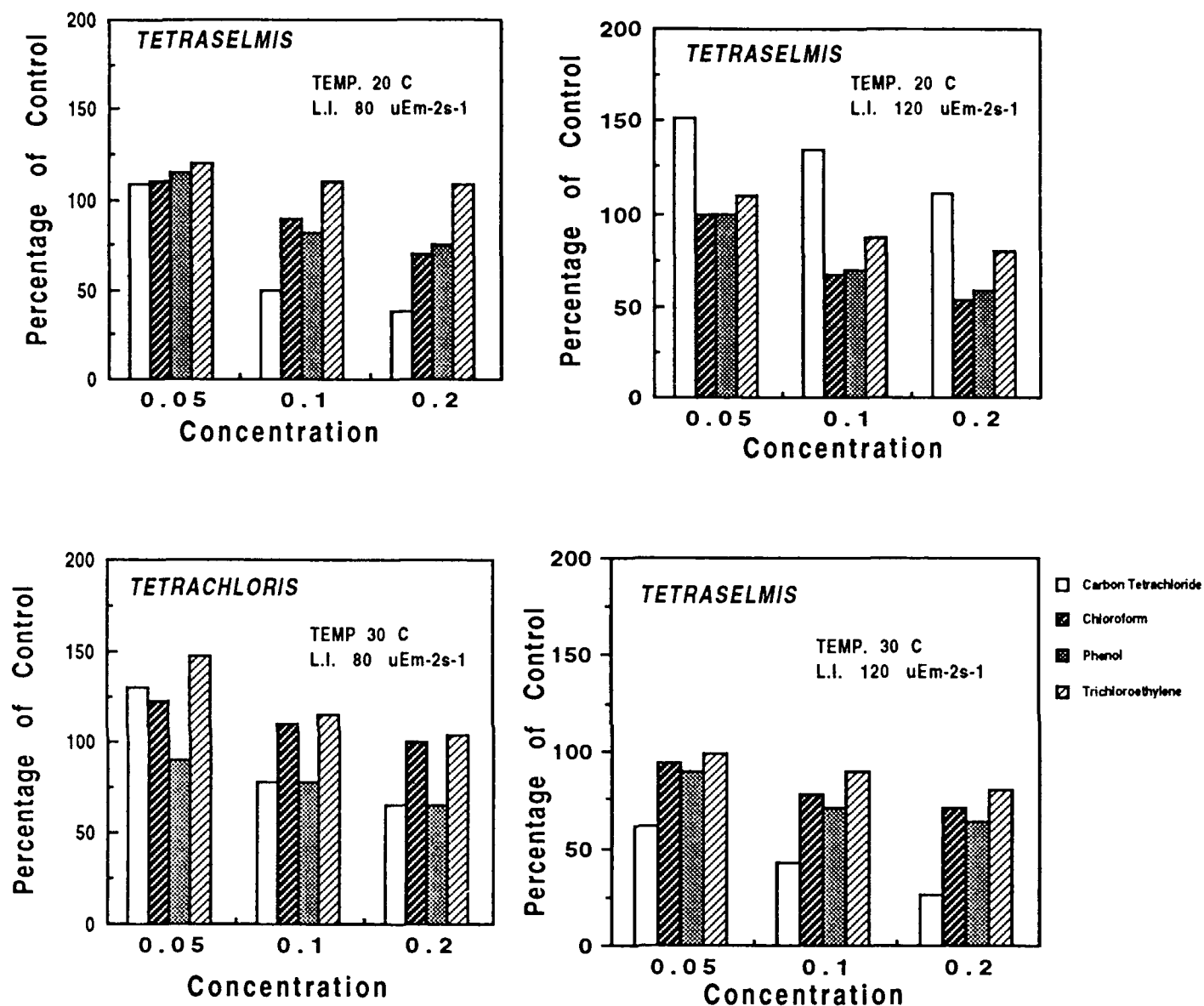


Figure 6: Effect of chemicals on growth of green alga, *Tetraselmis sp.* as a percentage of the control. Standard deviation did not exceed 2%.

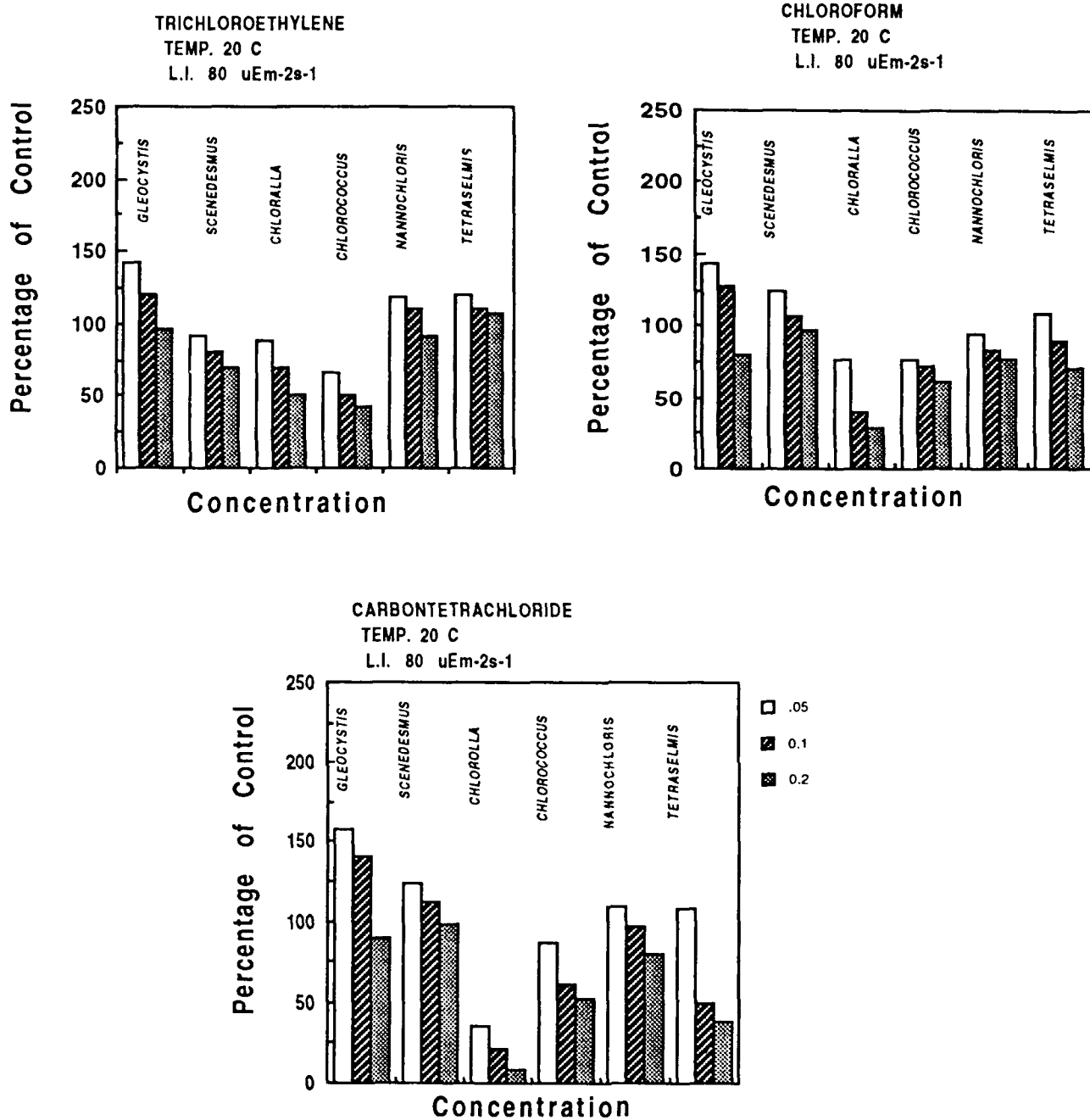


Figure 7: Response of green algal species to chemicals, at Temp 20 C and L.I.  $80 \mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

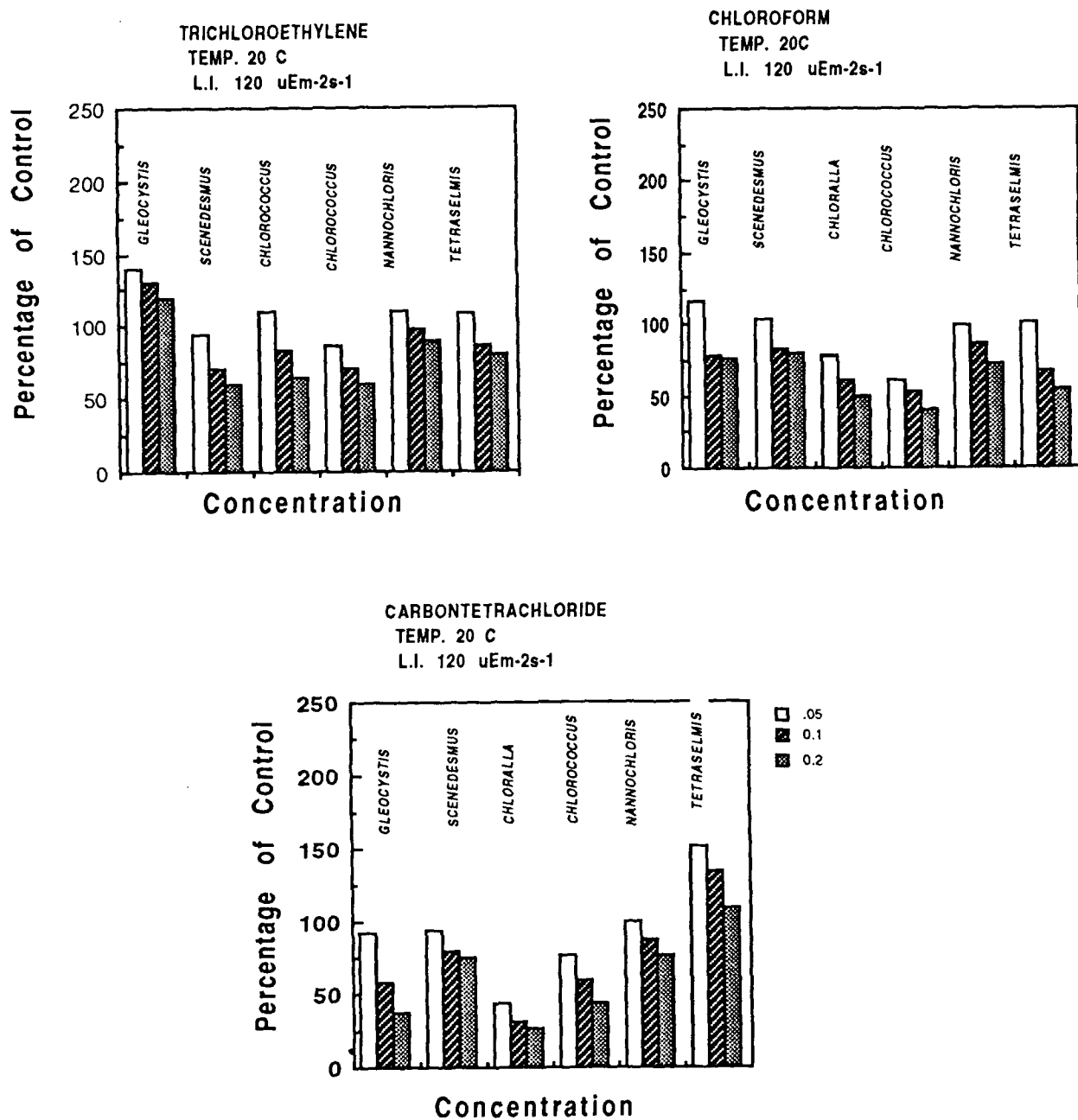


Figure 8: Response of green algal species to chemicals, at Temp 20 C and L.I.  $120 \mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

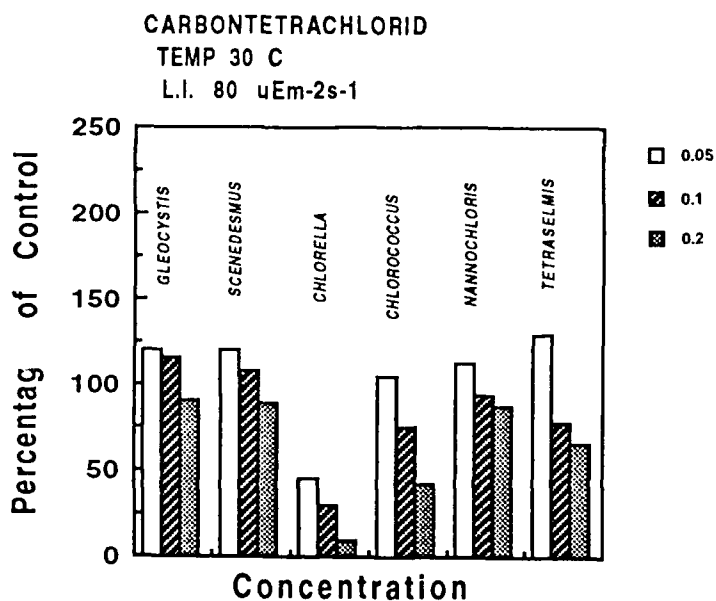
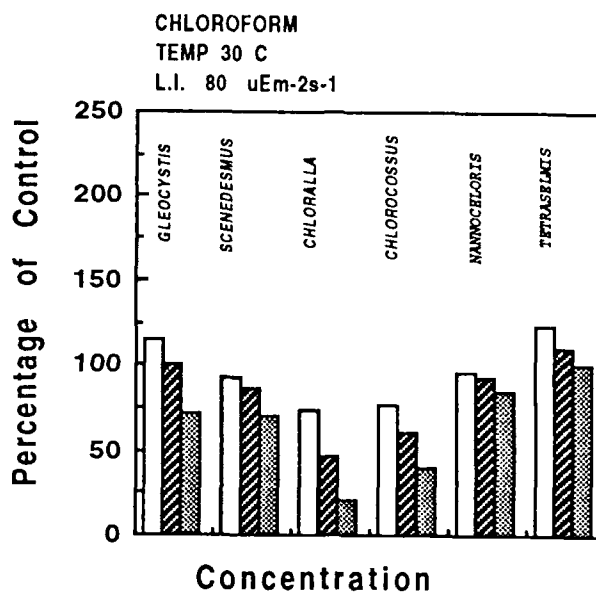
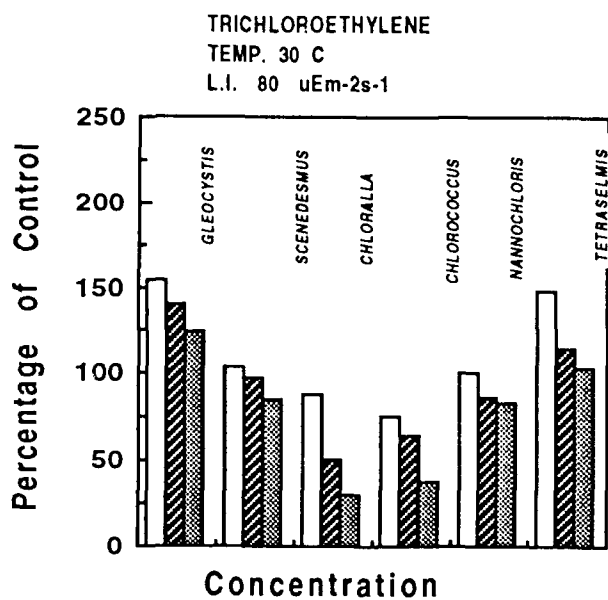


Figure 9: Response of green algal species to chemicals, at Temp 30 C and L.I. 80  $\mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.



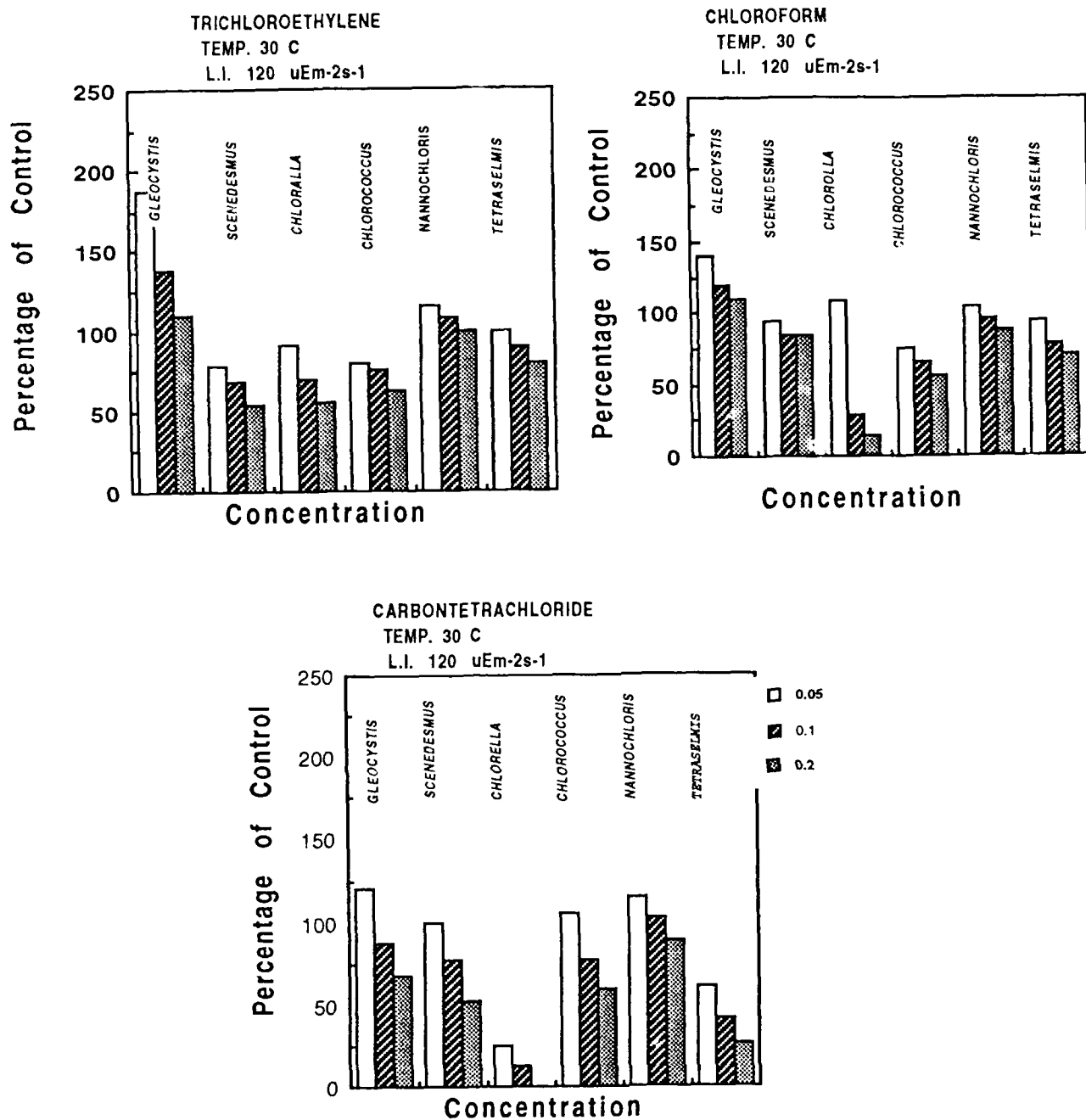


Figure 10: Response of green algal species to chemicals, at Temp 30 C and L.I.  $120 \mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

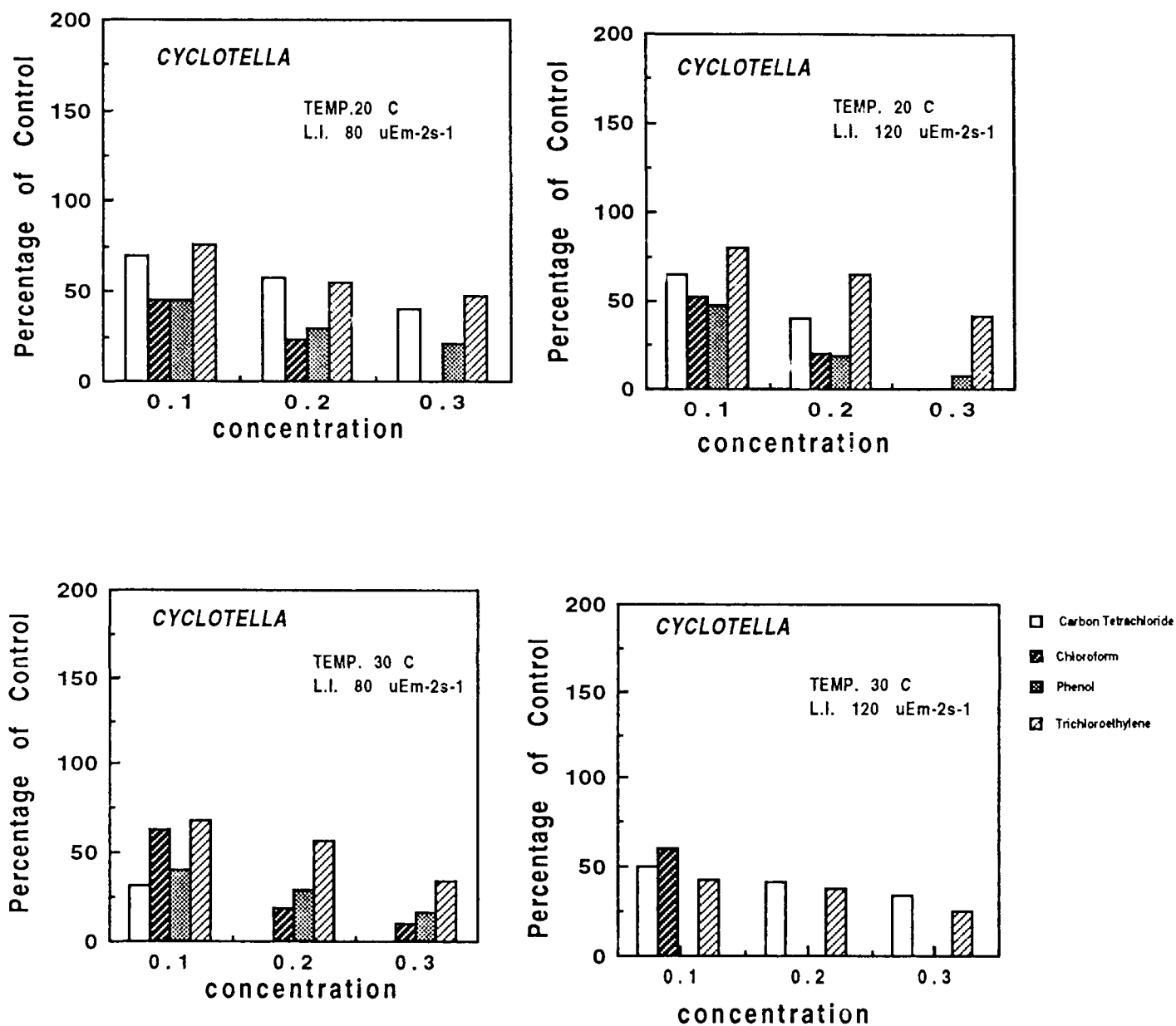


Figure 10: Effect of Chemicals on growth of diatom, *Cyclotella* sp. as a percentage of the control. Standard deviation did not exceed 2%.

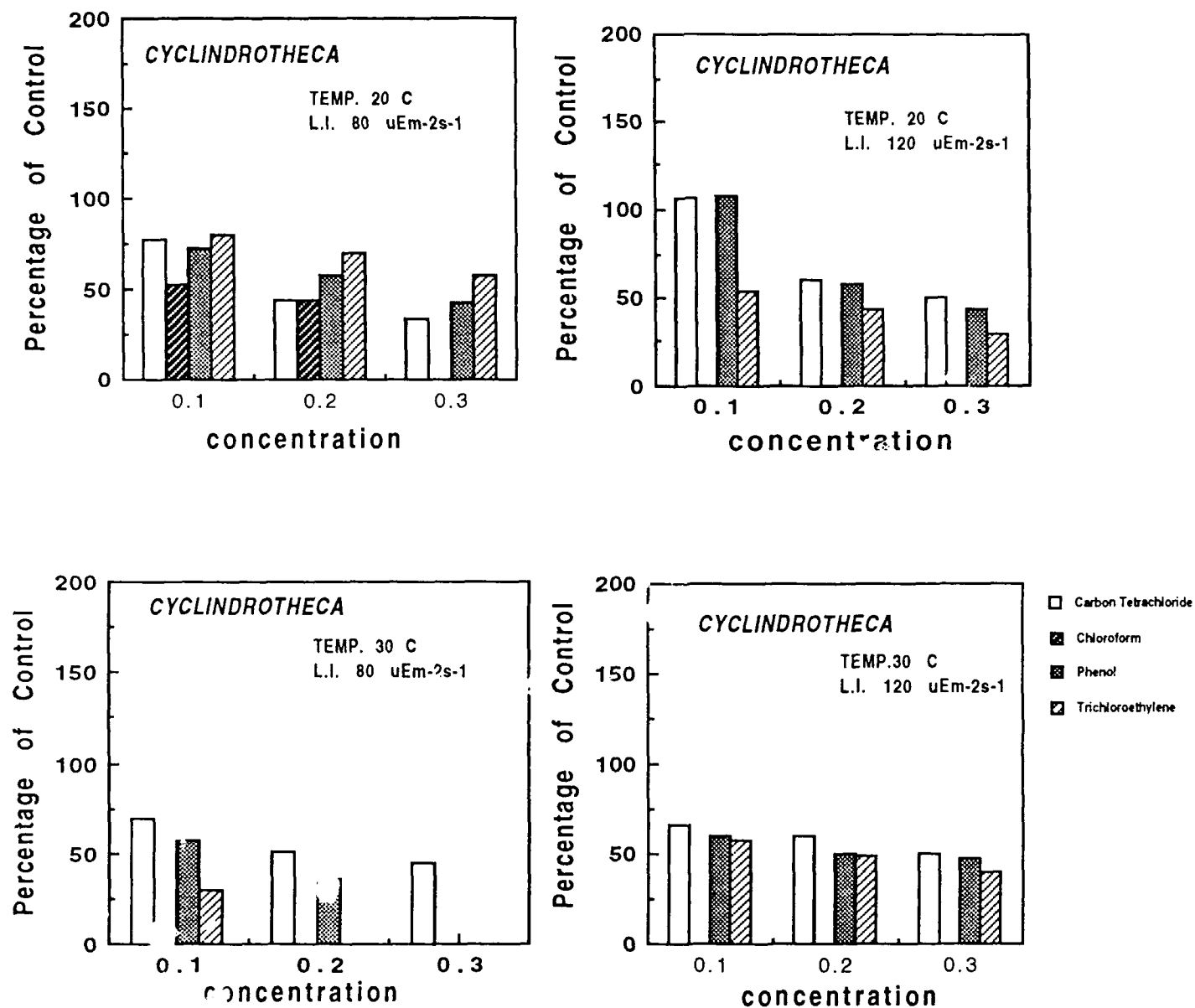


Figure 11: Effect of Chemicals on growth of diatom, *Cylindrotheca* sp. as a percentage of the control. Standard deviation did not exceed 2%.

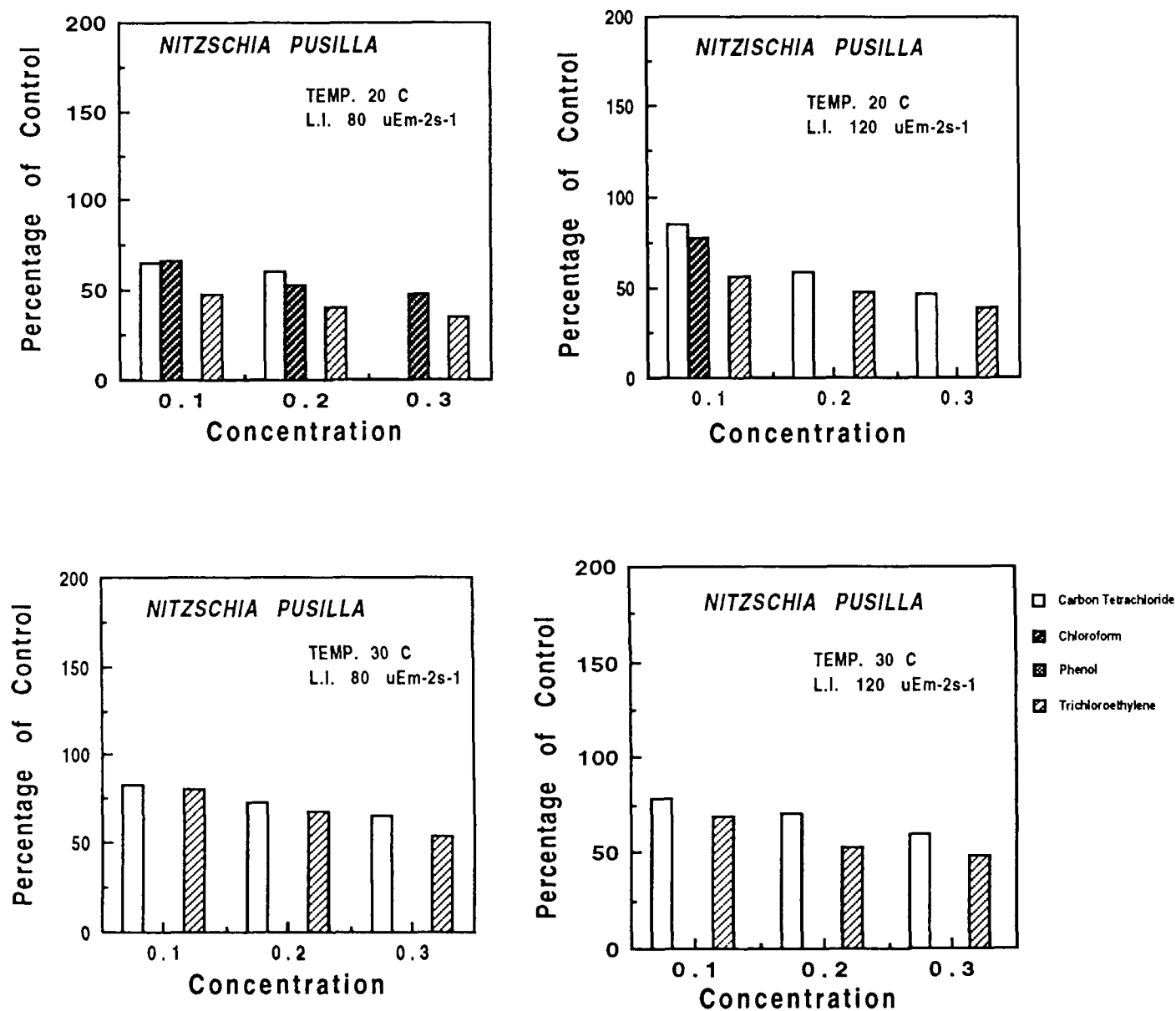


Figure 12: Effect of Chemicals on growth of diatom, *Nitzschia pusilla* sp. as a percentage of the control. Standard deviation did not exceed 2%.

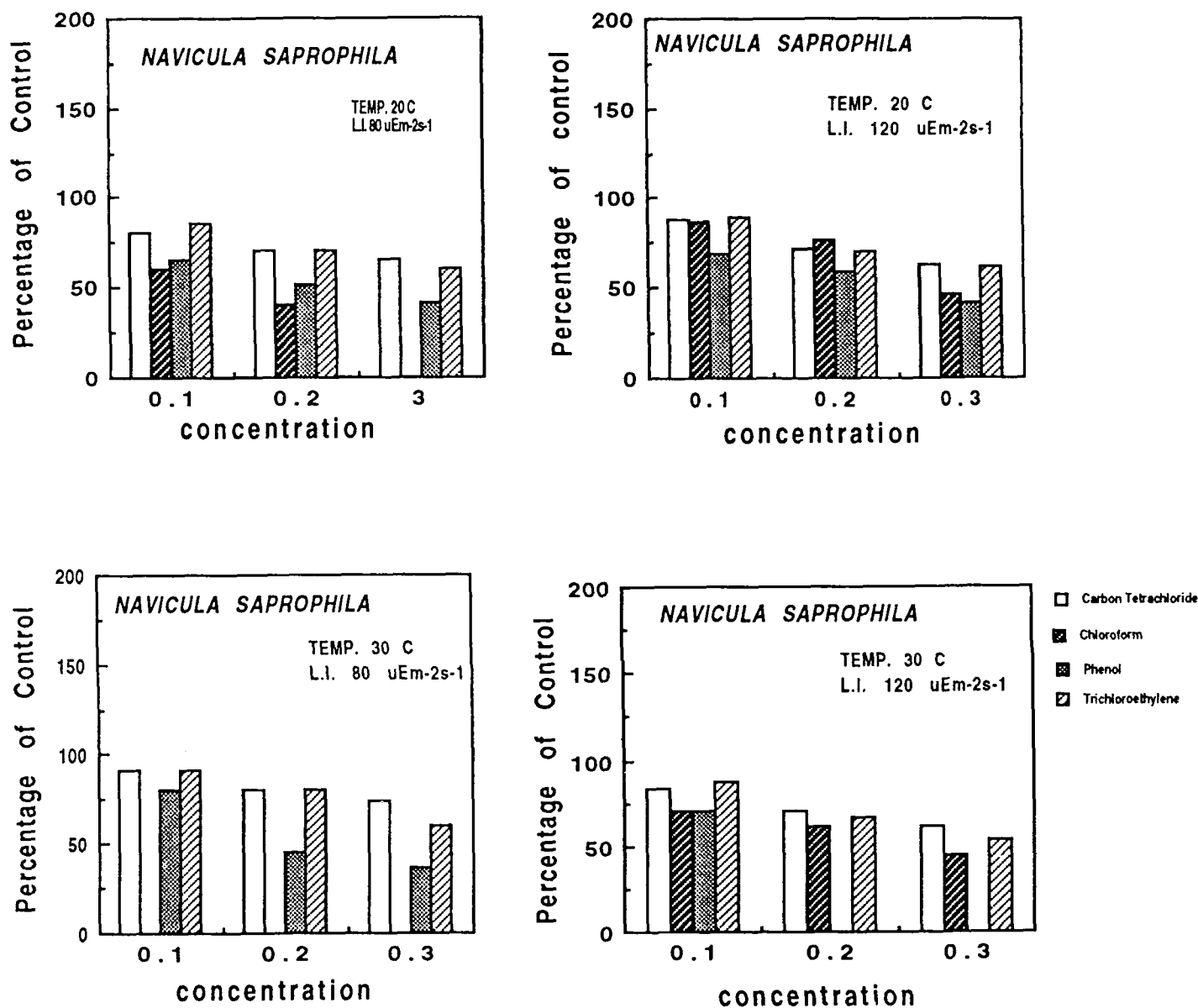


Figure13: Effect of Chemicals on growth of diatom, *Navicula saprophila* sp. as a percentage of the control. Standard deviation did not exceed 2%.

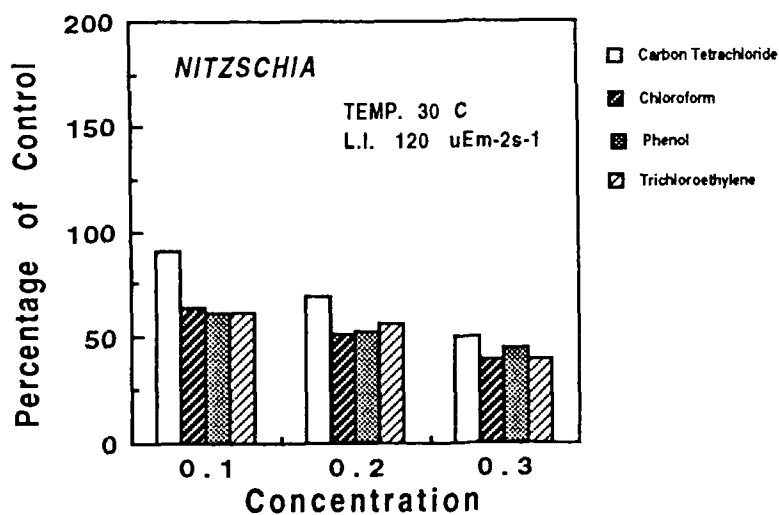
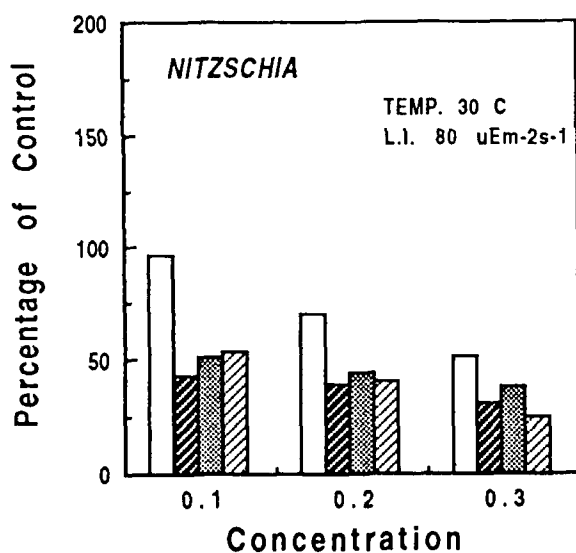
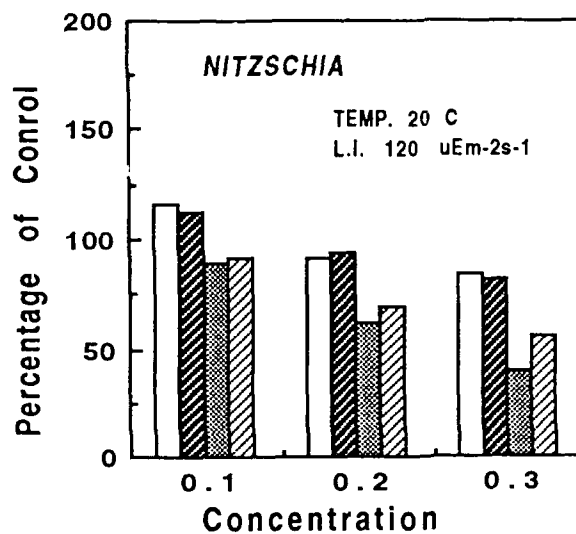
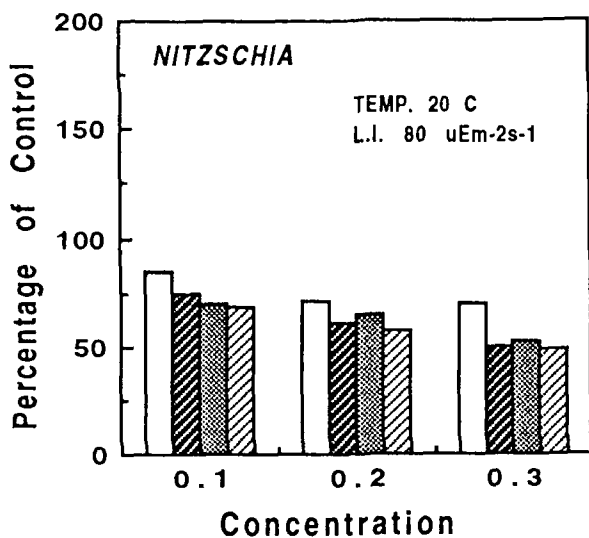


Figure 14: Effect of Chemicals on growth of diatom, *Nitzschia sp.* as a percentage of the control. Standard deviation did not exceed 2%.

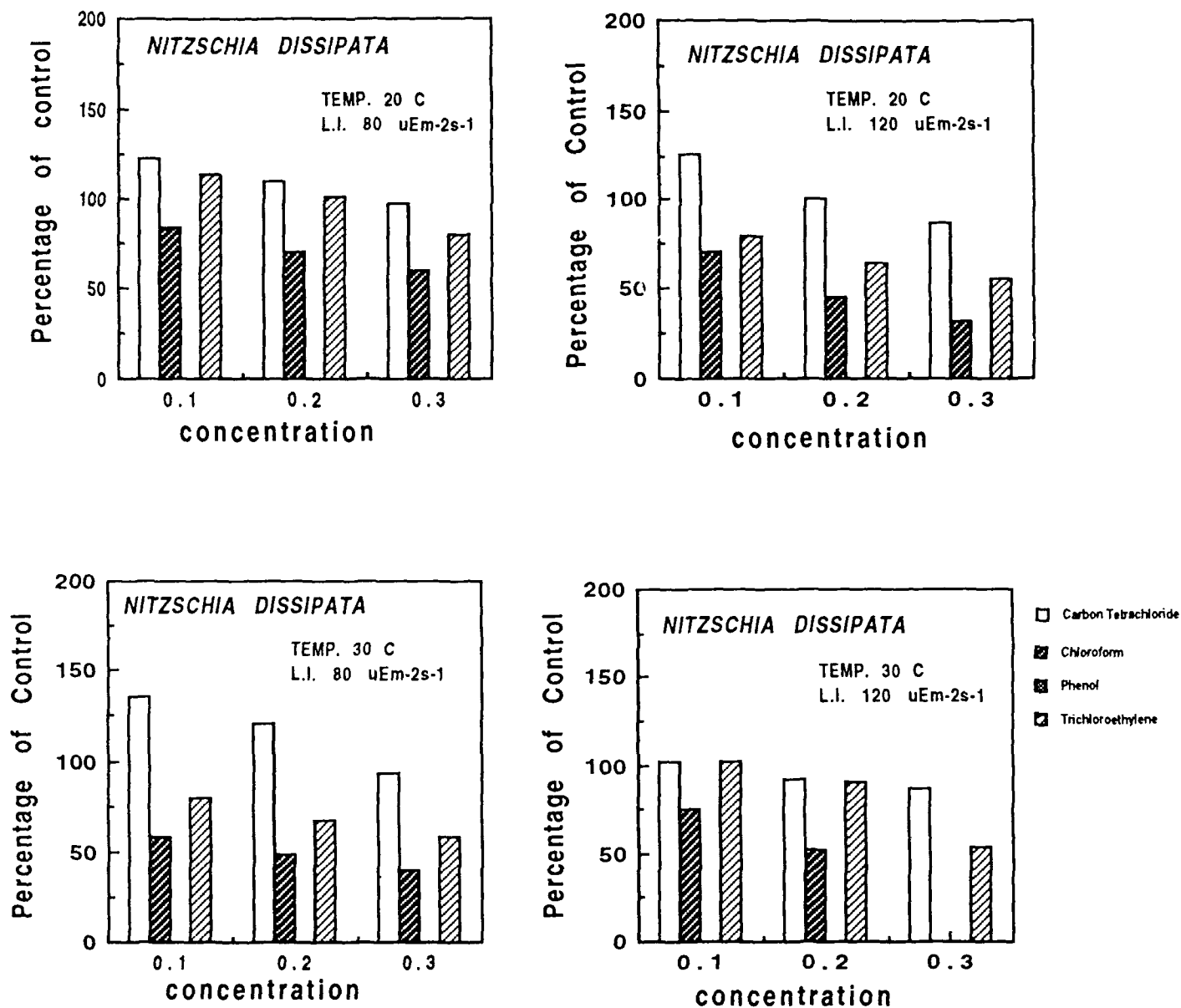


Figure 15: Effect of Chemicals on growth of diatom, *Nitzschia dissipata*. as a percentage of the control. Standard deviation did not exceed 2%.

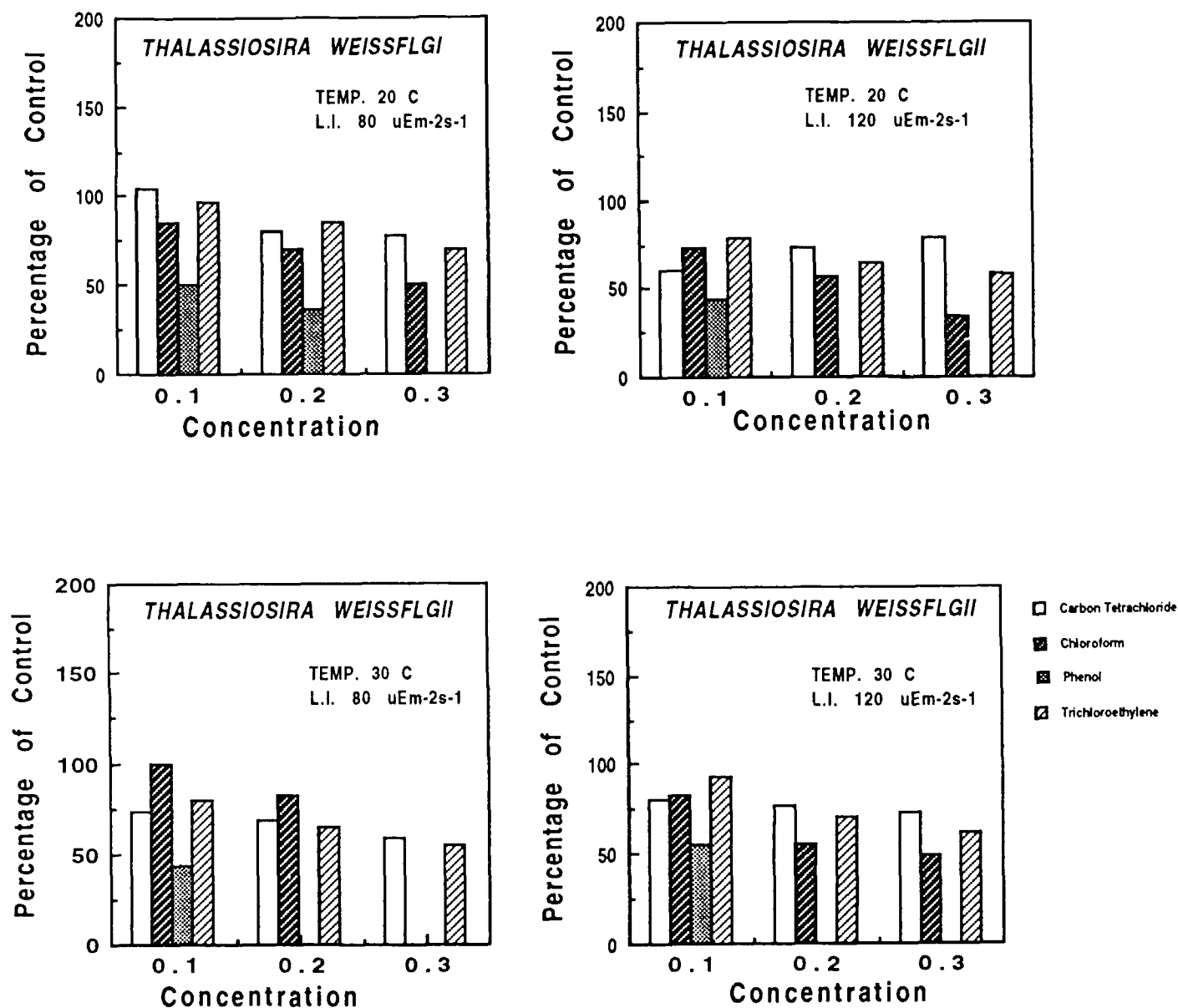


Figure 16: Effect of Chemicals on growth of diatom, *Thalassiosira weissflogii*, as a percentage of the control. Standard deviation did not exceed 2%.



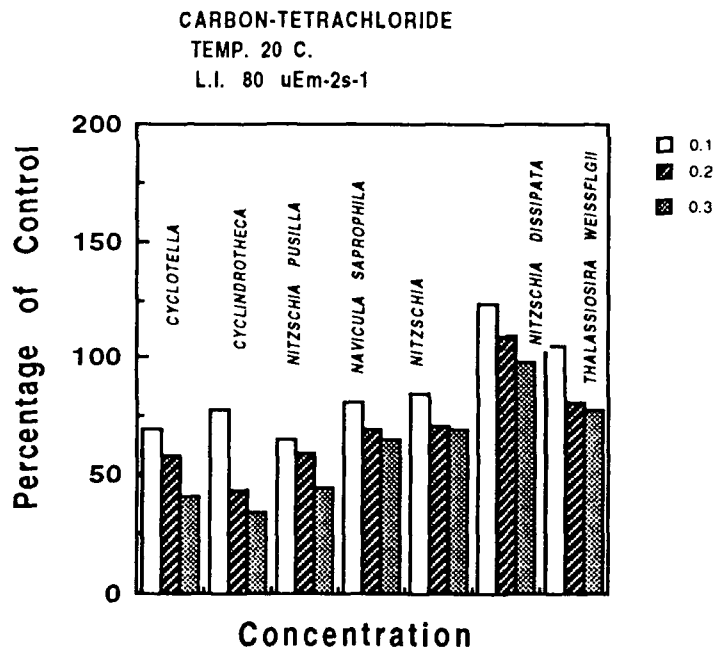
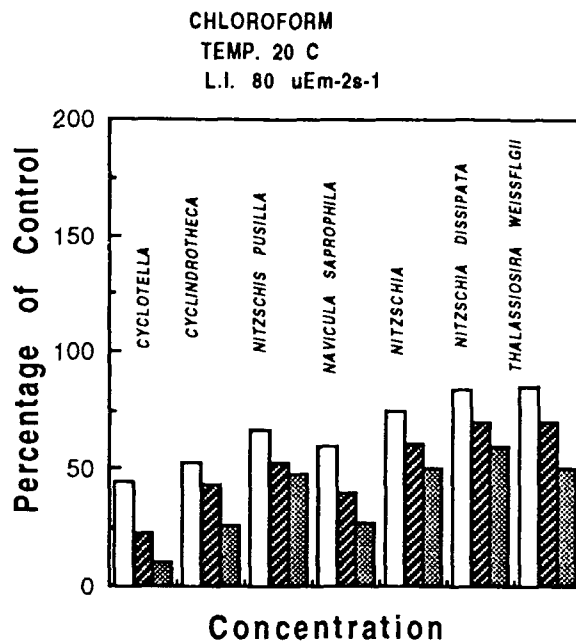
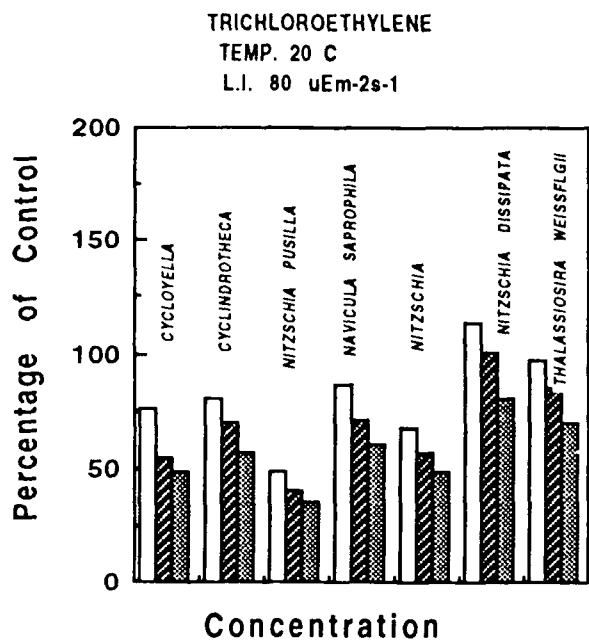


Figure 17: Response of diatom species to chemicals, at Temp 20 C and L.I. 80  $\mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

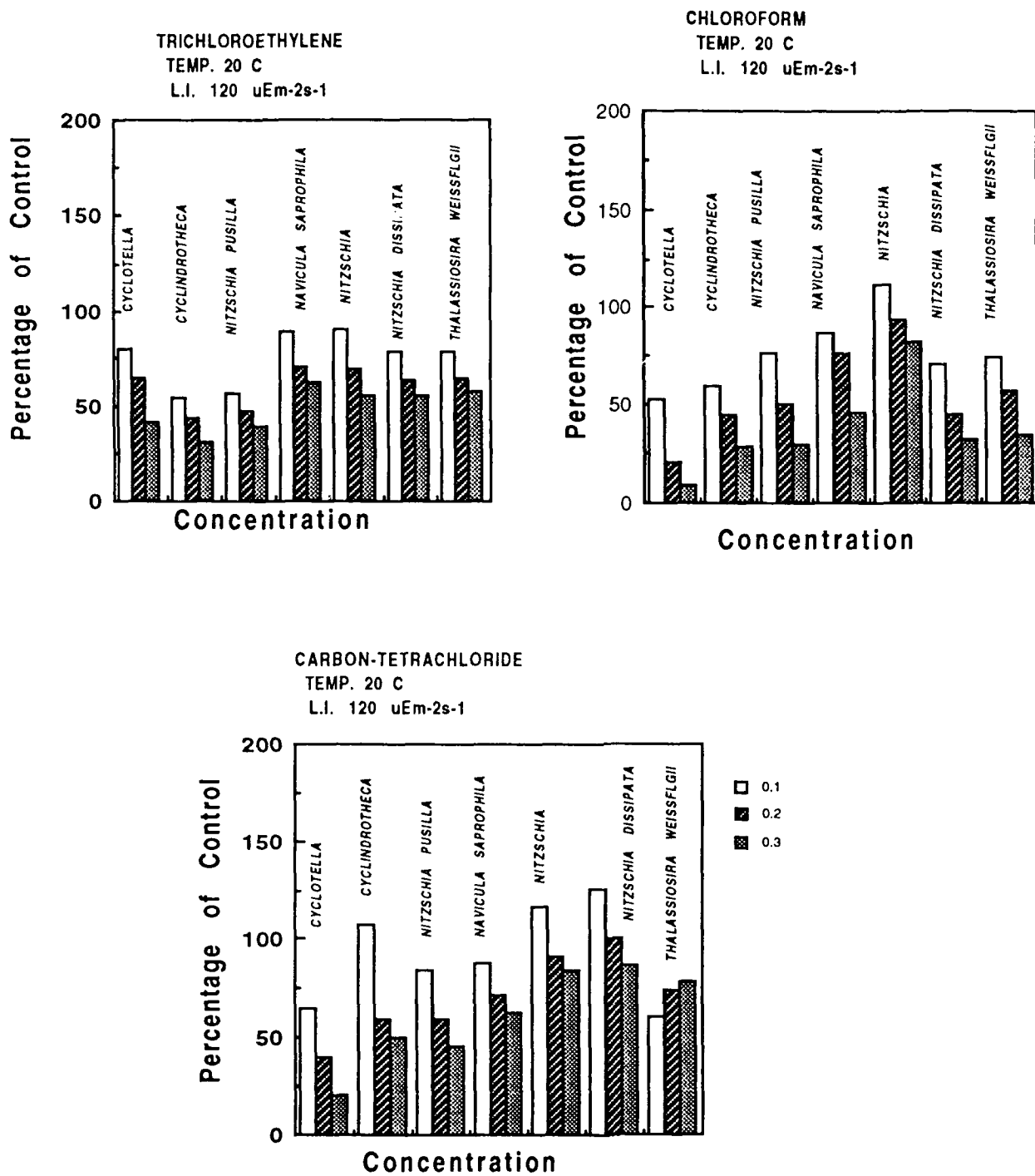


Figure 18: Response of diatom species to chemicals, at Temp 20 C and L.I.  $120 \mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

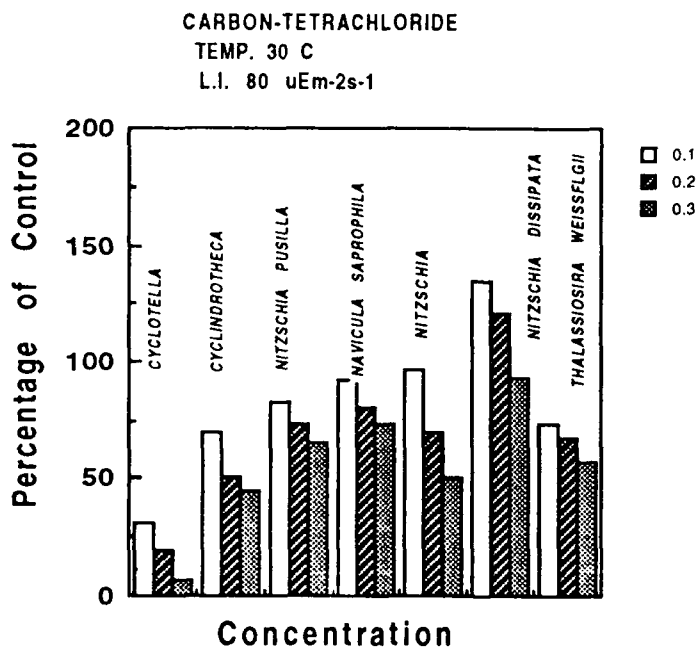
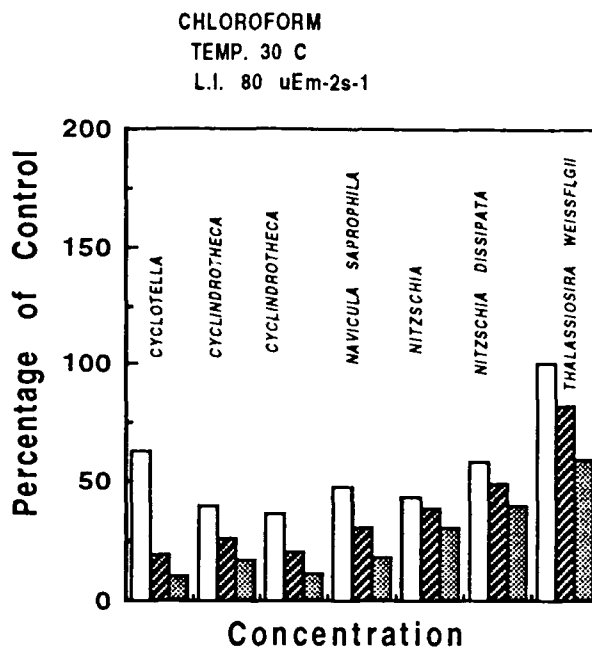
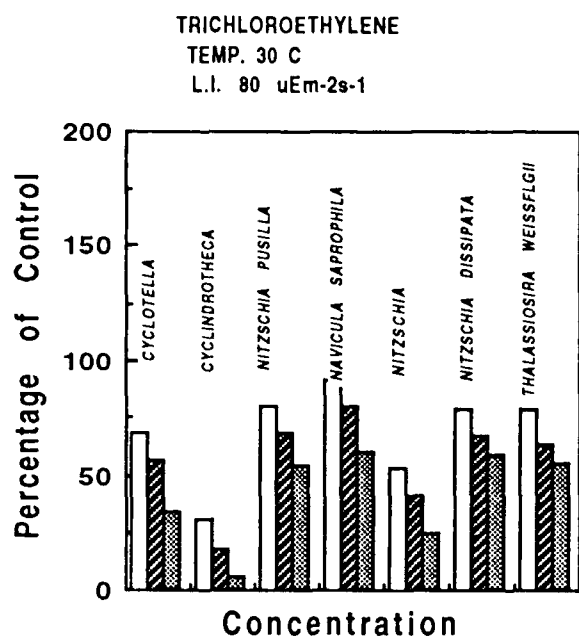


Figure 19: Response of diatom species to chemicals, at Temp 30 C and L.I. 80  $\mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

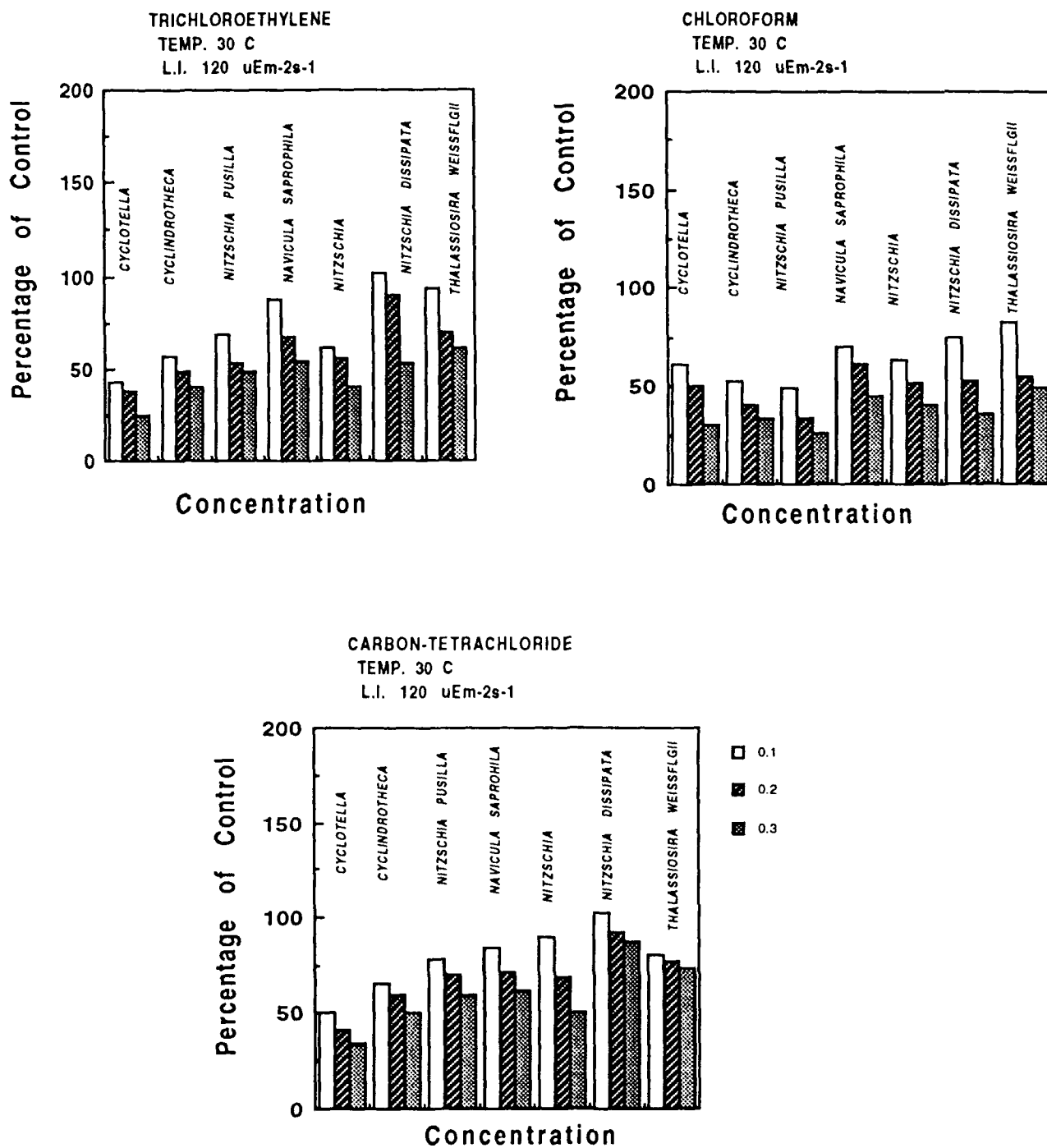


Figure 20: Response of diatom species to chemicals, at Temp 30 C and L.I.  $120 \mu\text{Em}^{-2}\text{s}^{-1}$ , as a percentage of the control.

## **PERSONNEL**

The following personnel have been involved in this project:

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