THE GROWTH PROMOTING EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) and 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T) ON *Microcystis aeruginosa*

by

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I. INTRODUCTION

Modern weed control practices are based almost exclusively on the use of herbicides. In the years since World War II, chlorinated herbicides have increased in use many fold. In 1969 alone, 49,000,000 lbs. of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 4,900,000 lbs. of 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) were produced (1). Production and use of herbicides have been carried out with little concern for their effects on the environment. Herbicides created within the last thirty years were developed and put to use before extensive environmental testing could be completed. This is evidenced by the small amount of literature available involving effects on microbial communities, particularly the algae (2).

The herbicides 2,4-D and 2,4,5-T have been eyed with suspicion in recent years by the Virginia State Water Control Board. Evidence has been gathered which correlates algal blooms with concomitant dumping or heavy sprayings of 2,4-D and 2,4,5-T (3). The blue-green alga, Microcystis aeruginosa, has been named as the blooming organism in one system receiving herbicidal contamination (4) and suspected in another (5).

Various studies have shown that very small amounts of indoleacetic acid will stimulate the growth of several algae (6-9). The compounds 2,4-D and 2,4,5-T have been shown to mimic the physiological effects of indoleacetic acid (10, 11, 12, 13).

In a study by Poorman (14), 2,4-D and 2,4,5-T were shown to increase the growth of Euglena gracilis significantly when incubated over a seven day period. Worth and McCabe (15) have also found 2,4-D to increase the growth of several bacteria.

It is the purpose of this study to assess the effect of 2,4-D and 2,4,5-T on the growth of the blue-green alga, Microcystis aeruginosa.

II LITERATURE REVIEW

Microcystis aeruginosa

Microcystis aeruginosa Kutz amend Elenkin [*Anacystis cyanea* Drouet and Daily (16)] is a planktonic blue-green alga (Cyanophyceae) of the order Chroococcales. It is known widely for the obnoxious character of its bloom (17, 18, 19). Individual cells measure from 1 to 6 microns in diameter and are spherical or ellipsoidal in shape (20). Under natural conditions the cells occur together in irregularly shaped gelatinous masses measuring up to a centimeter in diameter (21). Colonies are free floating due to the ability of the organism to form gas vacuoles (20). *Microcystis aeruginosa* does not fix nitrogen as do many other blue-green algae (20).

Reports of massive blooms of *Microcystis aeruginosa* are numerous (19, 22, 23). No conclusive studies are available which give an acceptable explanation for the occurrence of the blooms (19). Many investigators have tried to show stimulation of growth through nutritional spikes of various macro and micro nutrients (22, 24, 25, 26). Phosphorus and nitrogen have been studied extensively (24, 27, 28).

Gerloff *et al.* (28) reported phosphorus levels could be as low as 0.18 mg/l before growth was affected

appreciably in a unialgal culture of M. aeruginosa. The ratio of nitrogen to phosphorus could be as high as 75:1 and still yield maximum crops of the alga.

Barica (29) reported good correlation between high nitrogen levels during winter and subsequent Microcystis blooms the following summer. Studies by McLachlan and Gorham (24) showed M. aeruginosa will grow equally well on ammonium and nitrate as a nitrogen source. Urea was shown to be the only source of organic nitrogen tested that resulted in normal growth of the alga.

Control of pH is not critical to good culture growth between the pH levels of 6.5-10.0 (24, 25). However, recorded pH levels on natural blooms indicate that pH values between 9 and 10 are typical of the waters normally experiencing blooms (17, 22, 23).

Although the addition of sodium bicarbonate or sodium carbonate has been shown to be stimulatory in culturing a number of blue-green algae, M. aeruginosa has shown no apparent need for them in the laboratory (30). Subsequently, adjustments of pH in cultures of M. aeruginosa has been done with either sodium hydroxide or sodium carbonate (24, 28). The use of potassium hydroxide has been avoided due to an apparent low tolerance of potassium ion by the alga (31). Prescott (22) reports that in a

study of several Iowa lakes, Microcystis favored lakes with high levels of carbonate alkalinity.

Boyd (32) and Lange (33) have shown that Microcystis aeruginosa excretes one or more extracellular products which are capable of limiting growth of competing algae. These works suggest that M. aeruginosas ability to inhibit competing algae plus the ability to take full advantage of incident light energy via gas vacuole flotation (20) are two important factors in allowing it to take over a body of water.

The problems resulting from massive blooms of Microcystis aeruginosa include the death of fish, livestock, and poultry (18, 22). Death has been attributed to an extracellular polypeptide consisting of ten amino acids (22). In addition, death of life forms in the water has been attributed to the diurnal oxygen fluctuation due to bacterial decomposition of decaying algal mass (34).

Taste and odor problems also arise as a result of the die off of a bloom. This has been attributed to the relatively high content of protein and other nitrogen bearing substances within Microcystis (22). Decomposition of these materials produces a disagreeable "fishy" taste and odor in the water.

Stanier et al. (34) suggest that rapid die off of M. aeruginosa blooms may be attributed to bacterial or

viral invasion. Two phycoviruses have been found to exist for Microcystis (35). However, no evidence is yet available that proves the rapid die off is because of viral or bacterial infection (34).

Simmons and Armitage (23) did an extensive study on a chronic bloom of Microcystis aeruginosa on the Potomac River in 1972. Historical data showed that the Potomac River had increased dramatically in levels of total phosphorus, nitrogen, and organic carbon since the first studies in 1913 (19). The increases were attributed to rapid urbanization in the Washington, D.C. area. Blooms of Microcystis aeruginosa began appearing in 1959 at the mouth of the Anacostia River in Washington. The bloom continued each summer until the summer of 1973. The length of the Potomac River affected by the bloom had extended from year to year, reaching a maximum of approximately thirty miles in 1969 and 1970. At this time the oxygen demand placed on the system by the alga was greater than the total demand of all the wastewater plants in the Washington area (36). Maximum concentration of Microcystis was noted from July through October in 1972 at a mean concentration of 84,800 cells/ml in samples collected each week from a station ten to fifteen miles below Washington, D.C.

Eutrophic conditions existed on the Potomac River during the Simmons and Armitage study (23). Nutrient levels varied seasonally but were within ranges generally accepted to stimulate algal growth (37). Temperatures fluctuated between 20°C and 35°C during the summer months. The pH on the Potomac ranged from 6.3 to 10. The highest pH values occurred from June through August. The Potomac River in the area of the bloom is considered to be a fresh-water estuary. Flow is very slow with tidal effects. It takes 40 days to move 24.2 km downstream from Washington (38).

An EPA report (23) noted that the Microcystis bloom suddenly disappeared in 1973 on the Potomac. Phosphorus and nitrogen contents in the river were reported to be at levels as high as those prevalent during the bloom. Only the total organic carbon levels were appreciably lower. Since 1973, levels of phosphorus and nitrogen have been recorded as high or higher than those recorded during bloom conditions (39).

It is interesting to note that during the years of the bloom a herbicide formulator was discharging heavy concentrations of herbicide to the Potomac River (4). The plant is located a few hundred yards from the mouth of the Anacostia River, where the bloom was first reported.

Discharges of herbicide formulations of 2,4-D, 2,4,5-T silvex, atrazine, arsenate, dicamba, and methoxychlor have been recorded by the Virginia State Water Control Board in samples from the discharge pipe leaving the property of the formulator (4). From 1.5 to 2.0 million gallons of herbicide formulations are mixed each year at the facility. Empty or near empty railroad cars were cleaned and emptied onto the lot as a regular practice. Wastes spilled or drained onto the lot either percolated into the ground or were carried some 50 yards to the Potomac River by storm drains (4).

In an interview with an engineer from the Water Control Board (40), it was learned that the herbicides 2,4-D and 2,4,5-T made up a large portion of the formulations used by the company starting in the late 1950's and continuing until the early 1970's. Discharges of herbicides to the river were closely monitored by the Water Control Board beginning in the spring of 1974. Discharge since that time has been minimal (4).

It is interesting to note that the years of the discharge of 2,4-D and 2,4,5-T approximate the time frame of the Microcystis aeruginosa bloom. Also, the bloom was first sighted just a few hundred yards from the plant in 1959.

Other blooms have been reported by the Virginia State Water Control Board in recent years which indicate possible herbicide influenced stimulation (3).

Lake Chesdin in Chesterfield County, Virginia reportedly experienced a sudden algal bloom within a few days after the adjoining woodlands received a heavy spraying of 2,4,5-T and 2,4-D (41).

A pond in Orange, Virginia experienced a heavy bloom of M. aeruginosa downstream from a herbicide dealer that made a practice of disposing of emptied herbicide bags and containers onto the ground a few feet from the stream feeding the pond. The dealer sold 2,4-D, 2,4,5-T, atrazine, and paraquat to local farmers (5).

Phenoxy-Carboxylic Herbicides

All herbicides in the phenoxy-carboxylic herbicide family are characterized by an aromatic benzene ring with an oxygen molecule forming a bond between an aliphatic chain of one or more carbons and a terminal carboxyl group (43). Chlorine molecules and/or aliphatic chains are included on the ring. Distinction between the phenoxy-carboxylic herbicides is drawn from the length of the aliphatic chains and number of chlorine atoms on

the ring. Also, various salts and ester compounds are formulated from the parent acid formation.

The first formulation of phenoxy-carboxylic herbicides was achieved in Germany in the 1930's (44). Investigators were attempting to synthesize compounds related to the auxin, indoleacetic acid. After World War II, it was discovered that phenoxy-carboxylic herbicides were capable of selectively killing broad leaf plants while not effecting growth of cereal and grass crops (13). Rapid expansion of the use of phenoxy-carboxylic herbicides has been undertaken since World War II, particularly in the agricultural industry (1).

The most widely used phenoxy-carboxylic herbicides are 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (45).

2,4-D (2,4-Dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid)

The only structural difference between 2,4-D and 2,4,5-T is in the extra chlorine molecule on the 2,4,5-T molecule at the five position of the phenol ring. A wide variety of similarities between the two are pointed out throughout the literature; including mode of

action, metabolism, and absorption characteristics. Applications for the two compounds are similar, although 2,4-D may not be used for the control of woody plants.

The compound 2,4,5-T is a white solid chlorophenoxy herbicide. It has a molecular weight of 255.5, a melting point of 154°C, and specific gravity of 1.80. The compound is stable to 200°C at which point decomposition occurs. In water, 2,4,5-T will dissolve to 238 mg/l at 30°C. Under normal conditions of light and temperature 2,4,5-T is very stable (44).

The compound 2,4-D is a white, crystalline, odorless herbicide. It has a molecular weight of 221.0, a melting point of 135°C, specific gravity of 1.565, and is stable under normal light and temperature conditions (44).

The acid forms of 2,4-D and 2,4,5-T are most widely used for control of broad leaf weeds in fallow or non-crop areas (46). However, most dicotyledon plants are susceptible to 2,4-D and 2,4,5-T at herbicidal application rates of 0.25 to 4 pounds per acre (44, 47). Resistance of higher plants to the phenoxy herbicides, most notably

the grasses, correlates best with lack of a vascular cambium and pericycle (11). .

Application of chlorophenoxy herbicides is generally achieved by spraying with aerial or ground equipment. Most sprayings take place after emergence of the target weeds (44).

Much research has been done on the mode of action of the chlorophenoxy herbicides, particularly 2,4-D. However, no one has proposed a universally accepted explanation for the mode of action (11, 12, 13). In a review of 2,4-D literature by Hanson and Slife (11), death in susceptible plants is generally attributed to aberrant growth as a result of many recorded metabolic malfunctions. Higher plants having a susceptibility to 2,4-D and 2,4,5-T experience stoppage of meristematic cell growth, rapid proliferation of parenchyma cells in the roots and stems, cell elongation stops, and radial growth is increased. Also, normal growth of leaves stop and development of excessive vascular tissue and compacted mesophyll cells predominate. Roots no longer absorb water and nutrients effectively. Photosynthetic activity is slowed as the phloem cells become crushed by radial cell proliferation in the stem and roots. Combinations of these metabolic malfunctions lead to senescence and physiological breakdowns in the leaves and roots. Consequently, the plants do not properly

carry out autotrophic functions and death ensues (11, 13, 48).

Much has been written suggesting that the chlorophenoxy herbicides have the molecular structure to perform metabolic functions normally attributed to the auxin, indoleacetic acid (IAA) (12, 45, 46, 47). Indoleacetic acid is thought to play an important role in the formation of ethylene, enlargement and division of cells, increasing RNA and DNA synthesis, regulation of enzyme activity in cell metabolism, and a host of other metabolic functions (49).

Bidwell (49) describes 2,4-D auxin activity as being a result of the attractive forces at various points on the molecule (Figure 1). These forces enable 2,4-D to link up to substrates in the same manner as does IAA. Thus, 2,4-D is thought to be capable of interfering with IAA activity.

The herbicide 2,4-D has been shown to be more stable and less likely to undergo biological and chemical oxidation than IAA (10, 50). Overbeak (10) and Key et al. (51) suggest that 2,4-D can therefore cause susceptible plants to form hormonal imbalances resulting in aberrant responses and death.

Increased growth responses in higher plants by the chlorophenoxy herbicides has been well documented (52-55).

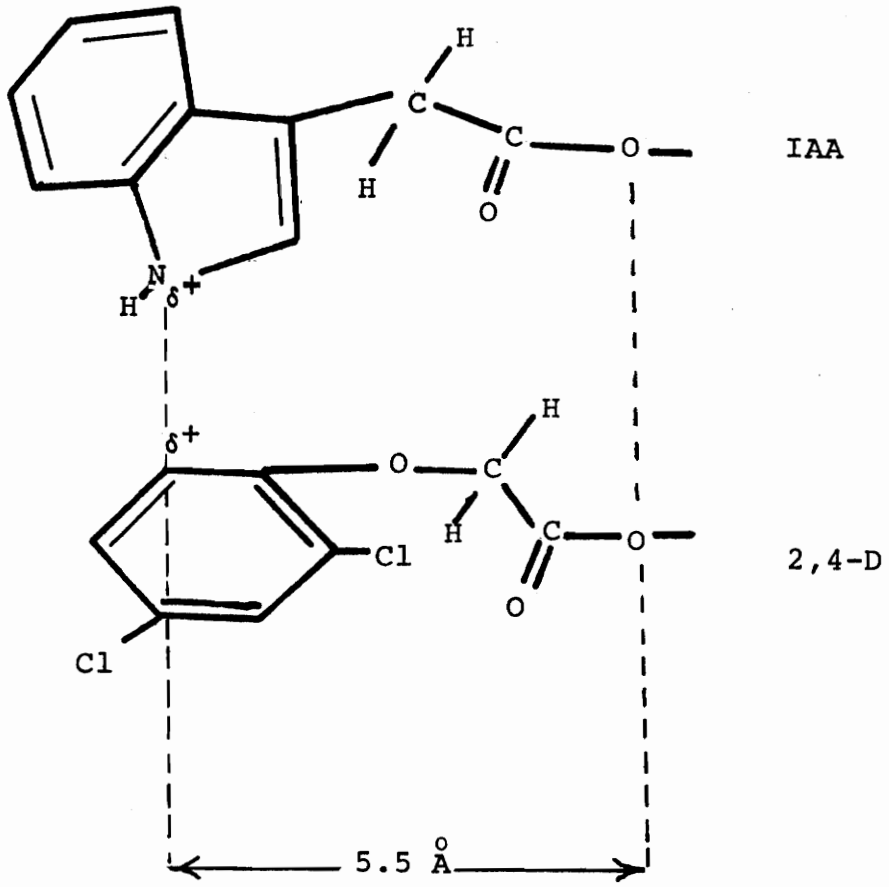

Figure 1. Indoleacetic acid and 2,4-dichlorophenoxyacetic acid (2,4-D) showing charge-distance relationship between the negative charge on the carboxyl and a fractional positive charge on the nucleus. [From Bidwell (49)]

All increased growth in the studies reviewed occurred in plants having a susceptibility to the toxic effects of 2,4,-D in high concentrations. Low concentrations of the herbicide resulted in increased growth.

Wedding et al. (56) increased green bean yield by 35% with applications of 10-20 mg/l of 2,4-D ester to plants two weeks after emergence. Increases in sugar beet root production was recorded at 44% over the control in another study (54).

Analysis by Sell et al. (57) of kidney bean stems which had undergone growth stimulation by 2,4-D revealed that protein and amino acids had increased over the control. Payne et al. (58) noted a protein increase of 27% in potato tubers treated with low levels of 2,4-D.

Stahler and Whitehead (59) showed the overall nitrogen increase experienced by sugar beets treated with sublethal levels of 2,4-D were high enough to be toxic to cattle.

Several explanations have been offered for the stimulated growth. Holmes and Abeles (60) suggested that increases were due to the IAA qualities of 2,4-D via enhanced ethylene production. Ethylene has been shown to increase cell division (61, 62). Imaseki and Pjon (61) found a significant increase in growth of etiolated rice seedlings with additions of 10 µl/liter of ethylene.

Ku et al. (62) found ethylene stimulation could be enhanced further by subjecting etiolated rice seedlings to high CO_2 levels.

In a study by Morgan and Hall (63) ethylene production in cotton was increased 26 times the control when sprayed with sublethal concentrations of 2,4-D.

Absorption of 2,4-D has been shown to take place readily in plants (46). Absorption in higher plants is enhanced at lower pH levels (45). Valentine and Bingham (64) showed that uptake of 2,4-D by the green alga, Scenedesmus, was enhanced as the pH dropped below 6. This study suggested that uptake was related to the ionization constant of 2,4-D (pKa about 3). Also Scenedesmus was shown to take up 2,4-D more readily than Euglena, Chlorella, and Chlamyclomonas. These algae were shown to be ineffective for uptake of 2,4-D. Scenedesmus was shown to take up 2,4-D rapidly for the first hour of the study. After the first hour, further uptake was negligible.

No literature was found which attempted to show effects of 2,4-D or 2,4,5-T on M. aeruginosa over an extended incubation period.

A study conducted on the toxic effect of 2,4-D and 2,4,5-T on 300 algal species including M. aeruginosa showed 2,4-D and 2,4,5-T have little value as an algicide after 24 hours of incubation. Concentrations of 2,4-D and 2,4,5-T used ranged from 10-250 ppm (42).

Bingham (65) showed 2,4-D to be toxic to three green algae cultured on agar at a concentration of 50 ppm over a fourteen day incubation period. Also, liquid media containing 25 ppm of 2,4-D was toxic to the algae over an extended incubation period.

Vance and Smith (2) point out that very few studies are available in the literature covering the effect of 2,4,-D and 2,4,5-T and other herbicides on lower plants and animals. The work that has been done in the area shows that the effects on species of bacteria and algae are unpredictable.

Worth and McCabe (15) found that 2,4-D effects on several aerobic, facultative, and anaerobic microorganisms ranged from toxic to stimulatory or had no effect on growth. Solutions of 2,4-D ranged in concentration from 10 µg/l to 100 mg/l. Organisms were enumerated every 24 hours of a 72 hour incubation period. Facultative organisms showing stimulation at lower concentrations were not affected at higher concentrations. Aerobic organisms showing stimulation at lower concentrations were inhibited at higher concentrations. Many organisms showed toxic effects at all levels and many were not affected.

Dean and Law (66) and Colmer (67) found no effect on two soil bacterium using 2,4-D.

Vance and Smith (2), Arvick et al (68) and Venkalaraman and Rajyalakshmi (69) found no toxic effects of 2,4-D on several species of blue green algae. All studies used concentrations of 2,4-D and 2,4,5-T greater than 20 mg/l.

Poorman (14) studied the effect of various concentrations of 2,4-D and 2,4,5-T on the growth of Euglena gracilis. After seven days of growth, the flasks containing 10 mg/l of 2,4-D and 2,4,5-T exhibited significantly more growth than the control. Media containing 100 mg/l of 2,4-D and 2,4,5-T inhibited growth of the alga.

Weddings et al. (70) showed Chlorella pyrenoidosa significantly increased solute uptake when grown in culture with low concentrations of 2,4-D. Sucrose and mannitol were assimilated well at 2,4-D concentrations of 10^{-7}M. Orthophosphate uptake was increased more by low concentrations of 2,4-D than were the carbohydrates.

IAA has been shown to stimulate growth in green and blue algae by several workers (7, 8, 9, 50).

In 1938, Pratt (9) stimulated growth of a culture Chlorella vulgaris significantly with the addition of 10-50 mg/l of indoleacetic acid in 15-26 days of incubation. A similar study by Brannon and Sell (8) increased

the dry weight of Chlorella vulgaris 3 1/3 times by adding 10 mg/l of IAA and allowing growth for fourteen days.

Bunt (71) found that an unidentified species of Nostoc would not grow well without the bacterium, Caulobacter, present. Normal growth was attained when 1 mg/l of IAA was introduced to the culture.

Ahmad and Winter (6) found that several species of blue-green algae were stimulated by additions of IAA in concentrations between 10^{-5}M and 10^{-10}M. Higher concentrations of IAA were inhibitory. Species of green algae showed stimulation at higher concentration and no significant effect at lower concentrations.

Runoff from spraying of 2,4-D can contribute significant amounts to streams (72, 73, 74). Barnett et al. (73) found 2,4-D in runoff after simulated rain storms on an experimental irrigation plot was as high as 4 mg/l after 2.2 lbs/acre application.

Manigold and Schultz (74) evaluated 20 streams in the mid-west with known form runoff contributions. Tests revealed 36 of the 322 samples taken contained measurable amounts of 2,4-D. The highest recorded amount was 0.35 µg/l.

Studies have shown 2,4-D and 2,4,5-T do not readily absorb to suspended particles in water (44, 72). Subsequently, 2,4-D does not settle out of the water column.

Disappearance of 2,4-D and 2,4,5-T in natural waters is associated with microbial decomposition (44, 72) and algal uptake (64, 65). Breakdown of 2,4-D by algae has been shown to occur rapidly. Metabolites of 2,4-D, including products of hydroxylation and ether cleavage, and 2,4-D were released back to the media (1, 64).

III. METHODS AND MATERIALS

Introduction

The purpose of these experiments was to test the effect of 2,4,5-T and 2,4-D on the growth of a non-axenic culture of Microcystis aeruginosa. Experiments were set up to test a wide range of herbicide concentrations on the alga under controlled conditions. Cell counts and fluorometry were used to assess the growth on all odd numbered days for thirteen days of growth. Various statistical methods were used to analyze the data.

Stock Culture

A non-axenic culture of Microcystis aeurginosa was obtained from the National Eutrophication Research Program, Pacific Northwest Water Laboratory, Environmental Protection Agency, 200 S.W. 35th Street, Corvallis, Oregon.

A 1 ml portion of the original stock culture was transferred to 60 ml of autoclaved Algal Assay Procedure (AAP) medium (21) in a 250 ml flask. Subsequent stock transfers were made every ten days.

Lighting and Temperature

A Warren/Sherer Series RI-LTP Lighted Bio-Incubator was used to maintain stock and experimental

cultures of the alga. Light intensity was maintained at 200 foot-candles of continuous cool-white fluorescent lighting. A Weston Electrical Instrument Corporation Model 756 illumination meter was used to calibrate light intensity. Temperature within the incubator was maintained at 24°C $\pm$ 1.0°C.

Preparation of Glassware

All flasks, beakers, centrifuge tubes, graduated cylinders, and bottles were washed with sodium carbonate and rinsed with hot tap water. The glassware was then rinsed with a fifty percent solution by volume of hydrochloric acid (HCl). After the acid rinse, the glassware was rinsed five times in tap water followed by five rinses in glass distilled water. Clean glassware was critical for good growth of *Microcystis aeruginosa*.

Volumetric pipettes were placed in a fifty percent HCl solution for at least 24 hours and rinsed in the same manner as other glassware.

Sterilization

All glassware, cotton plugs, and AAP media were sterilized at 15 psi and 121°C for 30 minutes in a Barnstead CES-20 steam autoclave.

The herbicides were not sterilized in the autoclave due to their instability at high temperature and pressure. Probes for the pH meter were rinsed in 10 percent HCl solution and stored in glass distilled water prior to use.

Culture Medium

The Algal Assay Procedure (AAP) medium (Table 1) was used exclusively in the tests performed. The AAP medium has been used successfully in a previous study to evaluate algicides (75).

Preparation of Herbicide Solutions

Technical grade 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) were obtained from the Dow Chemical Company.

Table 2 lists the concentrations of 2,4-D and 2,4,5-T examined in this study. Solutions were prepared by making serial dilutions into AAP medium from an AAP solution containing the highest concentration of the respective herbicide. The pH of herbicide test media and the control medium was adjusted to pH 8 prior to inoculation with 0.02N sodium hydroxide (NaOH).

Table 1

Algal Assay Procedure (AAP) Medium (21)

Macronutrients	mg/l	Element	mg/l
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
$MgCl_2$	5.700	Mg	2.904
$MgSO_4 \cdot 7\ H_2O$	14.700	S	1.911
$CaCl_2 \cdot 2\ H_2O$	4.410	C	2.143
$NaHCO_3$	15.000	Ca	1.202
		Na	11.001
		K	0.469

Micronutrients	µg/l	Element	µg/l
H_3BO_3	185.520	B	32.460
$MnCl_2$	264.264	Mn	115.374
$ZnCl_2$	32.709	Zn	15.691
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2\ H_2O$	7.260	Mo	2.878
$FeCl_3$	96.000	Fe	33.051
$Na_2EDTA \cdot 2\ H_2O$	300.000		

pH = adjusted to 8.0 with 0.02N NaOH

Table 2

Herbicide Concentrations Investigated

2,4-D		2,4,5-T	
Molar	mg/l	Molar	mg/l
2×10^{-3}	442.0	0.9×10^{-3}	230.0
10^{-3}	221.0	10^{-4}	23.0
10^{-4}	22.1	10^{-5}	2.3
10^{-5}	2.21	10^{-6}	0.23
10^{-6}	0.221	10^{-7}	0.023
10^{-7}	0.0221	10^{-8}	0.0023
10^{-8}	0.00221		

Algal Counts

Cell counts were made every two days beginning after one full day of growth using a Thomas Hemacytometer as described in Phycological Methods (76). Counts were made with a Bausch and Lomb microscope at 420 x magnification.

Fluorometry

In vivo fluorometric determinations were made using a Turner Model 111 fluorometer. Equipment and methodology used for measuring chlorophyll are described by G. K. Turner Associates (77, 78).

Filters used in the fluorometer included a primary filter at 430 nm and a secondary filter at >650 nm.

Data were reported in relative fluorometric units (21). Fluorometric analyses were run in conjunction with cell counts.

Sub Culture Inoculation

Five 250 ml Erlenmeyer flasks containing 60 ml of medium were inoculated at each test level. Cells used in the inoculation were obtained from stock cultures in the logarithmic phase of growth. Inoculum was prepared by suspending the sedimented cells from centrifuged stock culture in glass distilled water containing 15 mg/l of

sodium bicarbonate ($NaHCO_3$). The cells were centrifuged again at 2000 x gravity. Sedimented cells were suspended a second time in the bicarbonate solution. Cells in this solution were pipetted into the test media to give an initial cell count of 50 x 10^3 cells/ml.

Statistical Analysis

An analysis of variance was performed on the cell count and fluorometric data. The test was set up to take each individual data period as a separate incident. Variances at each time interval were used in a t-test as described by Dunnett (80). The Dunnett test is designed to compare several individual sample means to a control mean. The test was computed at $\alpha = 0.05$.

A least-squares linear regression was computed over the log growth portion of cell growth at each test level and on the graphs of maximum cells per ml versus herbicide concentration. Correlation coefficients were computed in conjunction with each linear regression (79).

Doublings per day were computed on the cell growth (76).

IV. RESULTS AND DISCUSSION

2,4-Dichlorophenoxyacetic Acid (2,4-D)

Table 3 gives the mean cells per ml at each test period for all concentrations of 2,4-D and the control. The letter "b" associated with values presented in Table 3 indicates those days and concentration of herbicide at which the number of algal cells are significantly greater than the control at a $\alpha = .05$ level. These data indicate cell production was greatest at 10^{-3}M 2,4-D. Statistically significant cell counts at $\alpha = .05$ were found at this level between days 5 and 13. It is also evident from this table that cell production at lower concentrations of 2,4-D was progressively less.

Figures 2 and 3 show cell growth at 10^{-3}M and 10^{-4}M 2,4-D, graphically. It can be seen from these graphs that logarithmic growth occurs from days 3 to 7. Least-squares linear regression analyses were computed over the log phase of growth and doublings per day were determined for the entire growth period. These data are presented in Tables 4 and 5, respectively, and indicate

Table 3

Mean Cells per ml--2,4-D Experiment[a]

Time	Control	2 x 10^{-3}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Day 1	5.2	5.3	4.9	6.4	5.5	5.5	5.6	5.6
	±0.7	±0.3	±0.6	±0.3	±1.2	±0.7	±0.8	±0.8
Day 3	11.9	5.7	17.7	19.7	11.8	20.3	18.6	16.0
	±5.0	±0.7	±1.0	±2.8	±8.0	±2.2	±0.4	±4.8
Day 5	33.3	6.4	79.8[b]	77.2[b]	29.3	63.7	67.5	45.6
	±18.2	±0.7	±4.2	±20.1	±29.7	±8.3	±4.9	±18.5
Day 7	82.8	8.8	292.0[b]	204.9[b]	98.5	176.7[b]	149.8[b]	109.3
	±32.0	±2.2	±26.4	34.2	±59.5	±30.4	±11.3	±39.4
Day 9	190.6	4.9	596.2[b]	453.4[b]	304.2[b]	377.8[b]	346.2[b]	243.0
	±41.7	±4.0	±21.8	32.4	±106.5	±45.0	±80.9	±64.6
Day 11	388.0	--	748.9[b]	615.2[b]	425.9	536.0	487.0	347.8
	±52.8		±52.1	±21.6	±155.1	±69.0	±70.6	±107.8
Day 13	460.8	--	908.4[b]	794.6[b]	596.9	678.1	577.8	471.9
	±85.3		±19.9	±49.9	±114.8	±104.2	95.8	±161.9

[a]Numbers in table must be multiplied by 10^4 for actual values.

[b]Denote levels significantly greater than control at $\alpha = .05$.

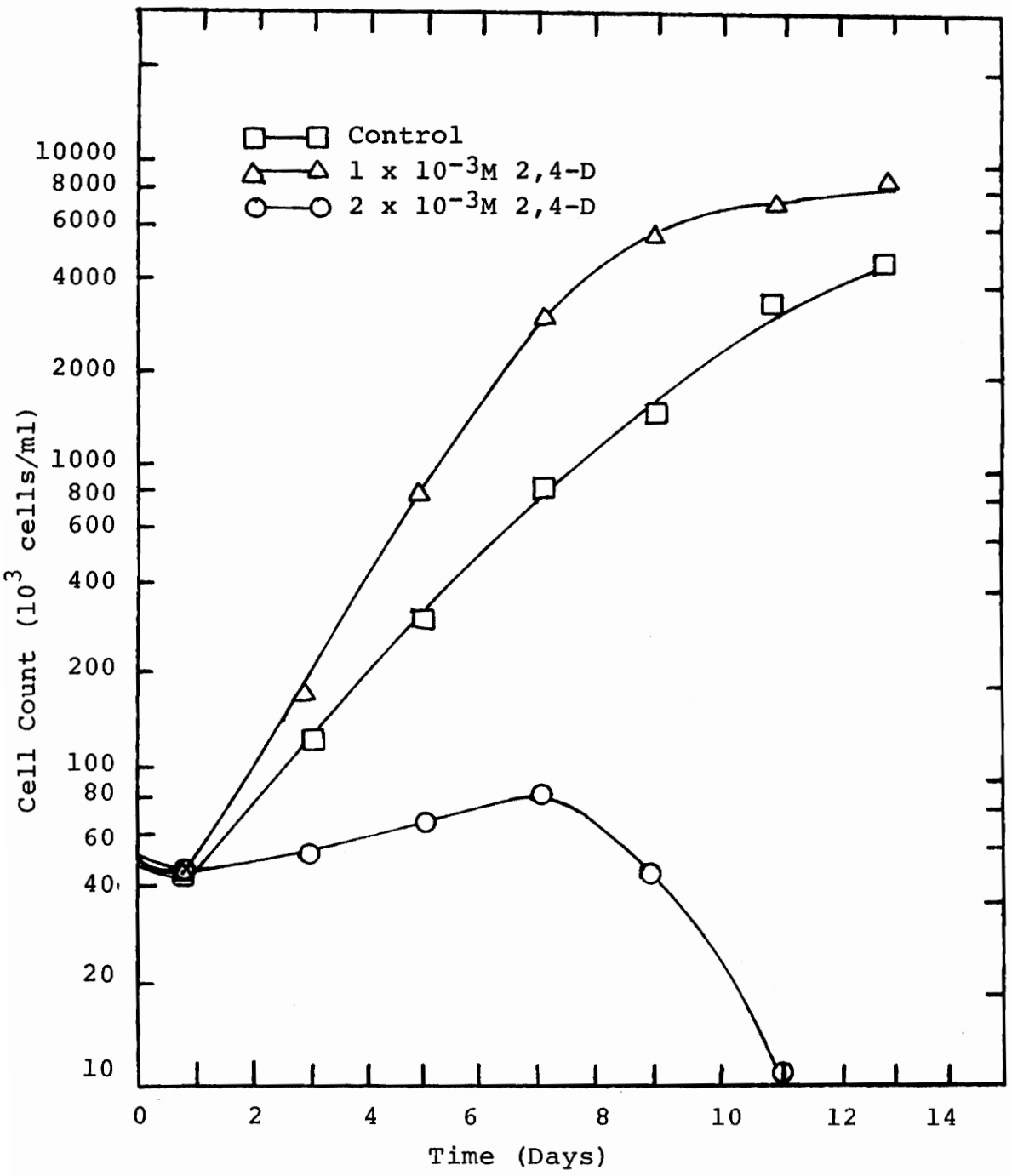

Figure 2. The growth of Microcystis aeruginosa in 2,4-D.

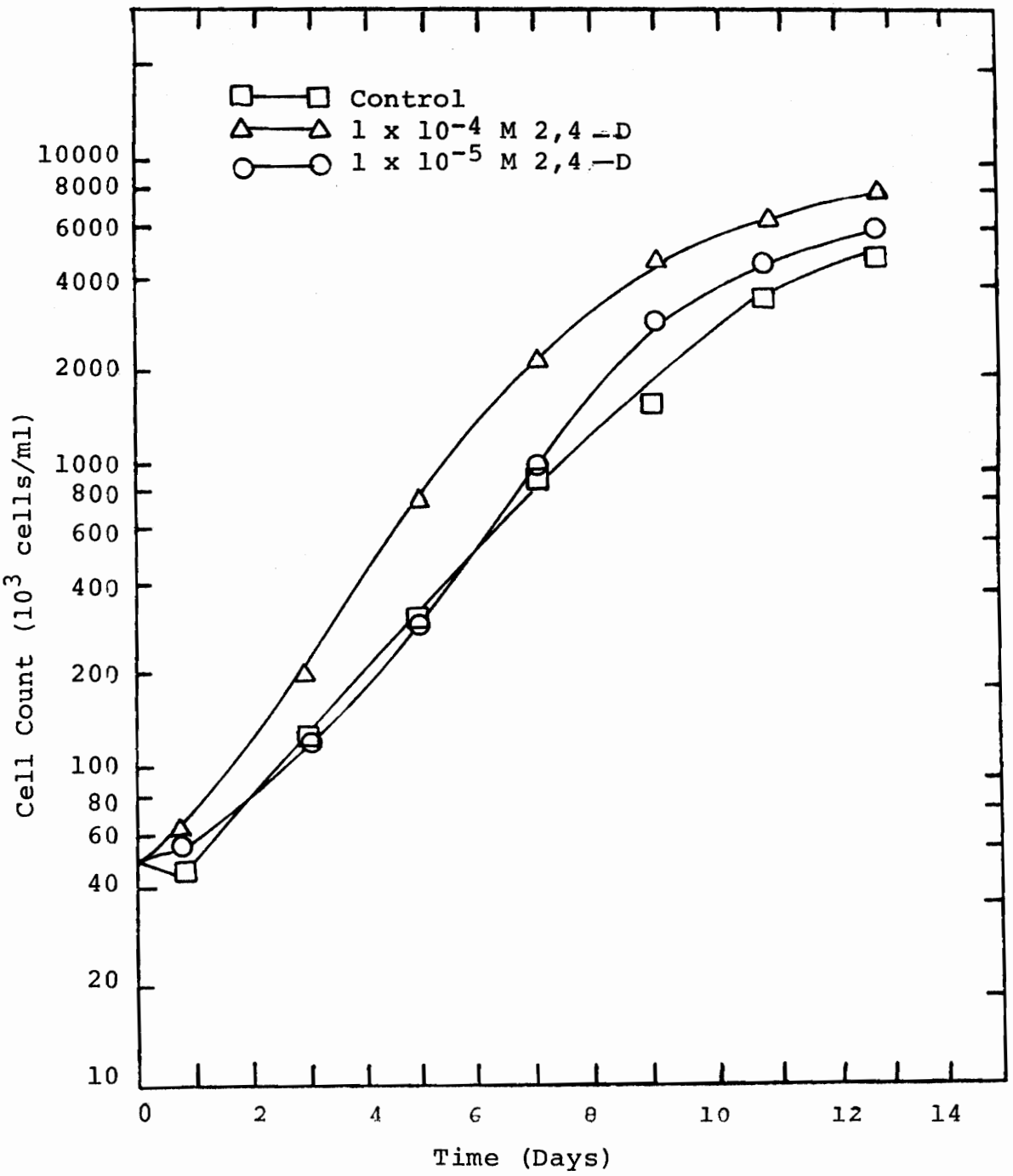

Figure 3. The growth of Microcystis aeruginosa in 2,4-D.

Table 4

Doublings per day--2,4-D[a]

Time	Control	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Day 1	0.056	--	0.356	0.137	0.137	0.163	0.163
Day 3	0.597	0.926	0.811	0.550	0.941	0.865	0.757
Day 5	0.742	1.009	0.985	0.656	0.824	0.929	0.755
Day 7	0.657	0.935	0.704	0.874	0.735	0.575	0.630
Day 9	0.601	0.514	0.572	0.813	0.547	0.604	0.578
Day 11	0.512	0.164	0.066	0.242	0.252	0.246	0.258
Day 13	0.122	0.139	0.184	0.244	0.114	0.123	0.220

[a]Expressed as logarithm to base-2 units of increase per day.

Table 5

Least-Squares Linear Regression Equations and Correlation Coefficients--2,4-D Experiment[a]

	Equation	Correlation coefficient
Control	y = 17.75x - 46.0	r = 0.9715
10^{-3} M 2,4-D	y = 68.00x - 211.0	r = 0.9522
10^{-4} M 2,4-D	y = 46.38x - 131.0	r = 0.9749
10^{-5} M 2,4-D	y = 21.63x - 61.5	r = 0.60
10^{-6} M 2,4-D	y = 39.18x - 108.7	r = 0.966
10^{-7} M 2,4-D	y = 32.85x - 85.55	r = 0.987
10^{-8} M 2,4-D	y = 23.25x - 59.25	r = 0.978

[a]Computed over logarithmic growth phase between days 3-7.

the rates of growth in 2,4-D treated cells exceed that of the control. Also, slope values for the linear regression analyses show greater values at 10^{-3}M than 10^{-4}M indicating decreased effect on growth rate with decreasing herbicide concentration. However, doublings per day data indicate that the rate of growth in 2,4-D treated cells drops well below the control between days 9 and 13.

Concentrations of 2,4-D lower than 10^{-4}M show similar results with one exception. The 2,4-D concentration at 10^{-5}M did not conform to the growth pattern established at all other concentrations of 2,4-D. This can be attributed to poor replication with the test group. However, 10^{-6}M and 10^{-7}M levels of 2,4-D were similar in cell growth response to the higher concentrations of 2,4-D. Mean cell growth in Table 3 shows these concentrations of 2,4-D produced larger cell populations than did the control from day 3 to 13. However, cell growth was significantly different from the control only on days 7 and 9. Mean cell levels at 10^{-5}M 2,4-D became significantly greater than the control at day 9. Maximum mean cell per ml counts at 10^{-5}M, 10^{-6}M, and 10^{-7}M 2,4-D were greater than the control, but were not statistically larger at $\alpha = .05$. Figures 3 and 4 graphically illustrate the mean cells per ml data for these three herbicide concentrations.

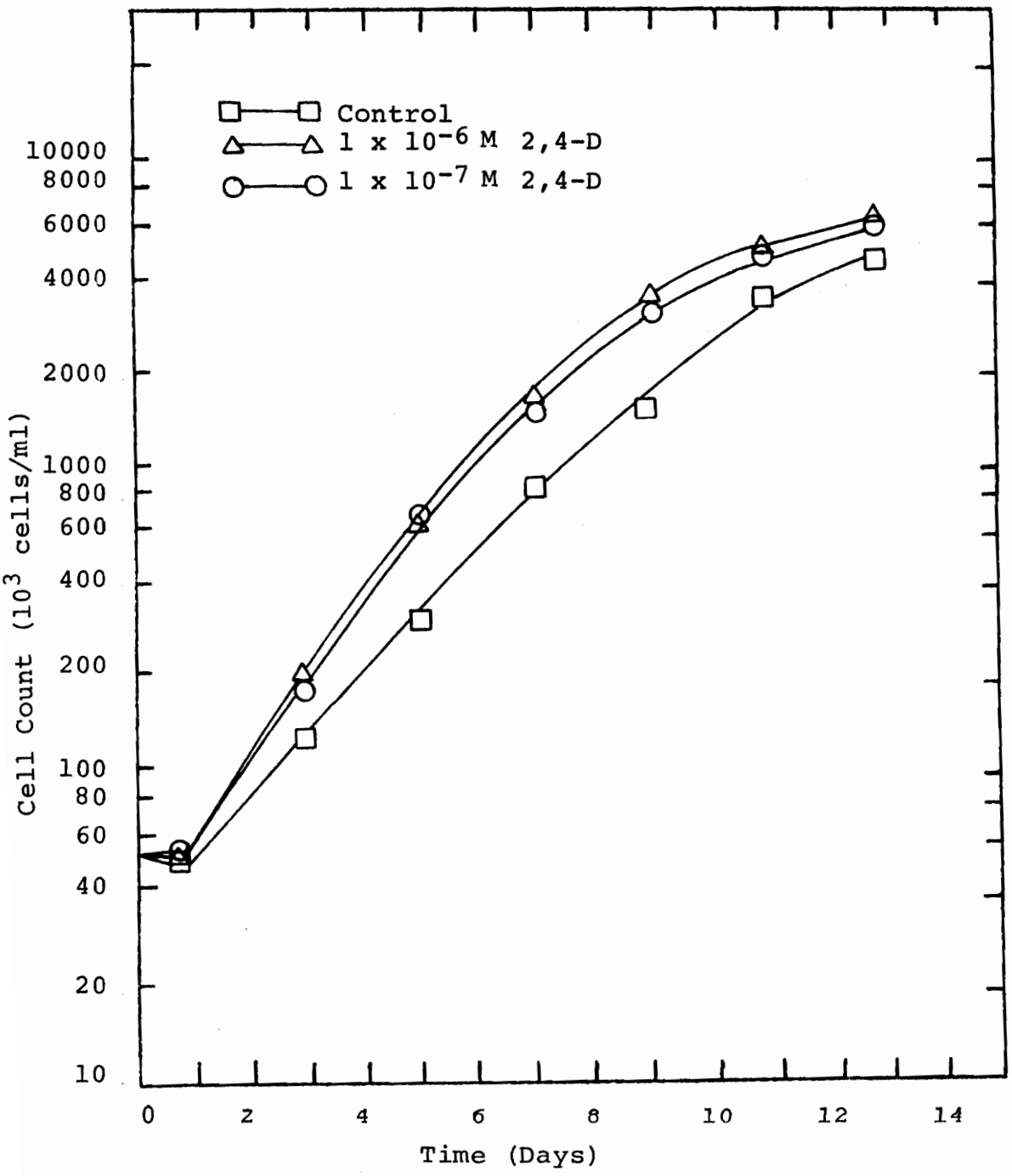

Figure 4. The growth of Microcystis aeruginosa in 2,4-D.

Doubling per day information and least-squares linear regression data presented in Table 6 and 7, respectively, indicate the rate of increase in cell growth during log phase is higher in the 2,4-D treated cells at 10^{-6}M and 10^{-7}M than in the control cells. However, the doubling rate data for 10^{-7}M 2,4-D shows a decrease in the doubling rate on day 7 below the control. The cells in 10^{-6}M 2,4-D remained greater than the control through day 7 and declined below the control on day 9.

Table 3 and Figure 5 show that the growth response of Microcystis to 10^{-8}M 2,4-D was similar to the control. Statistical analysis at the $\alpha = 0.05$ level presents no evidence to indicate growth promotion.

Doubling rates during the test period at 10^{-8}M 2,4-D were approximately equal to the control, although it appeared that growth in the control was somewhat less at day 3 and greater at day 11.

It would appear from an overview of all the cell growth data that the effect of 2,4-D on non-axenic Microcystis aeruginosa was to increase the rate at which the alga normally grows and becomes senescent. Also, maximum yields were increased over the control with 2,4-D application. Figure 6 illustrates that a linear relationship exists between herbicide concentration and maximum

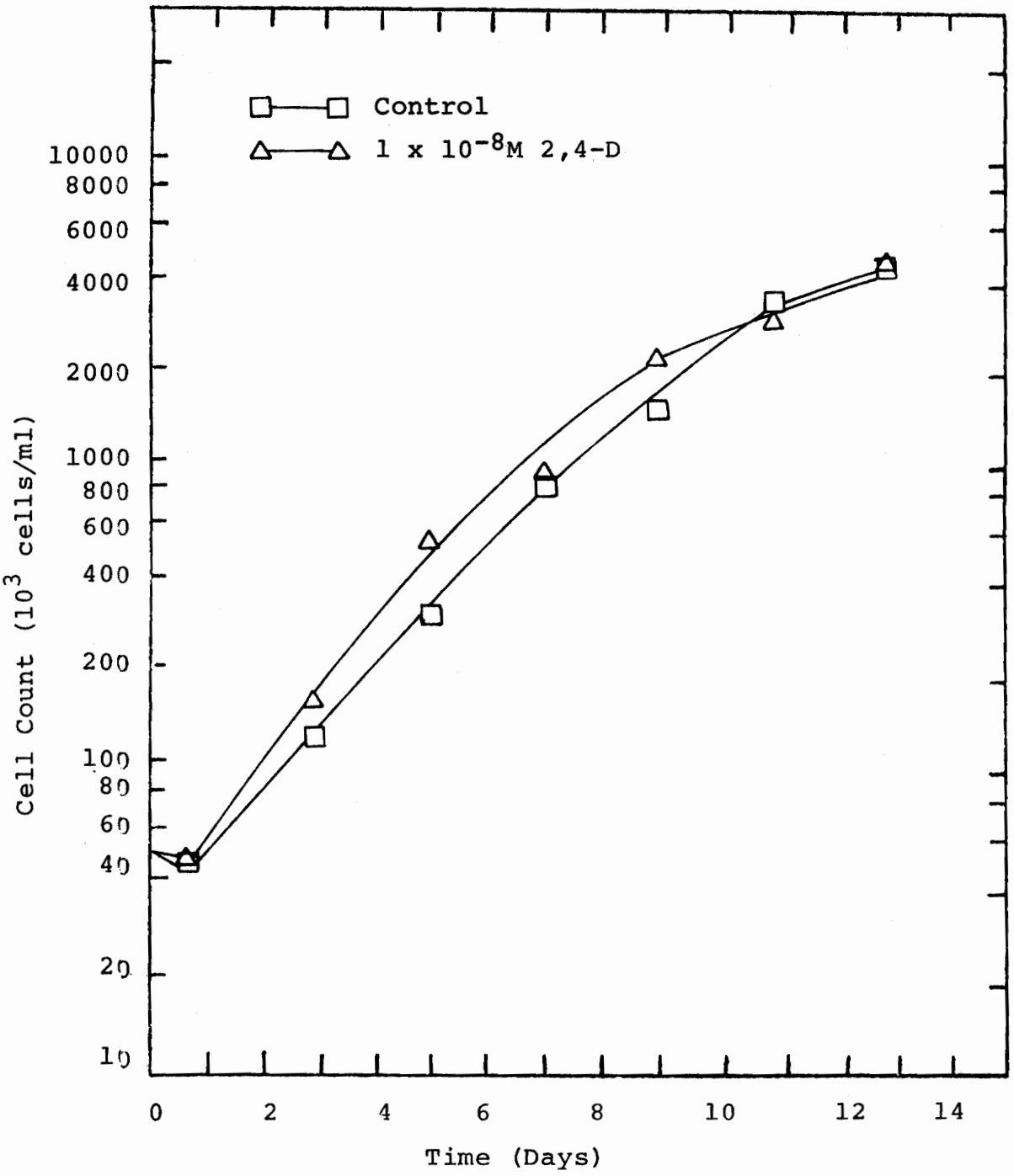

Figure 5. The growth of Microcystis aeruginosa in 2,4-D.

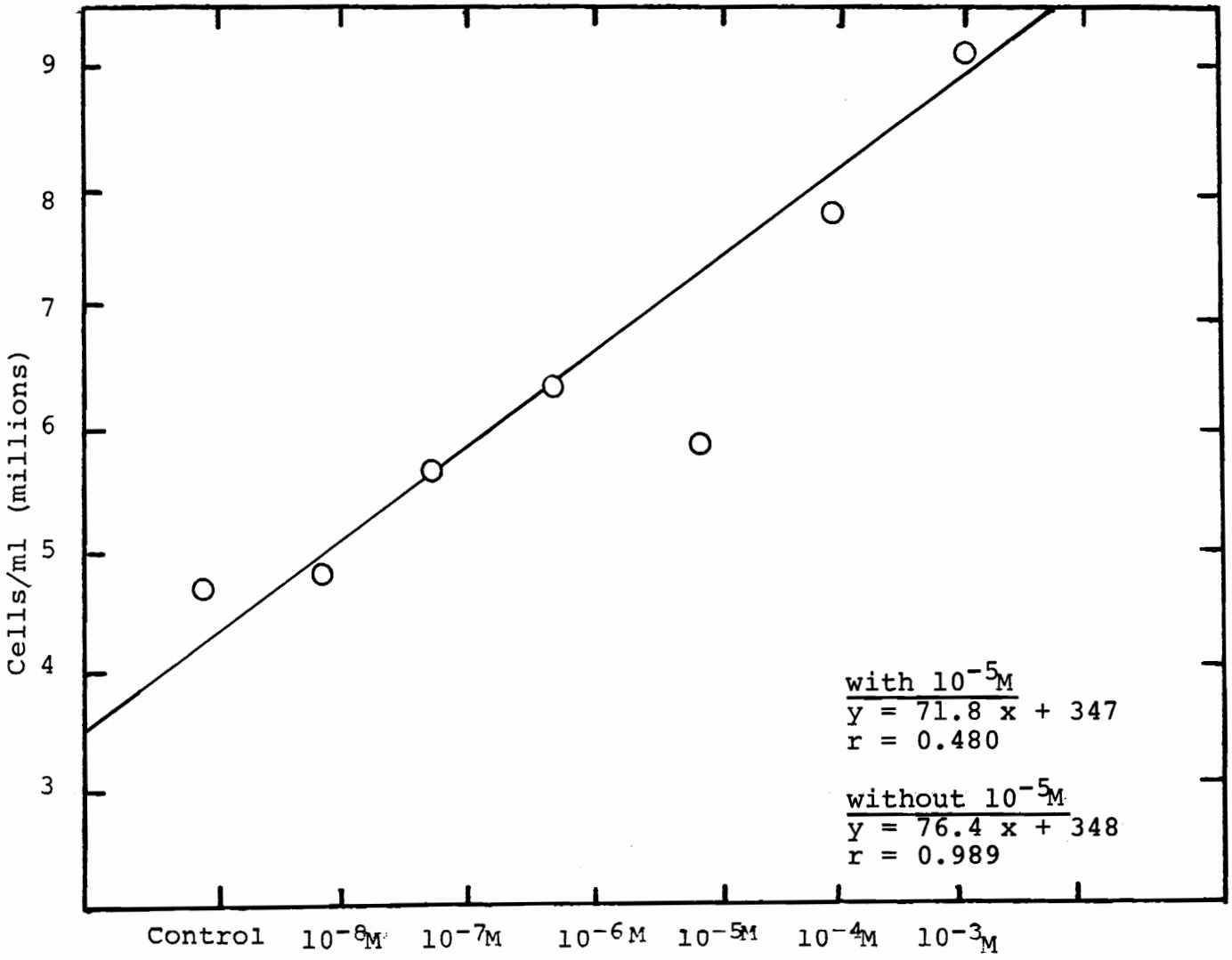

Figure 6. Maximum cells per ml values plotted as a function of increasing 2,4-D concentrations.

cell growth. The increase of overall growth may be attributed to higher growth rate during the log phase of growth. The doubling rate in the control exceeded that of the herbicides after day 7 and continued to be larger through day 11. This tended to reduce the difference between maximum cells per ml in the control and the herbicide treated cultures.

Bingham and Valentine (64) showed Scenedesmus assimilated more 2,4-D during the first hour of incubation than at any other time during a 24-hour incubation period. There is not enough evidence available through this study to conclude 2,4-D was readily assimilated; however, the data do suggest this may be the case.

Fluorometric analyses indicate that 2,4-D may decrease fluorescing pigment in the test alga (Table 6). Since this disallows direct correlations to cell count data it was decided to discuss it separately. However, the fluorometric data does support much of what was found in the cell count data due to increased numbers of fluorescing cells.

The highest concentration of 2,4-D used, 2×10^{-3}M, had very little increase in relative fluorescence activity. No fluorescence could be detected after 9 days of growth indicating death of the culture.

Table 6

Cells/Relative Fluorometric Unit Comparisons in 2,4-D Experiment[a]

Time	Control	2 x 10^{-3}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Day 1	11.8	10.8	10.2	13.3	11.4	11.4	13.3	13.0
Day 3	9.0	10.1	11.5	9.7	9.0	10.3	9.9	9.9
Day 5	10.4	10.2	12.1	13.5	8.7	12.0	13.0	10.4
Day 7	12.3	10.4	19.3	20.5	15.0	19.3	18.2	15.4
Day 9	18.3	10.1	29.2	32.5	28.0	27.2	26.8	21.1
Day 11	32.3	--	36.9	40.8	35.9	41.1	37.5	29.0
Day 13	26.0	--	44.5	38.8	32.4	31.2	32.5	26.2

[a]Numbers in table must be multiplied by 10^3 for actual value.

The 10^{-3}M test level produced the highest fluorometer readings of any group of samples (Table 7). Statistical analysis showed 10^{-3}M 2,4-D to be significantly greater than the control at the $\alpha = 0.05$ level between days 5 and 11. Relative fluorescent values remained within 1.2 units of the maximum fluorescent reading of 204.0 from days 9-13. This may indicate no change in chlorophyll over this time frame. However, since fluorometric values may contain positive interference due to pheophytin pigment (77), no definitive statement can be made in this respect.

Figure 7 gives a graphical representation of fluorometric data for 10^{-3}M and 10^{-4}M levels of 2,4-D. The log phase of fluorometric data occurs between days 1 and 7, after which data increases at a slower rate.

Figures 7, 8 and 9 graphically present fluorometric data found in Table 4. The rates of increase over the time period between days 1-7 are appreciably greater than the control for concentrations of 2,4-D at 10^{-6}M, 10^{-7}M, and 10^{-8}M. The graphical representation for 10^{-5}M 2,4-D in Figure 8 does not significantly differ from the control. After day 7, the rate of fluorescent activity slows in the 2,4-D treated cells and the control begins to decrease the difference. On day 13, fluorescence

Table 7

2,4-D--Fluorometric Analysis

Time	Control	2 x 10^{-3}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
1	4.4	4.6	4.8	4.8	4.8	4.4	4.2	4.4
	±0.89	±1.1	±1.3	±0.7	±1.1	±0.54	±0.4	±0.54
2	13.2	5.2	15.4	20.2[a]	13.0	19.6	18.8	16.2
	±4.76	±1.3	±0.89	±2.2	±7.8	±1.14	±0.8	±4.9
3	32.0	6.1	65.8[a]	57.0[a]	33.6	53.0[a]	51.6[a]	43.6
	±10.9	±0.3	±4.6	±7.9	±20.8	±6.1	±4.5	±15.1
4	67.2	8.2	150.6[a]	99.6[a]	65.6	91.2	82.2	70.8
	±16.0	±3.1	±13.8	±5.4	±25.5	±12.7	±3.4	±21.3
5	103.8	6.7	204.0[a]	139.2[a]	108.6	138.6[a]	129.0	115.0
	±15.2	±3.0	±6.0	±9.6	±25.0	±20.2	±11.0	±28.4
6	119.8	--	202.8[a]	150.6[a]	118.6	130.2	129.6	118.8
	±10.4		±3.4	±9.8	±21.8	±16.9	±10.9	±30.5
7	177.0	--	204.0	204.6	184.2	201.0	177.6	180.0
	±18.4		±7.9	±18.7	±31.9	±25.0	±18.9	±47.8

[a]Denote levels significantly greater than control at $\alpha = .05$.

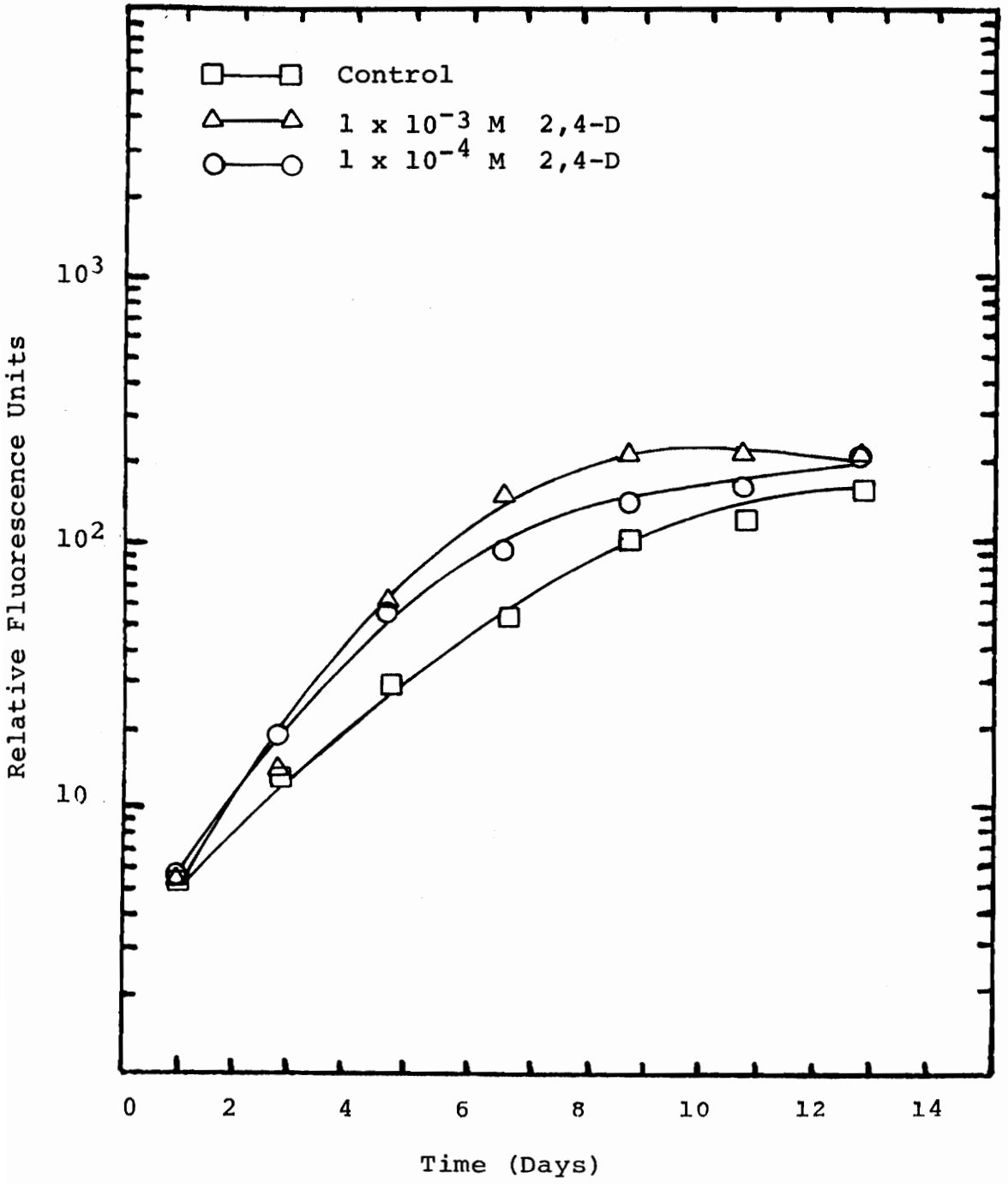

Figure 7. Increases in relative fluorescence units of Microcystis aeruginosa in 2,4-D.

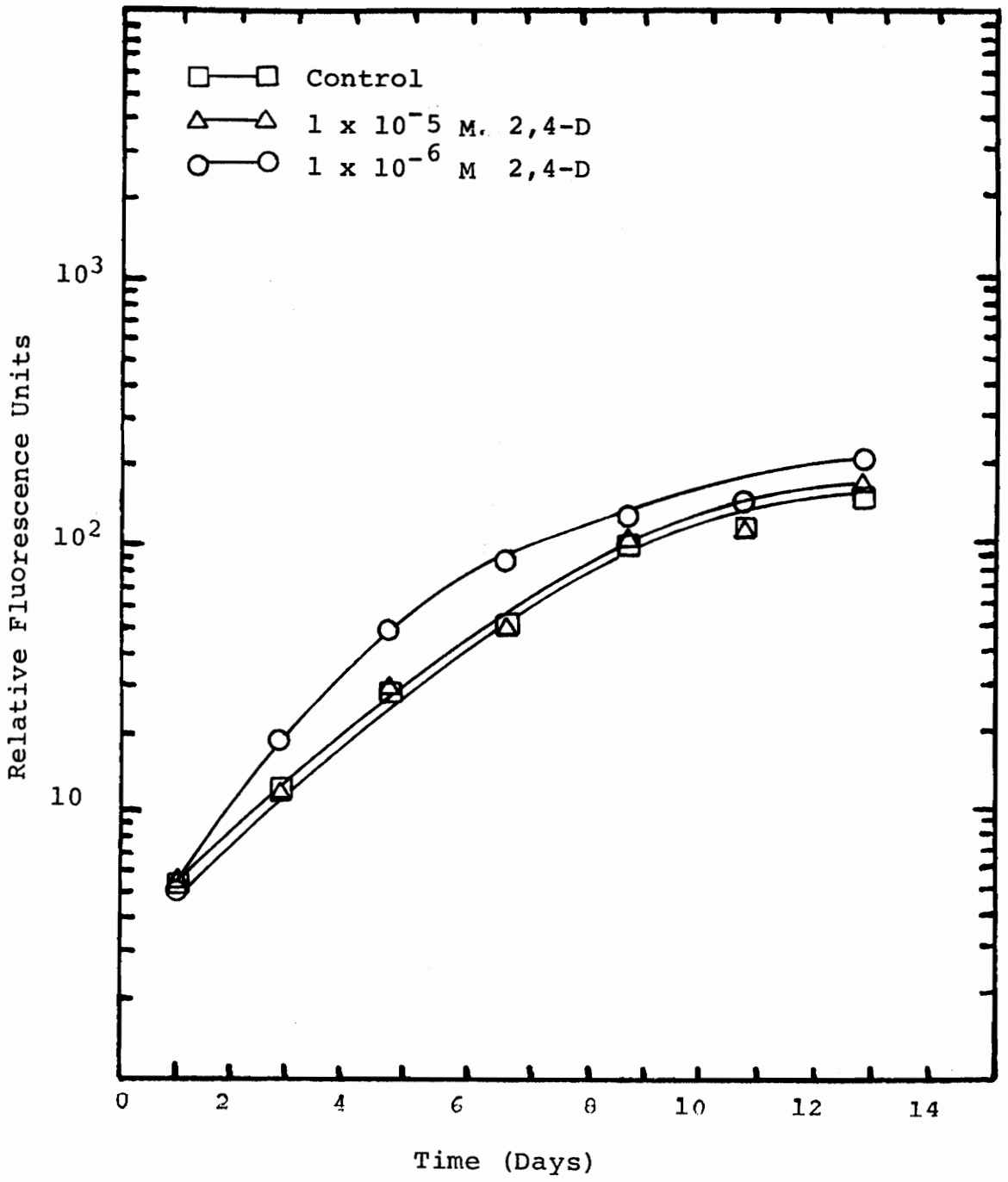

Figure 8. Increases in relative fluorescence units of Microcystis aeruginosa in 2,4-D.

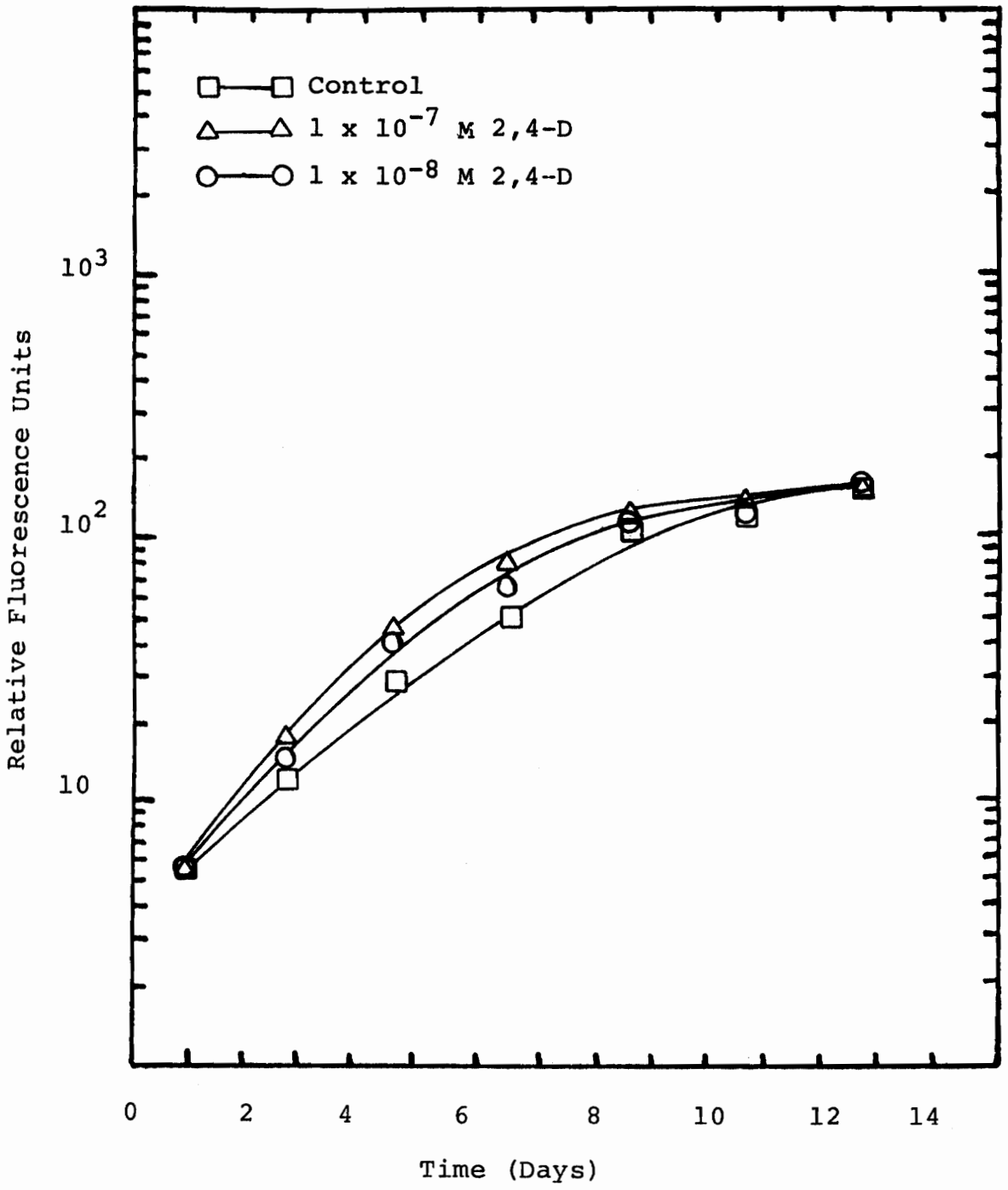

Figure 9. Increases in relative fluorescence units of Microcystis aeruginosa in 2,4-D.

values of the control nearly equal the 10^{-7}M and 10^{-8}M values. This correlates with findings established in the cell count data.

Table 6 presents calculated values for cells per relative fluorometric unit in the 2,4-D experiment. It is obvious from these data that cells exposed to 2,4-D levels other than 2 x 10^{-3} M and 10^{-8}M, contained less fluorescing pigment per cell than did the control, i.e. more cells per relative fluorometric unit. While the control had progressively higher values of cells per relative fluorescing unit as time increased, 2,4-D treated cells showed consistently higher values than the control. Also, cells per relative fluorescence unit values in 2,4-D treated cells from 10^{-3}M - 10^{-7}M were similar in number at each time interval. This would indicate 2,4-D effectively decreased chlorophyll and/or pheophytin within the individual cells. Studies have shown 2,4-D reduces chlorophyll in some plants (48, 56). Weddings et al. (56) suggested that chlorophyll in Chlorella pyrenoidosa was destroyed or its synthesis inhibited by 2,4-D resulting in decreased photosynthesis. It is interesting to note that chlorophyll and/or pheophytin levels appeared to be affected similarly over concentrations of 2,4-D from 10^{-3}M-10^{-7}M, while cell counts decreased over the concentration

range from 10^{-3}M-10^{-7}M. This would suggest that the decrease in the per cell chlorophyll and/or pheophytin does not alter the growth promoting effect of 2,4-D.

2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T)

Table 8 presents the mean cell per ml data for each herbicide test level and the control. Statistically significant growth above the control at a $\alpha = 0.05$ level is indicated with the letter b. The largest growth promoting effect occurred at the highest concentration of 2,4,5-T. Cell counts decreased with decreasing concentrations of the herbicide. However, growth was only significantly greater than the control at 2,4,5-T concentrations between 0.9 x 10^{-3}M and 10^{-6}M. Cell growth at concentrations of 2,4,5-T 10^{-7}M and 10^{-8}M were similar to the control and cell growth at 10^{-6}M was similar to the control for all but day 9.

Graphical representation of the data in Table 8 appears in Figures 10, 11, and 12. It is apparent from these figures that the log phase of growth occurred between days 3 and 7. Least squares linear regression analyses over this time period are presented in Table 9. It is clear from the linear regression equations that values for the slope during the log phase at 2,4,5-T

Table 8

Mean Cells per ml.--2,4,5-T Experiment[a]

Time	Control	0.9 x 10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Day 1	10.1	10.1	9.9	9.8	9.7	9.7	10.0
	±0.3	±0.3	±0.3	±0.4	±0.5	±0.2	±0.5
Day 3	33.7	59.1[b]	46.5[b]	40.8[b]	36.5	33.3	34.0
	±1.2	±2.2	±6.5	±5.7	±4.8	±0.5	±0.8
Day 5	72.2	244.6[b]	114.3[b]	97.4[b]	70.7	69.0	67.3
	±6.5	±8.0	±4.4	±3.0	±2.1	±2.2	±3.1
Day 7	196.3	578.0[b]	289.9[b]	230.3[b]	192.4	194.3	201.5
	±8.8	±6.5	±27.9	±16.1	±7.6	±10.5	±14.3
Day 9	326.9	836.8[b]	445.7[b]	391.3[b]	368.5[b]	324.2	323.6
	±11.6	±55.6	±42.9	±13.5	±18.7	±11.2	±11.3
Day 11	478.0	879.7[b]	572.2[b]	507.5	473.2	465.0	481.0
	±15.6	±82.9	±11.4	±10.5	±27.9	±13.7	±17.0
Day 13	645.2	780.2[b]	772.5[b]	657.5	654.8	646.1	643.0
	±61.9	±40.4	±58.5	±50.2	±43.0	±35.2	±40.8

[a]Numbers in table must be multiplied by 10^4 for actual values.

[b]Denotes levels significantly greater than control at $\alpha = .05$.

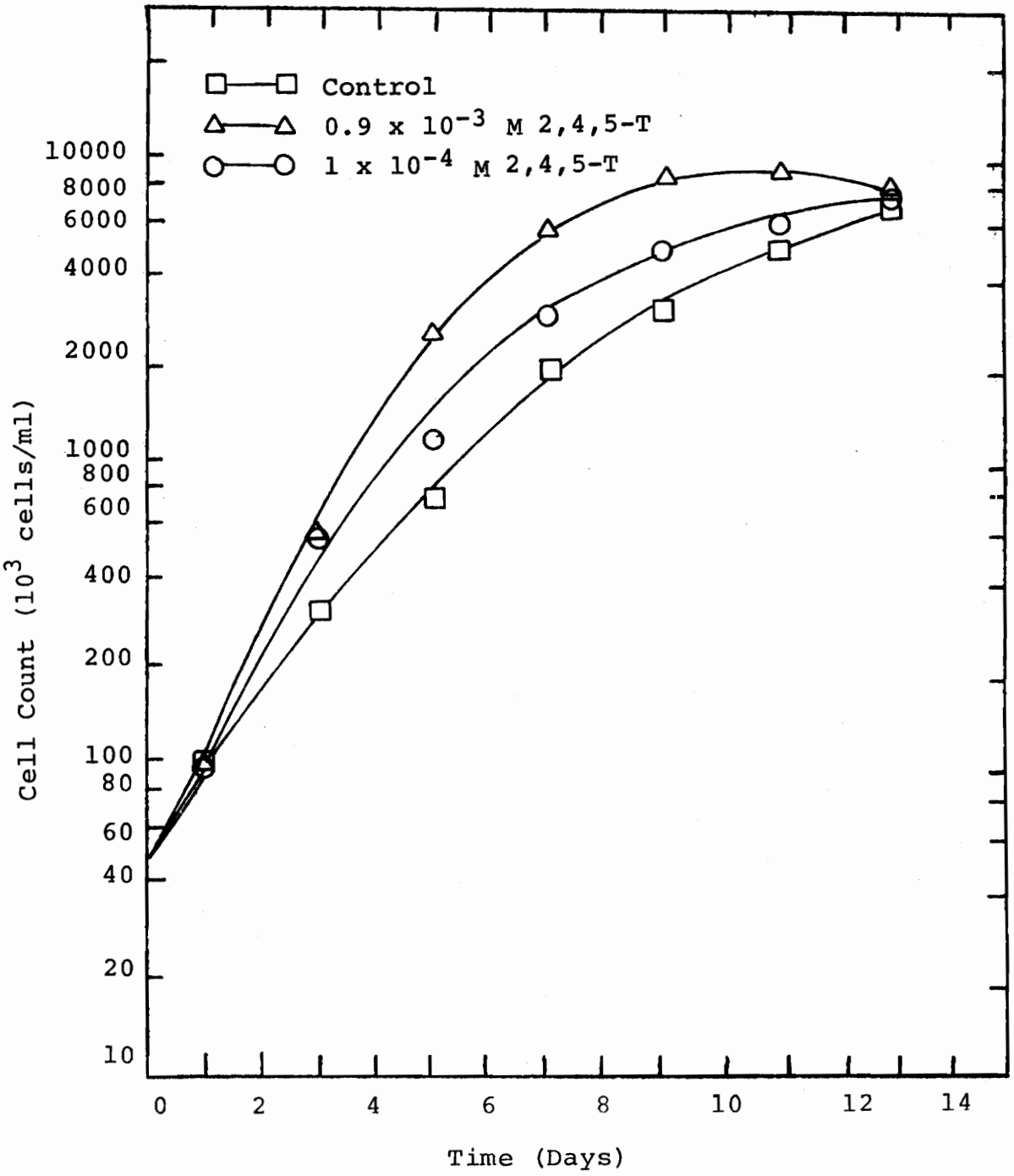

Figure 10. Growth of Microcystis aeruginosa in 2,4,5-T.

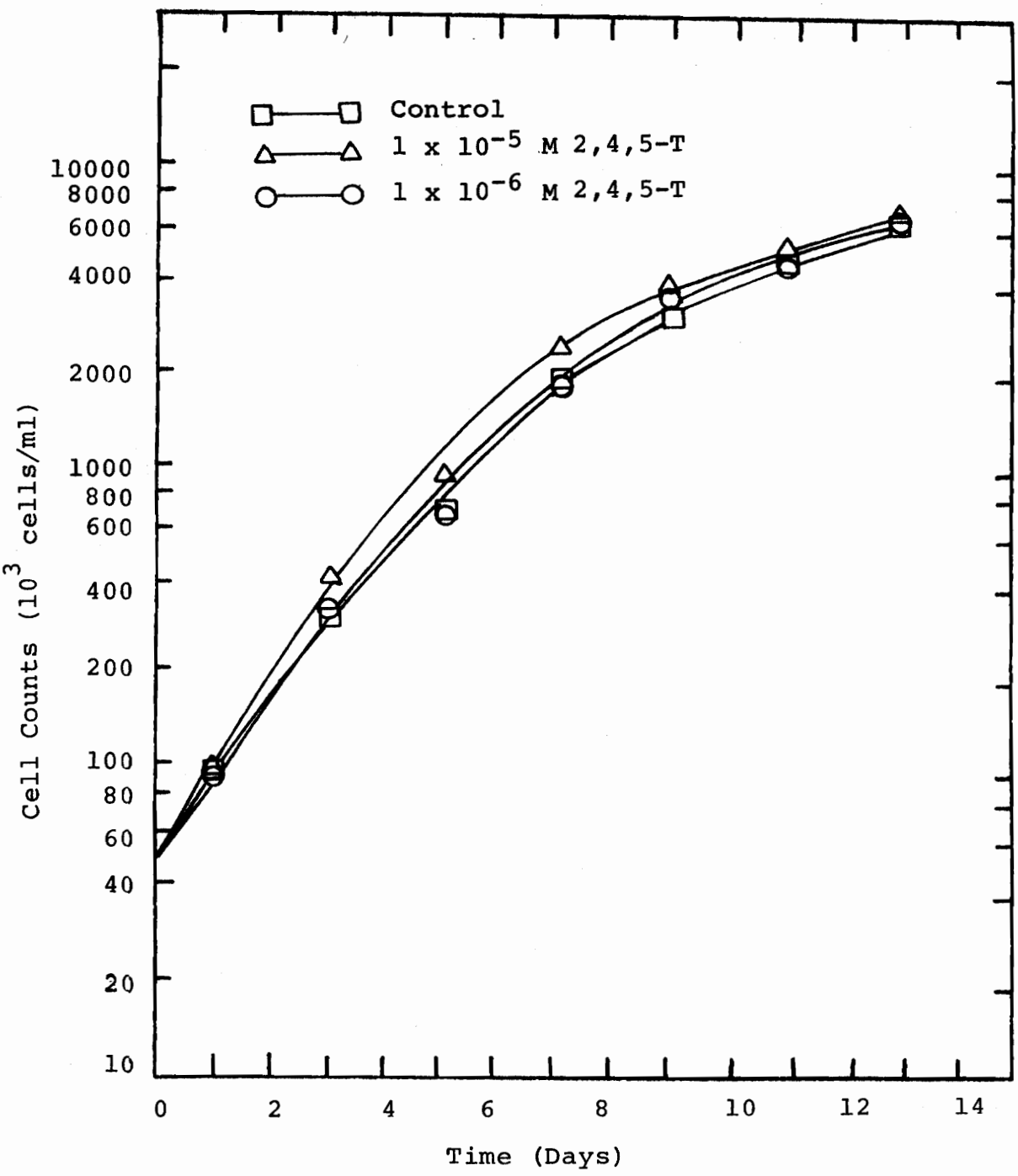

Figure 11. Growth of Microcystis aeruginosa in 2,4,5-T.

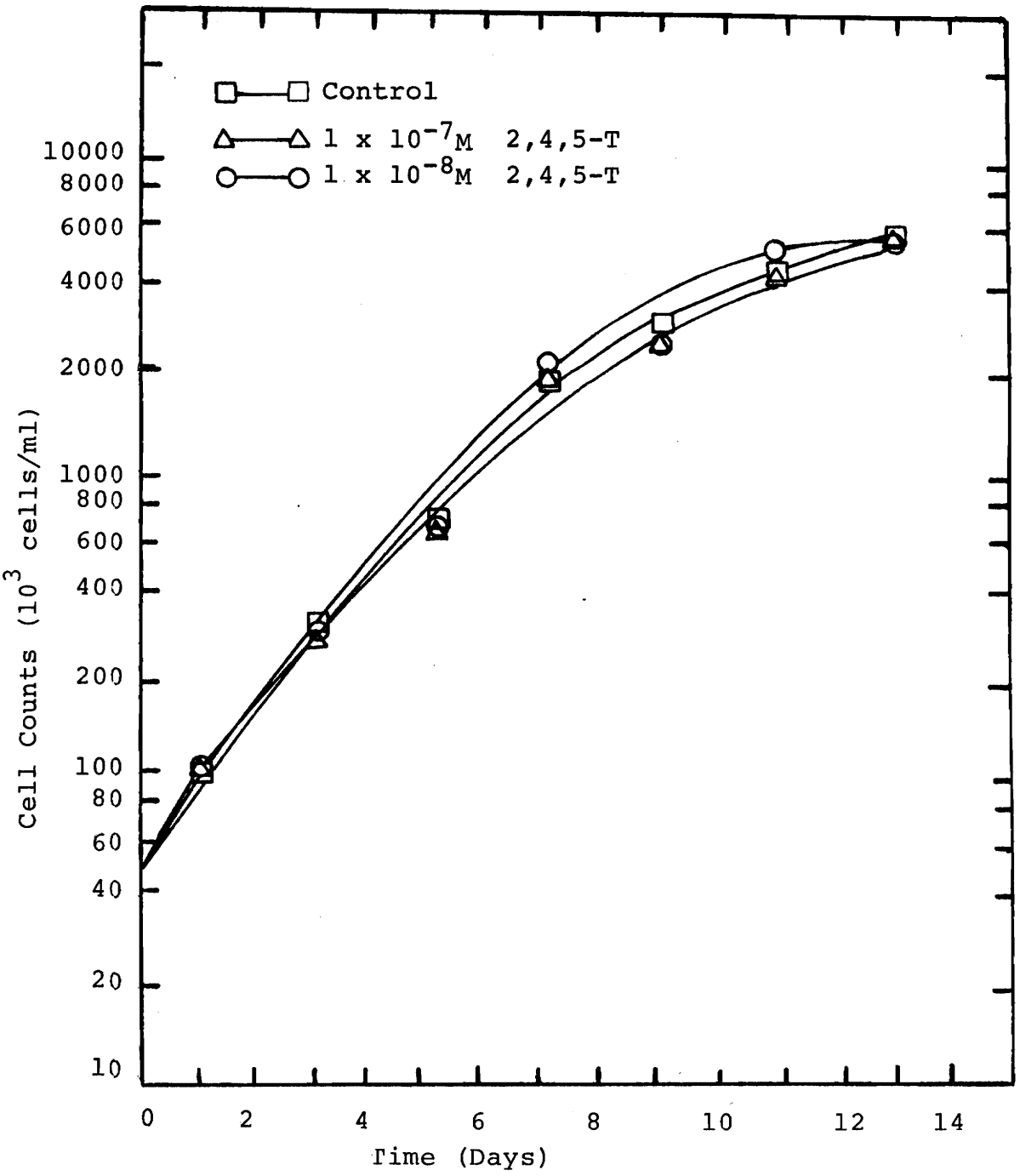

Figure 12. Growth of Microcystis aeruginosa in 2,4,5-T.

Table 9

Least-Squares Linear Regression Equations and Correlation Coefficients--2,4,5-T Experiments[a]

	Equation	Correlation coefficients
Control	y = 40.75x - 103	r = 0.9539
0.9×10^{-3}M 2,4,5-T	y = 130.75x - 358	r = 0.9846
10^{-4}M 2,4,5-T	y = 60.75x - 153	r = 0.9674
10^{-5}M 2,4,5-T	y = 47.75x - 115	r = 0.9723
10^{-6}M 2,4,5-T	y = 39.325x - 96	r = 0.950
10^{-7}M 2,4,5-T	y = 40.25x - 102	r = 0.9509
10^{-8}M 2,4,5-T	y = 42.15x - 109.45	r = 0.9459

[a]Computed over logarithmic growth phase between day 3-7.

concentrations of 0.9 x 10^{-3}M, 10^{-4}M, and 10^{-5}M were greater than the control. Doubling rate data in Table 10 also show greater doubling values for these herbicide concentrations between days 3 and 5. However, doublings per day fall below the control on day 7 for 0.9 x 10^{-3}M, 10^{-4}M, and 10^{-5}M of 2,4,5-T. The doubling rate at 0.9 x 10^{-3}M 2,4,5-T decreased more rapidly than all other test levels.

Cells per relative fluorescent unit data are reported in Table 11. Data in this table indicate that the correlation between cells grown in 2,4,5-T and control cells were good for most days. However, cells grown at 0.9 x 10^{-3}M 2,4,5-T apparently became senescent in a shorter time period than untreated cells and began losing fluorescing pigment by days 11 and 13. This resulted in an inconsistency in correlation between the cells per unit of relative fluorescence at 0.9 x 10^{-3}M 2,4,5-T and the control. The loss of green pigment was visibly noticeable in the 0.9 x 10^{-3}M 2,4,5-T flasks. The cultures became yellow-green in color by day 13.

Fluorometric data in Table 12 show mean relative fluorometric values and the statistically significant increases in relative fluorescence over the control at $\alpha = 0.05$. Figures 13 and 14 graphically illustrate these

Table 10

Doublings per day--2,4,5-T[a]

Time	Control	0.9×10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Day 1	1.014	1.014	0.985	0.970	0.956	0.956	1.000
Day 2	0.869	1.274	1.115	1.028	0.955	0.889	0.882
Day 5	0.549	1.024	0.648	0.627	0.476	0.525	0.492
Day 7	0.721	0.620	0.671	0.620	0.722	0.746	0.792
Day 9	0.367	0.266	0.310	0.382	0.468	0.369	0.341
Day 11	0.274	0.036	0.181	0.188	0.179	0.260	0.287
Day 13	0.216	--	0.216	0.186	0.233	0.237	0.209

[a]Expressed as logarithm to base-2 units of increase per day.

Table 11

Cells per Relative Fluorometric Unit Comparisons in 2,4,5-T Experiments[a]

Time	Control	0.9×10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
Day 1	8.5	8.4	8.5	8.4	7.7	8.8	8.1
Day 3	10.4	10.2	11.1	11.1	12.1	10.8	10.9
Day 5	11.2	12.3	12.6	12.8	11.1	11.3	10.9
Day 7	16.8	15.8	20.7	19.0	16.3	16.8	17.0
Day 9	24.8	22.0	20.6	20.8	22.7	26.5	24.6
Day 11	19.4	25.1	21.5	20.4	20.5	19.2	19.3
Day 13	21.3	29.3	22.5	25.2	21.9	21.5	21.1

[a]Numbers in table must be multiplied by 10^3 for actual values.

Table 12

Fluorometric Analysis--2,4,5-T

Time	Control	0.9 x 10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M	10^{-8}M
1	11.8	12.0	11.6	11.6	12.6	11.0	12.4
	±1.1	±1.2	±0.9	±1.8	±1.1	±1.6	±1.5
2	32.4	57.8[a]	41.8[a]	36.8[a]	33.6	30.8	31.2
	±2.9	±3.3	±1.3	±2.2	±3.8	±1.6	±1.9
3	64.0	199.2[a]	91.0[a]	76.0[a]	63.6	61.0	61.2
	±5.3	±6.6	±4.6	±2.0	±2.9	±3.1	±2.7
4	116.4	366.0[a]	140.4[a]	121.2	118.2	115.8	119.4
	±7.2	±19.5	±6.8	±4.0	±5.4	±4.5	±8.6
6	246.0	350.0[a]	266.0	248.0	248.0	242.0	238.0
	±11.4	±12.2	±8.9	±16.4	±17.9	±8.4	±16.4
7	302.0	266.0	344.0[a]	306.0	300.0	302.0	304.0
	±31.1	±8.9	±32.1	±16.7	±18.7	±19.2	±16.7

[a]Denotes those levels significantly greater than control at $\alpha = .05$

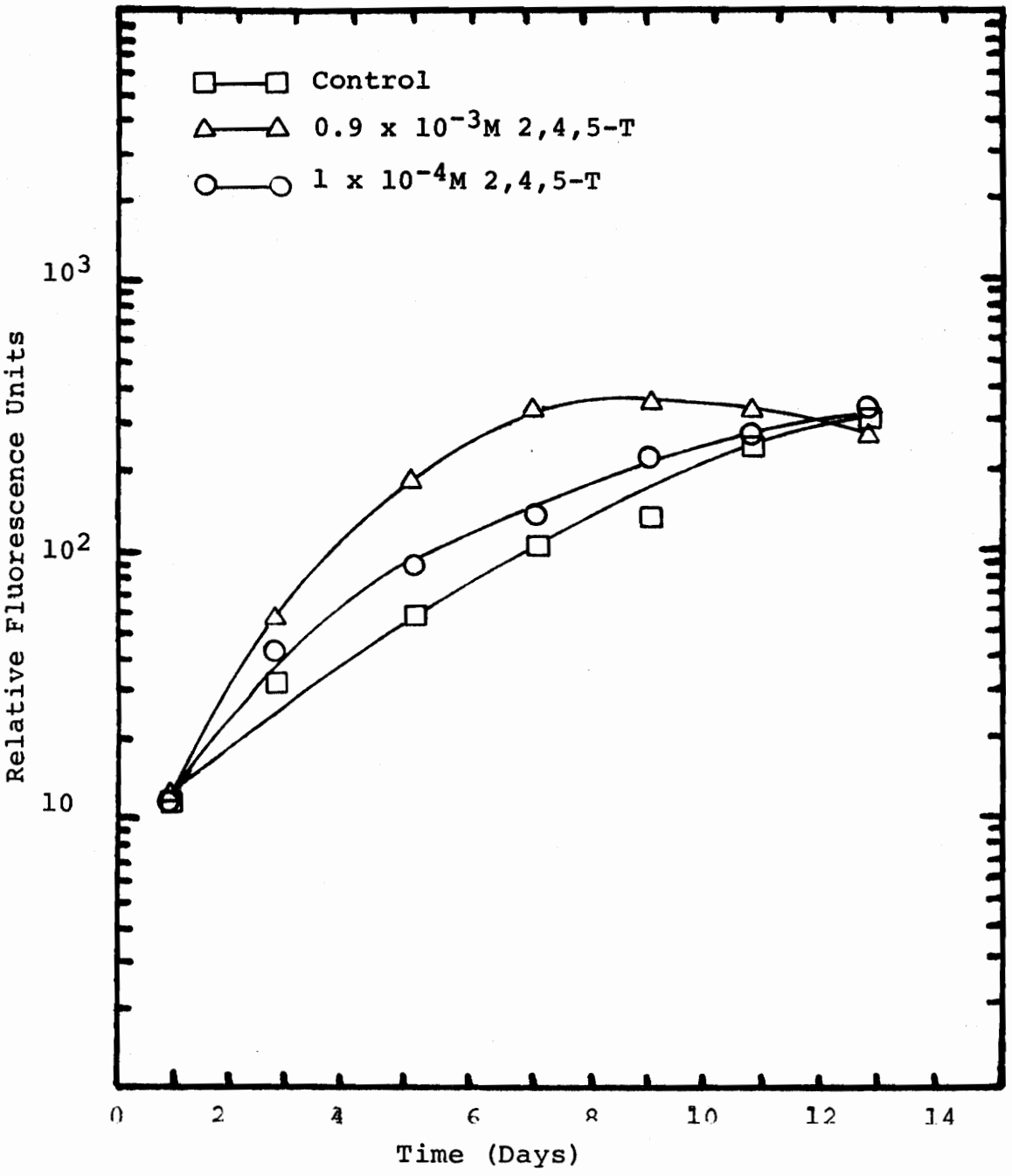

Figure 13. Increases in relative fluorescence units of Microcystis aeruginosa in 2,4,5-T.

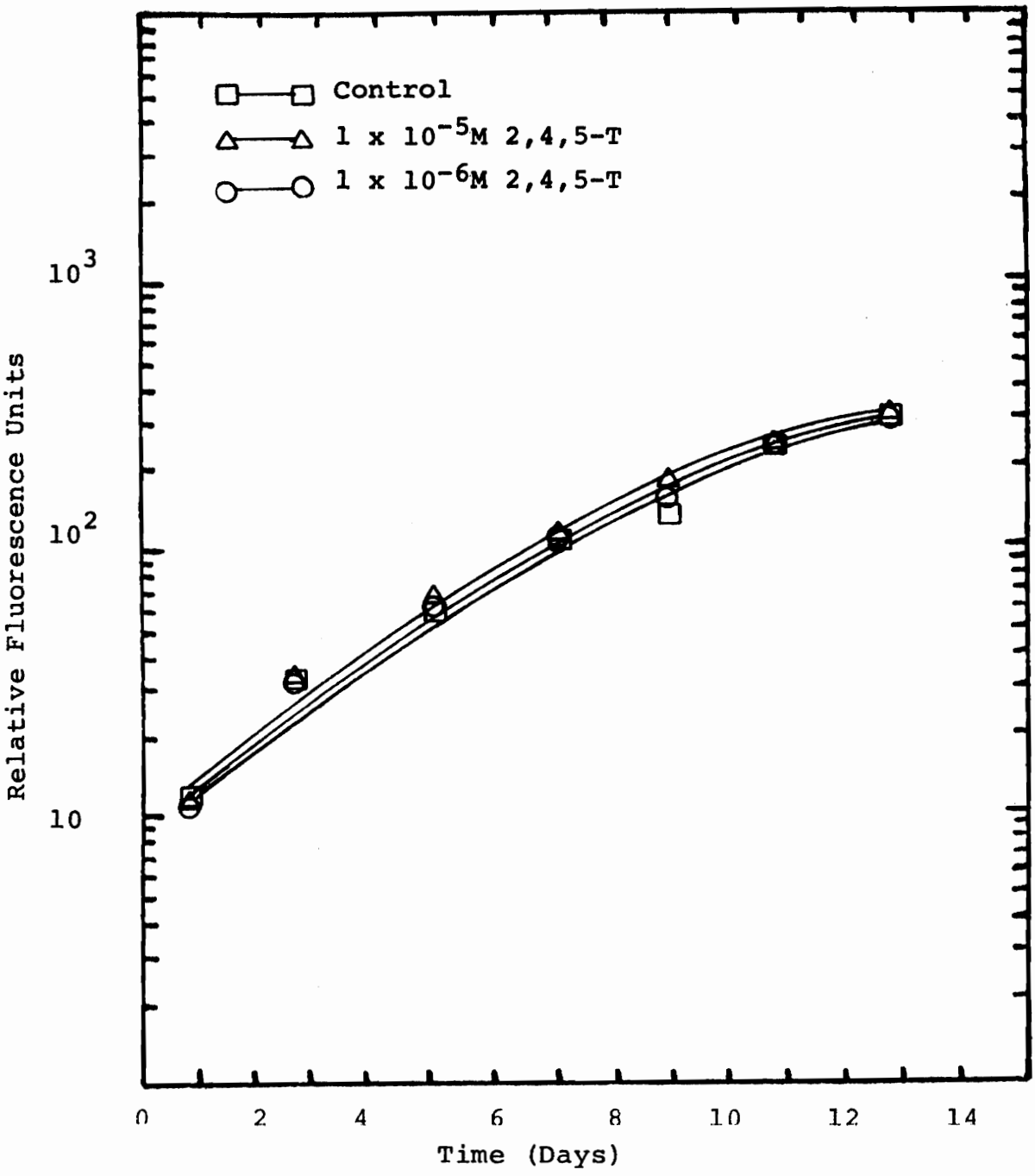

Figure 14. Increases in relative fluorescence units of Microcystis aeruginosa in 2,4,5-T.

data. At most 2,4,5-T levels in the concentration range between 0.9 x 10^{-3}M and 10^{-5}M, differences between 2,4,5-T treated cells and the control, as determined through fluorometric and mean cells per ml data, were statistically significant.

Figure 15 illustrates that a linear relationship exists between maximum cell counts and a range of concentration of 2,4,5-T between 0.9 x 10^{-3}M and 10^{-5}M.

An overview of data from 2,4-D and 2,4,5-T experiments indicate similarities for rates of growth and maximum yields. A linear relationship existed between maximum cell growth and concentration for both heribicides. Cells treated with 2,4-D and 2,4,5-T grew at a higher rate than did the controls over the log phase. Also, the rate of growth decreased with decreasing concentrations in both herbicides. The growth promoting effect of 2,4,5-T was not apparent in the lower concentrations of the experiment, whereas, 2,4-D did promote growth at the lower levels.

Values presented in Table 13 show pH data after 13 days of growth for both experiments. These data offer no evidence indicating pH fluctuation was a factor in growth of the alga.

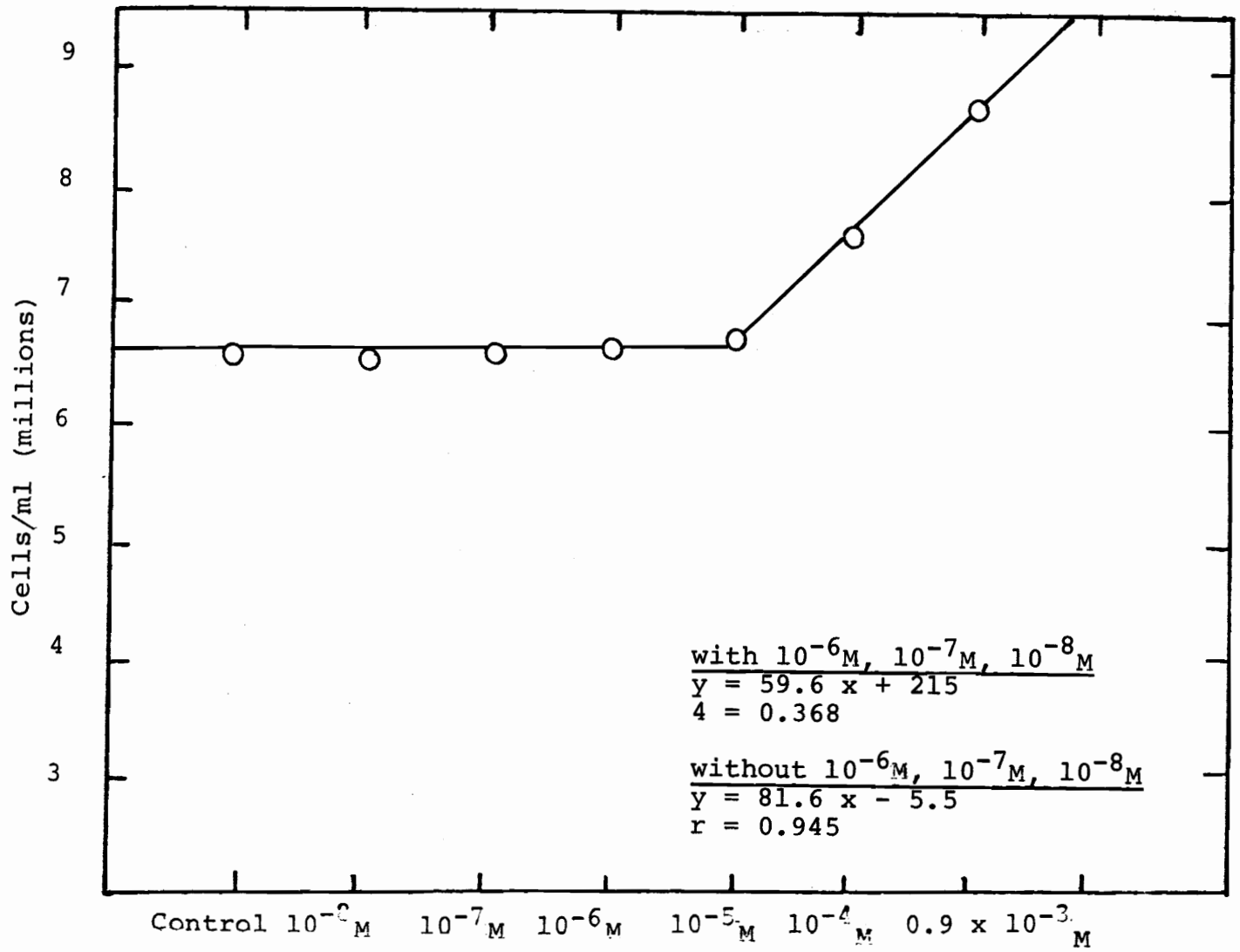

Figure 15. Maximum cells per ml values plotted as a function of increasing 2,4,5-T concentrations.

Table 13

Final pH Values

2,4-D Test Finals		2,4,5-T Test Finals	
Test Level	Final pH	Test Level	Final pH
Control	7.7	Control	8.3
2×10^{-3}M	8.0	0.9×10^{-3}	7.2
1×10^{-3}M	7.6	1×10^{-4}	8.4
1×10^{-4}M	7.6	1×10^{-5}	8.4
1×10^{-5}M	7.6	1×10^{-6}	8.3
1×10^{-6}M	7.5	1×10^{-7}	8.4
1×10^{-8}M	7.6		

V. CONCLUSIONS

Tests on the effects of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) on a non-axenic culture of Microcystis aeruginosa under the conditions of this experiment has resulted in the following conclusions:

1. The herbicide 2,4,-D promoted the growth of a non-axenic culture of M. aeruginosa. The effect decreased as 2,4-D concentration decreased from 10^{-3}M (221 mg/l) to 10^{-8}M (0.002 mg/l).

2. A linear relationship existed between maximum mean cell growth and concentrations of 2,4-D ranging from 10^{-8}M to 10^{-3}M.

3. Cultures of non-axenic M. aeruginosa treated with 2,4-D doubled at a higher rate than control cultures during logarithmic growth. The doubling rate of 2,4-D treated cells decreased below the control as cultures approached maximum yield.

4. Concentrations of 2,4-D at 2 x 10^{-3}M (442 mg/l) have a toxic effect on non-axenic M. aeruginosa.

5. It appeared that cells treated with 2,4-D between the concentration range of 10^{-3}M to 10^{-7}M contained

less chlorophyll and/or pheophytin per cell than did the control.

6. The herbicide 2,4,5-T promoted the growth of a non-axenic culture of M. aeruginosa. The effect decreased as 2,4,5-T concentration decreased from 0.9×10^{-3}M (230 mg/l) to 10^{-5}M (2.29 mg/l).

7. A linear relationship existed between maximum mean cell growth and concentrations of 2,4,5-T ranging from 0.9×10^{-3}M to 10^{-5}M.

8. Cultures of non-axenic M. aeruginosa treated with 2,4,5-T in the concentration range of 0.9×10^{-3}M to 10^{-5}M had a higher doubling rate than control cultures during logarithmic growth. The doubling rate decreased below the control cultures in this concentration range as cultures approached maximum yield.

9. Chlorophyll and/or pheophytin were apparently not affected by 2,4,5-T in the non-axenic M. aeruginosa cultures.

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VITA

Henry Wilmore "Buck" Cox, Jr. was born in Fredericksburg, Virginia on January 16, 1950. He grew up in Fredericksburg and graduated from James Monroe High School in 1968. In 1973, the author received his Bachelors Degree in Biology from Virginia Polytechnic Institute and State University. After working for two years with the Virginia State Water Control Board, he returned to VPI & SU to begin work on his Masters degree in Environmental Science and Engineering. After completion of this degree, Mr. Cox will take part in a research project funded by the National Science Foundation in Antarctica. He will begin work for the Virginia State Water Control Board upon his return from Antarctica.

Henry Wilmore Cox, Jr.

THE GROWTH PROMOTING EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) and 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T) ON *Microcystis aeruginosa*

by

Henry Wilmore Cox, Jr.

(ABSTRACT)

Microcystis aeruginosa is known widely for the obnoxious nature of its bloom. Problems resulting from blooms of the alga include: death of fish and other aquatic life, clogging of filter systems in water treatment plants, taste and odor problems, and death of cattle and water fowl via extracellular polypeptides. Blooms of this nuisance alga have historically occurred in waters receiving pollution from sewage effluents or runoff from agricultural lands.

Studies have shown that 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) mimic the metabolic activities of the plant hormone indoleacetic Acid (IAA). IAA has been shown to stimulate the growth of some green and blue-green algae.

Recent evidence suggests that blooms of *Microcystis aeruginosa* were triggered when the herbicides 2,4-D and

2,4,5-T were introduced to lakes and rivers.

The results of this study showed that batch cultures of non-axenic Microcystis aeruginosa containing 2,4-D and 2,4,5-T exhibited significantly more growth than controls over a wide range of herbicide concentrations. Growth in 2,4-D treated cells was statistically significant from the control at $\alpha = 0.05$ at concentrations as low as 10^{-7}M (0.020 mg/l) and 10^{-5}M (2.5 mg/l) for 2,4,5-T. Also, a plot of maximum cell yield values versus herbicide concentrations resulted in a linear relationship.