THE EFFECT OF 2,4-D, ATRAZINE, AND DIURON ON ALGAL PHYSIOLOGY AND POPULATION DYNAMICS

by

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DEDICATION

To my son, Jefferson

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I. INTRODUCTION

The ecology of the planktonic aquatic communities has been shown to be complex. This is particularly true in attempting to explain conditions governing the dominance of a single algal species in bloom proportions (36). Nutrient loading is most often cited as a major factor. However, this alone cannot reliably predict the dominance and succession of one species over others (36). The literature suggests that bacteria, fungi, viruses, and zoöplankton can play important roles in controlling algal populations (36,37). Other studies indicate that certain algal species release extracellular products that inhibit the growth of competing species of algae and bacteria (19,61,70,80). Additionally, environmental factors such as light, temperature, turbidity, and current complicate growth conditions (37). This list is by no means exhaustive, for many other variables can serve to complicate the population dynamics of the planktonic community (37,80).

Whitton (106) suggested that the whole algal community could be used as a means of characterizing aquatic environments and toxic stress to those environments. Studies by Cairns and Plafkin (27), and Cairns and Dickson (26) showed clearly that community structure shifted when stress was applied. Using chlorine as the stressing agent, a well-diversified community of benthic organisms shifted to a community dominated by a few tolerant species. The biomass of those tolerant species increased under less competitive conditions. This response has been shown to be typical for a variety of ecosystems (25,27).

The effect of herbicides on community structure has received very little attention. Most of the work done with herbicides has been directed toward their effect on individual species under controlled conditions within the laboratory. An excellent study conducted by Boyle (14) on the effect of 2,4-D on the ecology of experimental ponds suggested that the increase in phytoplankton was attributable to losses of nutrients by the macrophyte population. Getsinger *et al*. (43) reported similar findings when applying 2,4-D to control water milfoil. Laboratory studies, on the other hand, suggest that 2,4-D has a wide range of effects on individually tested algal species (21,22,32-35,78, 84). The effects ranged from highly toxic to growth to stimulatory to growth in testing a variety of species. Similar results have been reported for other herbicides. Atrazine was reported to stimulate chlorophyll production and growth of test algae (39,88,96) while similar studies show atrazine inhibits growth (39,88). Additionally, Fitzgerald *et al*. indicated selected algae had a wide range of tolerance for the 200 toxicants tested. This information would suggest that herbicides in the aquatic environment may serve to alter the community structure and disrupt natural competitive processes. Under the appropriate conditions, remaining competing species could conceivably grow to excess under the reduced stress of limited competition.

The objective of this study was to define the effect of 2,4-D, atrazine, and diuron on the community structure of the algal population

of Smith Mountain Lake. Laboratory studies were conducted to more closely define the physiological effects of these herbicides on selected algae.

II. LITERATURE REVIEW

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

The herbicide, 2,4-D was first introduced after World War II for the selective control of broadleaved weeds. Monocotyledonous plants are not particularly affected by 2,4-D so it is commonly used on cereal crops, grass pastures, and lawns.

Several formulations of 2,4-D are available. The acid form is rarely used. The most commonly used formulations are salts and esters of the parent acid. The salts of 2,4-D are amine, sodium, and ammonium forms with potassium and lithium salts being two other lesser used varieties. The ester formulations are soluble in oil, but not water. However, in practice, esters of 2,4-D are made so that the compound may be emulsified in either oil or water. Commonly linked to 2,4-D through an ester bond are methyl, ethyl, isopropyl, and butyl groups.

The acid form of 2,4-D is a white crystalline substance. The molecular weight is 221.0 with a melting point of 140.5°C. The pKa of 2,4-D acid is 3.2.

The phenoxy-carboxylic herbicides, one of which is 2,4-D, are collectively known as auxin-type herbicides. Auxins are defined as those organic compounds that can promote the growth of higher plants along the axis of shoots while inhibiting root elongation when applied at concentrations less than 10^{-3} M (65). It appears that 2,4-D mimics

the activity of the natural plant auxin, indoleacetic acid (IAA). However, plants contain an enzyme called IAA-oxidase which breaks down IAA rapidly, but 2,4-D is very stable within the plant and remains biologically active in plants for a long period of time (10). Audus (10) showed that IAA was 450 times less active than 2,4-D at inhibiting root elongation. Van Overbeek (95) found that 1000 times more IAA was needed to elongate the shoot in the pea than the dissociated form of 2,4-D.

Several studies have indicated that IAA has a stimulatory growth effect on species of green and blue-green algae (15,79). Ahmad and Winter (3) reported that the growth of several species of blue-green algae were stimulated when concentrations ranging from 10^{-10} M to 10^{-5} M IAA were added. Higher levels were inhibitory. Green algae, on the other hand, needed higher concentrations of IAA before stimulated growth was evident.

Work by Audus (11) indicated that IAA triggers the production of ethylene. Studies have also shown that ethylene production can be enhanced by using 2,4-D (2). Ethylene is apparently produced in a pathway involving the enzymatic decomposition of methionine. Ethylene itself has been shown to be a plant hormone responsible for regulating several activities including stimulating growth (2).

The response of plants to 2,4-D has been reviewed extensively (8,11,65,71,77), but there are still several fundamental aspects concerning 2,4-D actions which are unclear. For example, it appears to

act as a plant auxin, but it is also able to initiate such effects as interference with the metabolic production of nucleic acids, inhibition of photosynthesis, and inhibition of oxidative phosphorylation (5).

The apparent effect of 2,4-D on photosynthesis occurs in the reaction which results in the splitting of water to two hydrogen molecules and singlet oxygen. This process is known as the Hill reaction. Inhibition of this process results in inhibition of the entire photosynthetic process. However, it appears that while 2,4-D can inhibit photosynthesis, it can also stimulate the process. Weddings *et al.* (104) showed a stimulation of photosynthesis in *Chlorella* with 2×10^{-3} M 2,4-D at pH 7. Decreasing the pH to 3.0 and 4.1 resulted in inhibition of the process. This would indicate that undisassociated molecules are more effective in inhibiting the photosynthetic process. Bingham (13) showed that 2,4-D uptake by *Scenedesmus quadricauda* seem to be associated with medium pH. Lower pH resulted in greater uptake. Bingham suggested 2,4-D uptake was related to the pKa of 2,4-D which is about 3.0. Hendrick *et al.* (48) showed the same stimulating effect on maize in low concentrations of 2,4-D (14.2 μg/3 ml). Ten times this concentration inhibited the process. In a similar study, Bertagnolli and Nadakavakaren (12) found that *Chlorella pyrenoidosa* produced more oxygen when 5×10^{-4} M 2,4-D was administered. Oxygen production was inhibited at about 5×10^{-3} M 2,4-D levels. Glycolate and glycolate dehydrogenase concentrations were significantly increased with 2,4-D. Because glycolate dehydrogenase is rate limiting in the photorespiratory

pathway, the authors suggested that the photorespiratory pathway was receiving the excess oxygen in production of glycolate.

Both stimulation and inhibition of respiration have also been noted in plants receiving 2,4-D applications (11). Klotz and Duysen (59) noticed increased respiration levels in *Chlorella pyrenoidosa* grown under heterotrophic conditions. Stimulated growth was also noted. No effect was reported for *Chlorella* grown under mixotrophic and autotrophic conditions at low levels of 2,4-D.

Stimulated respiration and growth have been seen in several studies of higher plants receiving 2,4-D doses (11). Inhibition of respiration has been demonstrated using high concentrations of 2,4-D ($> 5 \times 10^{-3}$M). However, lower doses may be stimulatory (11,105).

In an extensive review of the literature available in 1958, Woodford *et al*. (108) reported that the activity of nine plant enzymes was stimulated with 2,4-D. Included were the enzymes: catalase, IAA oxidase, invertase, pectin methyl esterase, peroxidase, phosphatase, glucose-6-phosphate dehydrogenase, polyphenol oxidase, and phosphorylase (108). It seems likely that the stimulatory activities induced by 2,4-D may be due to heightened activity of some enzyme or enzymes. No literature to this effect was found however.

Several authors have shown 2,4-D produces a variety of effects on crop yields in higher plants (11). Wedding *et al*. (104) actually increased shelled green bean yield by 35 percent with 10 to 20 ppm additions of 2,4-D. Also, lima bean production and snap bean production

were increased by as much as 70 percent with 5 to 20 ppm 2,4-D. However, production was not always increased. Losses of 75 percent over control values were also recorded for the same applied-concentration range. Miller et al. (69) showed increases in production using 0.5 to 1.0 ppm of 2,4-D. Higher concentrations resulted in decreases in production.

Like higher plants, the literature concerning 2,4-D and growth response of algae reveals that there are both stimulatory and inhibitory responses. Elder et al. (35) found high concentrations of 2,4-D (> 22 mg/l) did not affect the growth of 18 genera of freshwater algae. Lembi and Coleridge (63) found an appreciable decrease in growth of Scenedesmus sp., Ankistrodesmus sp. and Pedeastrum sp. with 110 mg/l of 2,4-D. Cullimore and McCann (34) found the growth of four species of green algae were significantly decreased in only 1 mg/l of 2,4-D. Butler et al. (21,22), Singh, (84) Cullimore and McCann (33), Hawxby et al. (46), and Arvick et al. (7) all found that 2,4-D did not appreciably affect the growth of a wide range of algae and cyanobacterium. Voight and Lynch (98) reported a wide range of sensitivity of three algae to varying concentrations of 2,4-D. Stimulatory effects of 2,4-D were reported by Poorman (78) on test alga, Euglena gracilis. Growth was stimulated over a range of concentration from 1.0 to 50 mg/l of 2,4-D. Hendrick et al. (48) stimulated growth of Scenedesmus quadricauda by 50 percent over control dry weight values when grown in 500 mg/l of 2,4-D for 14 days. Growth response to 50 mg/l showed only a 6 percent

increase. Klotz and Duysen (59) and Cox *et al*. (32) showed stimulated growth of *Chlorella pyrenoidosa* with varying concentrations of 2,4-D, while Cox and Boardman (33) reported increases in the growth of *Microcystis aeruginosa* using a range of 2,4-D concentrations from 2 μg/l to 221 mg/l. In the latter study, growth increased with increasing 2,4-D concentration and maximum cell production correlated linearly with log increases in molar concentration. A concentration of 442 mg/l had a toxic effect on growth.

In a study on the effect of 2,4-D on a wide variety of aerobic, anaerobic, and facultative bacteria, Worth and McCabe (109) reported both stimulatory and inhibitory responses. Most anaerobic organisms were slightly inhibited by 2,4-D. The reaction of aerobic organisms varied from total inhibition to stimulation. The aerobic bacterium *Rhizobium trifolii* was inhibited in concentrations exceeding 25 mg/l and stimulated at lesser concentrations. Facultative organisms were not significantly affected by 2,4-D in concentrations exceeding 25 mg/l. However, growth stimulation was reported at concentrations less than 25 mg/l.

Studies by Hansen and Slife (45) indicated that stimulated growth occurred concomitant with increases in RNA synthesis and subsequently increased synthesis of proteins. Key *et al*. (58) previously discovered that increased RNA synthesis occurred before the production of DNA and protein and cell division were enhanced in soybean seedlings. These increases can perhaps be regulated by increased enzyme activity as a

result of the stimulatory effects of 2,4-D. Studies by Arens and Stout (6) indicated that the ribonucleic acid polymerase activity of maize was stimulated by 2,4-D treatment. Enhancement increased with increasing 2,4-D concentrations from 10^{-7} to 10^{-3} M. Similar findings have been reported for IAA treated plants (53).

Several authors have shown that some bacteria and fungi are capable of utilizing 2,4-D as a source of carbon (11,65,72). A mechanism for bacterial degradation of 2,4-D was suggested in a paper by Loos (65).

Degradation of 2,4-D in the soil is almost totally due to microorganisms (11,56). Bacteria play a large role in the degradation process as indicated in a kinetic study of bacterial decomposition by Audus (11). The types of metabolic reactions which occur include: cleavage of the ether linkage, dehalogenation, ring cleavage, ring hydroxylation, and ester hydrolysis.

Audus (11) was the first to suggest that the initial step in the breakdown of 2,4-D was hydrolysis of the acetic acid side chain which releases glycolate and the phenolic ring. Studies of the decomposition of 2,4-D in an activated sludge system indicated that the biodegradation followed zero-order kinetics. Approximately 68 percent of the 2,4-D was used in respiration, while 32 percent was incorporated into microbial cells as a reserve source of energy. Acclimatized cells were more efficient at degrading 2,4-D than non-acclimatized cells, a fact which indicated that enzyme activation was necesssary for decomposition (47).

This would explain the initial lag period in decomposition reported by other authors (11,89).

Photochemical decomposition of 2,4-D involves the loss of halogen atoms. Two common products include 4-chloro-2-hydroxy-phenoxyacetic acid and 2-chloro-4-hydroxy-phenoxyacetic acid. In addition, fission of the ether linkage in 2,4-D can occur, producing 2,4-dichlorophenol and 4-chlorocatechol.

Several authors have shown that plants are capable of metabolizing 2,4-D (11). Luckwill and Lloyd-Jones (66,67) showed that the process involves a decarboxylation of the acetic acid side chain in several plants. Other authors have suggested amino acid complexes and other conjugates are formed (11). Valentine and Bingham (92), found that *Scenedesmus* produced several metabolites with (3-hydroxy-2,4-dichlorophenoxy) acetic acid being the major constituent.

Extensive research has shown that 2,4-D is noncumulative, nonpersistent, and readily biodegradable (101). Studies have also shown that 2,4-D does reach natural waters in appreciable levels. Brooker and Edwards (18) surveyed 20 canals in the U.S.A. receiving ditchbank application of 2,4-D. Concentrations in a range from 50 μg/l to 365 g/l were recorded. However, in a Tennessee Valley Authority study (107) it was discovered that when 2,4-D was applied at common rates for the control of water milfoil, 2,4-D levels of 2 mg/l were routinely detected in the water column.

Perhaps the most thorough ecological study of 2,4-D effects was conducted by Boyle (14). The dimethylamine salt form of 2,4-D was sprayed over experimental ponds at an application rate of 5 and 10 kg/ha. Twelve ponds were drained the previous winter to assure that each pond would be comparable to others in relative states of succession. Three groups of four ponds each were randomly selected to serve as a control group and to receive two 2,4-D application rates. Bluegill sunfish were stocked in each of the ponds seven days prior to treatment. Results showed that the macrophyte community was unaffected by the 2,4-D with the exception of arrowhead (*Sagittaria montividensis*). This species died out completely. It reappeared in large numbers within four to six weeks after treatment, coinciding with the disappearance of most of the 2,4-D in the water column.

Pond water treated with 2 mg/l of 2,4-D had significantly greater total numbers of phytoplankton during July, August, and September (14). Also, $H^{14}CO_3$ uptake was significantly increased in the phytoplankton community by 1 and 2 mg/l of 2,4-D in laboratory studies. The increased uptake was cited as a possible cause for increased phytoplankton growth. Boyle (14) also hypothesized that increased growth could be attributed to loss of nutrients by the macrophyte population. Some correlation was noted between application rate nutrient availability, chlorophyll a, and production of oxygen.

Other investigations have reported similar results. Brooker and Edwards (18) and Wojtalik *et al.* (107) noted decreases in pH and oxygen

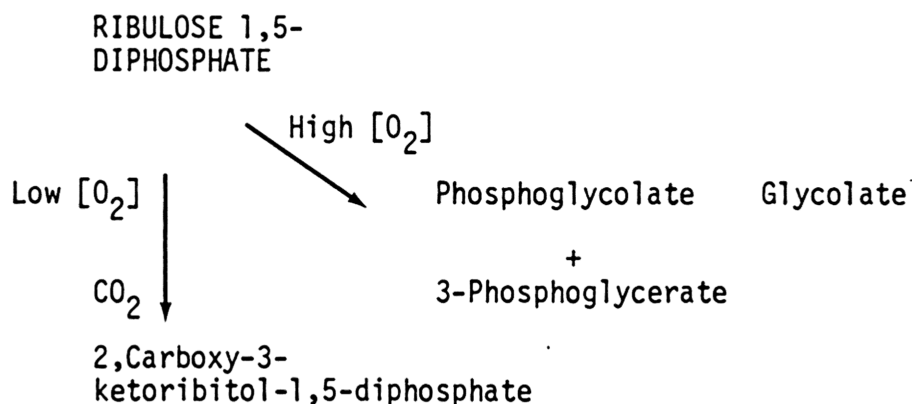
and increases in nutrient following 2,4-D application to rooted aquatic plants. No plankton blooms occurred. However, Getsinger _et al_. (43) did report a phytoplankton bloom following an application of 2,4-D to control water milfoil.

It is important to note at this point that no literature was located which attempted to assess the changes within the algal phytoplankton community as a result of 2,4-D application.

Photorespiration - Glycolate Production and 2,4-D Effects

The fixation of CO_2 during in the formation of glucose in plants (Calvin Cycle) occurs when ribulose 1,5-diphosphate reacts with CO_2 to form an intermediate, 2-carboxy-3-ketoribitol-1,5-diphosphate. This intermediate is presumably enzyme-bound. Upon hydration, the intermediate forms two molecules of 3-phosphoglycerate. The enzymes, involved in this reaction, ribulo-sediphosphate carboxylase, is inhibited by molecular oxygen. This inhibition is thought to be the mechanism for controlling the fixation of CO_2 when the partial pressure of oxygen is high and that of CO_2 is low. Studies have shown that, under high oxygen tension, molecular oxygen can actually replace CO_2, resulting in the formation of phosphoglycolic acid and 3-phosphoglyceric acid. It is the phosphoglycolate that is the precursor to free glycolate in an enzymatic hydrolysis reaction. This apparent waste of photoreduced substrate has been reported to be at a rate as high as five

times that observed under dark respiration conditions. The preceding set of reactions may schematically be represented as follows (62):



Glycolate has been discovered in natural waters by several investigators. Miller *et al.* (69) showed that glycolate was photoassimilated by *Anabaena flos-aquae* and a species of *Oscillatoria*. Fogg *et al.* (38) suggests that increased growth of planktonic algae may sometimes be due to the photoassimilation of dissolved organic matter, particularly glycolate. Watt (100) reported that 92 percent of the extracellular products released *in situ* by a natural population of algae was glycolate. This may partially explain the correlation of abundant blue-green algae growth to large concentrations of dissolved organic matter (37).

Bertagnoli and Nadakavakaren (12) reported that 5 x 10^{-4} M 2,4-D stimulated both net oxygen uptake and production in synchronously grown cultures of *Chlorella pyrenoidosa*. Oxygen production and uptake were inhibited by 5 x 10^{-3} M levels. Glycolate content in the culture media increased significantly in 2,4-D treated samples. This study suggested

that increased glycolate production was due to increases in oxygen partial pressure that occurred when the 2,4-D was applied. Furthermore, 2,4-D was partitioned from isolated chloroplast fractions while other fractions failed to show measurable amounts of 2,4-D. This would indicate that the chloroplast is the site for 2,4-D action in *Chlorella pyrenoidosa*. This supports evidence that 2,4-D is capable of may be interfering with the photorespiration process.

Diuron 3-(3,4-dichlorophenyl)-1,1-dimethyurea)

Diuron is a white crystalline solid and is odorless. It has a molecular weight of 233.1 and a melting point of 158°C. Diuron will decompose at 189°C. It is stable under normal conditions of moisture and redox potential and will not hydrolyze unless exposed to acid or alkaline conditions at elevated temperatures. Diuron is extremely soluble in acetone (53,000 ppm) but only slightly soluble in water (42 ppm).

Formulations of diuron are usually sold as wettable powders under the trade names Karmex, Krovar, Marmer, Viondiuron, DCMU, or Diurex. The chemical is applied for selective control of weeds in agricultural crops at rates of 0.6 to 4.0 pounds per acre (lbs/a). Application rates for general weed control and perennial weed control are from 4 to 16 lbs/a and from 16 to 48 lbs/a, respectively.

Michigan, Oklahoma, and Texas have registered diuron for aquatic use. Canada has also approved use of diuron for aquatic use at rates of 1 to 3 ppm.

Urea is the basic chemical nucleus of the family of compounds known as the urea herbicides. Two methyl groups and a dichlorophenyl group are substituted on the urea molecule in the formulation of diuron (5).

It is generally understood that the principal mode of action of diuron is in the inhibition of the Hill reaction results in an inhibition of photosynthesis. The Hill reaction is the process by which water is oxidized in non-cyclic photosynthesis (Photosystem II), releasing singlet oxygen, H+, and electrons for reduction of chlorophyll in the light. Apparently, diuron blocks the system by preventing electrons from reducing the chlorophyll. The chlorophyll becomes oxidized as the electrons within its structure are passed on through the photosynthetic cycle. This eventually results in the "bleaching" often noted in studies of this chemical (95). Further studies show that the actual site of inhibition is between plastoquinone and a quencher which is the primary electron acceptor in non-cyclic photosynthesis (20). Since the splitting of water releases hydrogen for the reduction of NADP this process is also inhibited. Loss of ATP and NADPH from photosystem II results in shutting of the energy and reducing agents necessary for carbon fixation (8).

Zweig *et al.* (110), showed 3×10^{-5} M diuron to have only a slight effect on cell growth of *Chlorella* over a 48 hour period. However, it

brought about bleaching and death of all cells within seven days. Cells began dying after 90 hours of contact time. Oxygen levels decreased over 48 hours of contact time, as did chlorophyll concentrations. Hutber et al. (52) found that the growth of four, blue-green algae was suppressed when they were exposed to 0.01 to 0.5 mg/l diuron. Other authors have reported similar laboratory findings (60,97).

Field studies (53)in which diuron was applied to ponds indicated that many algae and rooted aquatic plants were controlled well for periods up to six months. Concentrations of diuron ranged from 0.07 to 2.0 ppm. The algae that were controlled included: Anabaena sp., Chara sp., Chlorella vulgaris, Nitella sp., Oedogonium sp., Oscillatoria sp., Spirogyra sp., and Microcystic aeruginosa. In some cases regrowth of the target algal species was considerably different than in controls. No literature was located in which effects on species diversity were noted. However, reports of reduced growth in fish after application of diuron have been recorded. It was not known if the growth problem was due to decreased food supply or to direct toxic effects.

Toxic effects to fish were noted in laboratory studies over a range of concentration from 0.1 to 12.0 ppm diuron. The most significant effects were noted to be endocardial changes. In pond studies the effect in the endocardial area was more pronounced than in laboratory studies using similar concentrations of diuron (53).

Effects of diuron on pond invertebrate populations were studied by Punjin (81). The results indicated that zooplankton were not

immediately affected; however, populations did decrease several weeks after application. No explanation was given. In other studies both non-toxic and toxic effects to several individual species of invertebrates were noted (83).

Few studies have been conducted on the effect of diuron on bacteria and fungi (53). Degradation of diuron and other urea compounds by the fungus, Rhizoctonia solani have been documented. The diuron was apparently broken down into three metabolites by the fungi (105). Some metabolites derived from the degradation of diuron have been identified as chloroanilines and chloroazolbenzenes (42).

Demethylation appears to be the primary means of detoxifying diuron in plants. The removal of one methyl group lowers phytotoxicity, whereas removal of two methyl groups essentially totally detoxifies the chemical (8).

Photodecomposition of diuron has been reported by Comes and Timmons (31). Diuron activity was decreased when exposed to summer sunlight by 73 percent to 82 percent after 25 and 60 days, respectively.

Very little information is available concerning the fate of diuron in the aquatic environment. Studies on structurally similar urea herbicides (monuron and neburon) show rates of loss in a simulated aquatic environment to be slow. Using an application rate of 40 pounds per acre, Frank (40) discovered that the levels of monuran and neburon in the water column were not significantly different after 8 and 16 weeks, respectively. Losses of the two herbicides after these periods

were significant, but approximately 39 percent of the neburon and 15 percent of the monuron still remained after 2.5 years. McCraren _et al._ (68) reported that diuron was not detected in the water column of test ponds after 28 days in a study on chronic effects of diuron on blue gills. Detectable levels were found in plants for 90 days and in sediments for 122 days after treatment. Ponds received from 0.5 to 3.0 ppm applications of diuron.

Van der Weig (94) calculated expected half-life values for diuron in a model ecosystem. The calculated values were: 17 days for 0.2 ppm, 48 days for 0.4 ppm, and 70 days for 0.8 ppm.. In pond studies, the half-life value for a treatment of 0.1 ppm was 11 days. Significant accumulation of diuron in fish was also reported.

Atrazine (2 chloro-4-ethylamino-6-isopropylamino-S-triazine)

Atrazine, a member of the substituted diamino-S-triazine family of herbicides, is commonly used for selective control of preemergence and early postemergence seedling grasses and broadleaf weeds.

The triazine herbicides vary in the group substituted at the second, fourth, and sixth positions on a ring structure composed of three nitrogen atoms and three carbon atoms arranged alternately around the ring. Amino groups are always attached at the fourth and sixth position on the ring.

Atrazine has a molecular weight of 215.5 and is only slightly soluble in water. Formulations of atrazine are commonly prepared as wettable powders of approximately 80 percent concentration. Recommended application rates for nonselective control of vegetation range from 5 to 40 pounds per acre.

The apparent mode of action of atrazine is in inhibiting the Hill reaction and interfering with the fixaction of CO_2 (5). Several authors have noted decreases in net photosynthesis and CO_2 uptake in algae caused by triazine herbicides (11,87,93).

Studies by Valentine and Bingham (93) indicated that photosynthesis, as measured by $H^{14}CO_3$ uptake, was inhibited by atrazine in concentrations ranging from 0.01 to 1.0 mg/l in four test algae. Funderbank and Lawrence studies (41) indicated that inhibition of the Hill reaction and photosynthesis in duckweed occurred at 0.2 mg/l atrazine.

While inhibition of the photosynthetic process and growth is a frequent response to atrazine (11), several authors reported increased growth and chlorophyll content in experiments with several test algae (88,96). Torres _et al_. (88) reported an inhibition of chlorophyll production at concentrations of atrazine above 1 mg/l. Stimulation of chlorophyll production was noted using 0.1 and 0.5 μg/l. In two studies, the growth of _Chlamydomonas_ in the presence of atrazine and simazine, a similar triazine herbicide, was increased. Vance and Smith (96) increased growth in _Chlamydomonas_ by using up to 200 mg/l of

simazine, while Foy and Hiranpradit (39) achieved stimulated chlorophyll production in test algae with both simazine and atrazine. Concentrations ranged from 0.052 to 0.104 mg/l. Chlorella was found to be most sensitive of the test algae to atrazine. Chlamydomonas was the next most sensitive and was inhibited by 85 percent at 0.05 mg/l. Scenedesmus was inhibited by 60 percent using 0.05 mg/l atrazine.

Some variability in the toxicity of atrazine to algae can be explained by the apparent ability of some algae to adsorb more of the chemical per unit weight than other species. Valentine and Bingham (93) showed that a Euglena culture adsorbed significantly less atrazine per unit dry weight of culture than did Scenedesmus and Chlamydomonas. Euglena was not as sensitive to atrazine as were the other test algae. Other authors have reported similar findings in tests with several families of phytoplankton algae (50,73,99).

Metabolism studies of atrazine have largely been confined to higher plants. Degradation and detoxification of atrazine in higher plants has been shown to take place by n-dealkylation, conjugation with peptides, and hydrolysis (11). Complete oxidation to CO_2 or incorporation of breakdown derivatives into cellular material has been shown (11).

Valentine and Bingham (93) suggested that the alga, Scenedesmus, did not metabolize atrazine after 24 hours of contact. This result could not be stated conclusively, however, because atrazine was toxic to the test algae and extremely small amounts of atrazine had to be used. This study with atrazine suggested a physiochemical uptake of atrazine.

Work by Wedemeyer (103) supported this contention and showed further that uptake in a physiochemical manner may be a very important means of decreasing pesticide residues.

Atrazine is apparently extremely persistent in the aquatic environment. Experiments have indicated that atrazine remained in the physical and biological portions of a pond for two years after application (1). Studies of degradation in the soils show that atrazine is more readily degradable there than in the water. Degradation by hydrolysis apparently precedes microbial degradation in the soil as the major detoxifying pathway (85). Soil persistence is generally accepted to be from three months to a year (5).

Studies have shown that runoff from agricultural sites is the primary mechanism by which surface waters become contaminated by atrazine (54). Concentrations of atrazine in finished potable water of several major cities were measured and found to range from 0.03 - 0.4 ppb. Of 92 streams tested in Canada, 77 percent were found to contain greater than 0.03 ppb and atrazine was found in every major watershed in Iowa in concentrations ranging up to 0.042 ppm. Runoff studies indicated that a maximum of six percent of the applied atrazine was transported from fields under the most favorable conditions. The average percentage of the compound in runoff was under two percent of that applied. The highest concentration found in runoff water was 0.48 ppm (91). Other studies supported these findings (11,44).

ATP and Energy Charge

All living cells contain chemical energy in the form of adenosine triphosphate (ATP). Because ATP is common to all cells, it becomes a very good indicator of biomass and available energy within environmental samples.

Through a phosphorylation process, adenosine diphosphate (ADP) is converted into ATP. Energy for phosphorylation is yielded from the degradation of energy rich molecules. The ATP delivers energy to specific acceptor molecules to carry out chemical, osmotic, or mechanical work for the cell. Upon delivery of energy, ATP undergoes a loss of a phosphate group, yielding ADP once again. ADP is produced through an energy-consuming phosphorylation of the single, phosphate-carrying molecule of adenosine, adenosine monophosphate (AMP). Normally, cells operate with far more ATP than ADP and AMP. When cells become stressed, ATP levels will decrease rapidly. An estimate for the half-time turnover rate for ATP in *E. coli* under favorable conditions is considered to be only seconds. As ATP levels decrease in response to stress, ADP levels rise. Reductions in stress lead to rapid increases in ATP and decreases in ADP to normal levels (62).

The adenylate energy change (EC) concept is defined by Atkinson *et al.* (9) to be equal to one-half the number of anhydride-bound phosphate groups per adenine moiety:

$$EC = \frac{[ATP] + 1/2\,[ADP]}{[ADP] + [ADP] + [AMP]}$$

Therefore, EC can be described as a linear measure of the metabolic pool of energy stored in the adenylate compounds. Theoretically, the range of EC ratio can range from 0.0 (all AMP) to 1.0 (all ATP). In actual laboratory tests, the EC has been found to stabilize at values around 0.8 to 0.9 in cultures of rapidly growing cells. At an EC value of 0.5, E. coli. cells died (29). E. coli cells in the stationary phase of growth had EC values between 0.6 and 0.7.

Karl (55) conducted experiments using EC as a means of measuring stress on organisms during filtration. The effect of filtering decreased the EC as increasing volumes of culture were forced through the filter. Further, when the total concentrations of adenylate compounds were summed, no significant changes were noted over the range of volumes filtered. However, ATP levels decreased significantly. The magnitude of the effect on EC was found to be related to the density, genera present in the sample, and initial EC level. Asymtotic EC values were noted as sample volume increased.

Karl (55) also reported a good correlation between increased productivity within natural samples and energy charge. EC values were found to range between 0.69 to 0.81 in natural samples. Maximum values were correlated with subsurface measurements of chlorophyll and ATP.

The use of ATP to estimate biomass in natural waters and waste treatment facilities has received some attention. Relatively constant

levels of ATP have been reported by numerous authors for both bacteria and algae when cultures were in the logarithmic phase of growth. Weber (102) has compiled a list of reported ATP concentrations for various organisms. Studies by Brezonik et al. (17) with two green algae and two blue-green algae indicated that there were fairly good correlations between ATP concentrations and the three conventional measures of algal biomass: chlorophyll a, dry weight, and cell numbers. Ratios of ATP to chlorophyll a for the test algae ranged from 0.09 to 0.35. While the results showed generally good correlations, significant variations were not uncommon (30). Wide variations in ATP values were noted when MLSS and COD values for an activated sludge did not change significantly (30). Similar results were reported by Hobbie et al. (49), Karl (55), and Traaganza (90) for natural waters. It is important to note that only Karl considered stress on the test organisms when reporting inconsistencies of the correlations. Stress could be due to environmental or laboratory factors, as demonstrated with the filter stress studies mentioned earlier. It seems likely that ATP values may vary considerably as a result of stress and, therefore, would not correlate consistently with other biomass parameters. Consideration of the other two adenylate compounds, ADP and AMP, would appear to be necessary before consistent correlations can be attained.

ATP as an indicator of toxicity has been studied by Kennicutt (57). Varying concentrations of chloroform, acetone, acrolein, and mercuric chloride were added to pond water and the response of ATP was monitored.

Tests were also conducted to determine the effects of pH over a range of 1 to 8. Mercuric chloride caused a nearly 100 percent loss of ATP after one hour exposure time at concentrations in the area of 1 mg/l. Chloroform reduced ATP levels by 40 percent after six hours. Acrolein was most effective in reducing ATP. At a pH of 1.0 ATP levels were decreased by 80 percent. The study concluded that ATP could be a valuable tool in assessing the impact of pollutants. Patterson _et al_. (76) reported that greater than 50 percent of the ATP pool in activated sludge was reduced within one hour after adding 15 mg/l of HgCl. Brezonik _et al_. (17) demonstrated that the ATP of a _Chlorella_ culture was significantly decreased when the cells were exposed to 100 ppb of mercury for only 80 minutes. Rapid responses of bacterial ATP to large pH changes and to the onset of anaerobiosis was reported by Brezonik and Patterson (16).

Patterson _et al_. (75) reported that the presence of compounds that inhibit luciferase activity could be determined by making serial dilutions of ATP standard solutions containing the suspected inhibitor. The presence of an enzyme inhibitor is confirmed if a parabolic relationship between calculated ATP concentrations and measured ATP levels results. Fractional dilutions of samples which do not contain luciferase inhibitors yield linear relationships between calculated and measured ATP values.

Algal Ecology

Biotic relationships within aquatic communities have been shown to be complex, particularly when attempting to explain conditions surrounding the dominance of a single algal species (36). Many factors come into play, and although nutrient loadings can be an important factor, they alone cannot explain the observed dominance and succession of one species over others (36). Many authors have suggested that bacteria, fungi, viruses, and zooplankton can play important roles in controlling algal populations (36,37). These works suggested that dominance of one or more algae is made possible when competing species are selectively fed on by zooplankton or decomposed by bacteria and fungi. Other studies indicate that certain algal species release extracellular products that inhibit the growth of competing species of algae and bacteria (19,61,70,80). Growth promotion by extracellular products, particularly glycolate, is now an accepted phenomena. A substantial amount of photosynthetically fixed carbon can be released by algae directly into waters as dissolved organic carbon. Fogg *et al.* (38) 0.05 mg/l of glucose, acetate, and glycolate have been found in eutrophic waters.

Blue-green algae have been shown to be particularly adept at photoassimilating these compounds (37). In fact, blue-green algae have been found to grow in the dark, although slowly, in the presence of organic substrates. More efficient growth is achieved when some light

is supplied. There appears to be two reasons for this. Blue-green algae lack a full complement of enzymes to operate the entire tricarboxylic acid pathway and the ability to repress and stimulate enzyme synthesis in response to increases and decreases in available organic substrate. Therefore, they are not able to take full advantage of substrate additions, using instead that which their limited supply of enzymes will allow. The Calvin cycle enzymes are present in sufficient amounts to account for the variety of metabolites required, although the control of the system is apparently due to enzyme activity and not to enzyme synthesis on demand.

Because no specialized membrane separates the enzymes responsible for photosynthesis, photorespiration, and dark respiration in blue-green algae, it is probable that interaction between these processes is unavoidable (37).

There are several reasons which explain why blue-green algae are able to favorably compete with other microorganisms. Often, blue-green algae possess gas vacuoles which allow them to float or move vertically and take maximum advantage of available light and nutrients. Blue-greens are able to survive over a broad range of temperature and osmotic potential. Another competitive advantage of blue-green algae over eukaryotes lies in their tolerance and sometimes preference for microaerophilic conditions. Blue-greens also frequently have a more efficient system in taking up and using available CO_2. Many possess an active bicarbonate uptake system, unlike competing eukaryotes. Luxury

uptake of phosphorus has been noted frequently in blue-green algae. Some nuisance blue-green algae, notably Anabaena flos-aquae and Gloetrichia echinulata, are capable of fixing nitrogen gas aqueous forms of nitrogen are in short supply, thus overcoming a major limiting nutrient problem (37).

Surface waters with a low pH, low hardness, and low alkalinity support very little algae growth. The algae that are present are most often green and Chrysophycean algae. On the other hand, waters with neutral to high pH (i.e. 8-10), high alkalinity, and high hardness levels are usually more supportive of algal productivity, particularly the productivity of blue-green algae.

Total dissolved solids seem to play a role in determining algal community structure. Larger celled algal species seem to predominate in waters in advanced stages of eutrophication (i.e. waste stabilization ponds). Smaller algae seem to predominate when surface waters contain low levels of total dissolved solids. The reason for this phenomenon may be due to the surface-to-volume ratios of the cells. Smaller algae with greater surface-to-volume ratios gain an advantage in uptake of a limited supply of nutrients and, therefore, gain a competitive edge.

Because carbon makes up as much as 50 percent of the dry weight of algal cells, it is apparent that availability of the proper inorganic carbon source is imperative. Inorganic carbon can be limiting to algal growth. This is particularly evident at high pH levels (> 10) where $CO_3^=$ is the predominant form of inorganic carbon and cannot be utilized by

algae. Certain algae can utilize only CO_2 or H_2CO_3, as is the case with <u>Chlorella pyrenoidosa</u>, because they lack an active bicarbonate uptake system. There are other algae that can utilize H_2CO_3, CO_2, or HCO_3^-.

Inside algae, the conversion of HCO_3^- to H_2CO_3/CO_2 is carried out by the enzyme, carbonic anhydrase, which is present in essentially all algae species. Blue-green algae do not possess the capacity for cytoplasmic streaming as do eukaryotic algae. This process may be important in moving the carbon to the point of need and, therefore, may play a role in competition among species. Cell size also plays a role. Smaller cells, like <u>Chlorella</u> (5 μm), can supply the carbon source to the point of need at a greater rate simply because the distance the CO_2 has to diffuse is shorter. Blue-green algae have their photosynthetic sites associated with the membrane of the cell. This allows them to optimize the transfer of carbon into the photosynthetic process.

Seasonal development of variation among algae species domination has been noted by many authors. For example, in temperate regions, diatoms show conspicuous increases in their numbers during spring and autumn. Green algae usually follow diatoms into early summer. Blue-green algae take over through the warmer months. As eutrophication or pollutants increase, the annual variation in algae production becomes far less obvious (106).

Some degree of predictability of succession has been gained from common physical-chemical properties of the water. However, no substantial data yet exist to reliably predict conditions for

populations and community structure changes. The problem is extremely complex because several environmental variables are capable of effecting stuctural changes (106). Whitton (106) suggested that the whole algal community could be used as a means of characterizing aquatic environments and toxic stress to those environments. His system has received very little attention to date because a sophisticated knowledge of taxonomy is required (106).

Bioassays

For years scientists have been increasing the species lists for the biological monitoring of surface waters and waste streams. Cairns (23) suggested that simply expanding the list of species tested to gain a more confident estimate of the effect on an ecosystem may not be adequate because the systems are very complex. This approach is further complicated by the difficulties of culturing several species in the laboratory and the difficulty of collecting a statistically valid number of test organisms from the environment (24). In a separate assessment of future needs in biological monitoring, Cairns (23) stated that the highest priority should be given to multispecies and system level tests.

System level studies on the environment have consistently shown that changes can take place not only in the variation of organisms present but in total shifts in predominant species present (25,27). Predictability of this situation cannot always be gained through a

single species assay of a system. Patrick et al. (74) showed in eighteen bioassays performed with an industrial waste that fish were more sensitive than diatoms in seven of the tests. Diatoms were more sensitive than fish in ten of the tests. Only one test was nearly identical for both species.

Changes in species diversity is a good indication of whether or not a system is stressed. Studies by Cairns and Plafkin (27), and Cairns and Dickson (26) showed very clearly that community structure shifted when stress was applied. When chlorine was applied, a well-diversified community of benthic organisms shifted to a community dominated by a few tolerant species. A simple method for determining diversity was described. The method allows for a numerical assessment of biological data. This method does not require competence in taxonomy. It basically requires that an investigator be able to distinguish between organisms based on differences of shape, color, and size. A more detailed description will follow in the materials and methods section.

Common Bioassay Test Procedures

Principle among the algal techniques utilized for toxicity monitoring is the "Algal Assay Procedure" (AAP) developed by the National Eutrophication Research Program. This procedure was developed not only to evaluate toxic materials, but also to assess the algal growth potential of natural waters. It is a batch-culturing test utilizing three test algae. Algae are grown in synthetic nutrient

medium under specified test conditions. Differences in growth due to additions of suspected toxicants are monitored by cell counts, chlorophyll analysis, and dry weight.

Another useful indicator of water quality is the autotrophic index. This technique relies on the analysis of chlorophyll a and ash-free dry weight of organic matter. The ratio of biomass to chlorophyll a results in a number known as the autotrophic index. The index normally number ranges from 50 to 200 and increases as water quality decreases (4).

Continuous cultures of algae in the lab have been used to test toxic materials (12,64). This approach is not common, and few articles were found in the literature. The major reason for this technique not being used more widely appears to be related to the difficulty and expense of operating a continuous culture (37). Toxicity tests conducted with the unicellular alga, *Selenastrum capricornutum* indicated that batch culture techniques were relatively easy, sensitive, and inexpensive. However, Lin (64) found a continuous technique to be useful for bioassays as well as for autecology studies. Examples of other commonly used organisms for both short and long term toxicity studies include polychaetes, shrimp, *Daphnia*, copepods, cray fish, and amphipods.

III. MATERIALS AND METHODS

Field Study Site

All field studies were conducted on Smith Mountain Lake at the 4-H Educational Center in Wirtz, Virginia. Smith Mountain Lake is the water source for a pump storage hydroelectric project. It receives roughly half of its supply of water from the Roanoke River drainage basin. This basin is characterized largely by forest land and the Roanoke metropolitan area. The 30 MGD Roanoke wastewater treatment plant discharges to this basin. The Blackwater River and Gill's Creek drainage basins supply the Lake with most of its remaining flow. These basins are largely agricultural and forest lands. Figure 1 illustrates where the 4-H Center is located on the lake.

Studies were conducted in a cove adjacent to the boat dock at the 4-H center. The area of the cove in which experiments took place received direct sunlight from approximately 9:00 a.m. to 7:00 p.m. The depth increased rapidly from the rip rap covered shoreline to a maximum of approximately 10 feet. The cove ran roughly from north to south from the main body of the lake and was roughly 80 feet wide on the average. The eastern shoreline was bordered by trees which shaded the cove in the early morning. The northern shoreline was grass covered and allowed for maximum afternoon sunlight. The cove afforded good protection from rough water created by wind and boat traffic.

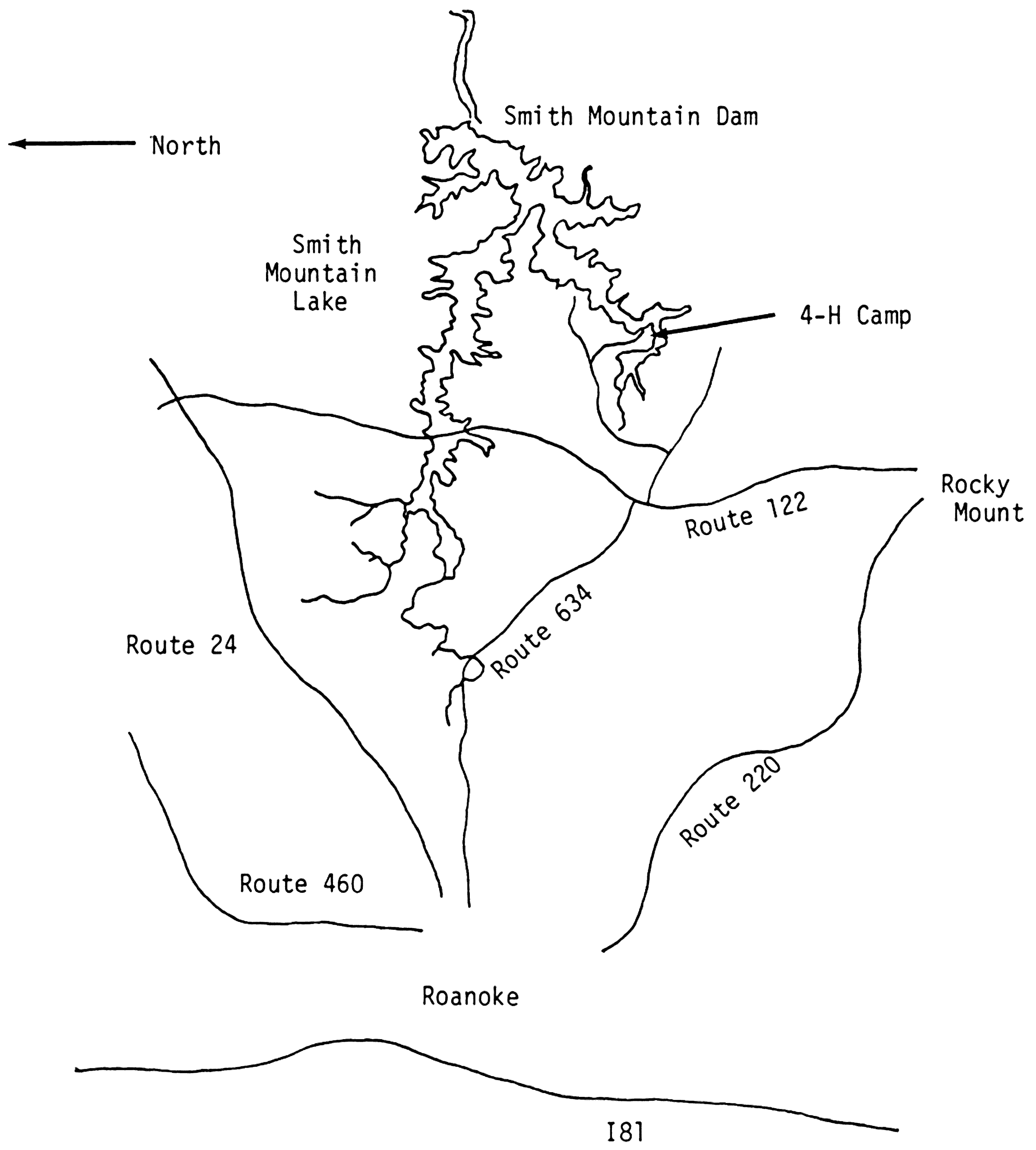


Figure 1. Smith Mountain Lake

Experimental Raft

A raft was developed to support the containers in which the herbicide studies were conducted (Figure 2). It was constructed using an aluminum frame with polystyrene inserts for flotation. Twelve Plexiglass cylinders 4 inches in diameter and 12 inches long were used to house one liter Whirl-pak bags. The cylinders had several grooves cut in the sides to allow currents within the lake to reach the bags. The bags were filled with appropriate amounts of lake water and chemical additions, suspended within the Plexiglas cylinders, and hung on the raft by steel leader line used commonly by fishermen. A Plexiglass sheet was cut to fit the top of the raft in order to protect the sample bags from rain.

Field Study Procedures for Raft Experiments

Prior to starting each field study, an 18 liter polyurethane carboy was filled with lake water. One liter of this water was poured into each of twelve Whirl-pak bags and the appropriate amounts of herbicides or nutrients were added to each bag before placing them in the Plexiglas cylinders and suspending them from the raft. In three field studies 100 ml of filter sterilized sewage was added to each of the Whirl-pak bags prior to the addition of 900 ml of lake water.

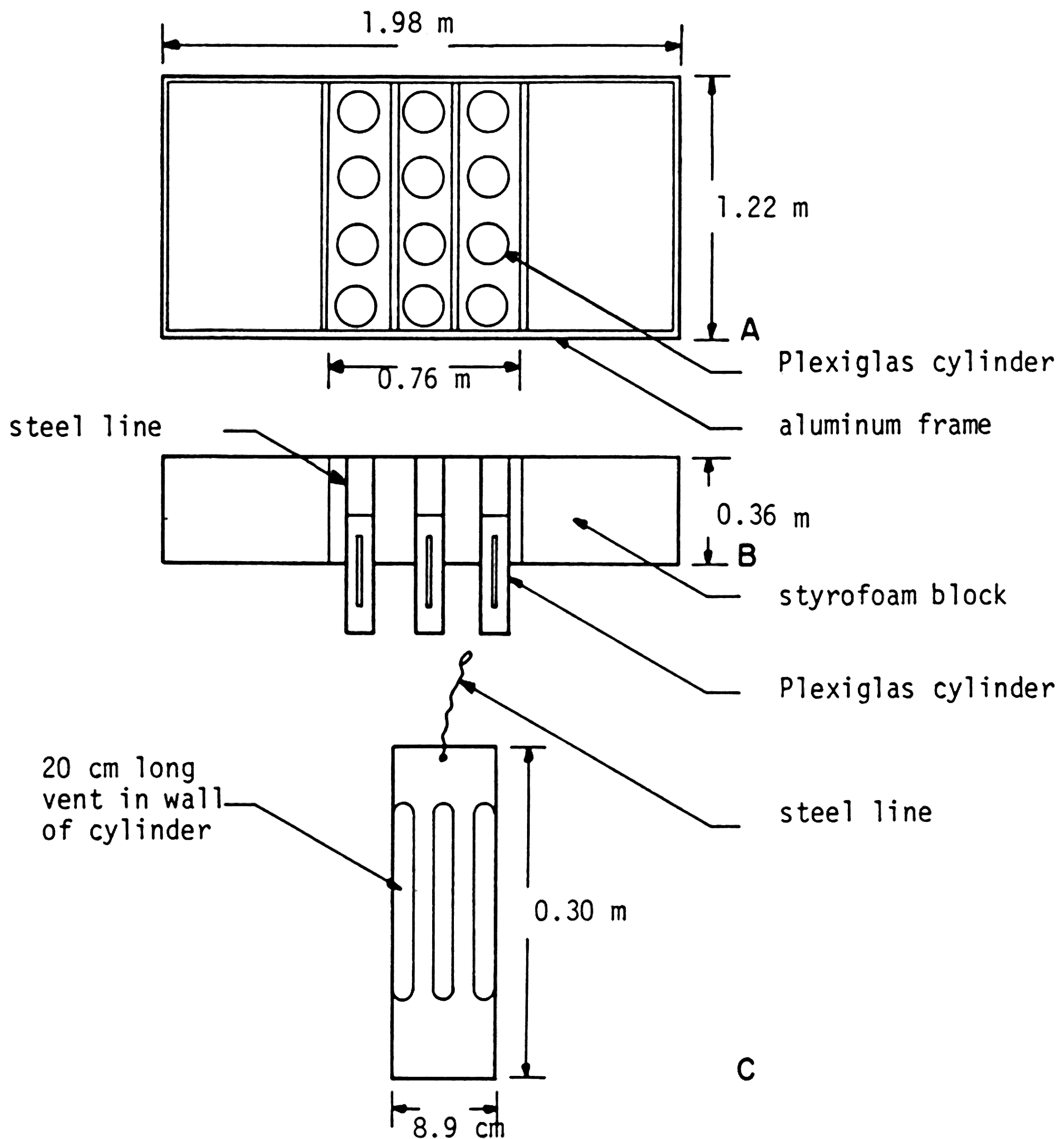


Figure 2. Schematic of the raft which was used in the field studies; A. plan view of raft, B. side view of raft, C. cylinder which was suspended from raft.

On sampling days, 120 ml of water was removed from each bag on the raft and placed in Whirl-pak bags. Samples were stored in the dark for immediate analysis of chlorophyll and extraction of ATP. Dissolved oxygen, pH and temperature were monitored. Ten ml of sample were taken from each container for microscopic studies and preserved with acid lugols.

Radiochemical Studies

Labeled sodium bicarbonate [$NaH^{14}CO_3$] was used for determination of the effect of the herbicides on the primary productivity of algae in the lake and laboratory. Sodium bicarbonate from New England Nuclear with a specific activity of 8.4 Ci/n mol was used in all studies. Individually sealed ampules contained 5.0 μCi/ml activity in sterile aqueous solution and 50 μg of $NaH^{14}CO_3$. To assure uniformity in experiments, the necessary number of ampules needed for an experiment were pooled in a single container prior to addition to the test chamber.

Field uptake studies involved the addition of 5 μCi of $NaH^{14}CO_3$ to 250 ml dilution bottles containing the appropriate concentration of the herbicide being tested. Two or three replicates were used for each concentration being tested. A dark bottle at each test concentration was tested concurrently with light bottles to compensate for uptake under dark conditions. Additionally, a light bottle fixed with acid Lugols solution compensated for effects due to adsorption. After a four

hour incubation period at a depth of two feet, the bottles were removed and the liquid filtered through a 0.22 μm membrane filter. The filters were fumed for 30 minutes in a glass desicator containing HCl. Filters were placed in borosilicate vials which contained 10 ml of ScintiVerse, a scintillation cocktail purchased from Fisher Scientific. The vials were then placed in a Beckman Model LS230 scintillation counter. Levels of ^{14}C were determined at a 2 percent error level with a full window setting and a counting period of one minute. The amount of carbon fixed was then calculated using the following relationship as cited in Standard Methods (4):

$$\text{mg carbon fixed/l} = \frac{\text{counts of filtered sample}}{\text{total activity added to sample}} \; \frac{250}{\text{volume filtered}} \; (\text{mg/l inorganic carbon})(1.064)$$

Laboratory $NaH^{14}CO_3$ uptake studies were carried out using the same procedures as previously described except that unialgal cultures were used. The cultures were cultured in 500 ml Erlenmeyer flasks and incubated in a Sherer RI-LTP Lighted Bioincubator. Dark studies were carried out in the Sherer Bioincubator at 24 $\pm$ 1.0°C. Aliquots of cultures were removed from the flasks in the incubators and quickly filtered and fixed in scintillation fluid. All studies with algae under lighted conditions were conducted at 24 $\pm$ 1.0°C and a light intensity of 200 foot candles. One million cells per ml were used in all laboratory uptake studies unless otherwise noted.

Studies using 2,4-dichlorophenoxy [2 - ^{14}C] acetic acid with a specific activity of 31 m Ci/mmol and a radioactive concentration of 5 μ Ci/ml were conducted using similar techniques as were employed in $NaH^{14}CO_3$ uptake studies. The only difference in the procedure was that the acid fuming step was not performed. The percentage of labeled 2,4-D added to sample vessels was 7.7 percent of the total amount of 2,4-D added. The amount of 2,4-D assimilated was calculated using the following formula:

$$\text{mg/l 2,4-D assimilated} = \frac{\text{counts of filtered sample}}{\text{tot. activity added to sples.}} \; \frac{\text{total volume}}{\text{volume filtered}} \; (\text{mg/l initial 2,4-D})(1.064)$$

Herbicide Preparation and Analysis

Technical grade 2,4-dichlorophenoxyacetic acid, diuron, and atrazine were used in this study.

Diuron and atrazine were prepared in stock solutions by dissolving them in acetone before adding sterile distilled water. The acetone was then evaporated on a hot plate under conditions of low heat and mixing. The solution was then adjusted to the desired volume with distilled water.

Preparation of 2,4-D stock solutions was accomplished by mixing the desired amount of 2,4-D in distilled water until it dissolved. Twelve hours were typically required for 200 mg of 2,4-D to dissolve in about one liter of distilled water.

To extract 2,4-D from water, 100 ml of sample was added to a 250 ml separatory funnel and saturated with Na_2SO_4. The pH was adjusted to 2 by adding 1.5 ml of 1 N H_2SO_4. Twenty ml of diethyl ether was then added to the solution and the solution was shaken for one minute. The water layer (bottom) was discarded after two more extractions with diethyl ether. This solution was then transferred to Pyrex 9825 (150 mm x 20 mm) test tubes and evaporated to near dryness with N_2 gas. Ten ml of a BF_3 methanol solution was added and the mixture was heated in a boiling water bath for two minutes. The liquid was then transferred to 60 ml separatory funnels, and 10 ml of H_2O and 10 ml of hexane were added. The funnels were shaken for one minute and the water layer was discarded. This washing was repeated two more times. The hexane layer was transferred to a centrifuge tube and the volume adjusted to 10 ml for analysis using a gas chromatograph.

Algal Culturing and Assay Techniques

Preparation of Glassware

All glassware for experimentation was washed in Alconox detergent prior to acid cleaning. The acid cleaning step consisted of immersing glassware in 30 percent HCl followed by five rinses in tap water and five rinses in distilled water. Glassware was then covered with aluminum foil and autoclave sterilized for 15 minutes at 15 psi and 121°C in a Barnstead CES-20 autoclave.

Incubation Conditions

The cultures were incubated in a Warren Sherer Series RI-LTP Lighted Bioincubator. Light intensities for continuous cultures; designated herein as R1, R2, and R3, were 140, 180, and 130 foot-candles, respectively. Batch- and continuous-flow cultures were incubated at 24 $\pm$ 1.0°C and 30 $\pm$ 1.0°C, respectively.

Culture Medium

ASM-1-TR and Chu 10 media were used throughout this study (86). The ASM-1-TR medium is excellent for the growth of blue-green algae, whereas Chu 10 is a good growth medium for many types of algae. Glass distilled water was used in the preparation of all media. The media were pH adjusted and autoclave sterilized prior to use.

Algal Counts

In the laboratory algae cells were counted using a Hemacytometer as described in Phycological Methods (86). In field experiments cells were counted using a special counting chamber in which the cells are allowed to settle, and a Wild M40 inverted microscope. The chamber and procedure are described in Phycological Methods (86). A Whipple grid installed in the eyepiece of the microscope aided in counting the algae. In general, the 200x and 400x magnification were used.

Preservation of Algae

Natural algae samples were preserved using an appropriate amount of acid Lugols solution (3 ml/liter samples) as described in Phycological Methods (86).

Sequential Comparison Index Procedure

Cairns and Dickson (26) demonstrated that a simple test of biological diversity could be used to assess pollutional effects on aquatic systems. The test is simple and results in numerical data which can be used to interpret damage to an ecosystem.

The test uses benthic organisms for estimating relative changes in diversity. Preserved organisms collected from a study site are randomly scattered over an enamel pan which has parallel lines drawn on the bottom. Any organisms which are clumped are shaken free and randomized once again. The investigator then simply compares each organism to the one next in line for differences. If the organism is the same, then it is considered part of a "run" of organisms and is counted accordingly. If the organism is different, then it becomes the first of a new "run."

The best method for tabulating sequences of organisms, is in the use of x's and o's; i.e., each organism in a given run would be designated by an x or an o. For example, if eight organisms lay in sequence and the first four were the same followed by three other similar organisms and the last organism of the eight looked like one of the first four, then the sequence would be marked xxxxooox. Even if the

last organism was altogether different from any of the others, an x would still have been recorded. The beginning of a new run is designated as such because it is only compared to the organism preceeding it.

To calculate the sequential comparison index (DI) one simply divides the number of runs by the total number of organisms counted. This procedure should be repeated using 50,100,150, and additional increments of 50 total organisms until the plot of relative DI values versus number of specimens counted becomes asymptotic. The point at which the relationship becomes asymptotic is then used in calculating a DI.

Statistical studies by Cairns and Dickson (26) showed that in order to be 95 percent confident that the mean DI is within 20 percent of the true DI value, the test must be repeated twice on the same sample. A DI_T value is calculated by multiplying the number of different kinds of organisms in the sample (taxa) by the DI value. A 95 percent confidence interval can then be placed around this number by calculating plus or minus 10 percent of the DI_T. If two separate samples are compared and their confidence limits do not overlap, then the samples are said to be significantly different.

The assumption was made in this work that the procedure for calculating statistical differences between samples of benthic organisms would hold true for comparisons of diversity changes in an algal community.

For the purposes of calculating an algal community DI in this project, samples of algae preserved with acid Lugols solution were settled in chambers for the Wild M40 Inverted microscope as described in Phycological Methods (86). A Whipple grid was used to establish parallel lines for the comparison of taxa within the field. A magnification of 400x was used in all comparative studies. Fields for doing various comparisons were chosen by randomly moving the stage. If clumps were visible within a field of view, another field was chosen at random. A strand of filamentous algae was counted as only one organism. Fungi, molds, bacteria (other than cyanobacterium), and zooplankton were not included in the diversity counts. In situations where waters contained few organisms, greater amounts of sample were allowed to settle in the chamber. Samples containing many organisms were diluted to an appropriate level before settling.

Isolation of Algal Clones from Lake Samples

Samples from Smith Mountain Lake were brought into the laboratory, spiked with Chu 10 media and ASM-1-TR media, and incubated in a Sherer Bioincubator at 24°C $\pm$ 1.0°C and 200 foot candles of light until growth was visible. Chu 10 and ASM-1-TR agar plates were prepared using aseptic techniques. The plates were then sprayed with a fine mist of both spiked lake water and pure lake water as described in Phycological Methods (86). After several days of incubation, individual colonies of algae were picked from the plates using glass micro-capillary tubes with

the aid of a Bausch and Lomb dissecting microscope. Individual colonies were then put into test tubes containing sterile Chu 10 or ASM-1-TR media for further incubation. Cultures were microscopically examined to determine if they contained only a single algal species when growth in the tubes was apparent. Cultures not in the unialgal state were again sprayed onto agar plates and the procedure was repeated.

Culture Maintenance

Stock cultures of all algal genera used in laboratory were maintained under low light conditions within the Sherer Bioincubator. Transfers of stock cultures for maintenance purposes were generally performed every two to three weeks. Log-phase cells were used in all batch culture experiments. Preparation of dense growth cultures for experimentation were prepared by centrifuging stock culture cells at 5000 RPM on a Beckman Model J-21C centrifuge for 15 minutes. Cell pellets were then washed with fresh culture medium and centrifuged twice prior to being added to an appropriate culture vessel for an experiment.

Batch Culture Experiments

All batch culture experiments were carried out in 500 ml Erlenmeyer flasks at 200 foot candles of light intensity and 24 $\pm$ 1.0°C. Two hundred ml of medium was used in all these experimnets unless otherwise stated. Log-growth phase cells were introduced into test flasks so as to begin each test with about 50,000 cells/ml. Volumes of test flasks

were adjusted as necessary to account for the dilution factor introduced by inoculation. The flasks were shaken manually on a daily basis.

Continuous Culture

A gravity fed continuous culture of *Chlorella sorokiniana* (obtained from R. Schmidt, Biochemistry Department, Virginia Tech) was established to test the effects of test herbicides on a number of physiological and growth parameters. The algae were cultured in 1000 ml, flat bottomed, Pyrex boiling flasks fitted with an overflow nipple at approximately the 500 ml mark, a glass top to accomodate a glass air delivery stem, an out-gas vent, and a medium delivery tube (Figure 3). A Whisper 700 aquarium air pump delivered air to the vessel through an activated carbon filter. Chu 10 media was dripped into the vessel using a Piggyback Benoset Microdrip device with a Cair clamp (Abbot Hospitals, Inc., North Chicago, Illinois). The Microdrip device was able to deliver precise amounts of liquid without being affected by head changes at the medium feed source. Algal medium flowed to the reactors from 10 l glass carboys. Overflow from the reactors was collected in 2000 ml glass vessels.

Establishing the continuous flow cultures was rather difficult. It was discovered that the best way to start the cultures was to decrease the light intensity to 50 foot-candles and operate under semi-continuous flow conditions for several days. Each day, approximately 20 percent of the reactor fluid was replaced. The air supply was gradually increased

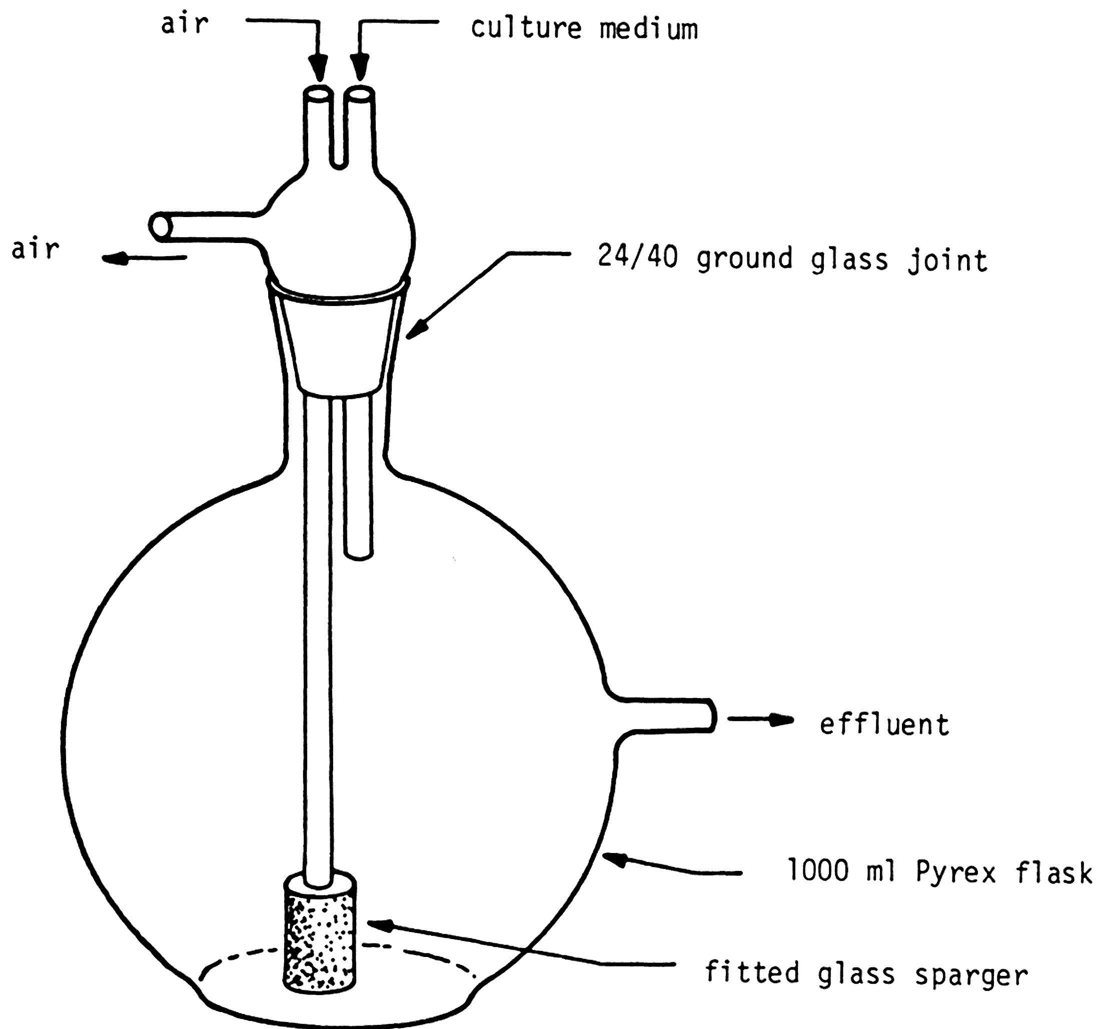


Figure 3. Schematic of the vessel used to culture algae in the continuous flow experiments.

each day from a low flow which did not keep the cells in suspension to a flow which completely mixed the cells. When the air flow was set low, the vessel was shaken each day to resuspend settled cells. Semi-continuous flow culturing techniques were continued until a relatively constant number of cells was recorded over a three to four day period. At this time, medium was added at a constant rate to provide each reactor with a detention time of five to seven days and light intensity levels were increased.

All of the algal medium needed for a given trial was prepared prior to beginning a continuous flow experiment. Herbicides were added to a portion of this medium and the portion was stored until the continuous culture reached steady-state. Steady-state was assumed when the total organic carvon (TOC), cells/ml, ATP, and fluorescence levels were relatively constant over a period of seven to nine days. A 10 percent deviation from the mean was considered within tolerance for steady-state.

Approximately 3.2 ml of sample was removed from each reactor on sampling days to test for TOC, ATP, ADP, AMP, relative fluorescence, pH, and energy charge. Cell counts were also conducted at this time.

<u>Short Term Continuous Flow System</u>

A continuous flow system for monitoring dissolved oxygen (DO), pH and fluorescence was designed to monitor short term toxic effects on algae (Figure 4). The system consisted of a specially designed reactor

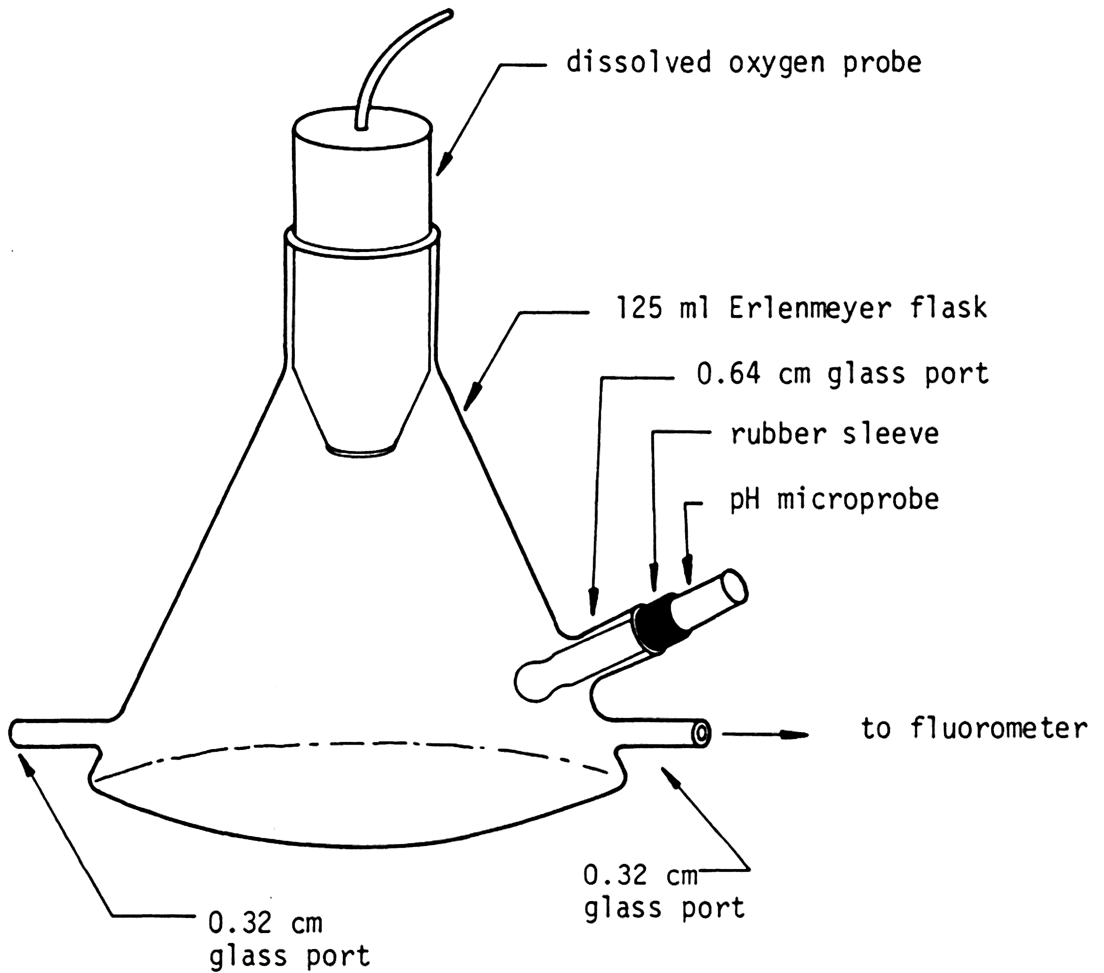
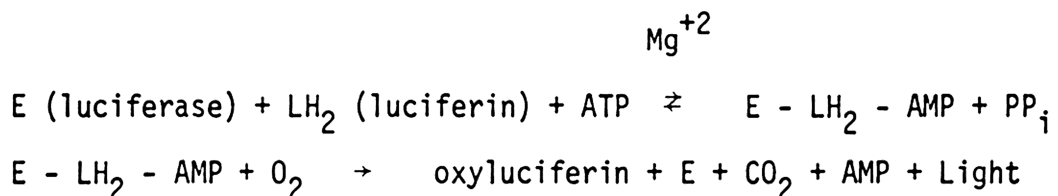


Figure 4. Schematic of the vessel used in the short term bioassay studies.

which sat under a fluorescent light in a water bath. The reactor was fitted with a high sensitivity YSI Model 5331 dissolved oxygen probe and a Fischer Model 13-639-92 pH micro probe. Fluid in the reactor was circulated through a Turner (Palo Alto, California) Model III fluorometer on a continuous flow basis. At the beginning of each trial the system was operated for a certain period of time to allow the system and various instruments to equilibrate. Suspected toxic chemicals were then injected into the reservoir and algal response to the toxicants was measured in terms of DO, pH, and fluorescence changes.

Adenosine Triphosphate (ATP) Measurement

Adenosine triphosphate (ATP) has been shown to be a fairly reliable index of biomass in the plankton community (102). Additionally, ATP can be associated only with viable plankton and not with nonliving particulates (51). The measurement of ATP requires the use of a bioluminescent chemical present in the tails of fireflies, called luceferin, and the enzyme, luciferase. The proposed steps in the reaction are:



$$\text{E (luciferase)} + LH_2 \text{ (luciferin)} + \text{ATP} \overset{Mg^{+2}}{\rightleftarrows} \text{E} - LH_2 - \text{AMP} + PP_i$$

$$\text{E} - LH_2 - \text{AMP} + O_2 \rightarrow \text{oxyluciferin} + \text{E} + CO_2 + \text{AMP} + \text{Light}$$

Light from the reaction can be measured and is directly related to the

amount of ATP present.

A Science Applications Incorporated (SAI), San Diego, California Model 2000 ATP Photometer with a 1 dram sample tube was used in all ATP measurements. This instrument is capable of measuring less than 1 picogram ATP.

Working standards of pure ATP (Sigma Chemical Company) were prepared just prior to use from frozen stocks. Stocks of 100 mg/ml ATP were prepared in 0.02 M Tris buffer at pH 7.75 and sealed in glass ampules before freezing at -20°C. Dilutions of stocks were made in 0.02 M Tris buffer at pH 7.75 to achieve a series of standard ATP solutions.

Vials containing "firefly enzyme" were obtained from Sigma (FLE-50). To prepare an enzyme solution for a test, 5 ml of water was added to a vial. The vial was stored at room temperature for at least three hours. The enzyme solution was then diluted to 45 ml by adding 20 ml of 0.04 M $MgSO_4$ and 20 ml of 0.02 M Tris buffer at pH 7.75. This mixture was centrifuged at 5000 RPM for 15 minutes and the supernatant was poured into a clean beaker and covered with aluminum foil. At this point the enzyme was ready for use. If stored in the dark at 15°C, the enzyme preparation was stable from 6 to 12 hours.

Extractions of ATP from plankton suspended in natural waters was accomplished by filtering aliquots (usually 50 ml) of lake water through 0.2 mμ Gelman GL/C filters. At no time were vacuum pressures allowed to exceed 5 psig. Filters were then quickly transferred to 5 ml of a boiling buffer solution containing 0.02 M Tris and 0.002 M EDTA at pH

7.75. After five minutes of boiling, the filters were placed in a freezer at -20°C until time for the measurement of ATP.

Laboratory algal cultures were dense enough to allow use of injection technique. This was accomplished by injecting 1 ml of algae culture directly into 9 mls of the boiling Tris buffer previously described. As before, the samples were allowed to boil for five minutes and then they were placed in a freezer at -20°C.

Because most samples contain inhibitors or interferences to light production and/or light transmission, it is important to include a standard addition of ATP to compensate for unknown factors. In this study the standard additions method was used to determine levels of ATP in all samples. Hence, three types of measurements were routinely made:

1) blank measurement - measures background light prior to injection;

2) sample measurement - measures ATP in the sample without the addition of a known amount of ATP;

3) internal standard - measures ATP in the sample with the addition of a known amount of ATP (usually 10 ng/ml ATP for laboratory cultures). This standard should produce three to five times the reading of the sample alone for maximum accuracy.

The results of these measurements which were performed in triplicate were used in the following formulas:

$$K = \text{constant} = \text{ng ATP/CPM} = \frac{\text{ng ATP added}}{(C_{ST} - C_B) - (C_{SA} - C_B)}$$

where $\quad CPM$ = counts per minute

C_{ST} = counts of standard

C_B = counts of blank

C_{SA} = counts of sample

and

$$\text{ng ATP/ml sample} = \frac{K\,(C_{SA} - C_B)}{\text{ml sample in photometer}}$$

Assays for ATP were performed after the completion of each field or laboratory study. This was done to assure consistency of technique in each study. One hundred microliters of sample and 100 1 of firefly enzyme solution were used in each assay over the period of this study.

Energy charge (EC) is a linear measure of the amount of metabolic energy stored in the adenine nucleotide pool. It can be determined by converting adenosine mono- and diphosphate to ATP and using an ATP photometer to measure the amount of ATP created. The relative amounts of the various adenylate compounds are then easily computed (55).

Four reagents were required in the converstion of AMP and ADP to ATP. The first reagent buffer was prepared by adding 29.4 ml of 0.075 M KH_2PO_4 and 45.6 ml of 0.075 M Na_2HPO_4 to a flask with 1.525 g $MgCl_2 \cdot 6H_2O$ and diluting the mixture to 500 ml. This preparation is hereafter

referred to as reagent A. Reagent B was prepared by adding 120.0 mg pyruvate kinase (PK) and 35.13 mg phosphoenol pyruvate (PEP) trisodium salt to 300 ml of reagent A. Reagent C contained 40 g ATP and 20 mg of myokinase (MK) in 200 ml of reagent B. Reagent D had 20 g AMP added to 100 ml of reagent C. All reagents were obtained from Sigma Chemical Co., St. Louis, Mo. The role of each reagent in the technique is given below:

1) Reagent A; no conversions, original ATP analyzed

2) Reagent B; ADP + PEP $\xrightarrow{PK}$ pyruvate + ATP

3) Reagent C; AMP + ATP $\overset{MK}{\rightleftarrows}$ 2 ADP

   ADP + PEP $\xrightarrow{PK}$ pyruvate + ATP

4) Reagent D; serves as a check on the efficiency of reagent C in reaction 3 above.

For each sample, standard, or blank four 13 cm by 100 cm tubes, labeled A through D, were prepared in triplicate. Two hundred microliters of sample were added to each tube with 50 μl of the appropriate reagent (A - D). The test tubes were then incubated for 30 to 60 minutes at 30°C and heat-deactivated for two minutes at 100°C after the initial incubation. The samples were cooled and assayed for ATP.

To calculate the amount of each adenylate compound present the following procedure was used:

1) Correct all sample, blank, and standard values by subtracting the corresponding end light values from them.
2) Plot a graph or calculate the least squares linear regression equation of standard net light emission vs. standard concentration. A separate standard curve must be produced for each of the reactions in the EC conversions (i.e. Tubes A - D).
3) Calculate the mean sample net light value and determine the sample extract concentration from the appropriate regression plot.
4) Calculate ATP: Equivalent to the result obtained from Tube A.
5) Calculate ADP: ADP = ATP Tube B - ATP Tube A
6) Calculate AMP:

$$AMP = (\text{ATP Tube C} - \text{ATP Tube B}) \frac{\text{AMP Internal Std.}}{\text{ATP Tube D} - \text{ATP Tube C}}$$

To calculate the adenylate energy charge (EC):

$$EC = \frac{ATP + 1/2\ ADP}{ATP + ADP + AMP}$$

Chlorophyll Analysis

All chlorophyll analyses were accomplished using a Turner (San Diego, California) Model III fluorometer. Filters to accomodate an excitation wavelength of 440 mm and an emission wavelength of 670 nm were used for chlorophyll analysis. A standard 13 x 100 mm cuvette was used with a cuvet adaptor kit for grab samples.

Chlorophyll was extracted from cells using a 1:1 mixture (v/v) of a solution of dimethyl sulfoxide (DMSO) and acetone (90 percent acetone, 10 percent distilled water). Natural samples and laboratory cultures were first filtered through a 0.22 m membrane filter which was soluble in both acetone and DMSO (spectral grade). Filters were then placed in 5 ml of the DMSO/acetone mixture and allowed to set in the dark for 15 minutes. Extraction of chlorophyll was complete at this time, and samples were then analyzed in the fluorometer. Each sample was analyzed before and after acidification with one drop of 50 percent hydrochloric acid. The acid converts all chlorophyll to pheophytin which is the only pigment that significantly interferes at these wavelengths. Hence, the acidified sample provides a reading which represents the sum of pheophytin and chlorophyll. The fluorescence due to the chlorophyll pigment alone can be determined from the equation:

$$F_{chi} = \frac{F_o\ [F_o/F_A - 1.0]}{0.7}$$

where

F_{chl} = fluorescence of chlorophyll

F_o = fluorescence before acidification

F_a = fluorescence after acidification

Calibration curves of fluorescence versus chlorophyll levels were established using this technique with pure chlorophyll a.

Concentrations of chlorophyll in natural and laboratory samples were then determined by entering the graph with fluorescence readings.

In laboratory samples, where cell growth was monitored frequently, *in vivo* determinations of chlorophyll fluorescence were used. This technique gave fast and reliable data for monitoring the growth of algae. Samples were taken directly from culture vessels and placed in cuvettes for the measurement of fluorescence. As mentioned earlier, the *in vivo* method was also used in the short term toxicity tests; but, in these tests, the fluormeter was fitted with a Turner Model III continuous flow door and fluorescence was monitored on a continuous basis. Sample was pumped through the apparatus using a peristaltic type pump at approximately 10 ml per minute.

The photosynthetic inhibitor, diuron, was used in conjunction with *in vivo* techniques in the continuous culture studies. Since the *in vivo* fluorescence measurements are a function of the amount of energy being channeled into photosynthesis, correlations between fluorescence and cell numbers are not always good. Diuron inhibits the flow of energy into the photosynthetic pathway so more light is emitted as fluorescence (62). *In vivo* fluorescence of samples was monitored both without diuron and in the presence of 2 mg/l diuron. Samples receiving diuron were allowed to sit for five minutes prior to the measurement of fluorescence.

Water Chemistry Analyses

The following chemical tests were performed in accordance with Standard Methods (4):

1. Alkalinity - titrimetric procedure; Section 403;
2. Dissolved oxygen - YSI probe method; Section 421 F;
3. Ammonia nitrogen - nesslerization method; Section 417 B;
4. Orthophosphate - ascorbic acid procedure, 10 cm cuvet used for low level determinations; Section 424 F;
5. Total phosphate - sulfuric acid/nitric acid digestion followed by ascorbic acid procedure, Section 424 C.

Analysis for total organic carbon (TOC) was conducted on a Dohrmann Division of Envirotech (Santa Clara, California) TOC Analyser, Model DC-50/52 series.

IV. RESULTS

Field Studies

Field Study I

The first raft experiment or Field Study I (FSI), was conducted over a six-day period beginning on June 14, 1981. The skies during the period of the study were generally cloudy. Nutrient analysis of lake water used for FSI indicated that the ammonia nitrogen levels were 0.63 mg/l. Orthophosphate-phosphorus and nitrate levels were below detectable levels (0.01 mg/l). Alkalinity was determined to be 36 mg/l (Table 1).

Dissolved oxygen levels in the lake water outside of the confines of the raft remained close to the levels noted inside the test vessels for the entire six day period. Raft values appeared to be slightly lower than outside lake water overall. On day six, raft dissolved oxygen (DO) values averaged 6.6 mg/l, while the lake DO level was 7.0 mg/l (Table 1).

Adenosine triphosphate (ATP) data indicated that 1.0 mg/l and 5.0 mg/l 2,4-D replicates had significantly less ATP than did the controls at the $\alpha = 0.05$ level after one day of growth (Table 2). At 10 mg/l 2,4-D, test vessels had less ATP than did the control replicates after one day, but the differences were not significant. After two days of growth, 10 mg/l 2,4-D replicates had significantly less ATP than did

Table 1. 2,4-D Study I - Field Data/No Nutrient Additions

Parameter	Day of Incubation				
	Day 0*	Day 1	Day 2	Day 3	Day 6
Water Temp.,°C	36	31	28	28	31
Air Temp.,°C	33	34	24	27	33
pH	7.92	7.90	7.90	7.85	7.80
D.O., mg/l**	7.0	7.3	7.3	7.6	7.0
Ortho-PO_4-P, mg/l	0.01				
Total-PO_4-P,mg/l	1.0				
NH_3-N, mg/l	0.63				
NO_3-N, mg/l	0.01				
Alkalinity, mg/l $CaCO_3$	36				
Weather	Clear	Partly Cloudy	Cloudy	Partly Cloudy	Cloudy

*June 14, 1981
**Dissolved oxygen (D.O.) levels of lake water around raft.

Table 2. Mean Values of ATP and Chlorophyll for Field Study I.

2,4-D (mg/l)	Day of Incubation			
	Day 1	Day 2	Day 3	Day 6
	ATP, ng/l:			
Control	559.8	1097.6	981.1	1695.2
1	194.8*	1233.9	1435.9	2029.2
5	334.9*	900.7	1458.5	2532.2
10	364.1	727.2*	1024.7	1995.2
	Chlorophyll, μg/l:			
Control	7.2	10.1	2.5	33.2
1	7.3	25.6	4.1	16.8
5	4.1	13.7	10.2*	25.8
10	4.3	17.1	5.6*	23.6

*Indicates significantly greater or less than control value at $\alpha = 0.05$ for n = 3 replicates.

controls at an α of 0.05. At 5 mg/l 2,4-D the level was less, but not significantly different. On the third and sixth days, all 2,4-D test means were greater than control means, but none were significantly greater at the $\alpha = 0.05$ level. It is important to note that as time progressed the variance within each test level became larger. This caused statistical problems in detecting significant differences between the test levels.

Statistical analysis of the chlorophyll data indicated that chlorophyll levels increased significantly ($\alpha = 0.05$) over control values on day 3 in the 5 mg/l and 10 mg/l 2,4-D tests. On day 2, values for chlorophyll were greater than control values at all 2,4-D test concentrations (Table 2). After six days of growth, all chlorophyll values for 2,4-D tests were below the control means. Interestingly, the fluctuations within chlorophyll data for all test levels followed similar patterns of increase and decrease over the days tested.

The overall lack of good correlation between ATP and chlorophyll in this study would suggest that the pool of photosynthetic and non-photosynthetic organisms was in a constant state of fluctuation in relationship to one another. However, the relative proportions of these two test data do give some information as to increase and decrease of the two populations. As such, the data would indicate that photosynthetic organisms experienced a relative gain over non-photosynthetic organisms in 2,4-D treated samples during the initial three days of study. This trend reversed itself on the sixth day of study.

Microscopic identification of the most predominate genera within the various reactors indicated that 2,4-D did effect a shift in algal populations. For example, the number of Synecchoccus sp. (coccoid) in reactors inoculated with 10 mg/l 2,4-D was considerably greater than the number noted in controls after one day of growth (Table 3).

Field Study II

The experimental raft for Field Study II was set out on June 22, 1981. The weather during the course of this nine day study was clear and very warm. Because the nutrient analysis indicated that orthophosphate-phosphorus levels were below detectable levels, 0.01 mg/l of orthophosphate-phosphorus was added to the test vessels. Ammonia concentrations were 0.45 mg/l and total phosphorus measured 0.3 mg/l in lake water. Nitrate levels were below detectable levels. Alkalinity was found to be 40 mg/l (Table 4).

Dissolved oxygen values in the lake water outside of the confines of the raft remained close to those values noted inside the test vessel for all test days except day 5. Values inside test vessels averaged 8.3 mg/l, while lake water dissolved oxygen concentrations reached a maximum of 8.9 mg/l (Table 4).

ATP levels in the 5 mg/l and 10 mg/l 2,4-D reactors were significantly ($\alpha = 0.05$) less than control ATP levels on day 1. Over the seven days of the study there appeared to be no real trend in the ATP data. However, after seven days of growth, the mean ATP

Table 3. Effect of 2,4-D on Algal Community Diversity

Genus Type	Fraction of Various Genus Types in Algal Community							
	Day 0				Day 1			
	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l
Synecchoccus sp (C)	0.44	same	same	same	0.39	0.45	0.39	0.60
Chlorella sp.	0.34	"	"	"	0.28	0.19	0.28	0.13
Glococystis sp.	0.08	"	"	"	0.15	0.16	0.11	0.11
Kirchneriella sp.	0.08	"	"	"	0.08	0.21	0.11	0.05
Synecchoccus sp. (R)	0.06	"	"	"	0.10			
Aphanocapsa sp.						0.06	0.11	0.11
Gloeocapsa Sp.								

(Continued on next page)

Table 3. Effect of 2,4-D on Algal Community Diversity (Continued)

	Fraction of Various Genus Types in Algal Community											
	Day 2				Day 4				Day 6			
	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l
Synecchoccus sp (C)	0.44	0.35	0.40	0.36	0.65	0.48	0.51	0.59	0.48	0.39	0.38	0.36
Chlorella sp.	0.36	0.29	0.34	0.35	0.06	0.10	0.08	0.08	0.08	0.03	0.11	0.09
Glococystis sp.	0.10	0.15	0.08	0.10	0.06		0.10	0.21	0.01	0.03	0.05	0.09
Kirchneriella sp.	0.08	0.11	0.09	0.12	0.18	0.25	0.20	0.08	0.36	0.52	0.41	0.40
Synecchoccus sp. (R)	0.05	0.10	0.09	0.07		0.08			0.07			
Aphanocapsa sp.					0.05		0.10	0.04		0.03	0.05	0.06
Gloeocapsa Sp.									0.01			

C = coccoid shape 1μ diameter
R = rod shape 2μ length

Table 4. 2,4-D Study II - Field Data/Addition of 0.01 mg/l Ortho-phosphate Phosphorus.

Parameter	Day of Incubation					
	Day 0*	Day 1	Day 2	Day 3	Day 5	Day 7
Water Temp.,°C	31	30	33	27	29	28
Air Temp.,°C	33	34	36	26	32	24
pH	7.50	7.65	7.55	7.50	7.65	7.65
D.O., mg/l	7.0	6.7	7.2	7.7	8.9	8.4
Ortho-PO_4-P, mg/l	0.01					
Total-PO_4-P, mg/l	0.3					
NH_3-N, mg/l	0.45					
NO_3-N, mg/l	0.01					
Alkalinity, mg/l as $CaCO_3$	40					
Weather	Partly Cloudy	Partly Cloudy	Clear	Clear	Clear	Cloudy

*June 22, 1981

concentrations in 2,4-D vessels were greater than controls bur not statistically greater (Table 5).

Chlorophyll levels at 5 mg/l 2,4-D were significantly greater than control levels after two days of growth. The 10 mg/l 2,4-D replicate means were greater than control values, but the difference was not significant at the $\alpha = 0.05$ confidence level. On day 5 all of the 2,4-D reactors contained more chlorophyll than the control. Levels of chlorophyll in the 5 mg/l and 10 mg/l 2,4-D reactors were significantly greater than control levels at the $\alpha = 0.05$ level. On day 7 chlorophyll in the 1 mg/l 2,4-D test reactor level was greater than the control, but the difference was not significant ($\alpha = 0.05$). Chlorophyll levels in both the 5 mg/l and 10 mg/l 2,4-D reactors were below control values on day 7 (Table 5). There were no additive effects obvious in 2,4-D treatments. Those vessels receiving 5 and 10 mg/l 2,4-D returned very similar data. On the other hand the containers receiving 1 mg/l 2,4-D were similar to control results.

Analysis of the major algae genera indicated that *Kirchneriella* sp. was predominant in 2,4-D treated samples on days 5 and 7 (Table 6). *Chlorella* sp. also was more prevalent in 2,4-D treated samples than in the control after seven days of study. *Fragillaria* sp., *Synecchoccus* sp., and *Kirchneriella* sp. were the most predominant algae in control samples after seven days of study. *Synecchoccus* sp. and *Fragillaria* sp. were not as predominant in 2,4-D treated samples (Table 6).

Table 5. Mean Values of ATP and Chlorophyll for Field Study II of 2,4-D.

2,4-D (mg/l)	Day of Incubation: Day 1	Day 2	Day 3	Day 5	Day 7
	ATP, ng/l:				
Control	2217.2	493.3	832.9	671.3	876.1
1	1499.7	499.8	970.5	757.7	1281.6
5	884.8*	590.7	1110.6	619.7	1671.1
10	707.1*	355.9	901.1	956.3	1353.9
	Chlorophyll, μg/l:				
Control	14.8	24.2	21.6	37.7	86.2
1	15.6	18.0	28.0	45.3	146.2
5	23.2	47.9*	20.0	132.5*	61.9
10	19.8	33.6	27.0	136.2*	44.6

*Indicates significantly greater or less than control value at $\alpha = 0.05$ for $n = 3$ replicates.

Table 6. Effect of 2,4-D on Algal Community Diversity in Field Study II.

Genus Type	Fraction of Various Genus Types in Algal Community — Day 2				Day 5				Day 7			
	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l
Synecchoccus sp.(C)	0.53	0.57	0.61	0.50	0.64	0.54	0.14	0.14	0.40	0.09	0.02	0.16
Chlorella sp.	0.32	0.23	0.22	0.32	0.20	0.19	0.25	0.30	0.07	0.21	0.43	0.27
Kirchneriella sp.	0.09	0.11	0.09	0.08	0.09	0.13	0.36	0.31	0.26	0.48	0.48	0.41
Gloeocystis sp.	0.03		0.03		0.03	0.02	0.09			0.07	0.01	
Synecchoccus sp.(R)	0.03	0.05		0.04		0.11		0.13	0.06	0.11	0.06	
Fragillaria sp.		0.04	0.04	0.06	0.03		0.06	0.12	0.22			0.10
Gloeocapsa sp.			0.03									
Microcystis sp.(C)												0.06

C = coccoid shape 1μ diameter
R = rod shape 2μ length

Figure 5 presents the calculated diversity indices, as per procedures cited by Cairns and Dickson (26). A 95 percent confidence interval is drawn through the control diversity index totals. Confidence limit lines for 2,4-D treated samples were not drawn in order to keep the graph from becoming cluttered. Because the confidence intervals of diversity indices for the controls and 2,4-D treatments overlap, it is not possible to state that any significantly different indices were noted. However, it is interesting to note that the predominating species changed in treated samples.

<u>Field Study III</u>

The experimental raft for Field Study III was set out on July 2, 1981. The skies were generally clear and the temperatures were moderate during this eight day study. Nutrient additions of orthophosphate-phosphorus and nitrate-nitrogen were made to achieve final experimental concentrations of 0.02 and 0.1 mg/l, respectively. Total phosphorus and ammonia-nitrogen levels were determined to be 0.4 and 0.05 mg/l, respectively. The alkalinity was 58 mg/l as $CaCO_3$ (Table 7).

Dissolved oxygen concentrations in the lake water outside of the confines of the raft were essentially the same as those inside the test vessels over the course of the experiment (Table 7).

ATP levels in both the 5 and 10 mg/l 2,4-D reactors mg/l were significantly greater than in the control vessels on days 2 and 5 at the

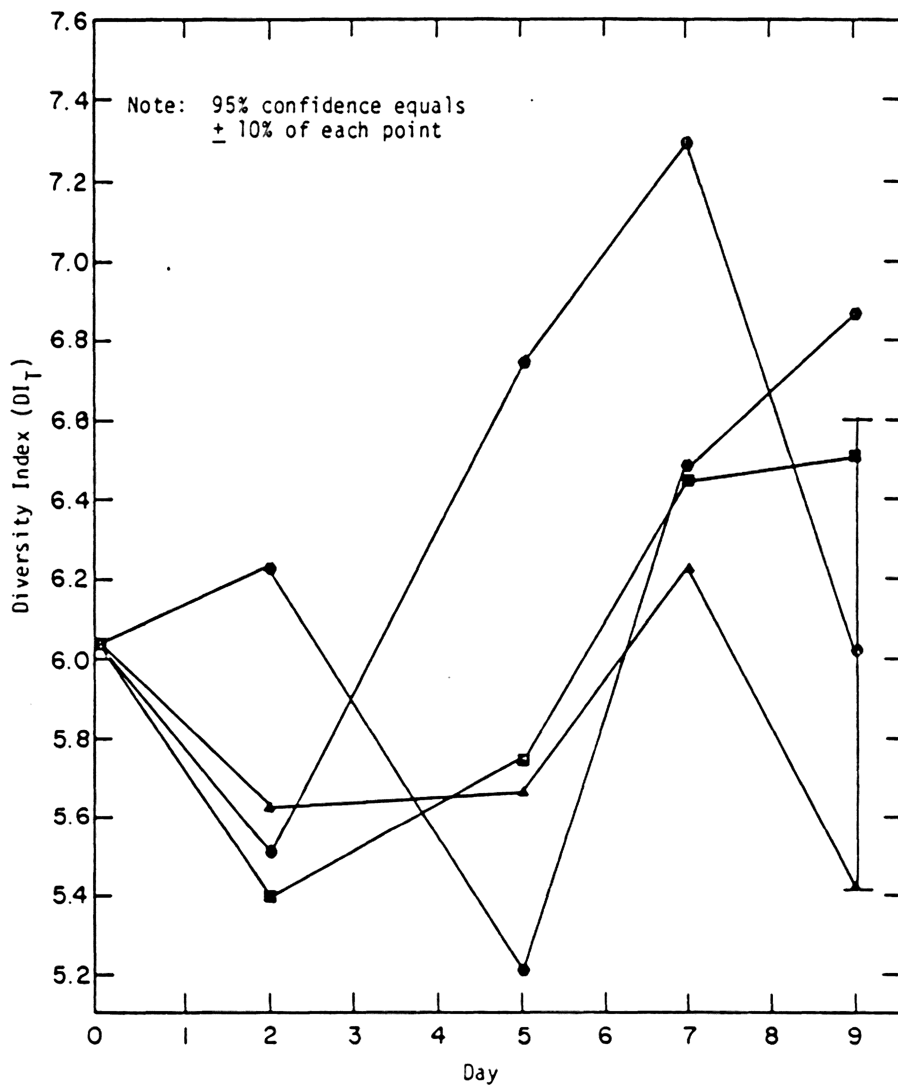


Figure 5. The effect of 2,4-D on diversity index in Field Study II; Control = ●, 1.0 mg/l 2,4-D = ■, 5.0 mg/l 2,4-D = ▲, 10.0 mg/l 2,4-D = ⬢

Table 7. 2,4-D Study III - Field Data/Addition of 0.02 mg/l and 0.1 mg/l Ortho-phosphate-P and Nitrate-N, Respectively.

Parameter	Day of Incubation			
	Day 0*	Day 2	Day 6	Day 8
Water Temp.,°C	26	25	26	27
Air Temp.,°C	27	24	28	31
pH	7.80	7.80	7.80	7.65
D.O., mg/l	8.6	8.4	8.0	9.2
Ortho-PO_4 -P, mg/l	0.020			
Total-PO_4-P, mg/l	0.4			
NH_3-N, mg/l	0.05			
NO_3-N, mg/l	0.1			
Alkalinity, mg/l as $CaCO_3$	58			
Weather	Partly Cloudy	Cool, Cloudy	Clear	Clear

*July 2, 1981

$\alpha = 0.05$ level. Concentrations of ATP in these reactors decreased below control values after 8 days of study, but the differences were not significant (Table 8). Once again the pattern of growth for 5 and 10 mg/l 2,4-D were similar and there appeared to be no additive effect due to 2,4-D (i.e. 10 mg/l twice amount of 5 mg/l).

Chlorophyll concentrations in all the 2,4-D vessels were slightly elevated above control concentrations on days 2 and 8 but the differences were not significant (Table 8). This is significant in light of the fact that ATP data were significantly greater than control values.

Microscopic examination of water from the various vessels indicated that there were some significant shifts in algal types when 2,4-D was administered. For example, Chlorella sp. began to appear on day 8 in the 2,4-D reactors but was not noted in the control. Also, rod-shaped Synecchoccus sp. was less prevalent after eight days in reactors inoculated with 5 and 10 mg/l 2,4-D than in the control (Table 9).

Field Study IV

The experimental raft for Field Study IV was set in place on July 10, 1981. Over the course of the eight day experiment, the days were warm and clear. No nutrients were added to the reactors used during this study.

Dissolved oxygen (D.O.) values in the lake water outside of the confines of the raft were essentially the same as those inside the test

Table 8. Mean Values of ATP and Chlorophyll for Field Study III of 2,4-D.

2,4-D (mg/l)	Day of Incubation: Day 2	Day 5	Day 8
	ATP, ng/l:		
Control	1714.2	704.6	2251.9
1	1675.9	930.6	2562.5
5	2330.0*	1882.7*	1592.7
10	2054.2*	1996.1*	1561.7
	Chlorophyll, μg/l:		
Control	13.5	11.6	23.5
1	19.1	15.0	26.3
5	22.3	9.3	25.9
10	19.7	7.1	28.9

*Indicates significantly greater or less than control value at $\alpha = 0.05$ for n = 3 replicates.

Table 9. Effect of 2,4-D on Algal Community Diversity in Field Study III.

Genus Type	Fraction of Various Genus Types in Algal Community											
	Day 0				Day 2				Day 8			
	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l
Synecchoccus sp.(C)	0.45	same	same	same	0.26	0.33	0.33	0.39	0.11	0.10	0.08	0.20
Synecchoccus sp. (R)	0.17	"	"	"	0.42	0.38	0.45	0.36	0.46	0.44	0.18	0.22
Fragillaria sp.	0.18	"	"	"	0.13	0.12	0.10	0.15	0.23	0.19	0.18	0.04
Gloeocystis sp.					0.10	0.05	0.10	0.05	0.05	0.08		
Microcystis sp.					0.08	0.12						
Nitzchia sp.	0.11	"	"	"			0.02	0.05	0.15			
Navicula sp.	0.09	"	"	"								
Chlorella sp.										0.19	0.36	0.31
Coelastrum sp.											0.20	0.23

C = coccoid shape 1μ diameter
R = rod shape 2μ length

vessels on day 4. Levels of D.O. were essentially the same in the control and 1 mg/l 2,4-D vessels on day 6, but the D.O. level was notably lower in the control than in the reactors inoculated with 5 and 10 mg/l 2,4-D. Average dissolved oxygen levels in the 5 and 10 mg/l 2,4-D vessels were 10.0 and 10.1, respectively, on day 6. The control vessel contained about 9.2 mg/l D.O. on day 6 (Table 10).

Although ATP levels in the reactors dosed with 5 and 10 mg/l 2,4-D were greater than those in the control on days 4 and 6, the only treatment that provided a significant difference was 10 mg/l 2,4-D after 6 days (Table 11). Microscopic examination of the samples did not reveal obvious differences in the types of algae predominating in the various vessels, with the possible exception of a day 8 observation in which 17 percent of the algae in a 1 mg/l 2,4-D reactor were *Kirchneriella* sp. None of the other vessels appeared to have any of this algal type (Table 12).

Field Study V

Field Study V (FSV) was started on July 10, 1981. There was cloudy cool weather early in the study, followed by warm, clear weather during the last three days. Each experimental vessel received one part of filter sterilized sewage for every nine parts of lake water to assure maximum eutrophic conditions. Values for orthophosphate-phosphorus and total phosphorus in the test vessels were 2.0 and 4.2 mg/l, respectively. The ammonia-N concentration was 9.1 mg/l and nitrate-N

Table 10. 2,4-D Study IV - Field Data/No Nutrient Addition

Parameter	Day of Incubation			
	Day 0*	Day 2	Day 4	Day 6
Water Temp.,°C	28	28	31	30
Air Temp.,°C	28	28	32	34
pH	7.80	7.80	7.85	7.80
D.O., mg/l	9.2	8.8	9.4	9.1
Ortho-PO_4-P, mg/l	0.01			
Condensed-PO_4-P, mg/l	0.06			
Total-PO_4-P, mg/l	0.30			
NH_3-N, mg/l	0.05			
NO_3-N, mg/l	0.10			
Alkalinity, mg/l as $CaCO_3$	52 mg/l			
Weather	Clear	Clear	Clear	Clear

*July 10, 1981

Table 11. Mean Values of ATP and Chlorophyll for Field Study IV of 2,4-D.

2,4-D (mg/l)	Day of Incubation		
	Day 2	Day 4	Day 6
	ATP, ng/l:		
Control	1373.9	560.8	2896.2
1	1528.9	482.8	2807.9
5	1259.5	660.0	3506.4
10	1334.7	712.3	5065.5*
	Chlorophyll, μg/l:		
Control	24.4	132.7	61.4
1	20.2	76.0	46.2
5	15.4	143.0	70.1
10	13.8	324.6*	129.7*

*Indicates significantly greater or less than control value at $\alpha = 0.05$ for n = 3 replicates.

Table 12. Effect of 2,4-D on Algal Community Diversity in Field Study IV.

Genus Type	Fraction of Various Genus Types in Algal Community											
	Day 2				Day 5				Day 8			
	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l
Synecchoccus sp.(C)	0.42	0.37	0.44	0.38	0.14	0.21	0.19	0.23				
Synecchoccus sp.(R)	0.27	0.32	0.30	0.34	0.57	0.48	0.47	0.44	0.44	0.48	0.51	0.43
Chlorella sp.	0.12	0.12	0.10	0.09					0.06	0.10	0.15	0.10
Fragillaria sp.	0.10	0.09	0.10				0.09		0.15		0.09	0.16
Kirchneriella sp.	0.09	0.10		0.07	0.10	0.09	0.16	0.15		0.17		
Gloeocystis sp.			0.03			0.08		0.08	0.07	0.03	0.04	0.05
Microcystis sp.				0.12	0.12	0.14	0.09	0.10	0.28	0.22	0.21	0.26
Unknown desmid			0.06		0.07							

C = coccoid shape 1μ diameter
R = rod shape 2μ length

levels were below detectable limits. The alkalinity was 62 mg/l as $CaCO_3$ (Table 13).

As expected, ATP and chlorophyll concentrations were greater in this study than in any of the other 2,4-D studies. No statistically significant differences were noted between any of the treatments and the control (Table 14). However, chlorophyll levels in the 2,4-D reactors were generally greater than those in the control vessels on days 5 and 7. Again the pattern of growth was similar in 5 and 10 mg/l 2,4-D for the duration of the test. Only in the reactor which received 1 mg/l 2,4-D were chlorophyll levels less than the control value after seven days (Table 14).

No outstanding differences were noted in the diversity of algae present in the control and 2,4-D treated reactors (Table 15).

Field Study VI

The experimental raft for diuron Field Study VI (FSVI) was set out on July 26, 1981. There was rainy or cloudy weather over the eight day period of the study. Each experimental vessel received a 1:10 dilution of filter sterilized sewage to assure eutrophic conditions. Values for orthophosphate-phosphorus and condensed phosphorus were 3.6 and 1.4 mg/l, respectively. The ammonia-N concentration was 9.0 mg/l as N and the level of nitrate-N was 0.7 mg/l. The alkalinity was 53 mg/l as $CaCO_3$ (Table 16). Dissolved oxygen values increased somewhat over the

Table 13. 2,4-D Study V - Field Data/1:10 Dilution of Filter Sterilized Sewage to Lake Water.

Parameter	Day of Incubation			
	Day 0	Day 2	Day 5	Day 7
Water Temp.,°C	29	27	32	31
Air Temp.,°C	29	24	34	32
pH	7.80	7.80	7.65	7.70
D.O., mg/l	10.0	8.6	8.5	9.0
Ortho-PO_4-P, mg/l	2.0			
Condensed-PO_4-P, mg/l	1.1			
Total-PO_4, mg/l	4.2			
NH_3-N, mg/l	9.1			
NO_3-N, mg/l	0.01			
Alkalinity, mg/l as $CaCO_3$	62 mg/l			
Weather	Cloudy	Cloudy	Clear	Clear

*July 10, 1981

Table 14. Mean Values of ATP and Chlorphyll Field Study V of 2,4-D.

2,4-D (mg/1)	Day of Incubation Day 2	Day 5	Day 7
	ATP, ng/1:		
Control	8085.5	21195.6	14041.4
1	5610.7	24968.9	12857.5
5	5020.0	20791.7	12190.1
10	6714.9	23028.0	17457.5
	Chlorophyll, $\mu g/1$:		
Control	433.1	489.1	495.7
1	425.1	890.2	406.3
5	385.1	713.4	583.9
10	426.4	700.4	612.6

*Indicates significantly greater or less than control value at $\alpha = 0.05$ for n = 3 replicates.

Table 15. Effect of 2,4-D on Algal Community Diversity in Field Study V.

Genus Type	Fraction of Various Genus Types in Algal Community							
	Day 2				Day 7			
	Control	1 mg/l	5 mg/l	10 mg/l	Control	1 mg/l	5 mg/l	10 mg/l
Synecchoccus sp.(C)	0.21	Same	Same	0.16	0.10		0.03	0.06
Synecchoccus sp.(R)	0.43	"	"	0.50	0.39	0.42	0.39	0.36
Oscillatoria sp.(Type I)	0.20	"	"	0.22	0.25	0.20	0.27	0.21
Oscillatoria sp.(Type II)					0.05	0.10		
Chlorella sp.	0.12	"	"			0.02		
Fragillaria sp.								
Kirchneriella sp.	0.04							0.05
Closterium sp.								
Chlamodomonas sp.								
Gloeocystis sp.							0.01	
Microcystis sp.				0.08	0.21	0.26	0.30	0.32
Scenedesmus sp.				0.08				

C = coccoid shape 1μ diameter
R = rod shape 2μ length

Table 16. Diuron Study VI - Field Data/1:10 Dilution of Filter Sterilized Sewage to Lake Water.

Parameter	Day of Incubation			
	Day 0*	Day 2	Day 5	Day 8
Water Temp.,°C	26	25	29	28
Air Temp.,°C	22	21	33	27
pH	7.80	7.80	7.72	7.75
D.O.	9.6	9.6	7.8	7.9
Ortho-PO_4-P, mg/l	3.6			
Condensed-PO_4-P, mg/l	1.4			
NH_3-N, mg/l	9.0			
NO_3-N, mg/l	0.7			
Alkalinity, mg/l as $CaCO_3$	53 mg/l			
Weather	Rainy	Rainy	Partly Cloudy	Cloudy

*July 26, 1981

course of the experiment in the test vessels, whereas lake oxygen values decreased (Table 16).

ATP levels in the 1.0 mg/l diuron reactors were significantly less than control values at the $\alpha = 0.05$ level on days 2 and 5. Significantly less ATP was found in the reactors receiving 0.01 and 0.1 mg/l diuron than in the control after five days of growth. All other diuron replicate values of ATP were below control values, but the differences, with the exception of that between the control and the 1.0 mg/l diuron vessel on day 8, were not significant. All ATP levels in the treated and control vessels increased over the days tested (Table 17)

Chlorophyll-levels were significantly less than control values in all diuron-treated vessels on days 5 and 8. The amount of chlorophyll in all treated and control vessels increased over the course of the experiment (Table 17). The effect of diuron was apparently an additive effect in that increases in diuron concentration resulted in greater decreases in growth. The effect on growth reached a maximum in less than two days.

Microscopic studies of treated and control reactor water indicated that there was a shift in the predominant type of algae. In the control, a rod shaped *Synecchoccus* sp. was predominant, but in the treated vessels a coccoid-shaped *Synecchoccus* sp. predominated. The shift was less noticeable as diuron levels were decreased (Table 18).

Table 17. Mean Values of ATP and Chlorophyll for Field Study VI of Diuron.

Diuron (mg/l)	Day of Incubation		
	Day 2	Day 5	Day 8
	ATP, ng/l:		
Control	4890.2	21829.1	16635.0
0.01	3368.0	9016.8*	10885.5
0.1	2794.2	4040.3*	5493.5
1.0	2237.2*	9472.9*	23660.4
	Chlorophyll, μg/l:		
Control	24.3	2108.5	7076.3
0.01	12.3	756.8*	3709.9*
0.1	11.3	18.8*	3232.6*
1.0	6.8	13.8*	317.9*

*Indicates significantly greater or less than control at $\alpha = 0.05$ for n = 3 replicates.

Table 18. Effect of Diuron on Algal Community Diversity in Field Study VI.

Genus Type	Fraction of Various Genus Types in Algal Community: Day 2				Day 5			
	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l
Synecchoccus sp.(C)	0.04	0.69	0.44	0.14		0.89	0.78	0.04
Synecchoccus sp.(R)	0.65	0.12	0.19	0.40	0.92		0.07	0.83
Chlorella sp.	0.22	0.10	0.19	0.31	0.06	0.05	0.07	0.04
Kirchneriella sp.	0.04			0.10	0.01	0.02	0.07	0.05
Chlamodomonas sp.						0.02		0.04
Gloeocystis sp.	0.05	0.04						
Masetonium sp.			0.07					
Anabaena sp.		0.05	0.12	0.05				
Coelastrum sp.					0.01			
Selenastrum sp.					0.01			
Fragillaria sp.							0.01	

C = coccoid shape 1μ diameter
R = rod shape 2μ length

Field Study VII

An experiment with atrazine in Field Study VII was initiated on July 28, 1981. The weather over the period of study was clear and warm during the initial seven days and cool and clear for the remainder of the study. Each experimental vessel received a 1:10 dilution of filter sterilized sewage to assure eutrophic conditions. Values for orthophosphorus-P and condensed phosphorus were 2.9 and 1.1 mg/l, respectively. The initial ammonia-N and nitrate-N levels were 7.3 and 0.6 mg/l, respectively. The alkalinity was 61 mg/l (Table 19).

Dissolved oxygen levels in the control and 0.01 mg/l atrazine vessels were consistently high throughout the period of study. Oxygen levels in the 0.1 mg/l and 1.0 mg/l atrazine treated vessels were low after three days. Levels of oxygen in the treated reactors increased to the level in the control after seven days of growth and increased above the control value after nine days.

ATP concentrations in all the atrazine reactors were significantly less ($\alpha = 0.05$) than the control ATP concentrations on day 9 and at the 0.1 mg/l atrazine level on day 3 (Table 20). It is important to note that the effect on growth was most predominant within three days. After three days growth increased in treated vessels. The effect of atrazine on ATP was apparently not an additive one. Greater concentrations of atrazine did not necessarily mean less ATP produced.

Chlorophyll levels in all the treated reactors were significantly less than control values after three days of growth. Chlorophyll values

Table 19. Atrazine Study VII - Field Data/1:10 Dilution of Filter Sterilized Sewage to Lake Water.

Parameter	Day of Incubation			
	Day 0*	Day 3	Day 7	Day 11
Water Temp., °C	31	30	27	25
Air Temp.,°C	32	33	25	23
pH	7.70	7.80	7.82	7.77
D.O., mg/l	9.1	9.3	8.2	9.3
Ortho-PO_4-P, mg/l	2.9			
Condensed-PO_4-P, mg/l	1.1			
NH_3-N, mg/l	7.3			
NO_3-N, mg/l	0.6			
Alkalinity, mg/l as $CaCO_3$	61			
Weather	Clear	Clear	Clear	Partly Cloudy

*July 28, 1981

Table 20. Mean Values of ATP and Chlorophyll for Field Study VII of Atrazine.

Atrazine (mg/l)	Day of Incubation Day 3	Day 7	Day 9
	ATP, ng/l:		
Control	10554.2	14315.1	28893.4
0.01	9870.8	14292.9	19002.8*
0.1	4275.7*	17936.7	11618.9*
1.0	8825.2	11655.8	11275.7*
	Chlorophyll, μg/l:		
Control	1798.1	329.9	1780.7
0.01	43.9*	182.2	691.7
0.1	105.0*	1024.5*	2133.2
1.0	54.9*	68.2	560.3

*Indicates significantly greater or less than control values at $\alpha = 0.05$ for n = 3 replicates.

for the 0.1 mg/l treatment level were greater than control values on days 7 and 9. In each of the other treated vessels chlorophyll levels were lower than in the control (Table 20). Note that the chlorophyll levels were initially less than control values in 0.1 mg/l atrazine, then significantly greater than controls on subsequent days. The 1.0 mg/l atrazine treatment was the most effective in decreasing chlorophyll levels. The 0.01 mg/l treatment was also effective.

Microscopic analysis of the reactor fluids indicated that the number of coccoid Synecchoccus sp. in treated samples increased between 3 and 5 days. Gloeocapsa sp. was the predominant type of algae in the control. After seven days of growth, Chlamydomonas sp. increased noticeably in the reactor receiving 1.0 mg/l of atrazine. Scenedesmus sp. levels began to increase in the vessels inoculated with 0.1 and 1.0 mg/l atrazine after 11 days (Table 21).

Field Study VIII

The experimental raft for diuron Field Study VIII was set out on August 10, 1981. The seven days of this study were clear and cool. No nutrient additions were made in this study. All nutrient values were low. Orthophosphate-P and nitrate-N concentrations were below detectable limits. The concentration of ammonia was 0.05 mg/l and the condensed phosphate-P level was 0.2 mg/l. The alkalinity was 42 mg/l (Table 22).

Table 21. Effect of Atrazine on Algal Community Diversity in Field Study VII.

	Fraction of Various Genus Types in Algal Community															
	Day 3				Day 5				Day 7				Day 11			
Genus Type	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l
Synecchoccus sp.(C)	0.01	0.71	0.55	< 0.01	0.05	0.82	0.48	0.04	0.01	0.12	0.02	< 0.01	0.16	0.06	0.15	0.22
Synecchoccus sp.(R)	0.21		< 0.1	0.28	0.02				0.23							
Chlorella sp.	0.08			0.28									0.02	0.11	0.08	0.11
Kirchneriella sp.			< 0.1	0.02												
Gloeocystis sp.	0.02		< 0.1		< 0.01		0.05	< 0.01	0.01		0.03			0.09		
Gloeocapsa sp.	0.68	0.28	0.44	0.42	0.82	0.12	0.47	0.96	0.72	0.49	0.91	0.99	0.66	0.25	0.50	0.57
Mesotaenium sp.	0.06					0.06				0.32						
Chlamodomonas						< 0.01				0.06		< 0.01				
Scenedesmus sp.											0.02			0.49	0.18	
Anabaena sp.					< 0.01				0.03							
Stauroneis sp.										0.01	0.02	< 0.01				
Navicula sp.												< 0.01				
Oscillatoria sp.		0.01											0.13			
Selanastrum sp.													0.04		0.09	0.08
Lyngbya sp.																0.02

C = coccoid shape 1μ diameter
R = rod shape 2μ length

Table 22. Diuron Study VIII - Field Data/No Nutrient Additions

Parameter	Day of Incubation			
	Day 0*	Day 3	Day 5	Day 7
Water Temp.,°C	25	25	25	28
Air Temp.,°C	25	25	25	27
pH	7.80	7.70	7.65	7.70
D.O.	8.7	9.2	9.4	9.0
Ortho-PO_4-P, mg/l	0.01			
Condensed-PO_4-P, mg/l	0.20			
NH_3-N, mg/l	0.05			
NO_3-N, mg/l	0.01			
Alkalinity, mg/l aS $CaCO_3$	42			
Weather	Clear	Cloudy	Clear	Clear

*August 10, 1981

Dissolved oxygen values within the experimental vessels were slightly below D.O. levels of the lake water adjacent to the raft (Table 22).

ATP levels in all treated vessels were significantly less (α = 0.05) than control ATP values for all days tested at the α = 0.05 level (Table 23). The effect of diuron on ATP was an additive one in that increases in diuron resulted in decreases in ATP concentration. Once again, the concentration of ATP decreased initially and began to increase with time in diuron treatments.

Chlorophyll levels in all treated vessels were below control values, with the exceptions of days 5 and 7 for the 0.01 mg/l. The values on these days were below control chlorophyll values, but not significantly (Table 23). Like ATP, chlorophyll levels decreased initially in all treatments of diuron then began a slow increase over the course of the study. This suggests some algal species were able to increase their numbers in the presence of this chemical. The effect of diuron on chlorophyll was additive.

Microscopic examination of reactor water indicated that diuron effected a shift in the predominant forms of algae. This was particularly evident as the concentration of diuron was increased. The predominant algal species after five days of growth were two _Synecchoccus_ sp. and a _Gloeocapsa_ sp. (Table 24). After seven days of growth, the two _Synecchoccus_ sp. algae predominated in the treated

Table 23. Mean Values of ATP and Chlorophyll for Field Study VIII of Diuron.

Diuron (mg/l)	Day of Incubation		
	Day 3	Day 5	Day 7
	ATP, ng/l:		
Control	1368.6	1512.5	1514.1
0.01	908.1*	1111.9*	1142.9*
0.1	776.6*	860.9*	869.2*
1.0	696.1*	585.8*	755.5*
	Chlorophyll, µg/l:		
Control	155.1	156.7	432.8
0.01	47.0*	139.8	322.3
0.1	9.9*	34.7*	45.5*
1.0	1.2*	9.2*	5.5*

*Indicates significantly greater than or less than control values at $\alpha = 0.05$ for n = 3 replicates.

vessels. The sequential index test indicated that significant diversity changes occurred in all the treated vessels for all days tested (Figure 6).

Field Study IX

Field Study IX with atrazine was started on August 23, 1981. Weather during the period of study was cool and cloudy for the majority of the days. No nutrient spikes were added to the sample vessels. Concentration values for orthophosphate-P and nitrate-N were below detectable levels. The initial concentrations of condensed phosphorus and ammonia were 0.22 and 0.05 mg/l, respectively. The initial concentrations of condensed phosphorus and ammonia were 0.22 and 0.05 mg/l, respectively. Alkalinity was 48 mg/l as $CaCO_3$ (Table 25). Dissolved oxygen levels within test vessels were similar to those noted in the lake over the period of study.

ATP concentrations within reactors treated with 0.1 and 1.0 mg/l atrazine were significantly greater than control values at the α = 0.05 level on day 3. Values for ATP remained greater than control values in the vessel receiving 0.1 mg/l atrazine for the remainder of the study. All other treated reactor values were less than control levels for the remainder of the study. ATP concentrations in the reactors inoculated with 0.1 mg/l atrazine increased over the seven day study period (Table 26). ATP also increased in the treatment containing 1.0 mg/l atrazine on day 3.

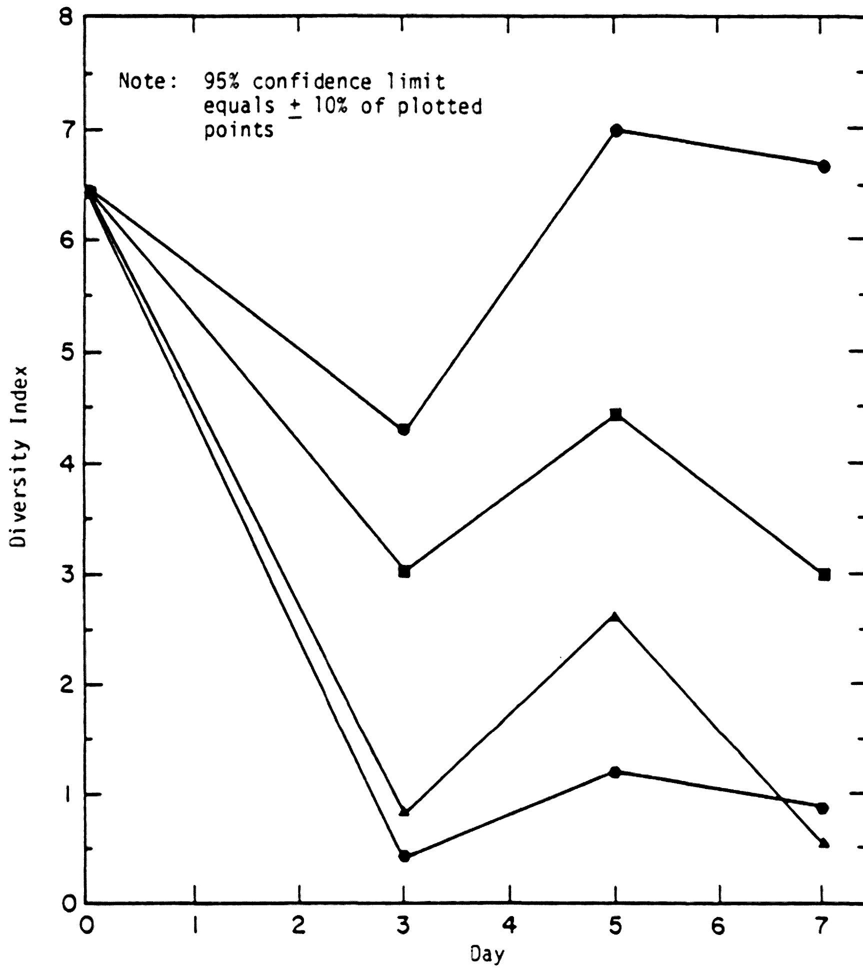


Figure 6. The effect of diuron on diversity index in Field Study VIII; Control = ●, 0.01 mg/l diuron = ■, 0.1 mg/l diuron = ▲, 1.0 mg/l diruon = ⬣.

Table 24. Effect of Diuron on Algal Community Diversity in Field Study VIII.

Genus Type	Fraction of Various Genus Types in Algal Community											
	Day 3				Day 5				Day 7			
	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l
Synecchoccus sp.(C)	0.45	0.71	0.43	0.42	0.35	0.43	0.11	0.49		0.33	0.40	0.23
Synecchoccus sp.(R)			0.10			0.03	0.16	0.10		0.61	0.47	0.12
Chlorella sp.			0.05		0.17				0.19	0.01	0.06	
Kirchneriella sp.			0.06		0.04		0.04	0.04				0.11
Gloeocystis	0.11			0.11						0.02		
Fragellaria sp.	0.06				0.09		0.11	0.03	0.26			0.11
Gloeocapsa sp.	0.34	0.25	0.36	0.38	0.35	0.50	0.58	0.34	0.26	0.03	0.06	0.43
Oscillatoria sp.		0.04				0.04			0.19		0.01	
Selanastrum sp.				0.04								
Navicula sp.	0.04			0.05								
Aphanocapsa sp.									0.10			

C = coccoid shape 1μ diameter
R = rod shape 2μ length

Table 25. Atrazine Study IX - Field Data/No Nutrient Additions

Parameter	Day of Incubation			
	Day 0*	Day 3	Day 5	Day 7
Water Temp., °C	27	27	26	26
Air Temp., °C	28	28	28	24
pH	7.85	7.70	7.70	7.70
D.O., mg/l	8.6	8.6	8.5	9.3
Ortho-PO_4-P, mg/l	0.01			
Condensed-PO_4-P, mg/l	0.22			
NH_3-N, mg/l	0.05			
NO_3-N, mg/l	0.01			
Alkalinity, mg/l as $CaCO_3$	48			
Weather	Partly Cloudy	Partly Cloudy	Partly Cloudy	Clear

*August 23, 1981

Table 26. Mean Values of ATP and Chlorophyll for Field Study IX of Atrazine.

Atrazine (mg/l)	Day of Incubation: Day 3	Day 5	Day 7
	ATP, ng/l:		
Control	379.5	1237.4	1297.3
0.01	327.1	788.8*	752.7
0.1	529.2*	1807.7	2643.5
1.0	514.0*	861.5*	1267.9
	Chlorophyll, μg/l:		
Control	64.8	148.2	146.3
0.01	76.8	99.6	68.9
0.1	112.8*	276.7*	329.0*
1.0	54.2	13.2*	59.6

*Indicates significantly greater than or less than control values at $\alpha = 0.05$ for n = 3 replicates.

Chlorophyll concentrations in reactors containing 0.1 mg/l atrazine were significantly greater than control values on all days tested. Chlorophyll levels in the reactors inoculated with 0.01 and 1.0 mg/l atrazine were below control values with the exception of day 3 for the 0.01 mg/l atrazine vessel. Chlorophyll concentrations on day 5 in the 1.0 mg/l atrazine reactor were significantly less than control values. Chlorophyll levels in the vessel containing 0.1 mg/l atrazine increased over the seven day period (Table 26). This, of course, would suggest that some algal species were able to increase their biomass in the presence of 0.1 mg/l atrazine. Whereas, in other treatments this effect was not noticeable. The effect of 0.01 mg/l atrazine was not as dramatic as it was in vessels receiving other treatments and followed control data more closely. Test vessels receiving 1.0 mg/l significantly affected chlorophyll levels.

Microscopic studies of the reactor fluids revealed that there was a large increase in the number of rod-shaped Synecchoccus sp. in atrazine-treated samples. This was particularly obvious early in the study. By day 7, samples of water from the reactors inoculated with 0.1 and 1.0 mg/l atrazine contained proportionally more coccoid forms of Synecchoccus sp. than did controls. Kirchneriella sp. and Chlorella sp. were quite numerous in control samples, but few to none of the algae were noted in treated samples (Table 27).

Diversity studies indicated that the diversity of algae in the 0.01 mg/l atrazine treatment was actually greater than control diversity to a

Table 27. Effect of Atrazine on Algal Community Diversity in Field Study IX.

Genus Type	Fraction of Various Genus Types in Algal Community											
	Day 3				Day 5				Day 7			
	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l	Control	1 mg/l	0.1 mg/l	0.01 mg/l
Synecchoccus sp.(C)	0.38	0.22	0.32	0.16	0.40	0.39	0.43	0.48	0.19	0.53	0.53	0.22
Synecchoccus sp.(R)		0.35	0.40	0.12		0.30	0.29			0.32	0.07	
Chlorella sp.	0.19	0.12	0.01	0.16	0.20		0.07		0.38	0.05	0.13	
Kirchneriella sp.	0.08	0.05			0.16	0.04	0.07	0.03	0.12			0.22
Gloeocystis								0.08				
Fragellaria sp.			0.04	0.16								0.15
Gloeocapsa sp.	0.29	0.26	0.23	0.40	0.13	0.24	0.14	0.29	0.21	0.05	0.20	0.34
Oscillatoria sp.	0.06				0.11	0.03		0.13		0.05	0.07	0.07
Chlamydomonas sp.									0.10			

C = coccoid shape 1μ diameter
R = rod shape 2μ length

statistically significant degree ($\alpha = 0.05$) after three days of growth. However, the diversity level decreased below control values for the remainder of the study. A marginally significant decrease was noted on day 5. No statistically significant differences were detectable on day 7 and 9. Statistically significant decreases in diversity were noted on days 3, 5, and 7 for the 0.1 mg/l atrazine treatment. Day 9 diversity was not significantly different from that of the control. The diversity of all samples at 1.0 mg/l atrazine was significantly below the control value (Figure 7).

Field C-Uptake (added as $H^{14}CO_3$) Studies of Atrazine and Diuron Spiked Lake Water

Table 28 presents data which show the effect of atrazine on the uptake of ^{14}C in Smith Mountain Lake plankton after a four-hour incubation period. Significantly less ^{14}C was taken up by plankton treated with 0.1 and 1.0 mg/l atrazine than by plankton in a control vessel. The statistical mean of ^{14}C assimilated at 0.01 mg/l atrazine was less than the control mean, but the difference was not significant at $\alpha = 0.05$.

The data in Table 29 show the effect of diuron on the uptake of ^{14}C by Smith Mountain Lake plankton over a four-hour incubation period. Uptake levels of ^{14}C in the control were significantly greater than any of the levels noted in reactors treated with diuron.

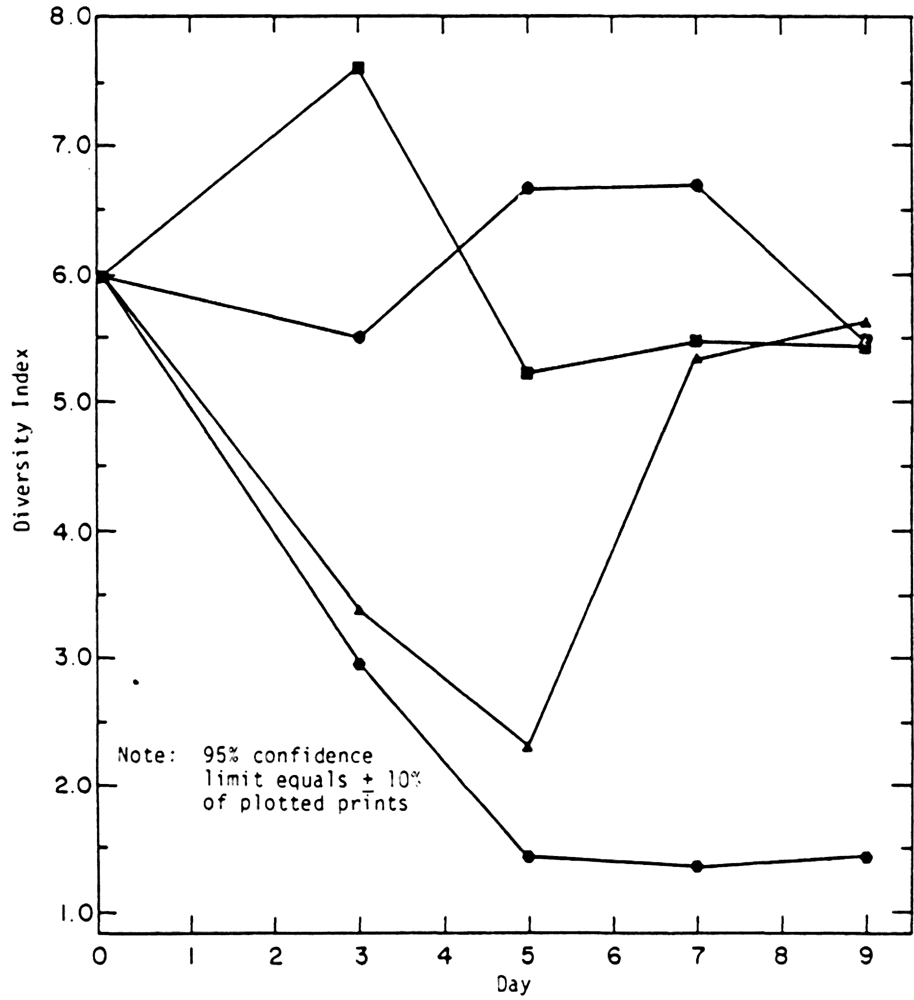


Figure 7. The effect of atrazine on diversity index in Field Study IX; Control = •, 0.01 mg/l atrazine = ■, 0.1 mg/l atrazine = ▲, 1.0 mg/l atrazine = ●

Table 28. The Effect of Atrazine on Primary Productivity in Smith Mountain Lake

Atrazine Concentration mg/l	Replicate	mg/l ^{14}C Fixed after 4 hours
Control	1	0.0115
	2	0.0114
	3	0.0116
0.01	1	0.0105
	2	0.0098
0.1	1	0.0056*
	2	0.0053*
1.0	1	0.0021*
	2	0.0019*

*Indicates significantly less than the control at $\alpha = 0.05$ level.

Table 29. The Effect of Diuron on Primary Productivity in Smith Mountain Lake

Diuron Concentration, mg/l	Replicate	^{14}C Fixed After 4 hours, mg/l
Control	1	0.0190
	2	0.0192
	3	0.0208
0.01	1	0.0145*
	2	0.0138*
0.1	1	0.0045*
	2	0.0042*
1.0	1	0.0025*
	2	0.0028*

*Indicates significantly less than control value at the $\alpha = 0.05$ level.

The effect of 0.2 mg/l diuron on ^{14}C uptake and dissolved organic carbon levels within the plankton community of Claytor Lake is shown in Table 30. Dissolved organic carbon mean values at every time period tested were less than control values after the initiation of the experiment. Significantly ($\alpha = 0.05$) less dissolved organic carbon was noted after incubation times of one hour and twenty minutes (10:20) and seven hours and forty-five minutes (16:45). In all cases significantly less ^{14}C was taken up in diuron spiked samples than in control samples.

Laboratory Studies

The Effect of 2,4-D on ^{14}C Uptake (added as $H^{14}CO_3$) by a Blue-green Algae Isolated from Smith Mountain Lake

Table 31 presents the results obtained from studies to determine the effect of 2,4-D on the uptake of ^{14}C in the dark by a species of *Synecchoccus* isolated from Smith Mountain Lake. The data indicate that significantly more labeled ^{14}C was fixed by algae treated with 2 and 10 mg/l 2,4-D after 240 minutes than algae in the controls. Average uptake values at all other time intervals were not significantly greater or less than control values. However, the mean value at 10 mg/l 2,4-D after 120 minutes was conspicuously larger than the corresponding mean value of the control (0.0579 mg/l to 0.0428 mg/l).

The effect of 2,4-D on the uptake of ^{14}C in the light is shown in Table 32. Significantly ($\alpha = 0.05$) more ^{14}C was taken up in 2,4-D

Table 30. The Effect of Diuron on Primary Productivity in Claytor Lake

Diuron Concentration	Time	Replicate	^{14}C Uptake, mg/l	Dissolved Organic Carbon, mg/l
Control	9:00	1	0.000	2.584
		2	0.000	2.394
	10:20	1	0.221	3.302
		2	0.221	3.064
	11:40	1	0.181	2.432
		2	0.157	2.628
	12:50	1	0.151	2.628
		2	0.120	2.276
	14:30	1	0.140	2.467
		2	0.152	2.320
	15:30	1	0.146	2.223
		2	0.151	2.216
	16:45	1	0.110	2.787
		2	0.123	3.070
0.2 mg/l	9:00	1	0.000	2.584
		2	0.000	2.394
	10:20	1	0.042*	2.212*
		2	0.037	2.277
	11:40	1	0.020*	2.256
		2	0.024	2.431
	12:50	1	0.023*	2.124
		2	0.022	2.288
	14:30	1	0.018*	2.048
		2	0.018	2.461
	15:30	1	0.019*	2.155
		2	0.016	2.076
	16:45	1	0.013*	2.328*
		2	0.012	2.003

*Indicates mean value of two replicates during time period is significantly less than control value at the α = 0.05 level.

Table 31. The Effect of 2,4-D on ^{14}C Uptake by *Synecchoccus sp.* in the Dark at pH 7.8

2,4-D Concentration	Time, minutes	**mg/l ^{14}C Fixed
Control	30	0.0251
		0.0242
		0.0226
	60	0.0354
		0.0304
		0.0259
	120	0.0402
		0.0450
		0.0431
	240	0.0747
		0.0884
		0.0749
2 mg/l	30	0.0273
		0.0292
		0.0289
	60	0.0321
		0.0309
		0.0301
	120	0.0384
		0.0444
		0.400
	240*	0.0873
		0.0920
		0.0915
10 mg/l	30	0.0259
		0.0190
		0.0243
	60	0.0276
		0.0236
		0.0243
	120	0.0741
		0.0589
		0.0407
	240*	0.1003
		0.1000
		0.1014

*Indicates significantly greater than control value at $\alpha = 0.05$ level.

**10^6 cells/ml in vessels.

Table 32. The Effect of 2,4-D on ^{14}C Uptake by Synecchoccus sp. Isolates from Smith Mountain Lake at pH 7.0

2,4-D Concentration	Time, Minutes	**mg/l ^{14}C Fixed Study 1	Study 2
Control	15	0.147	0.158
		0.143	0.168
		0.177	0.148
	30	0.243	0.308
		0.240	0.345
		0.226	0.302
	60	0.463	0.420
		0.348	0.413
		0.471	0.327
	120	0.806	0.809
		0.561	0.808
		0.913	0.674
	240	1.884	1.587
		1.798	1.821
		2.275	2.128
5 mg/l	15	0.191	0.161
		0.235	0.191
		0.193	0.196
	30	0.351	0.317
		0.335	0.337
		0.307	0.304
	60	0.594*	0.480*
		0.611	0.502
		0.523	0.471
	120	1.007*	0.894*
		0.985	1.133
		1.033	1.058
	240**	2.280*	1.938
		2.331	2.443
		2.317	2.377

*Indicates mean values at this time significantly greater than control values at α = 0.05 level.

**10^6 cells/ml in vessels.

spiked samples than in control samples after 60, 120, and 240 minutes in study 1 and after 60 and 120 minutes in study 2.

The Effect of 2,4-D on Growth of Algae Isolated from Smith Mountain Lake

Table 33 presents the effect of 2,4-D on the growth of a blue-green alga *Synecchoccus* sp. isolated from Smith Mountain Lake. Growth of the alga, as measured by cell counts, was significantly greater after 8 days at 20 mg/l 2,4-D and after 10 days at 0.002 mg/l than in control vessels. Fluorescence values increased significantly in cultures exposed to 20 mg/l 2,4-D on days 3, 5, 8, and 10.

The fluorescence of fluids taken from the reactor treated with 0.2 mg/l 2,4-D was significantly greater than that of the control on days 5, 8, and 10. Only on day 10 was fluorescense in the 0.002 mg/l 2,4-D reactor significantly greater than in the control.

ATP levels in the reactor treated with 20 mg/l 2,4-D were significantly greater than the ATP level of the control on days 5, 8, and 10. A significantly greater amount of ATP was noted in the 0.2 mg/l 2,4-D reactor on days 8 and 10. Only on day 10 was the ATP level in the 0.002 mg/l 2,4-D reactor greater, than in the control.

Control cell counts increased steadily for eight days. Cell number did not increase rapidly between days 8 and 10. Cell numbers in the 20 mg/l 2,4-D reactor increased rapidly for eight days and then decreased considerably between days 8 and 10. ATP and fluorescence values followed this same trend in both the control and 20 mg/l 2,4-D vessels.

Table 33. Growth Response of Smith Mountain Lake *Synecchoccus* sp. isolate to 2,4-D at pH 7.0

2,4-D Concentration	Days of Incubation								
	Day 1			Day 3			Day 5		
	Cells/ml (x 1000)	ATP (ng/ml)	Relative Fluores.	Cells/ml (x 1000)	ATP (ng/ml)	Relative Fluores.	Cells/ml (x 1000)	ATP (ng/ml)	Relative Fluores.
Control	475		162	417		153	921	1.5	250
	495		115	357		143	585	8.2	720
	630		234	385		200	840	4.4	175
	421		126	380		103	579	2.3	127
20 mg/l	372		152	758*		1167*	632	18.8*	3450*
	227		108	435		833	809	18.9	2700
	295		144	472		600	1003	18.3	3750
	754		210	680		800	961	30.7	3300
0.2 mg/l	281		138	727		711	683	3.3	630*
	312		150	449		733	598	6.3	1005
	2612		69	387		233	780	3.6	245
	306		108	556		150	774	3.4	210
0.002 mg/l	1522		120	404		216	606	1.6	160
	250		120	489		193	578	3.6	370
	2259		201	397		243	658	4.4	255
	395		159	370		183	698	1.8	145

(Continued)

Table 33. Growth Response of Smith Mountain Lake *Synecchoccus* sp. isolate to 2,4-D at pH 7.0 (Continued)

	Days of Incubation					
	Day 8			Day 10		
2,4-D Concentration	Cells/ml (x 1000)	ATP (ng/ml)	Relative Fluores.	Cells/ml (x 1000)	ATP (mg/ml)	Relative Fluores
Control	3580	2.4	90	4725	5.0	175
	3790	3.7	155	3744	4.0	140
	3636	1.9	125	4059	3.0	145
	4131	3.5	250	4488	3.5	85
20 mg/l	16205*	34.9*	3100*	8455*	23.5*	950*
	35254	52.2	3150	5405	18.5	1150
	9374	17.1	1550	4497	10.5	1050
	13398	22.6	3000	6393	27.5	1550
0.2 mg/l	5343*	19.5*	570*	9779	21.5*	1340*
	4241	8.2	480	3030	4.5	140
	4683	10.3	1350	3790	21.0	950
	4471	17.8	800	12805	52.0	4800
0.002 mg/l	4178	4.4	205	19524*	52.0*	2350*
	4038	11.8	185	5334	15.0	1100
	6011	15.6	540	13936	53.0	1900
	3463	3.2	115	6286	12.5	260

*Indicates mean is statistically greater than control mean at $\alpha = 0.05$.

Table 34. Short Term Assay of the Effect of 2,4-D on *Chlorella sorokiniana**

Time Minutes	2,4-D Concentration, mg/l	Dissolved Oxygen, mg/l	Relative Fluorescence
0	1.0	10.6	26
2		10.4	26
4		10.6	25
6		10.4	26
8		10.5	26
10		10.4	26
15		10.7	25
20		10.6	25
30		10.5	25
0	10.0	10.6	25
2		10.5	25
4		10.5	25
6		10.1	26
8		10.2	26
10		10.3	26
15		10.2	26
20		10.1	26
30		9.8	26
0	30.0	9.8	26
2		9.0	26
4		9.2	35
6		9.2	34
8		9.2	33
10		9.2	33
15		9.2	31
20		9.0	30
30		8.8	30

*10×10^6 cells/ml were used.

mg/l, at this treatment level and then remained relatively constant for the remainder of the 30 minute period.

Uptake Rate of 2,4-D by Chlorella sorokiniana

The data in Table 35 indicate that essentially all of the 2,4-D was taken up by Chlorella sorokiniana within five minutes. Analysis of 2,4-D levels in solution by gas chromatographic techniques confirmed that the uptake was rapid.

The Effect of Diuron on a Continuous Culture of Chlorella sorokiniana

The theoretical rate at which diuron was applied in this study is given in Table 36. Figure 8 shows that cell numbers decreased abruptly when diuron was introduced into the continuous culture on day 8. Cell numbers continued to decrease over the remainder of the experiment. Cell counts on days 14 and 15 were essentially equal.

The total organic carbon (TOC) data graphically depicted in Figure 9 show that the TOC decreased steadily following the introduction of diuron. The decrease from the average 8 day steady-state value of 50.1 mg/l to the lowest value registered on day 15 of 26.9 mg/l represents a 46 percent decrease.

Both ATP and energy charge decreased sharply when diuron was administered (Figures 10 and 11). A slight increase in both parameters was evident between days 14 and 15.

Fluorescence values were somewhat variable during what was considered to be a steady-state period of growth for the culture

Table 35. Uptake of 2,4-D (^{14}C label) by <u>Chlorella sorokiniana</u>*

Time Minutes	Replicate	Counts per Minute of Filtered Cells	^{14}Carbon Uptake, mg/l	Herbicide/Cell, mg/cell
0	1	0	0.00000000	0
0	2	0	0.00000000	0
0	3	0	0.00000000	0
5	1	3837	0.00324935	3.24935E-10
5	2	4255	0.00360333	3.60333E-10
5	3	4329	0.00366600	3.66600E-10
15	1	5799	0.00491086	4.91086E-10
15	2	4086	0.00346022	3.46022E-10
15	3	4067	0.00344413	3.44413E-10
30	1	4646	0.00393445	3.93445E-10
30	2	3901	0.00330355	3.30355E-10
30	3	5138	0.00435110	4.35110E-10
60	1	4113	0.00348308	3.48308E-10
60	2	3992	0.00338061	3.38061E-10
60	3	3676	0.00311301	3.11301E-10
120	1	4416	0.00373968	3.73968E-10
120	2	6646	0.00562814	5.62814E-10
120	3	4056	0.00343481	3.43481E-10
240	1	4115	0.00348477	3.48477E-10
240	2	4026	0.00340941	3.40941E-10
240	3	6433	0.00544777	5.44777E-10
360	1	4340	0.00367532	3.67532E-10
360	2	4426	0.00374814	3.74814E-10
360	3	4326	0.00366346	3.66346E-10

*1×10^6 cells/ml were used.

Table 36. Theoretical Increase of Diuron Concentrations in the Continuous Flow System

Day	Diuron Concentration, mg/l
8	0.0000
9	0.0014
10	0.0028
11	0.0043
12	0.0057
13	0.0071
14	0.0086
15	0.0100

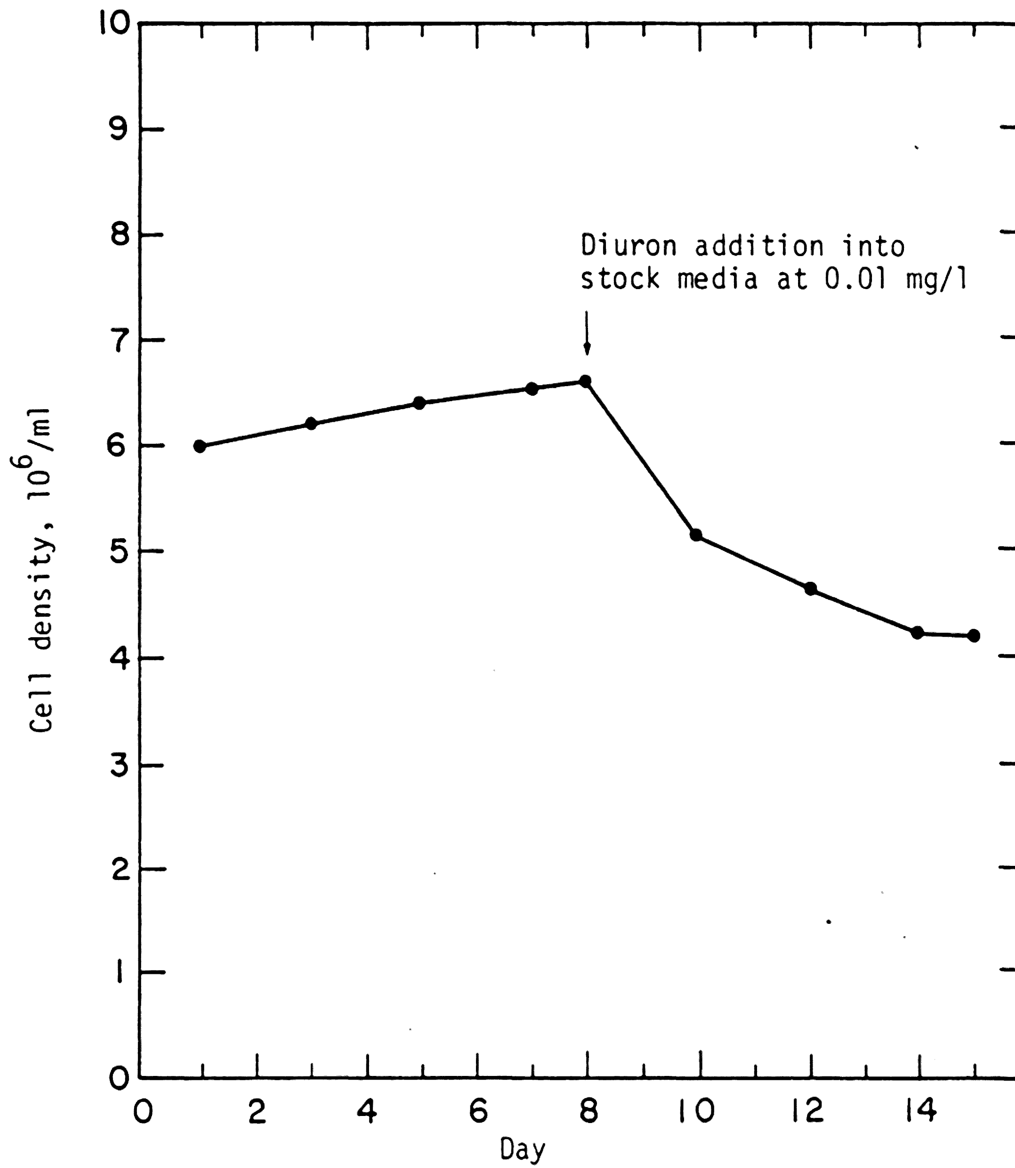


Figure 8. The effect of diuron on cell numbers in a continuous culture of Chlorella; diuron added on day 8.

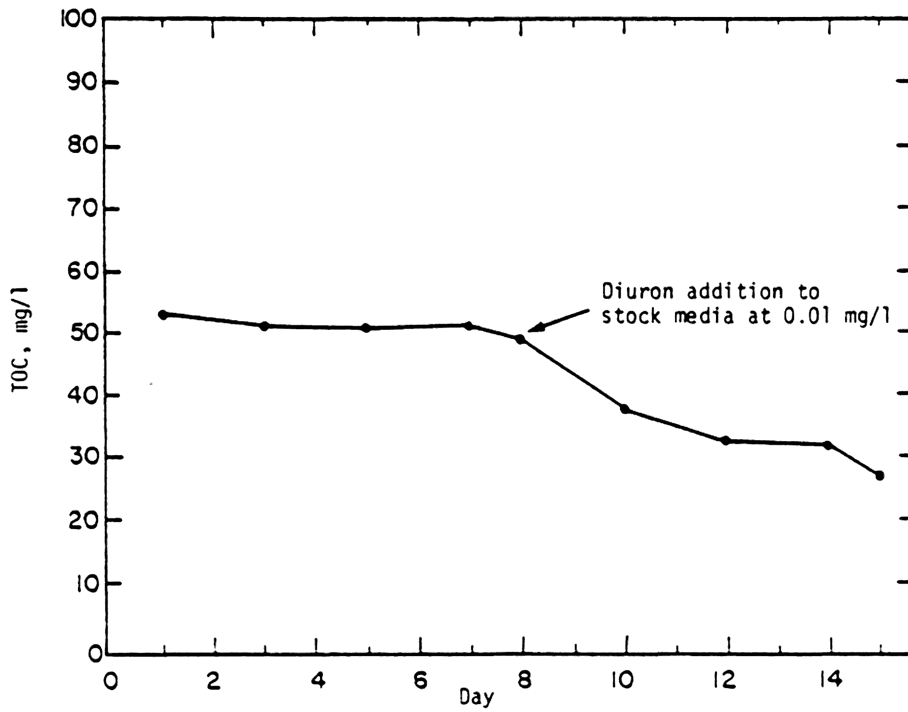


Figure 9. The effect of diuron on total organic carbon levels in a continuous culture of _Chlorella_; diuron added on day 8.

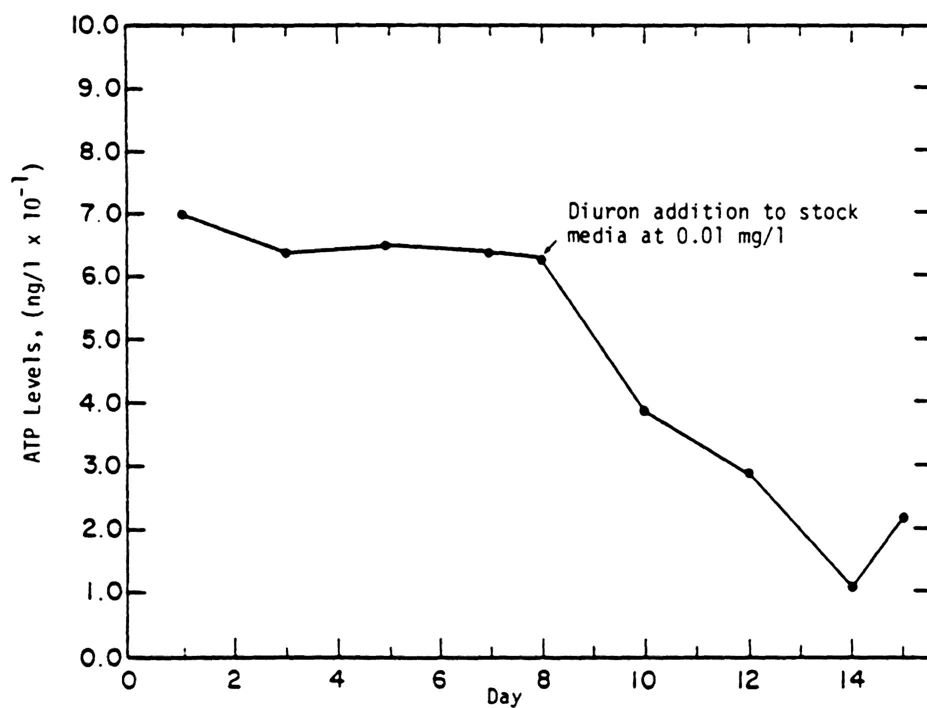


Figure 10. The effect of diuron on ATP levels in a continuous culture of Chlorella; diuron added on day 8.

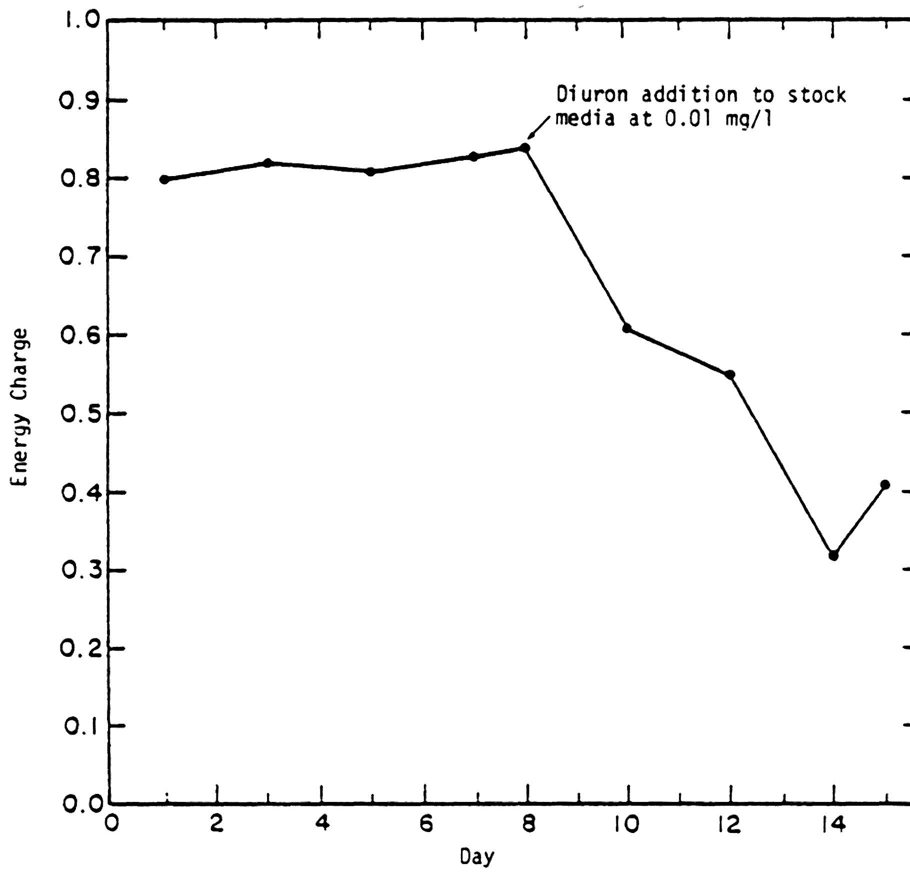


Figure 11. The effect of diuron on energy charge in a continuous culture of Chlorella; diuron added on day 8.

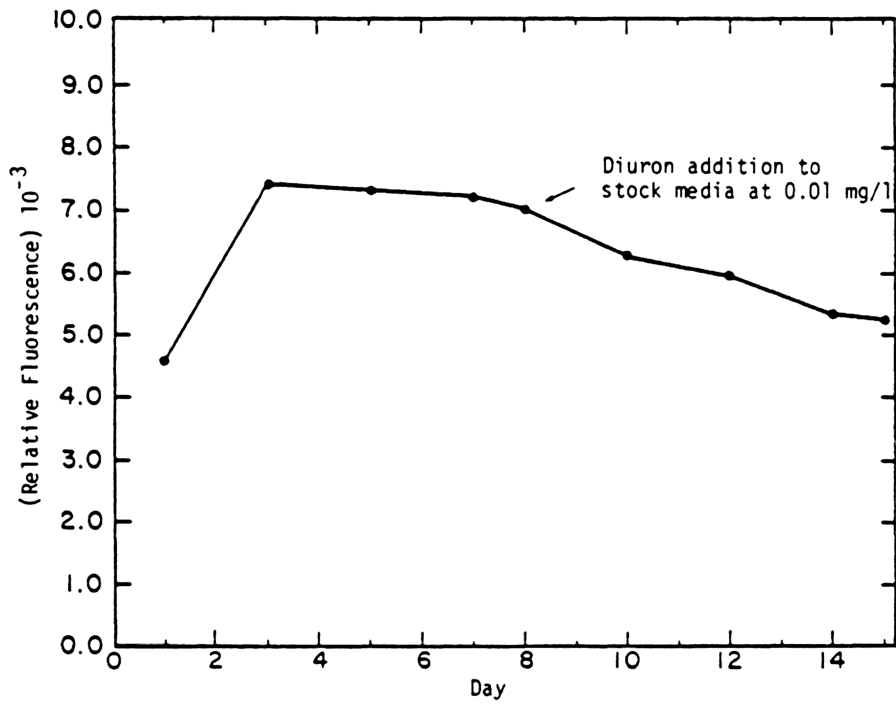


Figure 12. The effect of diuron on fluorescence in a continuous culture of Chlorella; diuron added on day 8.

(Figure 12). Fluorescence levesl increased sharply between days 1 and 3, while cell counts, ATP, and TOC values remained relatively constant. A steady decline in fluorescence was evident throughout the experiment after day 3. However, when 1 mg/l diuron was added to sample aliquots, prior to measurement of relative fluorescence, the data remained relatively constant through day 10. After day 10, relative fluorescence values began to drop off rapidly.

The Effect of Atrazine on a Continuous Culture of Chlorella sorokiniana

The theoretical rate of atrazine addition in this study is provided in Table 37. Figure 13 shows that cell numbers decreased sharply when atrazine was introduced into the continuous culture on day 7. Cell numbers continued to decrease over the following seven day period. Cells decreased in number from an average of 4.4 x 10^6 cells/ml over the seven days of steady state growth to a low of 1.7 x 10^6 cells/ml after seven days in the presence of atrazine.

Total organic carbon (TOC) levels appeared to decrease at a steady rate after the addition of atrazine (Figure 14). The average TOC over the seven days of steady state growth was 32.5 mg/l. The TOC decreased to a level of 17.5 mg/l after seven days in the atrazine containing reactor. This represents a 45 percent decrease in TOC over this period.

ATP and energy charge levels decreased after the introduction of atrazine. The decrease in energy charge appeared to be nearly linear once atrazine was added (Figure 15 and 16).

Table 37. Theoretical Increase of Atrazine Concentrations in the Continuous Flow System

Day	Concentration Atrazine, mg/l
7	0.000
8	0.014
9	0.028
10	0.043
11	0.057
12	0.071
13	0.086
14	0.100

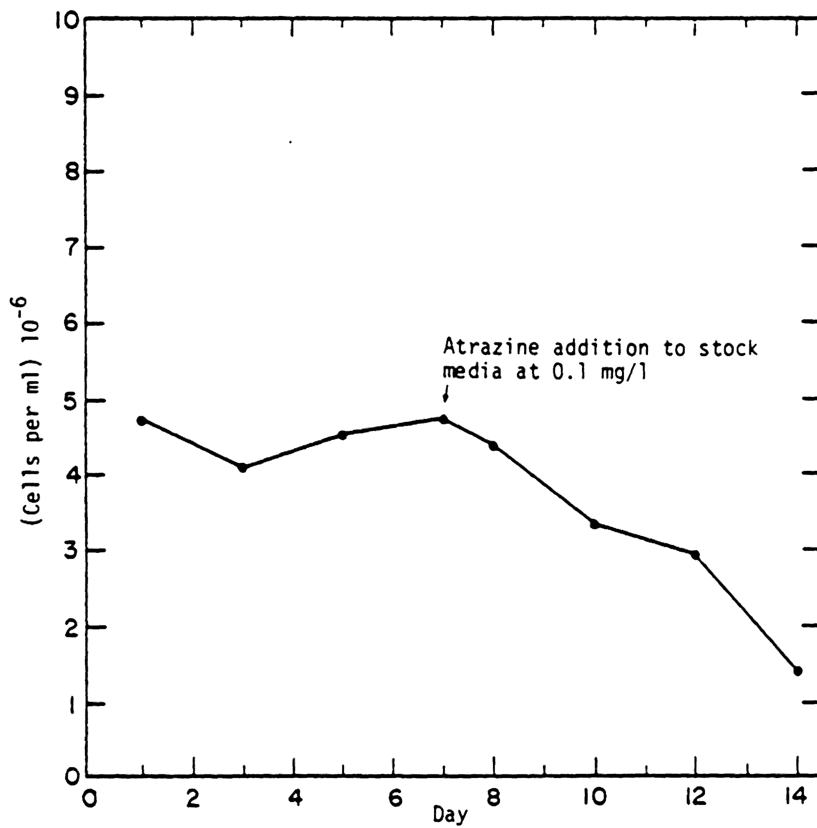


Figure 13. The effect of atrazine on cell numbers in a continuous culture of _Chlorella_; atrazine added on day 7.

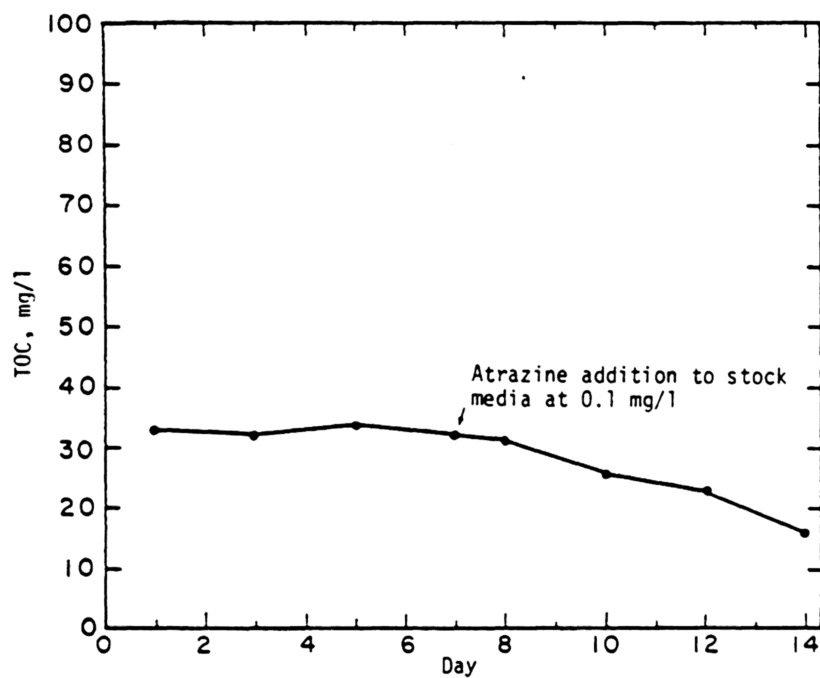


Figure 14. The effect of atrazine on total organic carbon levels in a continuous culture of *Chlorella*; atrazine added on day 7.

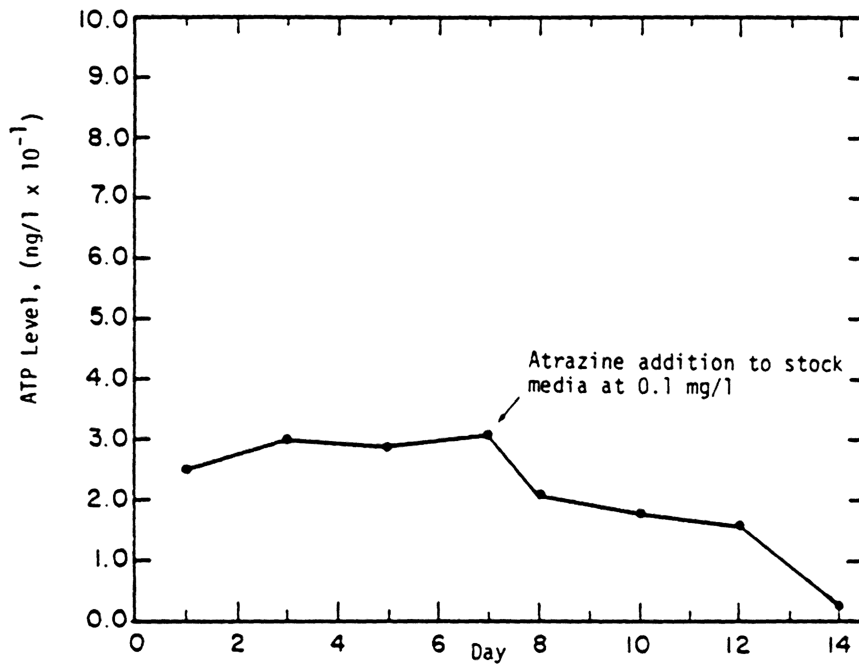


Figure 15. The effect of atrazine on ATP levels in a continuous culture of Chlorella; atrazine added on day 7.

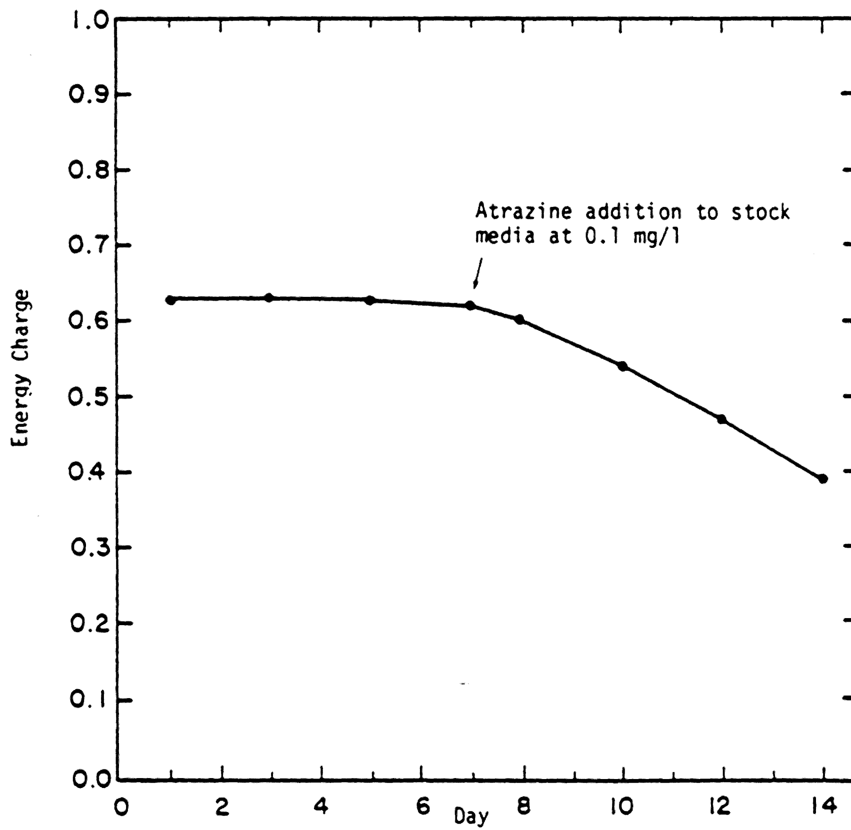


Figure 16. The effect of atrazine on energy charge in a continuous culture of *Chlorella*; atrazine added on day 7.

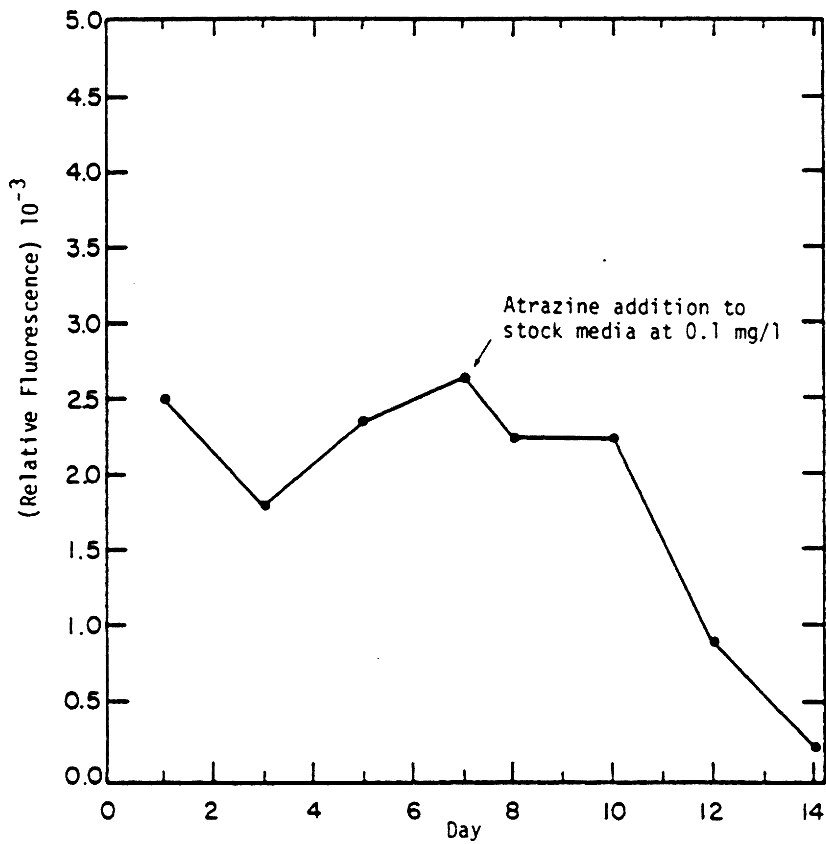


Figure 17. The effect of atrazine on fluorescence in a continuous culture of *Chlorella*; atrazine added on day 7.

The relative fluorescence data appeared to be somewhat erratic during the period in which the reactor was believed to be at steady state (Figure 17). However, if the value obtained on day 3 is ignored, the fluorescence level was quite stable up to day 7 at which time atrazine was added. After day 7 the fluorescence levels decreased dramatically in response to the addition of atrazine (Figure 17).

The Effect of 2,4-D on a Continuous Culture of Chlorella sorokiniana

The theoretical rate at which 2,4-D was administered in this study is provided in Table 38. Figure 18 graphically presents the cell count data for nine days of steady state growth and nine days of growth in the presence of increasing 2,4-D concentrations. The average number of cells over the eight days of steady state was 10,350,000 cells/ml. Cell counts decreased appreciably following the first day of 2,4-D input (day 9) and climbed steadily to a high of slightly over 14,000,000 cells/ml by day 14. The cell level at 14 days was therefore about 35 percent greater than the steady state mean cell level. The initial decrease in growth at 10 days was 26 percent less than the steady state cell level average.

Total organic carbon (TOC) values increased steadily after 2,4-D was added (Figure 19). The average TOC over the steady state period was about 38.5 mg/l. A high of 88.6 mg/l was reached on day 18 or the ninth day after the introduction of 2,4-D. This represents a 130 percent increase over the average steady state TOC level. Increases in TOC were

Table 38. Theoretical Concentration Increase of 2,4-D by Day In the Continuous Flow System.

Day	Concentration, 2,4-D, mg/l
9	0.0
10	2.9
11	5.7
12	8.6
13	11.4
14	14.3
15	17.1
16	20.0
18	20.0

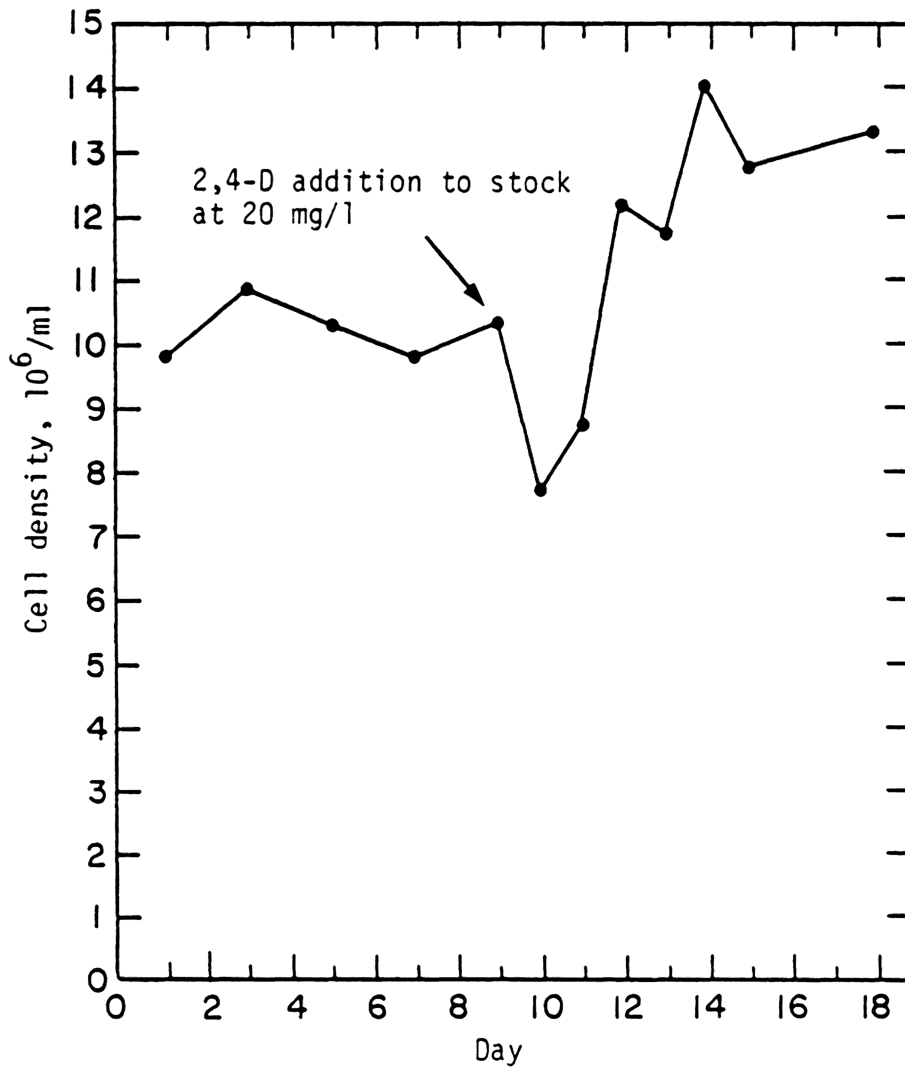


Figure 18. The effect of 2,4-D on cell numbers in a continuous culture of Chlorella; 2,4-D added on day 9.

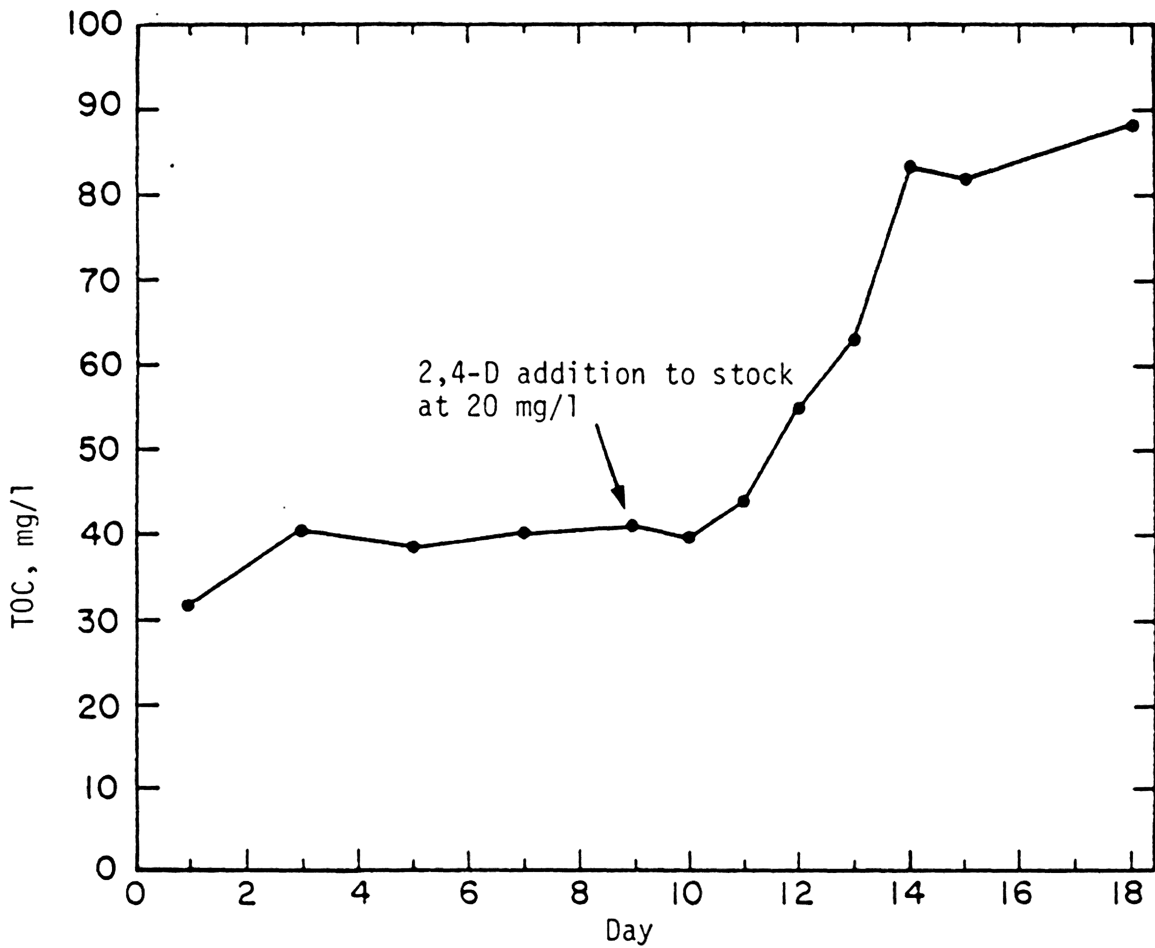


Figure 19. The effect of 2,4-D on total organic carbon levels in a continuous culture of *Chlorella*; 2,4-D added on day 9.

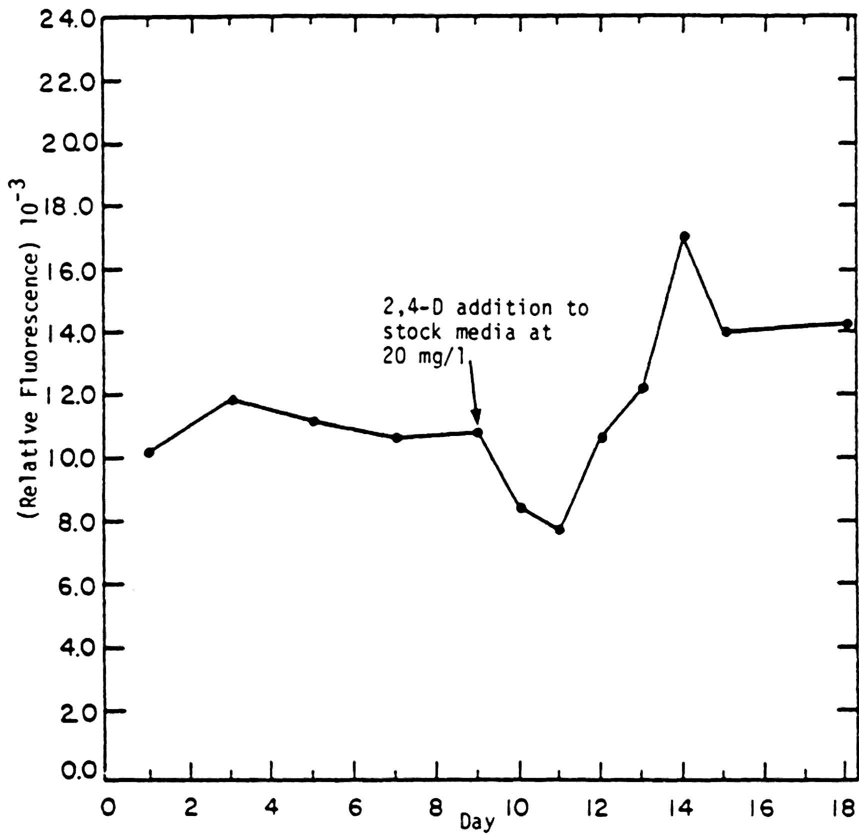


Figure 20. The effect of 2,4-D on fluorescence in a continuous culture of Chlorella; 2,4-D added on day 9.

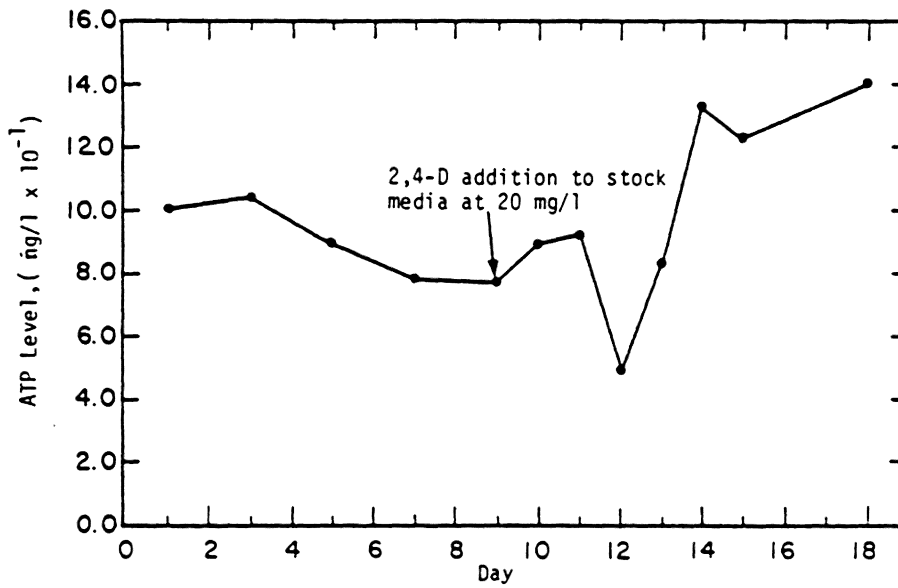


Figure 21. The effect of 2,4-D on ATP levels in a continuous culture of *Chlorella*; 2,4-D added on day 9.

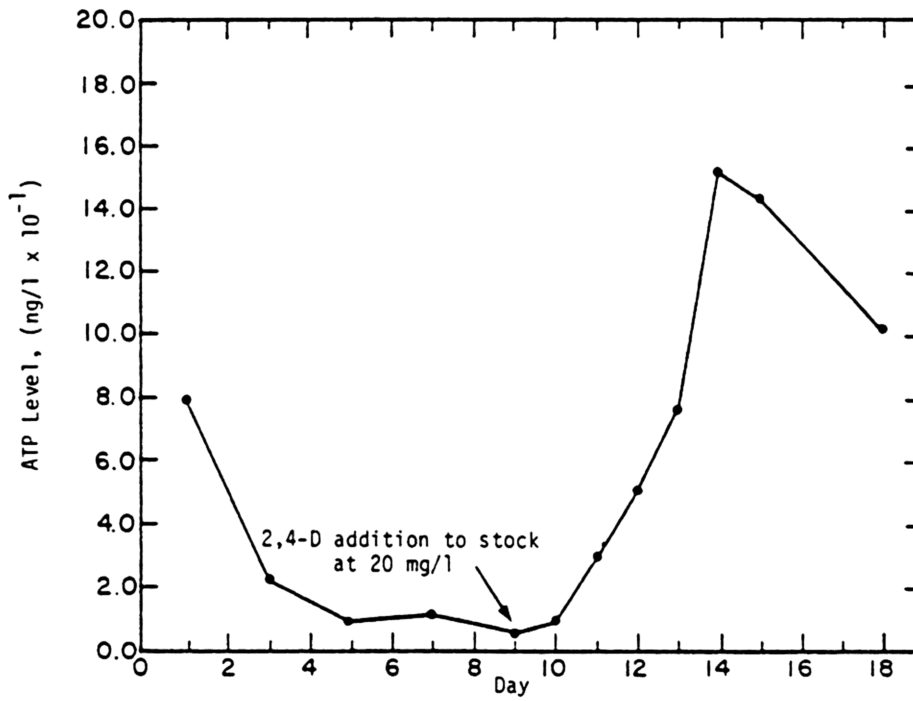


Figure 22. The effect of 2,4-D on AMP levels in a continuous culture of Chlorella; 2,4-D added on day 9.

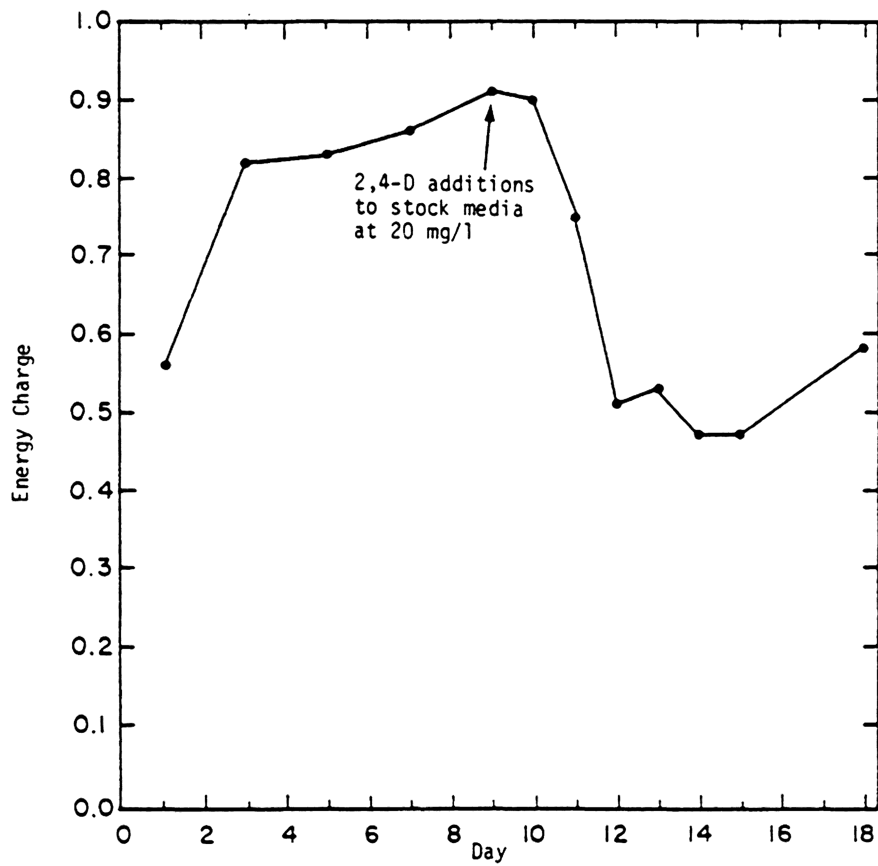


Figure 23. The effect of 2,4-D on energy charge in a continuous culture of Chlorella; 2,4-D added on day 9.

less dramatic after the fourteenth day. Note that there was somewhat of a lag period before TOC levels began to increase. The most rapid rate of increase in TOC values occured between days 11 and 14. The rate of increase during this time period was about 11.25 mg/l/day.

Similar patterns were noted in Figure 20 which presents the _in vivo_ fluorescence. Initial decreases in fluorescence were evident after the addition of 2,4-D, followed by linear increases and then a period in which the levels began to decrease. On day 14 fluorescence levels with and without diuron addition were, respectively, 66 and 55 percent greater than their corresponding average steady state levels.

ATP, AMP, and energy charge data are presented in Figures 21, 22, and 23, respectively. ATP variations with time follow roughly the pattern noted in graphs of the TOC, cell count, and fluorescence data. Following the introduction of 2,4-D, ATP levels increased slightly over a two-day period, but then there was a marked decrease in ATP on the twelth day. The level of ATP then increased rapidly through day 14 after which time ATP concentrations were rather stable. AMP levels steadily increased after the input of 2,4-D until day 14. The concentrations of AMP then decreased rapidly after 14 days. The energy charge decreased rapidly after 2,4-D was introduced on day 12. After 12 days, the energy charge was considerably lower than the steady state value, but was rather constant through to the end of the study.

V. DISCUSSION

Field Studies with 2,4-D

Additions of 2,4-D to test vessels at Smith Mountain Lake at times produced significant changes within the indigenous population (Table 2). While these population shifts were statistically significant, it is doubtful that they could be interpreted to be environmentally significant given the high concentrations of 2,4-D tested. The concentration of 2,4-D within natural water appears to rarely exceed 1 mg/l. A notable exception being in the use of 2,4-D to control aquatic weeds where concentrations in the range of 5 mg/l are common (101).

Significant increases were occasionally noted in the concentrations of ATP and chlorophyll in response to the introduction of 2,4-D were noted in those experiments in which the nutrients, orthophosphate and/or ammonia, were added to stimulate the overall growth of the phytoplankton community (Table 5).

In Field Study I (FSI) ATP values were initially significantly depressed in 2,4-D treatments. This would indicate that 2,4-D had an initial toxic effect to some constituents of the biomass or to the biomass as a whole. Mean ATP concentrations did increase above control values in all 2,4,-D treatments. This difference, however, was not statistically significant which can be largely attributed to a large variance within replicates. This tendency for greater variances with

time in the raft samples was consistently true for all field study experiments. This perhaps was attributable to the containerization effect on the biomass or to the relative position of the sample containers within the raft. However, the obvious trend for all 2,4-D treated samples in this study was toward greater overall ATP concentrations. While, statistically, the results are not clear, certainly the trend is significant. The increase in ATP above control values would suggest that 2,4-D may be enhancing the growth of biomass. This would be consistent with the literature in which 2,4-D has been shown to stimulate growth for numerous organisms (11). Studies suggest that 2,4-D can be used as a carbon source (11,65,72) or as a growth stimulant for plants, possibly in the role of a hormone (3,10-12,48,104,108).

The significant increase in chlorophyll levels in 5 and 10 mg/l on day 3 would indicate that 2,4-D had a stimulatory effect on the phytoplankton population (Table 2). It cannot be stated that this was a direct or indirect effect of 2,4-D. It is conceivable that nutrients released from dying organisms in response to toxicity of 2,4-D may have contributed to the increase in phytoplankton biomass. However, the literature does report numerous instances of chlorophyll increases in laboratory studies of algae in the presence of 2,4-D and this possibility cannot be ruled out (32,33,48,78). The increase in chlorophyll levels on day three in 5 and 10 mg/l 2,4-D cannot be attributed to a major shift in species distribution. Therefore, it must be assumed that the population as a whole increased. Reasons for this

phenomenon can only be speculated upon. It is possible that a decrease in competition from the non-photosynthetic population occurred during the initial toxic shock imposed by 2,4-D and that the entire population was able to take advantage of the opportunities. Of course, the availability of more nutrients may have also been a significant factor.

Field Study II (FSII) provided more definitive information about the effects of 2,4-D treatment than did FSI. However, as with FSI, the replicate data became more variable with time in all test vessels. This accounts for the difficulty in noting significant statistical differences even though the mean values appear to be widely different Table 5).

Day 1 ATP concentrations of the treated samples were significantly depressed below control values. This is consistent with FSI data for the initial measurements. This would indicate that 2,4-D was detrimentally affecting some life within the vessels. However, levels of ATP tended to recover over the period of time from day 3 to 7, suggesting tolerant organisms were increasing.

Greater chlorophyll values in treated samples containing all levels of 2,4-D once again indicated that phytoplankton were capable of increasing their biomass in the presence of 2,4-D (Table 5). In this study it was apparent that two species of green algae, Kirchneriella sp. and Chlorella sp. were able to take advantage of the 2,4-D application to the exclusion of a blue-green algae, Synecchoccus sp. (Table 6). Laboratory studies using Chlorella and Synecchoccus have shown growth

stimulation of both of these organisms in the presence of 2,4-D (32,33,59,104). However, other studies, including this one, often cite no effects on growth. While obvious changes in the algal community were occurring in 2,4-D treated samples, no significant changes were occurring as measured by diversity (Figure 5). The predominant species, _Synecchoccus sp._, was apparently out competed by species of _Chlorella_ and _Kirchneriella_ in the presence of 2,4-D. This is a significant finding in that it points out that 2,4-D is capable of shifting the natural succession of predominanting organisms within a community and increasing the biomass of the successful species. Further importance to this finding is evident in the fact that phytoplankton blooms have been reported following the application of 2,4-D (43). Changes in the types of algal predominating in a community structure could conceivably result in an overgrowth of a given species if natural competition barriers were eliminated (36,37).

Correlations between ATP and chlorophyll data for this study were not evident. That is, increases in ATP and chlorophyl did not necessarily coincide (Table 5). This would indicate that the relationship between the phytoplankton community and non-chlorophyll containing organisms cannot be delineated using information gathered from these tests. The relationships within the community are apparently too complex to lend themselves to interpretation by this means. Also, since ATP values were not dramatically increased to significantly greater levels than control values when chlorophyll values were

significantly increased (Table 5), the increases in biomass attributable to the stimulation of growth by 2,4-D were probably minimal. Additionally, the subsequent decrease in chlorophyll following the significant increase would suggest that 2,4-D did not have a lasting effect on the phytoplankton community. Any effects by 2,4-D were apparently short term and did not result in major departures in the populations of the natural microflora.

Data for Field Study III (Table 8) was similar to FSII. Significant increase in ATP was evident. Field Study IV was also similar to FSII in that significant increases in chlorophyll were recorded (Table 11). Microscopic data (Tables 9 and 12) would indicate that shifts occurred within the phytoplankon community in which dominanting species were altered in the presence of 2,4-D. Species of Chlorella, Coelastrum, Kirchneriella, and Synecchoccus dominated in the presence of 2,4-D and not in control samples. At no time, however, did any one species grow to the exclusion of all other species in bloom proportions.

One notable departure from data collected during FSII was evident in FSIV (Tables 5 and 11). The ATP in the reactors treated with 10 mg/l 2,4-D increased some sevenfold over controls between days four and six, while chlorophyll levels decreased by roughly twofold. The suggestion here is that the growth of non-photosynthetic organism(s) was significantly enhanced which may have posed limitations on the phytoplankton population. This is further evidence that the

complications of competition with the aquatic community makes prediction of a response difficult.

The addition of filter sterilized sewage to the reactors of FSV assured eutrophic conditions. No significant increases or decreases were noted in ATP or chlorophyll values at any 2,4-D test level (Table 14). Furthermore, no unusual growth patterns were noted through microscopic examination of reactor contents (Table 15).

Field Studies with Diuron

Field Study VI (FSVI) reactors received filter sterilized sewage in addition to herbicides to assure conditions conducive for maximum growth. ATP levels were significantly depressed on days 2 and 5 (Table 17) in all reactors inoculated with diuron. Concentrations of ATP did increase in each vessel after eight days, however, the concentrations remained significantly less than controls. The levels were still less than ATP control levels, however, in each treated vessel except for the reactor treated with 1 mg/l diuron. These data therefore demonstrate that, although diuron had a toxic effect on growth, the microorganisms were able to recover quite readily.

Chlorophyll levels were significantly depressed in all treated vessels on days 5 and 8 (Table 17). The effect decreased with decreasing concentrations of diuron. This was expected for diuron, because it is a widely used photosynthetic inhibitor and has been

reported to depress the growth of algae at the concentrations used (52). However, chlorophyll fluorescence did increase with time at all test concentrations indicating that some algal species may be resistant to diuron.

Microscopic examination of the treated and control waters indicated that diuron caused a shift in predominant algal forms (Table 18). A coccoid *Synecchoccus* sp. was predominate in treated samples, while a rod-shaped *Synecchoccus* sp. was predominate in the controls. The dominance shift increased with increasing diuron concentrations. Most of the algae in the reactor inoculated with 1.0 mg/l diuron after 5 days appeared to be *Synecchoccus* sp. The ATP concentration at this time was greater than in the control vessel, but chlorophyll levels were well below control levels. This would suggest that the dominant alga did not comprise the bulk of the biomass at this treatment concentration. However, the strong shift to a single, dominating species could exert a significant environmental impact in a natural system.

Field Study VIII (FSVIII) results indicated that diuron, at all concentrations tested, suppressed growth over the period of the study. Like FSVI, growth was depressed more at increasing diuron concentrations. Both chlorophyll and ATP concentrations were depressed significantly by addition of diuron (Table 23). Some recovery of chlorophyll was noticed in the 0.01 mg/l atrazine treatment on days 5 and 7, but the levels were still below control values.

Microscopic examination of the water indicated that the dominating algal species were changed dramatically in treated vessels (Table 23). Treated samples were largely dominated by blue-green algae. The most prolific species in the treated samples included *Synecchoccus* and *Gloeocapsa* (Table 24). Diversity studies (Figure 6) indicated that all levels of diuron tested significantly decreased the diversity of algae within the test containers. Diversity decreased with increasing concentrations of diuron.

Field Studies with Atrazine

In Table 26, one can see large apparent differences in statistical means yet no significant differences are noted. Again, as with other field studies, data variance became greater with increasing time. The large variances attributed greatly to the decrease in statistics as a useful tool for measuring differences. ATP and chlorophyll levels (Table 26) were, in general, less in treated vessels than in control vessels. This was not, however, always the result at a treatment level of 0.1 mg/l. In this case ATP values were lower than control values on days 3 and 9 but were slightly elevated on day 7. Chlorophyll values in the treated vessels were initially lower than control values but increased to concentrations significantly greater than control values on days 7 and 9. This finding is consistent with the work of others who have reported that chlorophyll production was inhibited at 1 mg/l

atrazine but was stimulated in the same study at 0.1 mg/l (88). Increased growth of test algae has also been reported by several authors (88,96). The decreases in ATP and chlorophyll values at the 0.01 mg/l atrazine treatment level noted in this work do not appear to be consistent with the findings of other investigators.

Microscopic examination (Table 27) of reactor water again indicated that the herbicide caused a shift in the dominant forms of algae. In the first few days of the study, Synecchoccus sp., was predominant at the two higher levels of atrazine studied, whereas Chlorella sp. was predominant at 0.01 mg/l atrazine. The control vessels contained mostly Gloeocapsa sp. However, after 7 days, the treated vessels contained mostly Gloeocapsa sp. The shift resulted in nearly total domination by Gloeocapsa sp. in the reactors inoculated with 0.1 and 0.01 mg/l atrazine. Examination of these reactors on day 11, however, indicated that the populations had reestablished themselves at a proportion similar to that of the control populations. This was not true at a 1.0 mg/l treatment.

The significant increase in growth of algae at the 0.1 mg/l atrazine treatment was apparently the result of an increase in the population of Gloeocapsa sp. This effect may have been the direct effect of atrazine on the algae. Studies have linked increases in the growth of algae to the presence of atrazine (88). However, it seems more likely that the increase in population was due to the increased availability of some nutrient or improved condition for growth as a

result of atrazine effects on the ecosystem. This is a difficult concept to prove experimentally, and it was not addressed within the scope of this research. However, this finding might prove useful in future studies of ecosystem dynamics. Certainly, the increase in the population of this algae and the apparent disruption of the algae population as a whole by atrazine poses some serious questions as to the effect of this chemical on the ecosystem.

The Effect of Diuron on a Continuous Culture of Chlorella sorokiniana

The effect of diuron on growth, as measured by ATP, energy charge, TOC, and cell counts, was immediate. Concentrations of diuron after one day in the continuous flow unit were calculated to be 0.0014 mg/l. Growth measurements (Figure 8) on this day would indicate that this level of diuron inhibited growth of the test algae. However, although growth was suppressed, viable algae were still plentiful until the end of the experiment. Cell numbers (Figure 8) decreased by approximately 30 percent over this time period, and it was apparent that the rate of loss was decreasing by the end of the eighth day. The energy charge (Figure 11) increased slightly between days 14 and 15, indicating that cells were apparently acclimating somewhat to the presence of diuron. However, the energy charge value of 0.42 on the final day of study indicates that the cells were still under stress. Chapman _et al._ (29) showed that _E. coli_ cells with an energy charge below 0.5 were dying.

However, other growth parameters indicated that the algal cells were still viable on the final day of the study.

The Effect of Atrazine on a Continuous Culture of Chlorella sorokiniana

Atrazine decreased the growth of *Chlorella sorokiniana* as was expected. Concentrations inhibiting the growth of this algae were consistent with values reported in the literature (39). Growth parameters decreased immediately in all cases after the first day of atrazine addition. The theoretical concentration of atrazine in the reactors after one day was 0.014 mg/l. Cell production decreased approximately 62 percent over the period of atrazine addition. Viable cells were still present after seven days at which time the atrazine level was 0.1 mg/l. Energy charge measurements declined steadily which indicates that the cells were being stressed.

Chlorophyll levels were most affected by the addition of atrazine. Fluorescence decreased below the steady state average by 91 percent after seven days. Since atrazine inhibits the Hill reaction, this result was anticipated (5).

The Effect of 2,4-D on a Continuous Culture of Chlorella sorokiniana

Energy charge measurements of the continuous flow culture of *Chlorella sorokiniana* indicated that the cells were under stress after

contacting 2,4-D. This was particularly obvious from measurements of the AMP pool. The AMP values increased steadily for five days after 2,4-D was added to the system. Interestingly, ATP values also increased over the study period after 2,4-D was introduced. Therefore, it is apparent that both cell anabolic and catabolic processes were increasing simultaneously. A brief decline was, however, noticeable on days 12 and 13. The increase in ATP occurs in synchrony with the large increases in TOC and cell numbers noted (Figures 18, 19 and 21).

This information suggests that both anabolic and catabolic processes within the cells were stimulated. The fact that the cells increased in numbers suggests that the influence of the energy producing cycles was overshadowing any stimulation of wasteful processes such as photorespiration. The dramatic increase in AMP concentrations suggests that energy consuming processes were operating at a rapid rate. These data support the hypothesis that 2,4-D was enhancing the effectiveness of the photosynthetic pathway. Increases in both ATP and AMP would indicate that energy was being produced and consumed at rates in excess of the rates established under steady state conditions.

The initial decreases noted in ATP, cell counts, and chlorophyll indicate that catabolic processes were initially overshadowing growth stimulation. This could mean that enzymes responsible for the stimulation of growth were not yet present in sufficient concentrations to overcome catabolic process increases. This would suggest that 2,4-D

may be responsible for stimulating enzyme production for an anabolic process.

Growth reached a maximum on day 14 when the theoretical concentration of 2,4-D was 14.3 mg/l (Figure 18). Fluorescence decreased after day 14 (Figure 20) while cell counts (Figure 18), TOC (Figure 19), and ATP values (Figure 21) appeared to have reached a steady state condition. It is possible that growth of the test algae was limited by something other than 2,4-D. However, it seems likely that concentrations of 2,4-D had reached a level in which growth stimulation was in a reasonable state of equilibrium with any detrimental effects of 2,4-D.

A 50 mg/l increase in TOC cannot be accounted for by an addition of 20 mg/l 2,4-D (8.6 mg/l of carbon). Valentine and Bingham (92) were able to show that a species of _Scenedesmus_ produced metabolites of 2,4-D. However, it appears that no one at this point has demonstrated that algae can utilize 2,4-D as a carbon source. This would suggest that the growth increase was probably due to stimulation of some metabolic process associated with energy production and/or utilization.

The Effects of 2,4-D on the Growth of a Blue-green Algae Isolate from Smith Mountain Lake

The growth of a non-axenic _Synecchoccus_ sp. isolate was significantly increased in three concentrations of 2,4-D (Table 33).

This result is consistent with the previous studies of Cox and Boardman (33).

Increased growth rates were apparent earlier in the study at higher concentrations than at lower concentrations of 2,4-D. This was also true for ATP and the relative fluorescence of test cells. Scenescence was obvious at the highest 2,4-D concentration (10 mg/l) tested after ten days of growth. This was not the case in the other two treatments. It was apparent that the concentration of 2,4-D used had little, if any, effect on the total biomass attained. At all levels of 2,4-D total biomass in the reactors, as measured by ATP, was essentially the same. However, at lower 2,4-D concentrations a longer period of time was required for the cells to reach the upper limit of growth. This would suggest that the effect of 2,4-D may have been to increase the activity of some enzyme system responsible for growth. The literature contains several reports of enzyme activity stimulation by 2,4-D (108). Included on the list of enzymes which are known to be affected are phosphatase and phosphorylase enzymes which take part in the regulation of energy flow in cells (62). Studies by Bertagnoli and Nadakavukaren (12) indicated that 2,4-D stimulated both net production and uptake of oxygen. This suggests that 2,4-D may influence the Hill reaction by stimulating an enzyme (or enzymes) involved in this process and cell respiration. Additionally, Weddings _et al._ (104) showed that 2×10^{-3}M 2,4-D stimulated photosynthesis in _Chlorella_. Higher concentrations had an inhibiting effect. This suggests that 2,4-D may have an effect on

more than one process associated with photosynthesis and/or respiration.

The data in Tables 31 and 32 indicate that 2,4-D increased the uptake of bicarbonate in _Synecchoccus_ sp. cells under both light and dark conditions. This suggests that 2,4-D affected either the process responsible for the uptake of bicarbonate or some pathway requiring carbon dioxide. Bertagnoli and Nadakavakaren (11) showed that a synchronously grown _Chlorella pyrenoidosa_ culture produced greater amounts of glycolate in the presence of 2,4-D. This study showed that the site for 2,4-D action was in the chloroplast. This suggested to the authors that by increasing the partial pressure of oxygen within the cell, 2,4-D was indirectly responsible for inhibiting the enzyme involved in fixing CO_2 in the Calvin cycle, ribulo-sediphosphate carboxylase. This enzyme is inhibited by high molecular oxygen partial pressures. Furthermore, under conditions of high partial pressure, molecule oxygen can replace CO_2 in the Calvin Cycle which results in the formation of free glycolate instead of glucose. This process is called photorespiration and it takes place normally during periods when plants cannot utilize all of the energy produced in the splitting of water. As molecular oxygen is utilized or diffuses out of the cell and respiration within the cell and bicarbonate uptake build CO_2 partial pressures, the Calvin Cycle can become operational. The concentrations of 2,4-D used in this study were over five times that utilized in Bertagnoli's research. However, the information suggests an interesting hypothesis. If 2,4-D caused similar responses in the _Synecchoccus_ sp., the partial pressure

of CO_2 would build due to increased uptake rates and the Calvin Cycle would become operational. The buildup of CO_2 would be further compounded if net respiration rates were greater than the production of oxygen in the reaction. Short-term assays conducted with Chlorella sorokiniana indicated that overall there was a measurable net decrease in oxygen when the cells were subjected to 2,4-D at concentrations exceeding 10 mg/l. This would indicate that respiration processes were operating at a higher rate than the Hill reaction which yields oxygen. This phenomena is widely recognized in the literature (5). Additionally, the results of short-term testing indicated that fluorescence values increased at 30 mg/l 2,4-D (Table 34). This would indicate that 2,4-D was causing cellular chlorophyll to waste energy as fluorescence (82). This is good supportive evidence that photorespiration was occurring as a result of a possible increase in the activity of the Hill reaction. After four minutes, fluorescence levels peaked and then began to decrease over the following thirty minutes of the study. This would suggest that recovery of the reduction cycle was taking place, possibly as a result of the increased availability of CO_2. Since photorespiration decreases the supply of ribulose-1,5-diphosphate, the demand for available glucose should increase within the cell. The increase in oxygen production by the Hill reaction results in an increase in the availability of reducing energy which can be utilized in the Calvin Cycle. The overall effect may be an increase in the rates of reactions in both the Calvin Cycle and photorespiration.

VI. SUMMARY AND CONCLUSIONS

Field Studies

The field study results showed that atrazine, diuron, and 2,4-D altered the population of the phytoplankton community within Smith Mountain Lake.

Studies using 2,4-D showed that the diversity of algae within the lake was not significantly altered under the conditions of these experiments. However, it was shown that the species dominating growth was often changed in the presence of 2,4-D. Genera that most often dominated in the presence of 2,4-D included *Kirchneriella*, *Synecchoccus*, and *Chlorella*. The change in species domination was normally associated with increases in chlorophyll concentration. Overall biomass, as measured by ATP, did not significantly increase above control concentrations when chlorophyll levels increased. However, there were significant increases and decreases in ATP which could not be attributed to the phytoplankton community. This indicates that 2,4-D was being utilized by some non-photosynthetic organisms as a carbon soruce and was toxic to others.

Atrazine significantly altered both species diversity and species dominance at 0.1 mg/l and 1.0 mg/l. However, species diversity did recover to control levels in 0.1 mg/l atrazine. Blue-green algae were

the predominant surviving organisms in the presence of atrazine. The number of the blue-green alga Gleocapsa significantly increased in the presence of 0.1 mg/l atrazine. Significant increases in ATP and chlorophyll concentrations correlated well with the increase in the Gleocapsa population.

Diuron significantly decreased both biomass (as measured by ATP) and chlorophyll levels in the field experiments at concentrations ranging from 0.01 mg/l to 1.0 mg/l. Blue-green algae were able to survive all concentrations tested and predominated the phytoplankton community. Species diversity was decreased at all the concentrations tested.

Physiological Responses of Unialgal Cultures

Growth of a continuous culture of Chlorella sorokiniana was stimulated by 2,4-D. Growth, as measured by TOC, increased 130 percent over steady state levels when the alga was exposed to 20 mg/l 2,4-D. Cell counts increased 35 percent and the relative fluorescence of chlorophyll increased 55 percent. Levels of both ATP and AMP increased significantly in these experiments. This indicates that both energy production and energy utilization were increasing in the 2,4-D treated cells. The energy charge of the culture, as indicated by the relative quantities of ATP and AMP, decreased rapidly in the presence of 2,4-D. This indicates that the cells were stressed despite increases in the population density.

In other continuous flow experiments it was not possible to stimulate growth by exposing cells to 2,4-D. However, as before, AMP levels always increased dramatically. Based on the results of this study no explanation may be offered for the inconsistencies of these experiments.

The results of studies to assess the effects of short-term exposures of algae to 2,4-D on oxygen production and utilization under well-lighted conditions using Chlorella sorokiniana revealed that consumption of oxygen was greater than production. This suggests that the respiration rate was greater than the photosynthetic rate. Additionally, fluorescence initially increased in the presence of 2,4-D, thus indicating that energy was either being supplied to the photosynthetic process at a greater rate or that the photosynthetic process was inhibited and, therefore, was wasting energy. This was a temporary effect and fluorescence returned to control levels indicating that the cells were able to recover.

Growth of a non-axenic, batch culture of Synecchoccus sp. isolated from Smith Mountain Lake was stimulated at 2,4-D concentration ranging from 0.002 mg/l to 20 mg/l 2,4-D. Initial studies using this Synecchoccus sp. showed significant increases in the uptake of $^{14}C(HCO_3)$ in the presence of 10 mg/l 2,4-D under both light and dark conditions. However, these results could not be reproduced in experiments which were performed four to six months later. Cultures of Navicula sp., Chlorella

sp. and Chlorocloster sp. were unaffected by 2,4-D under the same growth conditions.

Continuous flow studies with Chlorella sorokiniana indicated that growth was inhibited by diuron at concentrations ranging from 0.0014 mg/l and 0.01 mg/l.

Growth of a continuous culture of Chlorella sorokiniana was significantly inhibited by atrazine at concentrations in the area of 0.014 mg/l and 0.1 mg/l.

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APPENDIX A

FIELD AND LABORATORY DATA BASE

Table A1. Experimental Results from 10 days of Continuous Culture of Chlorella Pyrenoidosa.

1.	2.	3.	4.	5.	6.	7.	8.	9.
1	00	6175000	5300	49.5	13.6	0.6	4.6	0.75
2	00	2240000	2150	31.9	09.0	0.6	5.5	0.65
3	00	4000000	3700	31.9	08.8	0.1	5.3	0.62
1	01	5930000	5300	57.4	10.6	0.4	7.0	0.60
2	01	2550000	3000	28.8	06.2	1.3	3.8	0.60
3	01	4010000	4100	36.7	09.6	0.1	7.8	0.56
1	03	6200000	5100	45.7	09.6	1.0	7.0	0.57
2	03	1660000	1300	22.6	03.0	1.1	6.5	0.34
3	03	3980000	3900	30.9	12.4	0.1	7.1	0.63
1	07	4920000	6150	36.9	08.5	1.2	6.9	0.56
2	07	3220000	3600	25.7	08.7	3.4	6.6	0.55
3	07	4660000	6150	33.4	15.1	1.4	8.6	0.63
1	10	4720000	4300	32.1	08.4	1.1	6.8	0.59
2	10	1660000	1950	23.2	08.6	2.3	6.9	0.58
3	10	3240000	3300	24.7	14.8	1.3	8.8	0.63

Legend: 1. = Reactor No., 2. = Day, 3. = Cells/ml., 4 = Relative Fluorescence units, 5. = TOC (mg/l), 7. = ATP, ng/ml, 8. = ADP ng/ml, and 9. = Energy Charge

Table A2. Experimental Results from 10 days of Continuous Culture of Chlorella Pyrenoidosa. (Study two)

1.	2.	3.	4.	5.	6.	7.	8.	9.
1	00	4760000	6000	29.3	7.0	0.1	6.4	0.52
2	00	3140000	3000	20.6	4.6	0.2	1.2	0.78
3	00	5010000	5550	26.6	7.2	0.0	7.1	0.50
1	03	5800000	5250	32.4	7.9	0.7	3.2	0.70
2	03	3820000	3000	21.1	4.4	3.6	5.9	0.45
3	03	4300000	4200	24.5	9.9	0.0	3.3	0.75
1	04	5540000	5250	31.0	5.8	0.0	1.6	0.78
2	04	4600000	4050	20.0	5.3	0.1	8.3	0.39
3	04	6000000	6300	23.8	8.1	0.0	4.6	0.64
1	05	4760000	3000	27.1	7.3	0.8	6.5	0.53
2	05	4880000	3450	19.3	.	.	.	.
3	05	6700000	5550	28.4	6.3	5.8	0.0	0.76
1	07	7500000	7050	32.0	5.7	4.6	4.9	0.53
2	07	5120000	4350	22.8	2.5	3.5	7.1	0.32
3	07	5840000	6000	21.3	.	.	.	.
1	10	7680000	5850	31.6	.	.	.	.
2	10	3880000	2550	17.5	5.4	0.1	2.6	0.67
3	10	7750000	7350	23.8	7.8	0.0	3.1	0.72

Legend: 1. = Reactor No., 2. = Day, 3. = Cells/ml., 4 = Relative Fluorescence units, 5. = TOC (mg/l), 7. = ATP, ng/ml, 8. = ADP ng/ml, and 9. = Energy Charge

Table A3. Responses of Chlorella pyrenoidosa to 2,4-D in a Continous Flow Reactor.

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
00.0	1	01	07660000	08.70	09.60	40.7	09.2	0.1	07.6	0.54
00.0	2	01	03880000	03.90	05.10	26.2	02.8	0.6	05.9	0.33
00.0	3	01	09840000	10.20	11.85	31.8	10.1	0.3	07.9	0.56
00.0	1	03	10050000	06.00	09.55	27.8	10.6	0.1	02.6	0.80
00.0	2	03	04010000	03.90	06.00	28.2	12.0	0.1	02.6	0.81
00.0	3	03	10850000	11.90	15.75	40.7	10.5	0.2	02.2	0.82
00.0	1	05	07280000	07.65	09.15	31.2	09.8	0.1	04.1	0.71
00.0	2	05	03280000	03.90	04.95	23.1	06.0	0.1	01.8	0.77
00.0	3	05	10300000	11.20	14.20	38.8	09.0	0.4	00.9	0.83
00.0	1	07	06930000	06.30	09.00	32.9	08.1	0.1	00.9	0.90
00.0	2	07	03440000	03.95	04.80	26.1	05.2	0.1	00.6	0.87
00.0	3	07	09780000	10.70	14.20	40.4	07.9	0.7	01.1	0.86
00.0	1	09	07110000	05.90	08.75	31.0	08.2	0.1	02.2	0.79
00.0	2	09	03800000	03.75	04.80	24.2	05.3	0.1	01.3	0.81
00.0	3	09	10350000	10.90	14.30	41.2	07.8	0.6	00.5	0.91
03.0	1	10	06800000	06.00	09.15	31.4	07.5	0.7	04.2	0.63
03.3	2	10	02340000	01.95	03.95	23.2	04.4	0.2	00.9	0.77
03.5	3	10	07700000	08.55	11.45	39.9	09.0	0.2	00.9	0.90
06.0	1	11	04820000	04.35	06.00	33.2	08.0	0.3	06.3	0.56
06.5	2	11	02340000	02.10	03.45	21.2	04.4	0.1	02.8	0.61
07.0	3	11	08750000	07.80	11.45	44.3	09.3	0.6	02.9	0.75
09.0	1	12	04920000	05.25	06.15	33.0	05.1	0.6	04.5	0.53
09.8	2	12	04300000	03.45	05.25	23.1	03.3	0.6	02.5	0.56
10.5	3	12	12150000	10.80	15.15	55.3	05.0	0.9	05.0	0.51
12.0	1	13	05950000	08.40	10.15	35.1	05.5	1.1	04.9	0.53
13.1	2	13	04720000	04.35	04.80	34.2	03.2	0.4	02.8	0.53
14.0	3	13	11700000	12.30	23.40	63.5	08.4	0.9	07.6	0.53
15.0	1	14	05480000	08.85	10.35	43.3	06.5	0.1	08.8	0.43
16.4	2	14	04430000	04.05	04.95	42.1	03.8	0.1	08.6	0.31
17.5	3	14	14000000	17.10	20.70	83.6	13.4	0.1	15.2	0.47
18.0	1	15	05120000	06.00	08.55	41.0	08.2	0.1	16.9	0.33
19.7	2	15	04050000	03.75	04.80	41.6	05.9	0.1	23.4	0.20
20.0	3	15	12750000	14.10	20.70	82.3	12.4	0.9	14.3	0.47
20.0	1	18	05200000	08.55	11.55	43.7	08.6	0.8	10.0	0.46
20.0	2	18	04420000	06.00	06.45	43.1	06.1	0.4	13.8	0.31
20.0	3	18	13300000	14.40	18.50	88.6	14.1	0.1	10.1	0.58

Legend: 1. = Concentration, mg/l, 2. = Reactor no., 3. = Day, 4. = cells/ml., 5. = Relative Fluorescence (x 1000), 6. = Relative Fluorescence (x 1000) w/Diuron, 7. = TOC mg/l, 8. = ATP ng/ml, 9. = ADP ng/ml, 10. = AMP ng/ml, and 11. = Energy Charge.

Table A4. Growth Response of Smith Mountain Lake <u>Synecchoccus sp.</u> isolate to 2,4-D at pH 7.0.

1.	2.	3.	4.	5.	6.
01	1	1	00475	.	00162
01	1	2	00495	.	00115
01	1	3	00630	.	00234
01	1	4	00421	.	00126
01	4	1	00372	.	00152
01	4	2	00227	.	00108
01	4	3	00295	.	00144
01	4	4	00754	.	00210
01	6	1	00281	.	00138
01	6	2	00312	.	00150
01	6	3	02612	.	00069
01	6	4	00306	.	00108
01	8	1	01522	.	00120
01	8	2	00250	.	00120
01	8	3	02259	.	00201
01	8	4	00395	.	00159
03	1	1	00417	.	00153
03	1	2	00357	.	00143
03	1	3	00385	.	00200
03	1	4	00380	.	00103
03	4	1	00758	.	01167
03	4	2	00435	.	00833
03	4	3	00472	.	00600
03	4	4	00680	.	00800
03	6	1	00727	.	00711
03	6	2	00449	.	00733
03	6	3	00387	.	00233
03	6	4	00556	.	00150
03	8	1	00404	.	00216
03	8	2	00489	.	00193
03	8	3	00397	.	00243
03	8	4	00370	.	00183
05	1	1	00921	01.5	00250
05	1	2	00585	08.2	00720
05	1	3	00840	04.4	00175
05	1	4	00579	02.3	00127
05	4	1	00632	18.8	03450
05	4	2	00809	18.9	02700
05	4	3	01003	18.3	03750
05	4	4	00961	30.7	03300
05	6	1	00683	03.3	00630
05	6	2	00598	06.3	01005
05	6	3	00780	03.6	00245
05	6	4	00774	03.4	00210
05	8	1	00606	01.6	00160
05	8	2	00578	03.6	00370
05	8	3	00658	04.4	00255
05	8	4	00698	01.8	00145
08	1	1	03580	02.4	00090
08	1	2	03790	03.7	00155
08	1	3	03636	01.9	00125
08	1	4	04131	03.5	00250
08	4	1	16205	34.9	03100
08	4	2	35254	52.2	03150
08	4	3	09374	17.1	01550
08	4	4	13398	22.6	03000
08	6	1	05343	19.5	00570
08	6	2	04241	08.2	00480
08	6	3	04683	10.3	01350
08	6	4	04471	17.8	00800
08	8	1	04178	04.4	00205
08	8	2	04038	11.8	00185
08	8	3	06011	15.6	00540
08	8	4	03463	03.2	00115
10	1	1	04725	05.0	00175
10	1	2	03744	04.0	00140
10	1	3	04059	03.0	00145
10	1	4	04488	03.5	00085
10	4	1	08455	23.5	00950
10	4	2	05405	18.5	01150
10	4	3	04497	10.5	01050
10	4	4	06393	27.5	01550
10	6	1	09779	21.5	01340
10	6	2	03030	04.5	00140
10	6	3	03790	21.0	00950
10	6	4	12805	52.0	04800
10	8	1	19524	52.0	02350
10	8	2	05334	15.0	01100
10	8	3	13936	53.0	01900
10	8	4	06286	12.5	00260

Legend: 1. = Day, 2. = Concentration, 3. = Replicate, 4. = Cells/ml. (x 1000), 5. = Relative Fluorescence, and 6. = ATP Units.

Table A5. The Effect of 2,4-D on ^{14}C Uptake in *Synecchoccus* sp. in the Dark at pH 7.8.

1.	2.	3.	4.	5.
1	1	30	328	0.0251
1	2	30	296	0.0226
1	1	60	462	0.0354
1	2	60	339	0.0259
1	1	120	525	0.0402
1	2	120	587	0.0450
1	1	240	975	0.0747
1	2	240	1154	0.0884
1	1	720	6989	0.5358
1	2	720	8887	0.6813
2	1	30	357	0.0273
2	2	30	381	0.0292
2	1	60	393	0.0301
2	2	60	404	0.0309
2	1	120	488	0.0374
2	2	120	522	0.0400
2	1	240	1139	0.0873
2	2	240	1201	0.0920
2	1	720	8006	0.6137
2	2	720	7095	0.5439
10	1	30	339	0.0259
10	2	30	249	0.0190
10	1	60	361	0.0276
10	2	60	309	0.0236
10	1	120	967	0.0741
10	2	120	532	0.0407
10	1	240	1309	0.1003
10	2	240	1305	0.1000
10	1	720	9641	0.7391
10	2	720	8476	0.6498

Legend: 1. = Concentration (mg/l), 2. = Replicate, 3. = Time (minutes) 4. = Counts/Minute, 5. = Carbon Uptaken (mg/l).

Table A7. The Effect of 2,4-D on the Uptake of ^{14}C in *Synecchoccus* sp. at pH 6.5.

1.	2.	3.	4.	5.
1	15	1000000	2072	0.1588
1	15	1000000	2193	0.1681
1	15	1000000	1931	0.1480
1	30	1000000	4027	0.3087
1	30	1000000	4512	0.3459
1	30	1000000	3949	0.3027
1	60	1000000	5481	0.4202
1	60	1000000	6741	0.5168
1	60	1000000	4275	0.3277
1	120	1000000	10541	0.8081
1	120	1000000	10555	0.8092
1	120	1000000	8794	0.6742
1	240	1000000	20706	1.5874
1	240	1000000	23763	1.8218
1	240	1000000	27765	2.1286
1	480	1000000	27166	2.0827
1	480	1000000	48576	3.7241
1	480	1000000	35035	2.6860
5	15	1000000	2193	0.1681
5	15	1000000	2496	0.1913
5	15	1000000	2212	0.1695
5	30	1000000	4131	0.3167
5	30	1000000	4393	0.3368
5	30	1000000	3971	0.3044
5	60	1000000	6261	0.4800
5	60	1000000	6552	0.5023
5	60	1000000	6145	0.4711
5	120	1000000	8829	0.6768
5	120	1000000	14783	1.1333
5	120	1000000	13804	1.0583
5	240	1000000	25289	1.9388
5	240	1000000	31878	2.4439
5	240	1000000	31278	2.3979
5	480	1000000	27723	2.1254
5	480	1000000	38761	2.9716
5	480	1000000	29658	2.2737

Legend: 1. = Concentration, 2. = Time (minutes) 3. = Cells/ml., 4. = Counts per Minute, 5. = Carbons Fixed (mg/l).

Table A6. Energy Charge Response of Chlorella Sp. Isolate from Smith Mountain Lake to 2.0 mg/l 2,4-D at pH 7.65.

1.	2.	3.	4.	5.
000	00	26	16	0.69
005	07	27	21	0.62
015	15	01	23	0.61
030	13	19	23	0.59
060	00	28	18	0.69
120	00	18	17	0.74

Legend: 1. = Time, minutes, 2. = AMP (ng/l), 3. = ADP (ng/l), 4. = ATP (ng/l), 5. = Energy Charge.

Table A8. Sequential Comparison Index (SCI) Study of the Response of Smith Mountain Lake Algae Diversity to 2,4-D.

1.	2.	3.	4.
0	1	0.75	6.04
0	2	0.75	6.04
0	3	0.75	6.04
0	4	0.75	6.04
2	1	0.78	5.51
2	2	0.75	5.40
2	3	0.71	5.63
2	4	0.80	6.23
5	1	0.83	6.75
5	2	0.78	5.75
5	3	0.74	5.67
5	4	0.73	5.21
7	1	0.73	7.30
7	2	0.73	6.45
7	3	0.69	6.23
7	4	0.72	6.48
9	1	0.69	6.02
9	2	0.72	6.51
9	3	0.78	5.43
9	4	0.79	6.87

Legend: 1. = Day, 2. = Concentration (1 = control, 2 = 1.0 mg/l, 3 = 5 mg/l, 4 = 10 mg/l 2,4-D), 3. = Diversity Index, 4. = Diversity Index, Total.

Table A9. Sequential Comparison Index (SCI) Study of the Response of Smith Mountain Lake Algae Diversity to Atrazine.

1.	2.	3.	4.
0	1	0.85	5.97
0	2	0.85	5.97
0	3	0.85	5.97
0	4	0.85	5.97
3	1	0.78	5.49
3	2	0.84	7.60
3	3	0.67	3.37
3	4	0.59	2.95
5	1	0.74	6.66
5	2	0.74	5.21
5	3	0.46	2.30
5	4	0.35	1.42
7	1	0.74	6.68
7	2	0.68	5.47
7	3	0.67	5.33
7	4	0.34	1.34
9	1	0.76	5.47
9	2	0.64	5.42
9	3	0.65	5.62
9	4	0.32	1.42

Legend: 1. = Day, 2. = Concentration (1 = control, 2 = 0.01 mg/l, 3 = 0.1 mg/l, 4 = 1 mg/l), 3. = Diversity Index, 4. = Diversity Index, Total.

Table A10. Sequential Comparison Index (SCI) Study of the Response of Smith Mountain Lake Algae Diversity to Diuron.

1.	2.	3.	4.
0	1	0.80	6.45
0	2	0.80	6.45
0	3	0.80	6.45
0	4	0.80	6.45
3	1	0.71	4.29
3	2	0.66	3.03
3	3	0.28	0.84
3	4	0.22	0.44
5	1	0.70	7.00
5	2	0.63	4.44
5	3	0.43	2.63
5	4	0.40	1.21
7	1	0.80	6.70
7	2	0.50	3.00
7	3	0.18	0.56
7	4	0.21	0.89

Legend: 1. = Day, 2. = Concentration (1 = control, 2 = 0.01 mg/l, 3 = 0.1 mg/l, 4 = 1 mg/l Diuron), 3. = Diversity Index, 4. = Diversity Index, Total.

Table A11. Response of a Continuous Culture of _Chlorella pyrenoidosa_ to Diuron

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
0.00	01	1	7.10	7980000	3.90	7.80	3.8	1.5	0.0	0.85	32.9
0.00	01	2	7.12	9800000	4.35	8.55	4.1	3.5	0.0	0.77	46.2
0.00	01	3	7.12	9500000	4.05	8.55	6.5	1.6	0.0	0.90	42.9
0.00	03	1	7.34	4580000	2.10	4.20	3.8	0.7	1.2	0.73	31.6
0.00	03	2	7.25	5010000	3.60	5.70	5.1	1.0	0.3	0.77	42.9
0.00	03	3	7.34	6000000	4.60	7.70	7.0	1.2	1.3	0.80	53.2
0.00	05	1	7.45	5860000	6.00	9.60	5.4	1.0	1.0	0.79	31.9
0.00	05	2	7.47	5200000	6.45	8.40	5.6	0.7	1.1	0.82	44.1
0.00	05	3	7.50	6200000	7.45	7.65	6.4	1.5	1.2	0.82	51.3
0.00	07	1	7.75	5100000	4.35	5.70	3.9	0.8	1.0	0.83	30.2
0.00	07	2	7.78	4810000	5.80	7.95	6.3	1.5	1.3	0.72	44.3
0.00	07	3	7.69	6400000	7.35	7.85	6.5	1.3	1.8	0.81	50.8
0.00	09	1	7.75	5300000	4.45	6.00	4.8	1.1	0.9	0.80	31.3
0.00	09	2	7.69	5010000	5.90	7.95	6.1	1.4	1.0	0.81	43.1
0.00	09	3	7.70	6550000	7.25	8.00	6.4	1.2	1.3	0.83	51.2
0.01	10	1	7.78	4820000	3.45	4.65	4.2	0.7	0.6	0.79	29.8
0.01	10	2	7.74	5700000	5.70	7.45	6.1	1.2	1.1	0.80	43.6
0.01	10	3	7.74	6600000	7.05	8.10	6.3	1.2	0.9	0.84	49.0
0.03	11	1	7.45	4800000	3.30	4.95	3.8	0.4	0.7	0.82	24.2
0.03	11	2	7.43	4200000	3.60	5.40	3.9	0.5	4.6	0.46	35.5
0.03	11	3	7.51	5150000	6.30	8.15	3.9	0.2	2.6	0.61	37.9
0.06	13	1	7.48	3180000	1.95	3.00	2.6	0.8	2.3	0.52	19.2
0.06	13	2	7.52	3850000	3.20	4.45	3.1	0.9	2.1	0.68	13.4
0.06	13	3	7.59	4650000	6.00	7.70	2.9	1.8	2.0	0.55	32.4
0.08	14	1	7.54	3000000	2.25	2.70	1.4	0.4	2.5	0.37	18.5
0.08	14	2	7.53	4080000	3.15	3.90	2.7	0.8	2.7	0.56	17.8
0.08	14	3	7.56	4240000	5.40	6.00	1.1	0.6	2.6	0.32	32.2
0.10	15	1	7.53	1390000	1.95	2.10	0.9	0.3	4.1	0.20	17.3
0.10	15	2	7.51	3980000	3.15	3.30	2.6	0.4	3.6	0.15	17.1
0.10	15	3	7.49	4210000	6.45	6.60	2.2	0.4	3.2	0.41	26.9

Legend: 1. Concentration, 2. = Day, 3. = Replicate number, 4. = pH 5. = Cells/ml., 6. = Relative Fluoresence, 7. = Relative Fluorescence with Diuron, 8. = ATP, ng/ml, 9. = ADP, ng/ml, 10. = AMP, ng/ml, 11. = Energy Charge, and 12. = TOC, mg/l.

Table A12. Growth Response of Synecchoccus sp. Clones From Smith Mountain Lake to 2,4-D at pH 8.0.

1.	2.	3.	4.	5.	6.
01	0000	1	00050000	.	.
01	0000	2	00050000	.	.
01	02.2	1	00050000	.	.
01	02.2	2	00050000	.	.
01	10.0	1	00050000	.	.
01	10.0	2	00050000	.	.
02	0000	1	00050000	.	.
02	0000	2	00050000	.	.
02	02.2	1	00050000	.	.
02	02.2	2	00050000	.	.
02	10.0	1	00219000	.	.
02	10.0	2	00227000	.	.
03	0000	1	00209000	0180	.
03	0000	2	00143000	0105	.
03	02.2	1	00223300	0084	.
03	02.2	2	00050000	0040	.
03	10.0	1	00516000	0350	.
03	10.0	2	00816667	0470	.

Legend: 1.= Day, 2. = Concentration, mg/l, 3. = Replicate, 4. = Cells/ml, 5. = Relative Fluorescence, 6. = ATP

Table A13. Growth Rate Study for Synecchoccus sp. Clones From Smith Mountain Lake.

1.	2.	3.	4.
0	1	00719	.
0	2	00543	.
0	3	04942	.
1	1	42808	0.195
1	2	06443	0.195
1	3	01413	0.195
2	1	15216	1.206
2	2	03770	1.206
2	3	01773	1.206
3	1	02674	0.525
3	2	02301	0.525
3	3	02383	0.525
4	1	04608	0.771
4	2	04259	0.771
4	3	04106	0.771
5	1	24293	1.345
5	2	12168	1.345
5	3	12202	1.345

Legend: 1. = Day, 2. = Replicate, 3. = Cells, mg/l (x 1000)
4. = Doubling Time, Days.

Table A14. Growth Response of a Toxic Strain of *Synecchoccus sp.* to 2,4-D at pH 8.0

1.	2.	3.	4.	5.	6.
01	0000	1	00050000	.	.
01	0000	2	00050000	.	.
01	02.2	1	00050000	.	.
01	02.2	2	00050000	.	.
01	10.0	1	00050000	.	.
01	10.0	2	00050000	.	.
02	0000	1	00050000	.	.
02	0000	2	00050000	.	.
02	02.2	1	00050000	.	.
02	02.2	2	00050000	.	.
02	10.0	1	00205000	.	.
02	10.0	2	00173000	.	.
03	0000	1	00050000	0039	.
03	0000	2	00050000	0035	.
03	02.2	1	00227000	0058	.
03	02.2	2	00410000	0039	.
03	10.0	1	00442000	0035	.
03	10.0	2	00404000	0046	.
05	0000	1	01570000	0096	.
05	0000	2	01570000	0096	.
05	02.2	1	01710000	0102	.
05	02.2	2	01820000	0105	.
05	10.0	1	01450000	0099	.
05	10.0	2	01990000	0123	.
07	0000	1	01090000	0099	.
07	0000	2	01090000	0099	.
07	02,2	1	01930000	0090	.
07	02.2	2	01110000	0084	.
07	10.0	1	00946000	0108	.
07	10.0	2	01420000	0114	.

Legend: 1. = Day, 2. = Concentration, mg/l, 3. = Replicate, 4. = Cells, ml, 5. = Relative Fluorescence, 6. = ATP

Table A15. Growth Response of Chlorochloster sp. to 2,4-D at pH 7.0.

1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
03	1	1	014789	02.4	00094	07	6	2	042413	12.2	00670
03	1	2	013383	01.0	00087	07	6	3	032722	10.2	00750
03	1	3	012702	00.8	00129	07	6	4	062937	10.2	00590
03	1	4	014214	01.8	00108	07	8	1	036607	12.4	00590
03	4	1	013806	01.0	00159	07	8	2	020531	07.8	00330
03	4	2	013582	00.8	00240	07	8	3	037676	11.0	00610
03	4	3	014513	00.4	00165	07	8	4	030496	16.6	00640
03	4	4	015936	01.2	00150	09	1	1	068244	09.4	00810
03	6	1	014567	02.0	00114	09	1	2	080250	39.2	01410
03	6	2	014446	02.6	00111	09	1	3	063177	07.8	00930
03	6	3	013158	01.0	00108	09	1	4	061725	10.4	01200
03	6	4	013434	04.0	00150	09	4	1	060297	06.0	00960
03	8	1	011930	03.8	00129	09	4	2	058877	08.1	00610
03	8	2	013682	04.0	00120	09	4	3	073720	09.8	01200
03	8	3	011749	02.0	00043	09	4	4	111905	39.2	02400
03	8	4	014399	01.6	00126	09	6	2	057015	08.1	00780
05	1	1	030742	09.0	00640	09	6	3	099179	17.2	02400
05	1	2	032957	07.7	00660	09	6	4	090703	49.0	02340
05	1	3	035533	10.8	00750	09	8	1	012096	02.4	00052
05	1	4	041203	08.6	00570	09	8	2	029453	05.8	00204
05	4	1	031133	05.8	00560	09	8	3	045096	06.6	00660
05	4	2	037867	09.8	00710	09	8	4	051585	18.8	00770
05	4	3	036288	04.6	00670	12	1	1	178090	85.0	05000
05	4	4	030962	11.8	00590	12	1	2	043340	35.0	02010
05	6	1	032078	12.5	00510	12	1	3	151290	60.0	03000
05	6	2	035293	10.1	00530	12	1	4	222040	88.0	06300
05	6	3	040753	10.5	00720	12	4	1	220820	74.0	05200
05	6	4	038646	08.6	00470	12	4	2	431960	70.0	12600
05	8	1	028554	09.2	00570	12	4	3	185720	75.0	03200
05	8	2	028375	08.4	00340	12	4	4	145730	52.0	01770
05	8	3	028470	04.9	00450	12	6	1	125370	24.0	06200
05	8	4	027094	07.4	00450	12	6	2	129960	22.0	03500
07	1	1	038980	08.6	00810	12	6	3	199310	56.0	05400
07	1	2	011134	07.7	00120	12	6	4	210930	34.0	03500
07	1	3	001798	00.4	00015	12	8	1	157750	30.0	04000
07	1	4	016603	04.6	00370	12	8	2	161230	22.0	02200
07	4	1	042559	22.5	00900	12	8	3	233600	40.0	03600
07	4	2	074434	40.0	02040	12	8	4	233600	56.0	03400
07	4	3	017892	03.0	00138						
07	4	4	010936	01.8	00123						
07	6	1	019312	06.0	00195						

Legend: 1. = Day, 2. = Concentration, 3. = Replicate, 4. = Cells/ml (x 10), 5. = ATP, ng/l, 6. = Relative Fluoresence

Note: Under Concentration, 1 = Control, 4 = 10^{-4}M 2,4-D, 6 = 10^{-6}M 2,4-D, 8 = 10^{-8}M 2,4-D.

Table A16. Growth Response of *Chlorella sp.* Clones from Smith Mountain Lake to 2,4-D at pH 6.7

1.	2.	3.	4.	5.	6.
00	1	1	00050000	.	.
00	1	2	00050000	.	.
00	1	3	00050000	.	.
00	5	1	00050000	.	.
00	5	2	00050000	.	.
00	5	3	00050000	.	.
04	1	1	01240000	0051	004.0
04	1	2	01025000	0053	007.1
04	1	3	00950000	0053	006.4
04	5	1	01220000	0068	004.9
04	5	2	01070000	0057	006.7
04	5	3	01105000	0058	006.7
06	1	1	02580000	0141	025.2
06	1	2	02450000	0135	021.5
06	1	3	02760000	0123	024.3
06	5	1	02425000	0153	018.1
06	5	2	02150000	0159	014.9
06	5	3	02600000	0147	018.9
09	1	1	07900000	0410	022.8
09	1	2	07250000	0420	021.7
09	1	3	06000000	0340	018.0
09	5	1	06900000	0350	017.3
09	5	2	06650000	0340	016.4
09	5	3	05550000	0320	019.0

Legend: 1. = Day, 2. = Concentration, 3. = Replicate, 4. = Cells/ml, 5. = Relative Fluorescence, 6. = ATP, ng/ml.

Note: Under Concentration, 1 = Control, 5 = 10^{-5}M.

Table A17. Growth Response of Smith Mountain Lake <u>Synecchoccus sp.</u> Isolate to 2,4-D at pH 6.5

1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
00	1	1	00050000	-	-	06	6	1	08100000	01410	12.3
00	1	2	00050000	-	-	06	6	2	06920000	01350	11.8
00	1	3	00050000	-	-	06	6	3	08900000	01380	15.4
00	1	4	00050000	-	-	06	6	4	08200000	01500	09.9
00	1	5	00050000	-	-	06	6	5	08100000	01410	13.4
00	4	1	00050000	-	-	09	1	1	12600000	01110	10.8
00	4	2	00050000	-	-	09	1	2	13150000	01620	09.6
00	4	3	00050000	-	-	09	1	3	09850000	01290	12.3
00	4	4	00050000	-	-	09	1	4	09850000	01020	10.9
00	4	5	00050000	-	-	09	1	5	10100000	01150	11.0
00	6	1	00050000	-	-	09	6	1	12850000	00780	09.0
00	6	2	00050000	-	-	09	6	2	10800000	00600	11.5
00	6	3	00050000	-	-	09	6	3	11250000	00540	12.1
00	6	4	00050000	-	-	09	6	4	08750000	00750	08.7
00	6	5	00050000	-	-	09	6	5	08600000	00720	09.8
04	1	1	03310000	00670	04.2	15	1	1	26500000	00690	47.9
04	1	2	02740000	00510	04.3	15	1	2	26750000	00540	22.5
04	1	3	03570000	00600	04.0	15	1	3	24750000	00610	40.1
04	1	4	03690000	00610	03.8	15	1	4	23500000	00690	28.5
04	1	5	02440000	00490	03.7	15	1	5	24500000	00750	39.6
04	4	1	00000000	-	-	15	6	1	17125000	00690	00.3
04	4	2	00000000	-	-	15	6	2	21930000	00610	00.0
04	4	3	00000000	-	-	15	6	3	24500000	00640	00.0
04	4	4	00000000	-	-	15	6	4	20243000	00900	00.0
04	4	5	00000000	-	-	15	6	5	20500000	00690	00.0
04	6	1	03560000	00690	04.6						
04	6	2	04040000	00690	04.3						
04	6	3	03920000	00590	04.4						
04	6	4	02940000	00570	04.1						
04	6	5	04400000	00690	04.1						
06	1	1	08550000	01260	15.5						
06	1	2	10100000	01410	17.7						
06	1	3	08200000	01410	14.6						
06	1	4	07120000	01380	14.3						
06	1	5	08700000	01410	12.1						

Legend: 1. = Day, 2. = Concentration, 3. = Replicate, 4. = Cells/ml, 5. = Relative Fluorescence, 6. = ATP, ng/ml.

Note: Under Concentration, 1 = Control, 4 = 10^{-4} M, and 6 = 10^{-6}M.

Table A18. Growth Response of Synecchoccus sp. Clones From Smith Mountain Lake as Measured by Dry Weight at pH 6.5

1.	2.	3.	4.	5.
07	1	1	2419	026
07	1	2	2409	028
07	1	3	2419	028
07	4	1	2851	026
07	4	2	2850	024
07	4	3	2851	026
07	6	1	2250	016
07	6	2	2250	022
07	6	3	2250	022
09	1	1	4160	040
09	1	2	3024	030
09	1	3	3391	038
09	4	1	3726	042
09	4	2	3220	032
09	4	3	3435	028
09	6	1	3985	028
09	6	2	3385	028
09	6	3	3244	028
11	1	1	6651	050
11	1	2	6013	054
11	1	3	5648	056
11	4	1	2662	042
11	4	2	2432	040
11	4	3	2592	042
11	6	1	4201	048
11	6	2	4345	040
11	6	3	4157	046
13	1	1	.	062
13	1	2	.	054
13	1	3	.	068
13	4	1	.	060
13	4	2	.	070
13	4	3	.	068
13	6	1	.	116
13	6	2	.	054
13	6	3	.	068
15	1	1	.	074
15	1	2	.	080
15	1	3	.	052
15	4	1	.	084
15	4	2	.	076
15	4	3	.	074
15	6	1	.	068
15	6	2	.	070
15	6	3	.	056
17	1	1	.	078
17	1	2	.	178
17	1	3	.	084
17	4	1	.	094
17	4	2	.	096
17	4	3	.	096
17	6	1	.	076
17	6	2	.	076
17	6	3	.	076

Legend: 1. = Day, 2. = Concentration, 3. = Replicate, 4. = ATP, Relative Units, 5. = Dry Weight, mg/l.

Table A19. The Growth Response of Navicula sp. Isolate From Smith Mountain Lake to 2,4-D.

1.	2.	3.	4.	5.	1.	2.	3.	4.	5.
03	1	1	1917	032	07	6	2	2533	540
03	1	2	1283	032	07	6	3	4074	540
03	1	3	1497	032	07	6	4	6201	540
03	1	4	1504	032	07	8	1	3840	540
03	4	1	3692	057	07	8	2	4399	540
03	4	2	1371	057	07	8	3	4907	540
03	4	3	1996	057	07	8	4	5740	540
03	4	4	1509	057	10	1	1	4492	930
03	6	1	1936	159	10	1	2	5016	930
03	6	2	1775	159	10	1	3	4553	930
03	6	3	1878	159	10	1	4	3685	930
03	6	4	1518	159	10	4	1	5768	900
03	8	1	1792	038	10	4	2	7850	900
03	8	2	0973	038	10	4	3	8692	900
03	8	3	1230	038	10	4	4	5191	900
03	8	4	1080	038	10	6	1	5397	750
07	1	1	3025	480	10	6	2	4579	750
07	1	2	3027	480	10	6	3	2523	750
07	1	3	3463	480	10	6	4	3953	750
07	1	4	5546	480	10	8	1	4541	810
07	4	1	3078	450	10	8	2	5679	810
07	4	2	4042	450	10	8	3	7039	810
07	4	3	5126	450	10	8	4	6392	810
07	4	4	4500	450					
07	6	1	2452	540					

Legend: 1. = Day, 2. = Concentration, 3. = Replicate, 4. = Cells/ml.(x 10^{-3}), 5. = Relative Fluorescence.

Note: Under Concentration, 1 = Control, 4 = 20 mg/l, 6 = 0.2 mg/l, and 8 = 0.002 mg/l.

Table A20. The Effect of 2,4-D on ^{14}C Uptake in *Synecchoccus sp.* at pH 7.0

1.	2.	3.	4.	5.
1	0	100000	340	0.02607
1	5	100000	115	0.00882
1	15	100000	48	0.00368
1	30	100000	100	0.00767
1	60	100000	878	0.06731
1	120	100000	607	0.04654
1	240	100000	790	0.06057
1	0	500000	46	0.00353
1	5	500000	244	0.01871
1	15	500000	751	0.05758
1	30	500000	2477	0.18990
1	60	500000	2654	0.20347
1	120	500000	6589	0.50516
1	240	500000	10441	0.80048
1	0	1000000	547	0.04194
1	5	1000000	1219	0.09346
1	15	1000000	2455	0.18822
1	30	1000000	4587	0.35167
1	60	1000000	7839	0.60099
1	120	1000000	13409	1.02802
1	240	1000000	27969	2.14429
4	0	100000	208	0.01595
4	5	100000	17	0.00130
4	15	100000	60	0.00460
4	30	100000	31	0.00238
4	60	100000	231	0.01771
4	120	100000	217	0.01664
4	240	100000	135	0.01035
4	0	500000	43	0.00330
4	5	500000	291	0.02231
4	15	500000	820	0.06287

Legend: 1. = Concentration, 2. = Time (minutes), 3. = cells/ml., 4. = Counts/minute, 5. = carbon uptake mg/l

Note: Under concentration, 1 = control, 4 = 10^{-4} M, 5 = 10^{-5} M, and 6 = 10^{-6} M.

(Continued)

Table A20. The Effect of 2,4-D on ^{14}C Uptake in <u>Synecchoccus sp.</u> at pH 7.0 (Continued)

1.	2.	3.	4.	5.
4	30	500000	2460	0.18860
4	60	500000	1837	0.14084
4	120	500000	1788	0.13708
4	240	500000	12172	0.93319
4	0	1000000	119	0.00912
4	5	1000000	147	0.01127
4	15	1000000	306	0.02346
4	30	1000000	255	0.01955
4	60	1000000	330	0.02530
4	120	1000000	550	0.04217
4	240	1000000	760	0.05827
5	0	100000	490	0.03757
5	5	100000	29	0.00222
5	15	100000	39	0.00299
5	30	100000	77	0.00590
5	60	100000	696	0.05336
5	120	100000	452	0.03465
5	240	100000	391	0.02998
5	0	500000	33	0.00253
5	5	500000	216	0.01656
5	15	500000	717	0.05497
5	30	500000	333	0.02553
5	60	500000	2960	0.22693
5	120	500000	5942	0.45555
5	240	500000	11021	0.84494
5	0	1000000	473	0.03626
5	5	1000000	1431	0.10971
5	15	1000000	4250	0.32583
5	30	1000000	5003	0.38356
5	60	1000000	9288	0.71208
5	120	1000000	18469	1.41596

Legend: 1. = Concentration, 2. = Time (minutes), 3. = cells/ml., 4. = Counts/minute, 5. = carbon uptake mg/l

Note: Under concentration, 1 = control, 4 = 10^{-4} M, 5 = 10^{-5} M, and 6 = 10^{-6} M.

(Continued)

Table A20. The Effect of 2,4-D on ^{14}C Uptake in *Synecchoccus sp.* at pH 7.0 (Continued)

1.	2.	3.	4.	5.
5	240	1000000	34100	2.61433
6	0	100000	164	0.01257
6	5	100000	50	0.00383
6	15	100000	55	0.00422
6	30	100000	114	0.00874
6	60	100000	568	0.04355
6	120	100000	603	0.04623
6	240	100000	975	0.07475
6	0	500000	61	0.00468
6	5	500000	216	0.01656
6	15	500000	776	0.05949
6	30	500000	1724	0.13217
6	60	500000	2416	0.18523
6	120	500000	5538	0.42458
6	240	500000	1719	0.13179
6	0	1000000	371	0.02844
6	5	1000000	996	0.07636
6	15	1000000	2899	0.22226
6	30	1000000	4276	0.32783
6	60	1000000	7417	0.56864
6	120	1000000	16132	1.23679
6	240	1000000	31173	2.38993

Legend: 1. = Concentration, 2. = Time (minutes), 3. = cells/ml., 4. = Counts/minute, 5. = carbon uptake mg/l

Note: Under concentration, 1 = control, 4 = 10^{-4} M, 5 = 10^{-5} M, and 6 = 10^{-6} M.

Table 21. The Effect of 2,4-D on ^{14}C Uptake in in _Synecchoccus_ _sp._ at pH 8.0

1.	2.	3.	4.
1	15	950	0.0728
1	15	1374	0.1053
1	15	1091	0.0836
1	30	2009	0.1540
1	30	1981	0.1518
1	30	1903	0.1459
1	60	2815	0.2158
1	60	3707	0.2842
1	60	3705	0.2840
1	120	6005	0.4603
1	120	6321	0.4846
1	120	5857	0.4490
1	240	12223	0.9371
1	240	14652	1.1233
1	240	10791	0.8273
1	480	10720	0.8218
1	480	15523	1.1901
1	480	11921	0.9139
5	15	980	0.0751
5	15	1140	0.0874
5	15	914	0.0700
5	30	1376	0.1054
5	30	1781	0.1365
5	30	1517	0.1163
5	60	3103	0.2379
5	60	3864	0.2962
5	60	3104	0.2379
5	120	6880	0.5274
5	120	7357	0.5640
5	120	6758	0.5181
5	240	11600	0.8893
5	240	11893	0.9118
5	240	10124	0.7761
5	480	13572	1.0405
5	480	13102	1.0044
5	480	13502	1.0351

Legend: 1. = Concentration, 2. = Time (minutes) 3. = Counts/minute, 4. = Carbon fixed, mg/l

Note: Under concentration, 1 = control, 2 = 2.0 mg/l

Table A22. The Effect of 2,4-D on the ^{14}C Uptake in *Synecchoccus sp.* at pH 8.0.

1.	2.	3.	4.	5.
15	1	1	5688	0.4360
15	2	1	-	-
15	3	1	4982	0.3819
30	1	1	7765	0.5953
30	2	1	8181	0.6272
30	3	1	8399	0.6439
60	1	1	11287	0.8653
60	2	1	10586	0.8115
60	3	1	10393	0.7968
120	1	1	23355	1.7905
120	2	1	18260	1.3999
120	3	1	20006	1.5337
240	1	1	51770	3.9690
240	2	1	26563	2.0365
240	3	1	32941	2.5254
360	1	1	70066	5.3717
360	2	1	33690	2.5829
360	3	1	45586	3.4949
15	1	5	5309	0.4070
15	2	5	4718	0.3617
15	3	5	5191	0.3979
30	1	5	9797	0.7511
30	2	5	9344	0.7163
30	3	5	8533	0.6542
60	1	5	14330	1.0986
60	2	5	10650	0.8165
60	3	5	12636	0.9687
120	1	5	21206	1.6257
120	2	5	14105	1.0813
120	3	5	31263	2.3968
240	1	5	42037	3.2228
240	2	5	26602	2.0394
240	3	5	65806	5.0451
360	1	5	77600	5.9493
360	2	5	29964	2.2972
360	3	5	104950	8.0461

Legend: 1. = Time (minutes), 2. = Replicate, 3. = Concentration, 4. = Counts/minute, 5. = Carbon fixed, mg/l

Table A23. The Effect of 2,4-D on the ^{14}C Uptake in *Synecchoccus sp.* at pH 6.0.

1.	2.	3.	4.	5.
1	1	30	190844	14.631
1	2	30	190203	14.582
1	1	60	303888	23.298
1	2	60	285194	21.864
1	1	120	397561	30.479
1	2	120	437933	33.574
2	1	30	234618	17.987
2	2	30	215695	16.536
2	1	60	280344	21.493
2	2	60	329418	25.255
2	1	120	.	.
2	2	120	381135	29.220
5	1	30	225978	17.325
5	2	30	208156	15.958
5	1	60	286872	21.993
5	2	60	289488	22.194
5	1	120	363778	27.889
5	2	120	372414	28.551
10	1	30	217526	16.677
10	2	30	187081	14.342
10	1	60	266489	20.430
10	2	60	302864	23.219
10	1	120	389138	29.833
10	2	120	385592	29.562

Legend: 1. = Concentration, 2. = Replicate, 3. = Time (minutes), 4. = Counts/minute, 5. = Carbon fixed, mg/l.

Table A24. The Effect of 2,4-D on the ^{14}C Uptake in *Synecchoccus sp.* at pH 8.0.

1.	2.	3.	4.	5.
1	1	30	5611	0.43018
1	2	30	3660	0.28060
1	1	60	5141	0.39414
1	2	60	6512	0.49925
1	1	120	14986	1.14893
1	2	120	13208	1.01261
1	1	240	19970	1.53103
1	2	240	25848	1.98168
2	1	30	4501	0.34508
2	2	30	3633	0.27853
2	1	60	4902	0.37582
2	2	60	5941	0.45548
2	1	120	13689	1.04949
2	2	120	12224	0.93717
2	1	240	23600	1.80933
2	2	240	20496	1.57136
10	1	30	5790	0.44390
10	2	30	5790	0.44390
10	1	60	4873	0.37360
10	2	60	4873	0.37360
10	1	120	12066	0.92506
10	2	120	12066	0.92506
10	1	240	27116	2.07889
10	2	240	27116	2.07889

Legend: 1. = Concentration, 2. = Replicate, 3. = Time (minutes), 4. = Counts/minute, 5. = Carbon fixed, mg/l.

Table A25. The Effect of 2,4-D on Bicarbonate Uptake in Synecchoccus sp. at pH 6.3.

1.	2.	3.	4.	5.	6.
1	1	15	10000000	1929	0.147
1	2	15	10000000	1875	0.143
1	3	15	10000000	2321	0.177
1	1	30	10000000	3176	0.243
1	2	30	10000000	3136	0.240
1	3	30	10000000	2950	0.226
1	1	60	10000000	6048	0.463
1	2	60	10000000	4541	0.348
1	3	60	10000000	6155	0.471
1	1	120	10000000	10514	0.806
1	2	120	10000000	7329	0.561
1	3	120	10000000	11918	0.913
1	1	240	10000000	24576	1.884
1	2	240	10000000	10410	0.798
1	3	240	10000000	29683	2.275
1	1	480	10000000	57966	4.444
1	2	480	10000000	29644	2.272
1	3	480	10000000	57888	4.438
1	1	1440	10000000	153600	11.776
1	2	1440	10000000	118660	9.097
1	3	1440	10000000	165866	12.716
5	1	15	10000000	2501	0.191
5	2	15	10000000	3068	0.235
5	3	15	10000000	2519	0.193
5	1	30	10000000	4586	0.351
5	2	30	10000000	4375	0.335
5	3	30	10000000	4004	0.307
5	1	60	10000000	7760	0.594
5	2	60	10000000	7977	0.611
5	3	60	10000000	6823	0.523
5	1	120	10000000	13141	1.007
5	2	120	10000000	12857	0.985
5	3	120	10000000	13479	1.033
5	1	240	10000000	26645	2.042
5	2	240	10000000	30405	2.331
5	3	240	10000000	30227	2.317
5	1	480	10000000	57744	4.427
5	2	480	10000000	69737	5.346
5	3	480	10000000	59411	4.554
5	1	1440	10000000	157633	12.085
5	2	1440	10000000	186633	14.308
5	3	1440	10000000	167433	12.836

Legend: 1. = Concentration, mg/l, 2. = Replicate, 3. = Time (minutes), 4. = Cells/ml, 5. = Counts/minute, 6. = Carbon fixed, mg/l.

Table A26. Field Data - One/ 2,4-D Study

1.	2.	3.	4.	5.	6.	7.
1	1	1	6.9	219.86	7.525	0.091570
1	2	1	6.9	230.81	6.238	0.096133
1	3	1	7.1	221.12	7.842	0.092095
2	1	1	6.8	101.23	7.261	0.042160
2	2	1	6.7	125.73	6.535	0.052366
2	3	1	6.9	6.88	8.229	0.002867
3	1	1	7.0	147.93	7.496	0.061612
3	2	1	6.8	119.78	2.178	0.049890
4	1	1	7.0	210.20	1.257	0.087548
4	2	1	7.0	111.10	5.446	0.046273
4	3	1	6.7	115.60	6.481	0.048149
1	1	2	7.4	403.71	14.377	0.168146
1	2	2	7.0	456.68	7.261	0.190209
1	3	2	7.5	456.68	8.577	0.190209
2	1	2	7.4	511.82	19.484	0.213173
2	2	2	7.0	548.18	47.652	0.228317
2	3	2	7.3	420.65	9.803	0.175200
3	1	2	7.4	403.71	10.415	0.168146
3	2	2	7.3	315.78	17.306	0.131524
3	3	2	7.5	361.44	13.312	0.150540
4	1	2	7.2	315.78	14.377	0.131524
4	2	2	7.3	194.70	13.312	0.081094
4	3	2	7.4	362.16	23.486	0.150841
1	1	3	7.1	320.97	3.147	0.133685

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, mg/l, 5. = ATP, ng/l, 6. = Chlorophyll, µg/l, 7. = Dry Weight, mg/l.

Note: Under Concentration, 1 = control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2,4-D.

(Continued)

Table A26. Field Data - One (Continued)

1.	2.	3.	4.	5.	6.	7.
1	2	3	7.0	576.25	2.178	0.240009
1	2	3	7.1	280.14	2.124	0.116680
2	1	3	7.2	657.55	3.961	0.273869
2	2	3	6.9	456.07	4.084	0.189954
2	3	3	7.0	609.48	4.236	0.253847
3	1	3	7.2	590.99	8.577	0.246148
3	2	3	6.9	613.77	11.981	0.255633
3	3	3	7.3	545.41	9.984	0.227161
4	1	3	7.1	439.50	4.668	0.183050
4	2	3	7.0	384.90	4.425	0.160311
4	3	3	7.0	405.26	7.842	0.168791
1	1	6	6.5	529.44	104.301	0.220512
1	2	6	6.5	621.23	59.948	0.258740
1	3	6	6.6	883.57	25.414	0.368006
2	1	6	6.8	316.11	18.008	0.131659
2	2	6	6.5	1511.60	27.486	0.629583
2	3	6	6.6	607.24	4.972	0.252917
3	1	6	6.7	978.45	24.251	0.407525
3	2	6	6.6	1498.81	42.373	0.624255
3	3	6	6.7	561.41	10.892	0.233825
4	1	6	6.7	724.04	29.086	0.301561
4	2	6	6.7	1197.12	20.794	0.498599
4	3	6	6.5	442.13	20.794	0.184146

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, mg/l, 5. = ATP, ng/l, 6. = Chlorophyll, µg/l, 7. = Dry Weight, mg/l.

Note: Under Concentration, 1 = control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2,4-D.

Table A27. Field Data - Two/ 2,4-D Study

1.	2.	3.	4.	5.	6.	7.
1	1	1	6.8	18.672	2467.39	1.02767
1	2	1	6.6	13.654	2014.14	0.83889
1	3	1	6.7	12.137	2170.18	0.90388
2	1	1	6.8	16.101	1047.57	0.43631
2	2	1	6.8	14.159	2309.73	0.96200
2	3	1	6.7	16.519	1141.80	0.47556
3	1	1	6.9	29.045	1022.25	0.42577
3	2	1	6.8	19.308	1034.32	0.43080
3	3	1	7.1	21.365	597.93	0.24904
4	1	1	6.8	26.731	452.88	0.18863
4	2	1	6.8	14.159	981.89	0.40896
4	3	1	7.0	18.380	686.67	0.28600
1	1	2	7.4	29.911	347.30	0.14465
1	2	2	7.2	14.228	646.58	0.26930
1	3	2	7.4	28.591	486.04	0.20243
2	1	2	7.5	20.332	492.25	0.20502
2	2	2	7.4	13.226	619.01	0.25782
2	3	2	7.1	20.422	388.20	0.16168
3	1	2	7.4	42.735	348.65	0.14521
3	2	2	7.2	52.417	798.74	0.33267
3	3	2	7.3	48.495	624.59	0.26014
4	1	2	7.3	29.580	565.23	0.23542
4	2	2	7.4	33.536	180.18	0.07505
4	3	2	7.2	37.603	322.43	0.13429
1	1	3	7.7	15.816	820.54	0.34176
1	2	3	7.3	24.533	761.17	0.31703
1	3	3	7.6	24.533	917.12	0.38198
2	1	3	7.6	35.815	733.15	0.30536
2	2	3	7.6	17.542	666.04	0.27740
2	3	3	7.4	30.531	1512.25	0.62985

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, mg/l, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l.

Note: Under Concentration, 1 = control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2,4-D.

(Continued)

Table A27. Field Data - Two (Continued)

1.	2.	3.	4.	5.	6.	7.
3	1	3	7.4	20.831	703.33	0.29294
3	2	3	7.5	15.684	1323.87	0.55139
3	3	3	7.6	23.454	1304.50	0.54333
4	1	3	7.5	21.912	913.78	0.38059
4	2	3	7.3	36.760	765.14	0.31868
4	3	3	7.6	22.406	1024.41	0.42667
1	1	5	8.3	36.597	653.69	0.27226
1	2	5	8.2	28.885	670.18	0.27913
1	3	5	8.1	47.625	689.91	0.28735
2	1	5	8.2	22.124	939.82	0.39143
2	2	5	8.1	73.765	397.57	0.16559
2	3	5	8.4	39.909	935.68	0.38971
3	1	5	8.0	97.422	497.39	0.20716
3	2	5	8.6	153.731	798.74	0.33267
3	3	5	7.9	146.242	562.88	0.23444
4	1	5	8.3	100.816	1001.62	0.41718
4	2	5	8.4	176.449	920.81	0.38352
4	3	5	8.3	131.263	946.58	0.39425
1	1	7	8.1	28.513	570.00	0.23740
1	2	7	7.9	25.051	1354.50	0.56415
1	3	7	8.0	205.025	703.78	0.29313
2	1	7	8.2	136.574	1390.72	0.57924
2	2	7	8.0	180.533	1297.03	0.54021
2	3	7	8.1	121.513	1157.03	0.48190
3	1	7	8.2	29.481	1715.50	0.71450
3	2	7	8.3	87.0289	1211.80	0.504715
3	3	7	8.2	69.1205	2085.95	0.868796
4	1	7	8.3	20.6736	990.99	0.412748
4	2	7	8.3	55.0235	1049.73	0.437212
4	3	7	8.4	58.0901	2021.08	0.841780

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, mg/l, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l.

Note: Under Concentration, 1 = control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2,4-D.

Table A28. Field Data - Three/ 2,4-D Study

1.	2.	3.	4.	5.	6.	7.
1	1	2	8.6	19.0895	1777.57	0.74036
1	2	2	8.8	15.0506	1653.60	0.68873
1	3	2	8.5	6.4070	1711.44	0.71282
2	1	2	8.6	29.8230	1589.91	0.66220
2	2	2	8.2	14.6181	1638.20	0.68231
2	3	2	8.6	12.8320	1799.64	0.74955
3	1	2	8.4	34.9292	2753.51	1.14684
3	2	2	8.4	15.3115	2487.75	1.03615
3	3	2	8.2	16.6892	1748.74	0.72835
4	1	2	8.4	27.5369	2126.31	0.88561
4	2	2	8.6	19.4420	1835.14	0.76433
4	3	2	8.5	12.1139	2201.17	0.91679
1	1	5	8.2	6.4070	452.79	0.18859
1	2	5	8.0	11.4819	1126.22	0.46907
1	3	5	7.8	16.9213	535.05	0.22285
2	1	5	7.8	13.7386	875.50	0.36464
2	2	5	7.9	21.2651	1339.91	0.55807
2	3	5	8.0	10.1090	576.40	0.24007
3	1	5	8.1	2.6909	2447.12	1.01922
3	2	5	7.9	12.4936	2134.68	0.88910
3	3	5	8.1	12.5676	1066.31	0.44412
4	1	5	8.2	7.8422	2042.88	0.85086
4	2	5	7.9	9.0766	2033.69	0.84703
4	3	5	7.8	4.4929	1911.80	0.79627
1	1	8	8.8	11.1482	2272.70	0.94658
1	2	8	9.1	12.4936	2371.17	0.98759
1	3	8	8.9	46.8578	2111.80	0.87957
2	1	8	8.8	18.9600	1484.59	0.61833
2	2	8	9.0	24.0493	1484.59	0.61833
2	3	8	9.1	35.8151	4718.38	1.96520
3	1	8	9.1	20.7936	2207.57	0.91945
3	2	8	9.3	20.5001	970.81	0.40434
3	3	8	9.3	36.2660	1599.64	0.66625
4	1	8	9.0	16.0512	1904.14	0.79308
4	2	8	9.2	22.6347	1212.43	0.50498
4	3	8	9.3	48.0664	1568.38	0.65323

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2.4-D.

Table A29. Field Data - Four/ 2,4-D Study

1.	2.	3.	4.	5.	6.	7.
1	1	2	-	28.531	1615.14	0.67270
1	2	2	-	21.912	1037.39	0.43207
1	3	2	-	22.635	1469.10	0.61188
2	1	2	-	31.690	1594.32	0.66404
2	2	2	-	12.568	1213.15	0.50528
2	3	2	-	16.338	1779.28	0.74107
3	1	2	-	14.159	1134.14	0.47237
3	2	2	-	16.519	1141.62	0.47549
3	3	2	-	15.447	1502.79	0.62591
4	1	2	-	16.338	1324.95	0.55184
4	2	2	-	8.577	1342.34	0.55909
4	3	2	-	16.519	1336.67	0.55672
1	1	4	9.1	142.259	393.96	0.16409
1	2	4	9.6	112.864	540.54	0.22514
1	3	4	9.2	142.882	747.75	0.31144
2	1	4	9.2	59.650	396.58	0.16517
2	2	4	9.2	67.394	639.46	0.26633
2	3	4	8.8	100.910	412.34	0.17174
3	1	4	8.9	171.781	530.18	0.22082
3	2	4	9.3	171.781	566.04	0.23575
3	3	4	9.0	85.365	883.87	0.36813
4	1	4	9.2	274.476	565.23	0.23542
4	2	4	9.2	274.476	1181.53	0.49211
4	3	4	9.4	424.886	390.18	0.16251
1	1	6	9.2	54.615	2750.45	1.14556
1	2	6	9.2	37.740	3122.61	1.30057
1	3	6	9.4	91.900	2815.50	1.17265
2	1	6	9.4	50.743	2731.62	1.13772
2	2	6	9.8	65.351	2485.95	1.03540
2	3	6	10.2	22.489	3206.31	1.33543
3	1	6	10.8	70.324	3525.68	1.46844
3	2	6	9.4	80.126	3543.78	1.47599
3	3	6	10.0	59.905	3449.64	1.43677
4	1	6	9.5	122.884	6002.79	2.50016
4	2	6	12.0	163.117	4865.23	2.02637
4	3	6	9.7	102.983	4328.38	1.80277

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2.4-D.

Table A30. Field Data - Five/
2,4-D Study

1.	2.	3.	4.	5.	6.	7.
1	1	2	7.7	398.99	8617.2	3.5891
1	2	2	8.0	430.50	8931.9	3.7201
1	3	2	7.5	469.71	6707.5	2.7937
2	1	2	7.8	267.31	5261.5	2.1914
2	2	2	7.9	452.43	5495.8	2.2890
2	3	2	7.8	555.49	6075.0	2.5302
3	1	2	7.5	365.33	5582.1	2.3249
3	2	2	7.8	392.11	5283.9	2.2007
3	3	2	7.5	397.97	4195.0	1.7472
4	1	2	7.9	397.97	5549.1	2.3112
4	2	2	7.9	469.71	6119.9	2.5489
4	3	2	7.7	411.71	8475.9	3.5302
1	1	5	10.3	416.73	19373.2	8.0690
1	2	5	11.2	224.19	19199.0	7.9964
1	3	5	10.4	826.50	25014.6	10.4186
2	1	5	10.1	896.77	19756.7	8.2287
2	2	5	10.1	924.30	28190.1	11.7412
2	3	5	11.2	849.57	26959.9	11.2288
3	1	5	10.6	900.21	20736.9	8.6369
3	2	5	11.2	900.54	20686.5	8.6159
3	3	5	11.2	339.46	20951.7	8.7264
4	1	5	10.2	1012.95	18635.2	7.7616
4	2	5	10.2	321.51	28623.0	11.9215
4	3	5	10.6	766.79	21825.9	9.0905
1	1	7	9.1	540.41	14035.5	5.8458
1	2	7	9.0	414.57	13210.7	5.5023
1	3	7	9.7	532.15	14878.1	6.1967
2	1	7	9.6	790.98	10030.5	4.1777
2	2	7	9.0	173.28	7951.2	3.3117
2	3	7	8.6	254.70	14590.9	6.0771
3	1	7	9.6	718.86	15762.3	6.5650
3	2	7	8.8	556.52	12685.8	5.2836
3	3	7	8.8	476.52	8122.3	3.3829
4	1	7	9.6	849.57	17992.4	7.4938
4	2	7	8.8	572.53	16081.0	6.6977
4	3	7	8.6	415.87	18299.1	7.6216

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 1.0 mg/l 2,4-D, 3 = 5.0 mg/l 2,4-D, and 4 = 10.0 mg/l 2.4-D.

Table A31. Field Data - Six/ Diuron Study

1.	2.	3.	4.	5.	6.	7.
1	1	2	7.6	32.19	7624.4	3.1756
1	2	2	7.5	26.62	3485.8	1.4518
1	3	2	7.3	14.16	3560.5	1.4829
2	1	2	7.1	9.80	2739.2	1.1409
2	2	2	6.7	3.92	2983.3	1.2426
2	3	2	7.0	20.23	4381.8	1.8250
3	1	2	6.7	0.00	2602.9	1.0841
3	2	2	6.7	84.96	2657.4	1.1068
3	3	2	6.5	12.71	3122.4	1.3005
4	1	2	5.9	6.54	2588.9	1.0783
4	2	2	4.4	9.80	1951.6	0.8129
4	3	2	5.4	3.92	2171.2	0.9043
1	1	5	10.6	958.78	21349.4	8.8920
1	2	5	10.6	213.67	19391.2	8.0764
1	3	5	10.8	152.98	24746.9	10.3071
2	1	5	8.3	274.39	8635.9	3.5968
2	2	5	9.2	579.46	9404.5	3.9170
2	3	5	9.0	416.51	9010.2	3.7527
3	1	5	6.6	9.13	3593.3	1.4966
3	2	5	6.1	31.45	3763.3	1.5674
3	3	5	6.3	15.68	4764.1	1.9843
4	1	5	5.6	1.09	9315.9	3.8801
4	2	5	5.8	10.17	7432.4	3.0956
4	3	5	5.6	29.97	11670.4	4.8607
1	1	8	9.6	076.33	10199.3	4.2480
1	2	8	9.6	076.33	17688.9	7.3674
1	3	8	9.6	076.33	22016.9	9.1701
2	1	8	9.8	978.15	2878.0	1.1987
2	2	8	9.8	978.15	21145.8	8.8072
2	3	8	10.6	173.65	8632.9	3.5956
3	1	8	11.6	230.45	4762.7	1.9837
3	2	8	8.4	782.53	7817.9	3.2562
3	3	8	9.8	684.84	3899.8	1.6243
4	1	8	8.1	229.84	18480.3	7.6970
4	2	8	7.9	457.63	7466.1	3.1096
4	3	8	7.9	266.51	45034.8	18.7570

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 0.01 mg/l Diuron, 3 = 0.1 mg/l Diuron, and 4 = 1.0 mg/l Diuron.

Table A32. Field Data - Seven/ Atrazine Study

1.	2.	3.	4.	5.	6.	7.
1	1	3	12.8	3469.21	10466.4	4.3593
1	2	3	14.8	1045.97	9963.3	4.1497
1	3	3	14.5	879.33	11232.9	4.6785
2	1	3	13.2	50.97	5793.2	2.4128
2	2	3	13.0	54.46	10535.5	4.3880
2	3	3	12.4	26.14	13283.8	5.5327
3	1	3	5.6	106.20	5137.7	2.1399
3	2	3	5.8	169.91	2062.4	0.8590
3	3	3	6.0	38.94	5626.9	2.3436
4	1	3	2.3	118.94	9927.8	4.1349
4	2	3	3.4	19.48	6939.8	2.8904
4	3	3	2.6	26.14	9608.0	4.0017
1	1	7	8.5	617.11	16012.8	6.6693
1	2	7	8.0	278.26	9988.2	4.1601
1	3	7	8.3	94.59	16944.4	7.0573
2	1	7	8.6	26.55	12145.3	5.0585
2	2	7	9.4	302.35	17855.4	7.4368
2	3	7	8.6	217.84	12878.0	5.3637
3	1	7	9.8	1120.96	9939.6	4.1399
3	2	7	10.4	1374.50	22865.5	9.5235
3	3	7	10.3	577.97	21005.0	8.7486
4	1	7	7.7	14.88	12041.6	5.0153
4	2	7	8.7	102.69	15113.5	6.2948
4	3	7	8.0	87.14	7812.2	3.2538
1	1	9	9.2	505.67	29388.3	12.2402
1	2	9	9.5	4337.27	30963.7	12.8964
1	3	9	9.1	499.05	26328.2	10.9657
2	1	9	11.4	392.11	21369.0	8.9002
2	2	9	10.0	329.82	12645.9	5.2670
2	3	9	9.8	1353.21	22993.6	9.5768
3	1	9	10.8	165.56	7864.7	3.2756
3	2	9	10.8	5319.20	17845.9	7.4328
3	3	9	10.0	914.92	9146.0	3.8093
4	1	9	9.3	364.27	5279.7	2.1990
4	2	9	10.0	1076.19	12759.4	5.3143
4	3	9	12.2	240.49	15787.9	6.5757

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, µg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 0.01 mg/l Atrazine, 3 = 0.1 mg/l Atrazine, and 4 = 1.0 mg/l Atrazine.

Table A33. Field Data - Eight/ Diuron Study

1.	2.	3.	4.	5.	6.	7.
1	1	3	8.4	78.664	283.24	0.11797
1	2	3	8.4	56.685	411.08	0.17122
1	3	3	8.0	59.127	444.05	0.18495
2	1	3	8.5	95.071	260.63	0.10855
2	2	3	8.4	83.065	350.63	0.14604
2	3	3	8.3	52.281	370.00	0.15410
3	1	3	8.4	78.626	610.18	0.25414
3	2	3	8.4	116.473	471.26	0.19628
3	3	3	8.3	143.274	506.13	0.21080
4	1	3	8.3	49.014	635.05	0.26450
4	2	3	8.0	49.014	459.28	0.19129
4	3	3	8.3	64.698	447.75	0.18649
1	1	5	8.3	169.914	1571.17	0.65439
1	2	5	8.4	110.047	1224.86	0.51016
1	3	5	8.3	164.657	916.22	0.38160
2	1	5	8.3	97.604	406.31	0.16923
2	2	5	8.3	64.095	1021.62	0.42551
2	3	5	8.3	137.121	938.56	0.39091
3	1	5	8.1	218.844	1549.73	0.64546
3	2	5	8.0	331.284	2505.95	1.04373
3	3	5	8.3	279.943	1367.66	0.56963
4	1	5	8.0	8.169	500.36	0.20840
4	2	5	7.9	17.542	1377.93	0.57391
4	3	5	8.0	14.076	706.31	0.29418
1	1	7	9.2	78.664	1619.01	0.67432
1	2	7	9.1	109.622	1053.42	0.43875
1	3	7	9.2	250.826	1219.55	0.50794
2	1	7	9.1	83.383	886.04	0.36903
2	2	7	9.2	51.382	720.18	0.29996
2	3	7	9.1	71.886	651.98	0.27155
3	1	7	9.2	338.859	4949.19	2.06134
3	2	7	9.2	369.036	1445.86	0.60220
3	3	7	9.2	279.116	1535.32	0.63946
4	1	7	9.1	47.647	1204.68	0.50175
4	2	7	9.0	95.071	1425.05	0.59353
4	3	7	9.1	36.013	1174.23	0.48907

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, µg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 0.01 mg/l, 3 = 0.1 mg/l, 4 = 1.0 mg/l diuron.

Table A34. Field Data - Nine/ Atrazine Study

1.	2.	3.	4.	5.	6.	7.
1	1	3	7.9	153.644	1125.32	0.468694
1	2	3	7.9	149.461	1532.61	0.638333
1	3	3	7.9	162.064	1448.02	0.603100
2	1	3	8.2	33.463	1004.77	0.418489
2	2	3	7.9	102.049	924.23	0.384944
2	3	3	7.7	5.591	795.32	0.331249
3	1	3	8.0	1.176	572.16	0.238306
3	2	3	8.1	9.984	948.11	0.394887
3	3	3	7.8	18.539	568.02	0.236580
4	1	3	7.8	0.583	880.90	0.366895
4	2	3	7.8	.	730.00	0.304045
4	3	3	7.9	3.112	718.92	0.299430
1	1	5	8.9	142.275	1725.95	0.718856
1	2	5	9.0	146.611	1492.43	0.621598
1	3	5	8.8	181.418	1319.19	0.549442
2	1	5	8.9	138.243	1148.11	0.478187
2	2	5	8.9	173.432	1307.39	0.544527
2	3	5	8.7	107.830	880.09	0.366558
3	1	5	8.8	33.812	939.28	0.391210
3	2	5	8.7	30.633	585.14	0.243709
3	3	5	8.7	39.734	1058.29	0.440777
4	1	5	9.0	7.842	564.05	0.234929
4	2	5	8.5	12.494	577.57	0.240557
4	3	5	8.9	7.352	615.68	0.256429
1	1	7	8.7	536.332	1456.76	0.606739
1	2	7	8.8	363.063	1709.28	0.711915
1	3	7	8.7	399.156	1376.22	0.573194
2	1	7	8.6	424.784	1148.02	0.478150
2	2	7	8.6	255.541	1104.77	0.460139
2	3	7	8.6	286.610	1175.95	0.489781
3	1	7	8.7	48.825	694.50	0.289261
3	2	7	8.6	48.825	907.48	0.377964
3	3	7	8.5	38.789	1005.59	0.418826
4	1	7	8.4	8.649	555.68	0.231439
4	2	7	8.4	1.984	976.31	0.406632
4	3	7	8.6	5.872	734.59	0.305959

Legend: 1. = Concentration, 2. = Replicate, 3. = Day, 4. = Dissolved Oxygen, 5. = Chlorophyll, μg/l, 6. = ATP, ng/l, 7. = Dry Weight, mg/l

Note: Under Concentration, 1 = Control, 2 = 0.01 mg/l Atrazine, 3 = 0.1 mg/l Atrazine, and 4 = 1.0 mg/l Atrazine.

Table A35. Short Term Algal Toxicity Assay to 2,4-D.

1.	2.	3.	4.	5.
00	01	6.80	10.6	26
02	01	6.77	10.4	26
04	01	6.79	10.6	25
06	01	6.82	10.4	26
08	01	6.82	10.5	26
10	01	6.85	10.4	25
15	01	6.92	10.7	25
20	01	7.02	10.6	25
30	01	7.12	10.5	25
00	10	7.28	10.5	25
02	10	6.73	10.1	25
04	10	6.73	10.2	25
06	10	6.74	10.3	26
08	10	6.75	10.2	26
10	10	6.75	10.2	26
15	10	6.76	10.1	26
20	10	6.79	10.1	26
30	10	6.81	09.8	26
00	30	6.82	09.8	26
02	30	5.12	09.0	26
04	30	5.09	09.2	35
06	30	5.08	09.2	34
08	30	5.08	09.2	33
10	30	5.07	09.2	33
15	30	5.06	09.1	31
20	30	5.02	09.0	30
30	30	5.05	08.8	30

Legend: 1. = Time, minutes, 2. = Concentration, mg/l, 3. = pH, 4. = Dissolved Oxygen, mg/l, 5. = Relative fluorescence.

Table A36. Growth Response of Chlorella sp. Isolate From Smith Mountain Lake to 2,4-D at pH 7.0. (Continued)

1.	2.	3.	4.	5.	6.
07	1	1	03113	10.60	00280
07	1	2	03507	07.20	00300
07	1	3	03218	08.00	00320
07	1	4	03625	10.70	00340
07	4	1	02918	06.10	00190
07	4	2	03936	08.50	00220
07	4	3	02425	07.40	00220
07	4	4	03447	11.70	00180
07	6	1	02831	08.40	00250
07	6	2	03228	10.50	00260
07	6	3	04346	12.10	00240
07	6	4	03628	14.00	00250
07	8	1	05374	09.30	00350
07	8	2	06742	13.30	00320
07	8	3	03473	10.70	00280
07	8	4	05899	13.40	00370
10	1	1	05310	08.60	00510
10	1	2	06312	11.40	00510
10	1	3	16296	16.90	00540
10	1	4	17766	12.60	00420
10	4	1	03384	09.30	00410
10	4	2	05377	14.60	00400
10	4	3	05421	15.00	00360
10	4	4	07510	20.00	00480
10	6	1	03920	08.20	00270
10	6	2	04960	15.40	00320
10	6	3	04235	10.10	00360
10	6	4	04387	17.60	00340
10	8	1	06545	09.50	00350
10	8	2	05696	19.90	00520
10	8	3	05803	10.40	00400

Legend: 1. = Concentration, mg/l, 2. = Cells/ml (x 1000), 3. = ATP ng/ml), 4. = Relative Fluorescence.

Note: Under Concentration, 1 = control, 4 = 22.1 mg/l, 6 = 0.2 mg/l and 8 = 0.002 mg/l.

(Continued)

Table A36. Growth Response of Chlorella sp. Isolate From Smith Mountain Lake to 2,4-D at pH 7.0. (Continued)

1.	2.	3.	4.	5.	6.
10	8	4	06640	11.50	00490
12	1	1	10734	26.30	01410
12	1	2	10516	26.30	10440
12	1	3	11903	40.70	00750
12	1	4	10141	31.70	00630
12	4	1	10890	25.10	01140
12	4	2	09666	22.20	01200
12	4	3	08451	33.80	00960
12	4	4	09145	23.80	01050
12	6	1	07853	27.90	01260
12	6	2	09553	22.50	01200
12	6	3	11881	28.50	01500
12	6	4	08978	24.40	01320
12	8	1	12341	32.80	10320
12	8	2	07393	24.90	00940
12	8	3	11389	32.90	01650
12	8	4	06663	21.00	01200

Legend: 1. = Concentration, mg/l, 2. = Cells/ml (x 1000), 3. = ATP ng/ml), 4. = Relative Fluorescence.

Note: Under Concentration, 1 = control, 4 = 22.1 mg/l, 6 = 0.2 mg/l and 8 = 0.002 mg/l.

Table A36. Growth Response of Chlorella sp. Isolate From Smith Mountain Lake to 2,4-D at pH 7.0.

1.	2.	3.	4.	5.	6.
03	1	1	00303	00.15	00059
03	1	2	00632	00.07	00043
03	1	3	00418	00.20	00040
03	1	4	00608	00.08	00033
03	4	1	00421	00.28	00047
03	4	2	00631	00.13	00021
03	4	3	00483	00.20	00027
03	4	4	00508	00.27	00032
03	6	1	00774	00.13	00150
03	6	2	00437	00.13	00048
03	6	3	00494	00.06	00038
03	6	4	00476	00.04	00027
03	8	1	00369	00.04	00057
03	8	2	00337	00.14	00018
03	8	3	00576	00.04	00035
03	8	4	00317	00.08	00015
05	1	1	02468	04.00	00350
05	1	2	02564	04.30	00300
05	1	3	01673	03.00	00320
05	1	4	02082	04.10	00290
05	4	1	01761	03.20	00084
05	4	2	01768	04.00	00165
05	4	3	01168	03.10	00099
05	4	4	01645	01.00	00135
05	6	1	01025	02.80	00270
05	6	2	01397	03.40	00260
05	6	3	00900	02.90	00123
05	6	4	01555	03.40	00190
05	8	1	01308	02.70	00168
05	8	2	01724	03.20	00280
05	8	3	00908	02.20	00129
05	8	4	01306	03.00	00068

Legend: 1. = Concentration, mg/l, 2. = Cells/ml (x 1000), 3. = ATP ng/ml), 4. = Relative Fluorescence.

Note: Under Concentration, 1 = control, 4 = 22.1 mg/l, 6 = 0.2 mg/l and 8 = 0.002 mg/l.

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THE EFFECT OF 2,4-D, ATRAZINE, AND DIURON ON ALGAL PHYSIOLOGY AND POPULATION DYNAMICS

by

Henry Wilmore Cox, Jr.

(ABSTRACT)

Field and laboratory studies were conducted to determine the ecological and physiological responses of phytoplankton to environmentally significant concentrations of atrazine, diuron, and 2,4-D.

Field results would indicate that all three herbicides have a significant effect on species diversity and species dominance. Atrazine and diuron were particularly effective in decreasing diversity in concentrations of 10 ppb to 1.0 ppm. While total biomass as measured by ATP decreased significantly for both atrazine and diuron, total chlorophyll levels increased significantly in 0.1 mg/l atrazine. Decreases in chlorophyll were noted for all other concentrations tested in atrazine and diuron. Blue-green algae *Gloeocapsa* sp. and *Synecchoccus* sp. were the predominant genera in the 0.1 mg/l atrazine. 2,4-D tests also resulted in significant increases in chlorophyll over control replicates. Additionally, biomass as measured by ATP indicated significant increases over control values in 10 mg/l 2,4-D suggesting that 2,4-D may have stimulated the growth of algae or that select algae

within the test were able to take a competitive advantage in the presence of 2,4-D. This result was not consistent however.

Laboratory tests using continuous cultures of *Chlorella sorokiniana* indicated that both atrazine and diuron were effective toxicants in low concentrations. Diuron was an effective growth inhibitor in a range of concentration from 0.0014 to 0.01 mg/l. Atrazine was an effective growth inhibitor from 0.014 to 0.1 mg/l. 2,4-D stimulated the growth of continuously cultured *Chlorella sorokiniana* in a range of concentration from 8.6 to 20 mg/l as measured by cell counts, total organic carbon, *in vivo* chlorophyll fluorescence, and ATP. The data indicated that the effect was not due to the utilization of 2,4-D as a carbon source but to an actual enhancement of the photosynthetic process. The uptake of radioactively labeled carbon (^{14}C added as $H^{14}CO_3$) was significantly increased in the presence of 2,4-D. The literature contains several reports of enzyme activity stimulation by 2,4-D (108). The data supported the suggestion that 2,4-D may influence the Hill reaction and respiration by stimulating an enzyme (or enzymes) involved in these processes. The net result may have been an increase in the rates of reactions in both the Calvin cycle and photorespiration.