ABSTRACT

Kevin James Currie. Bioassay Determination of Species Specific Phytoplankton Responses to the Herbicide Atrazine and its Quantification in B. Everett Jordan Reservoir. (Under the direction of Dr. Donald E. Francisco)

Atrazine was quantified bi-weekly in samples from three locations in B. Everett Jordan Lake during March - July 1985 using gas chromatography. The presence in Segment 1 of atrazine and other previously identified Haw River constituents was verified by GC/MS. The highest concentrations were consistently found in Segment 1 (0.5-2.5 ug/L) and residue concentrations were generally higher in Segment 2 than Segment 3. Although atrazine concentrations declined rather rapidly following the field application runoff pulse in May, herbicide residue levels remained higher than those prior to that date. In vitro, natural population bioassays revealed species specific responses to atrazine. The population as a whole was severely inhibited at 50 ug/L atrazine. Results suggest low-dose (1 ug/L) growth stimulation for several members of the Cyanophyta. Several species of the Chlorophyta exhibited temporal growth lags at atrazine concentrations of 50 ug/L. However, maximum biomass was not severely depressed. Other species of green algae, Chlamydomonas in particular, exhibited resistance to the effects of atrazine at all doses. Competitive interactions between species affected individual responses to the toxicant. Species specific responses to atrazine levels commonly found in agricultural watersheds (0.25-10 ug/L) illustrate the potential of this important herbicide to alter the ecological basis of the food web.

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LITERATURE REVIEW

General Characteristics of Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a colorless crystalline powder in its pure form with a melting point of 173-175°C. At 25°C it has a vapor pressure of 4×10^{-7} mm Hg, and while relatively insoluble in water (70 mg/L), it is very soluble in organic solvents (ether 12,000 mg/L, methanol 18,000 mg/L and chloroform 52,000 mg/L). It is stable in neutral, weakly acidic or alkaline media and has a pKa of 1.68 at 22°C in water.

The herbicidal potential of atrazine was recognized in the early 1950's, and it was released for public use in 1959. Today, atrazine is the herbicide of choice for American corn and sorghum farmers. It is widely used for pre-and post-emergent control of germinating weeds and for non-selective control of weeds in non-cropped areas (Lewis et al. 1985). Atrazine does not biomagnify, has relatively low toxicity to mammals (acute oral LD_{50} for rats is 1750 mg/kg) but somewhat higher toxicity in fish (48 hour LC_{50} for rainbow trout is 10 mg/L). In 1976 41 million kilograms, as active ingredient, were applied to land in the United States (Eichers et al. 1978). In North Carolina alone, over 1.5 million kilograms are applied on an annual basis (Turner, Digiano, and DeRosa 1984).

Due to its low water solubility, atrazine is usually supplied as a wettable powder or as a suspension concentrate although a granular formulation is available for longer persistence. The usual method of application is whole field spraying. In North Carolina, application usually takes place with crop planting in April but occasionally, a second post-emergent treatment is also used (Lewis et al. 1985).

The amount of atrazine applied depends on the crop being grown and the soil type. Recommended rates of active ingredient for corn and sorghum (its principle application in North Carolina) are 2.0 - 3.0 and 1.6 - 2.4 pounds per acre, respectively. The range is due to soil type: The higher rates are used on heavy clay/organic soils while lighter loamy soils receive less herbicide. Atrazine is not recommended for sand or soils with less than 1% organic content. No-till agriculture requires slightly higher rates of herbicide application to penetrate the surface soil to the root zone where it has its action.

Fate of Field-Applied Atrazine

After application, atrazine will have one of six fates. It may be 1) taken up by target crops where it inhibits photosynthesis or by resistant crops where it is metabolized; 2) sorbed to the soil and hydrolyzed to inactive hydroxy-atrazine; 3) hydrolyzed in the soil-water to hydroxy-atrazine and remain soluble or become sorbed to the soil; 4) reversibly bound to soil without decomposition and later

released as active atrazine; 5) transported as active atrazine in runoff waters in soluble form or as a soil-bound colloid; or 6) degraded microbially producing a variety of atrazine analogs.

The potential impacts a field-applied herbicide might have on non-target organisms present in receiving rivers and lakes will be determined by the amount and form of the herbicide in runoff waters. This in turn, is primarily dependant on field moisture and the soil's physical-chemical characteristics. Atrazine is transported through the soil to absorbing plant roots by mass flow and molecular diffusion (Lavy 1968). Without adequate field moisture atrazine's phytotoxicity towards target plants is severely diminished (Harrison et al. 1976) and the molecule is increasingly subject to sorption by clay and organic materials in the soil (Dao et al. 1978).

Application rates for atrazine are based on the percentage of clay and organic material contained by receiving soils. The relationship between atrazine activity and percentage kaolinitic clay in North Carolina soils has been described as weak (Harrison et al. 1976), while Anderson et al. (1980) and Smit et al., as cited by Nel and Reinhardt (1984), found a strong negative relationship between atrazine availability and percentage organic material found in soils.

In most soils the organic material is intimately bound to the clay, probably as a clay-metal-organic complex. Humus (the organic fraction) has been shown to have about four

times the cation exchange capacity of clay (Klingman, Ashton, and Noordhoff 1975). Then, the relative sorptive capacity of the soil will be determined by the degree to which the clay fraction is coated with organic material.

Soil pH determines, to a great extent, the degradative mechanisms operating on atrazine and the sorptive capacity of the clays and humus present there. At pH values close to 7 atrazine is extremely stable, however a decrease in availability of atrazine with decreasing pH is generally recognized. According to Armstrong et al. (1967), the most important chemical mechanism for degradation of atrazine at low pH is hydrolysis to hydroxy-atrazine. It was suggested that sorption takes place between the ring nitrogen of atrazine and a protonated-COOH group of the organic matter/clay complex. Hydrogen bonding of the ring nitrogen causes loss of Cl^- and subsequent replacement with OH^-. Under high pH conditions, direct nucleophilic substitution of OH^- for Cl^- is thought to take place. Mechanisms of inactivation include sorption to organic/clay complexes via hydrogen bonding or protonation in the soil solution and subsequent ion exchange. Both mechanisms are reversible and result in complexes that are subject to transport in runoff water.

Smit et al., as cited in Nel and Reinhardt (1984), describe an inorganic soil fraction (Fe.Al.OH) which they label the soil amorphous component. It was suggested that sorption of atrazine to this component complexed with clay

delayed decomposition of the herbicide because atrazine was sorbed in the anionic form without hydroxylation at the sorption site. The complexed atrazine is biologically inactive, but their data suggest that atrazine is released in the active form when the pH rises. Soils with a large amorphous fraction could accumulate atrazine in this manner and release it a long time after application, during field liming, for example. This hypothesis is supported by the work of Kells et al. (1980). Wijayaratne and Means (1984) have found that active atrazine is released from colloid complexes under oxidizing conditions. There is general agreement that atrazine does carry over to the next growing season in soil (Armstrong 1967; Wu 1980; Khan and Saidak 1981; and Nel and Reinhardt 1984); however, it is apparently released in runoff during the next growing season because it does not accumulate over time (Wu 1980).

Smit et al., as cited by Nel and Reinhardt (1984), have found a positive correlation between phosphorus concentration and atrazine availability in soils containing a large amorphous component. It was hypothesized that phosphorus competes with atrazine for the negative binding sites in these soils.

A delay similar to the one outlined above has been reported (Wu et al. 1983) for experimental watersheds. During the growing season (May-August, 1977), less than ten percent of the total atrazine discharge to receiving streams occurred. Significant atrazine discharge did not occur until

January of 1978 which indicates that atrazine was either bound reversibly or remained uncomplexed for at least eight months and then was transported to receiving streams without decomposition. It should be noted that this runoff occurred after an extremely dry growing season and that this single storm event produced greater than 50% of the total atrazine discharged on an annual basis (Wu et al. 1983).

S-triazines are susceptible to biological degradation in the soil. Early researchers thought this to be the primary mechanism of atrazine inactivation. Non-sterile soils added to aqueous solutions of simazine have been shown to have greater degradative capacity than sterile soils (Burnside et al. 1961). Fungi (Kearney 1966) and bacteria (Kaufman et al. 1965) have been implicated in a variety of metabolic transformations. However, when compared to the evidence for the relatively rapid chemical degradative mechanisms operating, microbes play a relatively minor role in the inactivation of atrazine.

There is general agreement that a small percentage of applied atrazine actually makes its way to aquatic environments. Values of between 0.1% to 3.0% are present in the literature (Hall et al. 1972; Frank et al. 1979; Muir and Baker 1978; Hormann et al. 1979; Wu 1980; and Glotfelty et al. 1984). Atrazine transport in streams occurs mainly in solution. Solution-atrazine comprised 58% to 99% of the total detected (Ritter et al. 1974; Leonard et al. 1979; Frank et al. 1979; Wu 1980; and Glotfelty et al. 1984).

Estuarine colloids have been shown to be 10 to 35 times greater in atrazine sorptive capacity than sediment or soil organic matter on an organic carbon basis (Means and Wijayaratne 1982). However, a significant decrease in sorptive capacity occurred when the ambient pH of 7.98 was increased to 9.0 or decreased to 5.0. It was suggested that colloids could play an important role in atrazine transport.

The half-life of atrazine in various environments is expected to be highly variable, reflecting the different conditions present (pH, soil type, organic carbon, and salinity). In distilled water, hydrolysis to hydroxy-atrazine reaches a minimum at pH 7.0. The half-life under these conditions has been calculated to be approximately 1800 years (Plust et al. 1981). Essentially no atrazine degradation occurred in synthetic sea water after four months (Ballantine et al. 1978). Armstrong et al. (1967) found a ten fold increase in hydrolysis of atrazine upon addition of sterile soil to aqueous medium. A three to twelve day half-life (degradation plus sorption) was determined by Jones et al. (1982) using a 2:1 water/soil estuary mixture, whereas Ballantine et al. (1978) found the half-life to be 30 days using a 10:1 water/soil estuary mixture from Chesapeake Bay. Less work has been done with fresh water degradation. The half-life of atrazine in fresh water/sediment mixtures has been found to be between 95 days to greater than three years (Armstrong et al. 1967).

Atrazine Concentrations In Streams And Lakes

The above mentioned mechanisms for degradation as well as transport of atrazine and its degradation products to aquatic environments illustrate the complex set of factors that determine the concentration of atrazine and its degradation products in streams and lakes. As might be expected, concentrations of atrazine are highest near its source so the greatest immediate impact will be on submerged plants and periphyton in streams. Glotfelty et al. (1984) found 300 ug/L atrazine in "edge of field" runoff after the first significant rainfall in the Wye River Estuary system. Variation is to be expected with season and soil conditions. Wu (1980) reported "edge of field" values for the growing season (May-August, 1977) to be from <0.08 ug/L to 52 ug/L.

As atrazine is transported downstream, it may settle out if sorbed to suspended material and be diluted such that non-target impacts in larger streams and rivers should reflect lower concentrations. Conversely, if sorbed to colloids, atrazine may be transported without significant decrease in its concentration. In the main body of the Wye River Estuary, Glotfelty et al. (1984) reported upstream atrazine values of 15 ug/L, decreasing downstream to 1 ug/L near the Chesapeake Bay. Frank et al. (1979) reported atrazine values at the mouths of rivers entering the Great Lakes similar to those found upstream. Atrazine concentrations ranged from < 0.02 ug/L to 33 ug/L with a mean concentration (n=92) of 1.6

ug/L. Muir, Yoo, and Baker (1978) have reported similar concentrations in five Quebec watersheds.

Lakes and bays may serve as reservoirs for atrazine inputs via bioaccumulation and colloid or sediment sorption, although concentrations reported are generally lower than in streams. Samples taken in June and July 1980 from the Chesapeake Bay never exceeded 1.3 ug/L (Kemp et al., as cited by Glotfelty et al. 1984). This reflects the dry conditions that growing season and the low concentrations found in the Wye River (generally < 0.05 ug/L) reported by Glotfelty et al. (1984) for that year. In contrast, concentrations in an Iowa reservoir were as high as 9.4 ug/L during May-June 1974 (Richard et al. 1975). The highest atrazine residues will generally be found shortly after the first significant runoff event following spraying. However, during drought years, significant discharge events may be missed with summer sampling. Wu et al. (1983) found "edge of field" concentrations of atrazine exceeding 100 ug/L in runoff sampled during January and approximately 20 ug/L before crop spraying in May.

Ecological Impacts of Atrazine

Many of the studies mentioned above were conducted to assess the extent of atrazine pollution of aquatic systems due to concern over its impact on non-target organisms, mainly fish and rooted aquatic plants. A "safe" value using chronic and acute testing methods for estuarine zooplankton,

crab, shrimp, minnow and oyster populations of 9 ug/L atrazine was found by Ward and Ballantine (1985). Frank et al. (1979) state that atrazine concentrations entering the Great Lakes "do not pose a threat to Great Lakes water quality as defined by water quality objectives for non-persistent organics." Glotfelty et al. (1984) "can find no evidence that atrazine entering the Chesapeake Bay via the Wye River Estuary causes significant harm to the submerged aquatic vegetation found there."

Algal Sorption Of Atrazine

Currently, there are no regulations governing the introduction of non point-source pollutants from the perspective of phytoplankton impacts. However, the potential impacts a photosynthesis inhibitor might have on algae has been of concern and much work has been done in this area.

The fact that atrazine is not biomagnified in aquatic ecosystems is to be expected of a non-lipophilic herbicide. Residues are, therefore, not concentrated in predators. However, evidence for bioaccumulation in algae (which have large surface/volume ratios and occasionally high biomass in lake ecosystems) has been reported (Streit 1979). Similar evidence is provided by the work of Valentine and Bingham (1976). They found that adsorption of ^{14}C atrazine was complete after six hours in aqueous media containing 10 ug/L to 1.0 mg/L atrazine although more atrazine was removed on a percentage basis from the lower concentration (42%) than from

the higher concentration (20%). They found no difference with light or temperature conditions and concluded that the atrazine removal mechanism operating in Scenedesmus quadricauda must be physio-chemical in nature. This same experimental procedure was used with Chlamydomonas vulgaris and 2.5 mg/L atrazine (Veber et al. 1981). Within one hour, 90% of the atrazine present was adsorbed to cells. Upon longer exposure, adsorbed atrazine was released back to solution and subsequently taken back up by the end of 96 hours. This same result was observed in flasks in which essentially no growth occurred (5.0 mg/L atrazine) indicating that uptake of atrazine isn't dependant on cell growth. In contrast Butler, Deason, and O'Kelley (1975b) concluded that algae did not remove atrazine from media in which 21 algal isolates were grown for two weeks.

Uptake in higher plants has been shown to occur through the root system and phytotoxic action takes place by inhibition of photosythesis at photosystem II. Algae are susceptible to this same inhibition. It has been shown that atrazine does not inhibit respiration (Galloway and Mets 1981) or cause any permanent damage to the photosynthetic cell organelles (Boger 1976). Disregarding genetic resistance for the moment, plant resistance is conferred by the ability to metabolize atrazine to an inactive molecule along one of several pathways (Nel and Reinhardt 1984). These degradative mechanisms have been shown to be unimportant in algae (Butler, Deason, and O'Kelley 1975b;

Valentine and Bingham 1976), and in fact species of filamentous algae, considered a nuisance at high densities, have been successfully controlled in ponds by addition of 0.5 to 1.0 mg/L atrazine (Walker 1964).

Effects Of Atrazine On Algae - Single Species Bioassays

Many investigators have provided evidence that atrazine has an effect on the growth of algae. A representative sample of results is presented in Table 1.

Nitrogen fixation by Cyanophyta is an important factor in the availability of nitrogen in the soil, especially in rice cultivation. Rohwer and Flueckiger (1979) found that neither growth nor nitrogen fixation by Anaebaena cylindrica was affected at concentrations of atrazine between 0.22 - 22.0 ug/L. However, at 2.2 mg/L both functions were essentially static. This conflicts with the results of Stratton (1984) who found EC_{50} values of greater than 100 and 55 mg/L atrazine for nitrogen fixation in A. cylindrica and A. inaequalis respectively. These discrepancies may be due to differences in incubation periods.

Atrazine was found to delay growth of Chlorella pyrenoidosa by extending the "lag phase" of growth (Gonzalez et al. 1985). While cell division was inhibited by treatment compared to controls, chlorophyll synthesis was more strongly inhibited. In addition, it was found that atrazine dosing caused the ratio of chlorophyll a to chlorophyll b to

Table 1. Representative algal species and the effects of atrazine demonstrated *in vitro*, at the doses and conditions reported.

Species	Conc. (ug/L)	Effect/ Condition	Reference
Anaebaena cylindrica	0.22-22.0	no effect (2 week growth)	Rohwer and
A. cylindrica	2200	v. limited growth	Flueckiger(1979)
A. cylindrica	0.22-22.0	no effect (Nit. Fix.)	"
A. cylindrica	220	50% of Controls (Nit. Fix.)	"
A. cylindrica	2200	effectly inhibited (Nit. Fix.)	"
A. cylindrica	500	EC50 (photosynthesis)	Stratton (1984)
A. cylindrica	3600	EC50 (growth rate)	"
A. cylindrica	1200	EC50 (growth yield)	"
A. cylindrica	370	EC50 (photosynthesis)	Stratton and
A. inaequalis	50	EC50 (photosynthesis)	Corke (1981)
A. inaequalis	30	EC50 (growth yield)	Stratton (1984)
A. inaequalis	100	EC50 (growth rate)	"
A. variablis	100	EC50 (photosynthesis)	"
A. variablis	4000	EC50 (growth)	"
A. variablis	5000	EC50 (growth rate)	"
Oscillatoria lutea	1.0-1000	7%, 0% (of control growth)	Torres and O'Flakerty (1976)
Bumilleriopisis filiformis	1500	(growth equal to controls Chl a content/cell increase 33%)	Boger (1976)
Vaucheria geminata	1.0-1000	2%, 100% decrease Chl a	Torres and O'Flakerty (1976)
Euglena gracilis	100-10,000	75% of controls - no growth	Valentine and Bingham (1976)
Nitzschia sp.	1000	2 week - no growth	Butler *et al.* (1976)
Ankistrodesmus braunii	60	EC50 (growth and Chl a)	Burrell *et al.*(1985)
Cladophora sp.	500-1000	growth controlled in pond	Walker (1964)
Chlamydomonas reinhardtii	5000	growth prevented	Loeppky (1969)
C. reinhardii	100-1000	no inhibition/growth prevented	"
C. eugametos	5000	no effect	"
Chlorella pyrenoidsa	500	EC50 (photosynthesis)	Stratton (1984)
C . pyrenoidosa	300	EC50 (growth)	"
C. pyrenoidosa	200	EC50 (growth rate)	"
C. pyrenoidsa	100-10,000	no effect/ complete inhibition	Valentine and Bingham (1979)
C. pyrenoidsa	100-1000	slight inhibition/ total inhibition	Wells and Chappell (1965)
C. pyrenoidsa	54, 108	35% and 65% growth decrease	Gonzalez *et al.* (1985)

Table 1. (Cont'd) Representative algal species and the effects of atrazine demonstrated *in vitro*, at the doses and conditions reported.

Species	Conc. (ug/L)	Effect/ Condition	Reference
C. pyrenoidsa	20,40, 200,400	15,25,30,75, 100% inhibition	Gramlich and Frans (1964)
C. pyrenoidsa	500, 1000	70%,95% growth inhibition	Virmani et *al.*(1975)
C. vulgaris	25	EC50 (cell growth and Chl a)	Burrell et *al.*(1985)
C. vulgaris	1.0	50% decrease in Chl a	Torres and
C. vulgaris	0.5	156% increase in Chl a	O'Flaherty (1976)
Chloroccum hypnosporum	0.5-50	no effect	"
C. hypnosporum	5000	growth reduced "slightly"	Virmani et *al.*(1975)
Nanochloris oculata	100	46.2% - 54% inhibition (depending on light and temp.)	Karlander et *al.* (1983)
Scenedesmus	100-10,000	slight effect - complete inhibition	Valentine and Bingham (1976)
S. quadricauda	300	EC50 (photosynthesis)	Stratton (1984)
S. quadricauda	100	EC50 (growth yield)	
S. quadricauda	20	EC50 (growth rate)	
Stigeoclonium tenue	1.0-1000	99.8%, 92% of controls	Torres and O'Flakerty (1976)

decrease relative to controls. Ridley (1977) suggests that chlorophyll b is subject to less photo-destruction than chlorophyll a because of efficient energy transfer to chlorophyll a. Virmani, Evans, and Lynn (1975) reported a 70% and 95% initial growth reduction for Chlorella pyrenoidosa by 0.5 and 1.0 mg/L atrazine respectively. These results at non-lethal doses of atrazine were interpreted as an indication that cell walls adsorb the herbicide initially, inhibiting growth but reducing the concentration of herbicide remaining free in the media, which in turn allowed growth equal to controls. Similar results and conclusions were presented for atrazine doses of 0.2 mg/L (Gramlick, and Frans 1964). Chlorococcum hypnosporum was not affected by doses of 50 ug/L atrazine. This result was explained as a failure of atrazine to penetrate the thick cell wall of this species (Torres and O'Flaherty 1976).

Some reports indicate that sub-toxic levels of triazine herbicides increase growth and nitrogen content of certain plant species (Ashton and Crafts 1981). Increases in growth of Chlamydomonas eugametos grown in simazine, another triazine-Hill reaction inhibitor, may result from increased nitrate uptake (Vance and Smith 1969). Boger (1976) found a 33% increase, relative to controls, in chlorophyll a content per cell and oxygen evolution in Bumilleriopsis filiformis grown in atrazine-free media after being grown in 1.5 mg/L of the herbicide. Cell growth was approximately the same as controls. Chlorophyll a was used to measure the low-dose

response of Chlorella vulgaris (Torres and O'Flaherty 1976). Atrazine dosage of 0.5 ug/L produced a response that was 156% of control values.

The wide range of effects reported in Table 1 and the text above illustrates the species specific nature of the action of atrazine as well as the serious lack of standard testing conditions. Karlander, Mayasich, and Terlizzi (1983) have pointed out that temperature and light, in addition to atrazine concentration, are important to the toxicity exhibited by the herbicide. Toxicity was maximized when conditions were optimal for rapid growth. Their results for atrazine inhibition of Nanochloris oculata ranged from 46.2% at 15°C and 0.208 mW/cm to 54% at 25°C and 1.352 mW/cm.

Mechanisms Of Inhibition And Resistance In Algae

As early as 1964, species specific differences in tolerance to atrazine were postulated as being due to herbicide/receptor site binding (Gramlick and Frans 1964). Herbicide resistance has been induced in Chlamydomonas reinhardtii by growing it on atrazine-fortified medium. This culture showed atrazine resistance compared to the non-induced precursor culture under autotrophic conditions (Galloway and Mets 1981). More recent work has provided evidence that resistance in Chlamydomonas reinhardtii can be established by a mutation of the chloroplast gene which codes for a protein of photosystem II (Erickson et al. 1984). This protein is part of the secondary stable electron acceptor

(complex B) which receives electrons from the primary stable electron acceptor (complex Q) in photosystem II. It has been proposed that atrazine binds to the protein of complex B preventing electron transfer from complex Q and that resistance is conferred such that atrazine does not bind to this protein (Galloway and Mets 1984). Erickson et al. (1984) have sequenced this protein and shown that a single amino acid change in mutant C. reinhardtii cells results in resistance to the effects of the herbicide on electron transfer. It was also demonstrated that in the absence of atrazine, electron transfer from Q to B is inhibited in mutant cells. This effect has been established by others (Galloway and Mets 1984). An additional note on the species specific differences seen in atrazine resistance: Gillham (1978) points out that C. reinhardtii is the only alga for which gene recombination and therefore mutation possibility during sexual reproduction has been observed. This would indicate that chloroplast gene recombination may be important in conferring atrazine resistance via mutation in algae.

Atrazine/Atrazine-Analog/Solvent Interactions And Toxicity

Many of the studies cited above used relatively high concentrations of atrazine. The usual method of preparing atrazine/water solutions is to dissolve the herbicide in a water soluble organic solvent such as acetone and dilute this with water. Many researchers do not provide details of herbicide formulations or additions. Butler, Deason, and

O'Kelley (1975a) state the concentration of acetone they used was 0.5% or less, which did not affect growth. Stirring media overnight containing these same concentrations allowed acetone to evaporate and experiments indicated that acetone had no effect on growth (O'Kelley and Deason 1976). It has been suggested that acetone increases cell permeability and subsequent herbicide uptake by disruption of membrane structure and transport systems (Stratton, Burrell, and Corke 1982). It was argued that synergistic or antagonistic interactions between solvent and herbicide can mask the effects of the herbicide and lead to erroneous conclusions regarding its toxicity. Furthermore, it was suggested that the solvent used in bioassays should react additively with the herbicide and inhibition values should be calculated by subtraction from controls. Data provided by Stratton and Corke (1981) indicate that atrazine and acetone interact additively in experiments with Scenedesmus quadricauda at 0.1% and 0.2% acetone but synergistically above 0.2%. Solvent/herbicide interactions were additive at 0.1, 0.2 and 0.6% acetone, antagonistic at 0.4% but synergistic at 0.8 and 1.0% for Chlorella pryenoidosa. It was suggested that stimulation of photoactivity in the acetone controls (30-40% at 1.0% acetone) is the result of solvation of selected membrane components by acetone and the increased permeability to CO_2 that would result.

It is well established that much of the atrazine applied to crops degrades in the plant and receiving environment to

several of its analogs, primarily hydroxy-atrazine. Few studies have been conducted to assess the degree of metabolite pollution in fresh water. Atrazine/deethylated-atrazine ratios of between three and ten have been reported for Ontario rivers feeding the Great Lakes (Frank et al. 1979). Only recently have studies of atrazine analog toxicity been undertaken. Atrazine was found to be seven to ten times more inhibitory than the most effective metabolite towards blue-green algae (Stratton 1984). The order of inhibition by metabolites tested was; deethylated > deisopropylated > diamino > hydroxy-atrazine. The latter two metabolites were relatively non-toxic, having EC_{50} values greater than 10 mg/L. This same toxicity sequence has been reported for non-target submerged vascular plants (Jones and Winchell 1984).

Effects Of Atrazine On Algae - Population Bioassays

The ability of atrazine to inhibit cellular functions and growth in algae is widely accepted. Therefore, its presence will have an impact on these non-target organisms. Toxicity testing with single species can only produce information about the response of that organism to the toxicant. This information may not be valid in predicting any but immediate effects in a dynamic aquatic ecosystem in which interspecific competition is operating. This has been illustrated by Mosser, Fisher, and Wurster (1972). Thalassiosira pseudonana and Dunaliella teriolecta were grown

in mixed culture in the presence of DDT or PCBs. Pure culture bioassays with these toxicants had established D. teriolecta and T. pseudonana as resistant and sensitive, respectively. T. pseudonana established dominance over D. teriolecta in control cultures but was not able to compete with the resistant D. teriolecta in the presence of either of the pesticides. This was the case even at toxicant concentrations shown to have no effect on either organism in pure culture.

The ecosystem approach to toxicity testing has, to date received little attention. This is probably due to the enormity of the task and difficulty of its interpretation. DeNoyelles, Kettle, and Sinn (1982) exposed duplicate ponds to 20 and 500 ug/L atrazine; two additional ponds served as controls. Total biomass, as measured by cell counts and ^{14}C uptake decreased sharply during the first few days in the 500 ug/L ponds but by Day 30 equaled control ponds. The immediate decrease was interpreted as a direct result of atrazine inhibition of all algal species in addition to the secondary effect of zooplankton grazing on the stressed population. Later, opportunistic members capable of growing in the presence of atrazine dominated the ponds, bringing total autotroph biomass equal to controls. The lower, 20 ug/L dose of atrazine did not result in a biomass much different than the controls. Species that grew well in the 500 ug/L ponds after initial inhibition were; Mallomonas spp. (predominately M. pseudocoronata), Cryptomonas marsonii, and

C. erosa. Those that experienced decline were; Coelastrum spp., Oocystis spp., Scenedesmus spp., Staurastrum tetracirum, and Tetraedron minimum. Species that were present in all ponds were; Dinobryon divergens var. Schauinslandii, Kirchneriella lunaris var. irregularis, Synedra acus, Senedesmus radians, and Uroglenopsis americana. Additional atrazine exposure of subsamples from each pond after Day 42 resulted in less inhibition for populations from the higher atrazine-dosed ponds as measured by fluorescence increases. This was taken as evidence of resistance to atrazine by the species present. Resistance for one particular species, Cryptomonas marsonii, was demonstrated in the laboratory. Growth after 19 days in 500 ug/L atrazine was not significantly different than that in control flasks. Definite responses attributable to the effects of atrazine could not be shown for organisms in the food web of higher order than the zooplankton.

Another interesting study has been conducted to determine what effect atrazine might have on periphyton community structure in flow-through microcosms. Species enumeration was conducted prior to, during, and after treatment with 100 ug/L atrazine (Hamala and Kollig 1985). The pretreatment community was composed of (as percentage of total); Chlorophyta 71%, Bacillariophyta 19%, Cryptophyta 3% and Cyanophyta 2%. Following atrazine addition, the composition relative to controls for treatment and recovery periods, respectively, was as follows; Total count 23 and

25%, Chlorophyta 15 and 26%, Bacillariophyta 19 and 75%, Cyanophyta 216 and 83%, and Cryptophyta 9 and 8%. During treatment Ankistrodesmus falcatus, Chlamydomonas spp., Cosmarium reinschii, Scenedesmus dimorphus, Staurastrum manfeldtii, and Stigeoclonium lubricum all experienced large density decreases. In contrast Chroococcus minor increased to 224% of control values.

Community productivity, as measured by oxygen production decreased throughout the treatment period. In contrast, community respiration in the atrazine microcosms was similar to controls, with slight increases during treatment attributed to heterotrophic activity. It was suggested that the rapid recovery of community productivity following treatment indicated the effect of atrazine was algistatic rather than algicidal. No evidence for induced resistance could be found for the treated microcosms upon additional atrazine treatment after the recovery period. In contrast to this study, Lynch, Johnson, and Adams (1985) could find no significant or lasting effects on primary productivity or community respiration in a similar study using 25 ug/L atrazine.

To date, there is little information available concerning the concentration of atrazine residues in lakes and reservoirs. Most sampling has focused on initial runoff periods. There is a large body of literature concerning the direct effects of atrazine on individual algal species. However, natural interspecific competition will greatly

affect the individual responses of a community of phytoplankton to a toxicant. Efforts to quantify the temporal, species specific responses of natural phytoplankton populations are apparently absent from the literature. Such an analysis could reveal not only the direct effects a toxicant might have but also the indirect secondary effects such as nutrient competition and interspecific inhibition.

The objectives of this study were to:

I. Document the presence and concentration of atrazine at three locations in B. Everett Jordan Lake, N.C. from March through July of 1985.

II. Utilize <u>in vitro</u> bioassay techniques to:

A. Evaluate the direct and secondary effects of atrazine on individual species within a phytoplankton community.

B. Determine whether dose/response inhibitions or stimulations exist for atrazine-treated phytoplankton species.

C. Evaluate the effect of atrazine on species specific growth kinetics.

D. Determine whether an atrazine action level exists for natural communities of phytoplankton.

METHODS

Atrazine Sampling In Jordan Lake

Water samples were taken from Stations H16, NH17, and NH15 (Figure 1) in conjunction with bi-weekly water quality sampling trips from March 1985 through July 1985. Atrazine samples were composites of the euphotic zone (defined as the zone from the surface to a depth at which 1% of surface irradiation remains). This depth was chosen to represent the zone most likely to contain phytoplankton. Approximately 20 liters of lake water were pumped (Jabsco Inc.) directly into clean carboys. All glassware used for atrazine analyses was soaked in Micro (International Products Corp.) overnight and rinsed with at least six aliquots of glass distilled dionized water. Samples were usually transferred to the laboratory within four hours for resin adsorption which was always complete within 36 hours of sampling. No attempt was made to keep these large volumes of water on ice in the field. However if sampling-day resin adsorption was not feasible, the carboys were held at 4°C until the following day.

Adsorption Of Organics From Water Samples

Amberlite XAD resin has been used to concentrate dissolved organic materials from water with many methods. Junk et al. (1974) described in detail a method for extracting a variety of organic compounds added to water. A

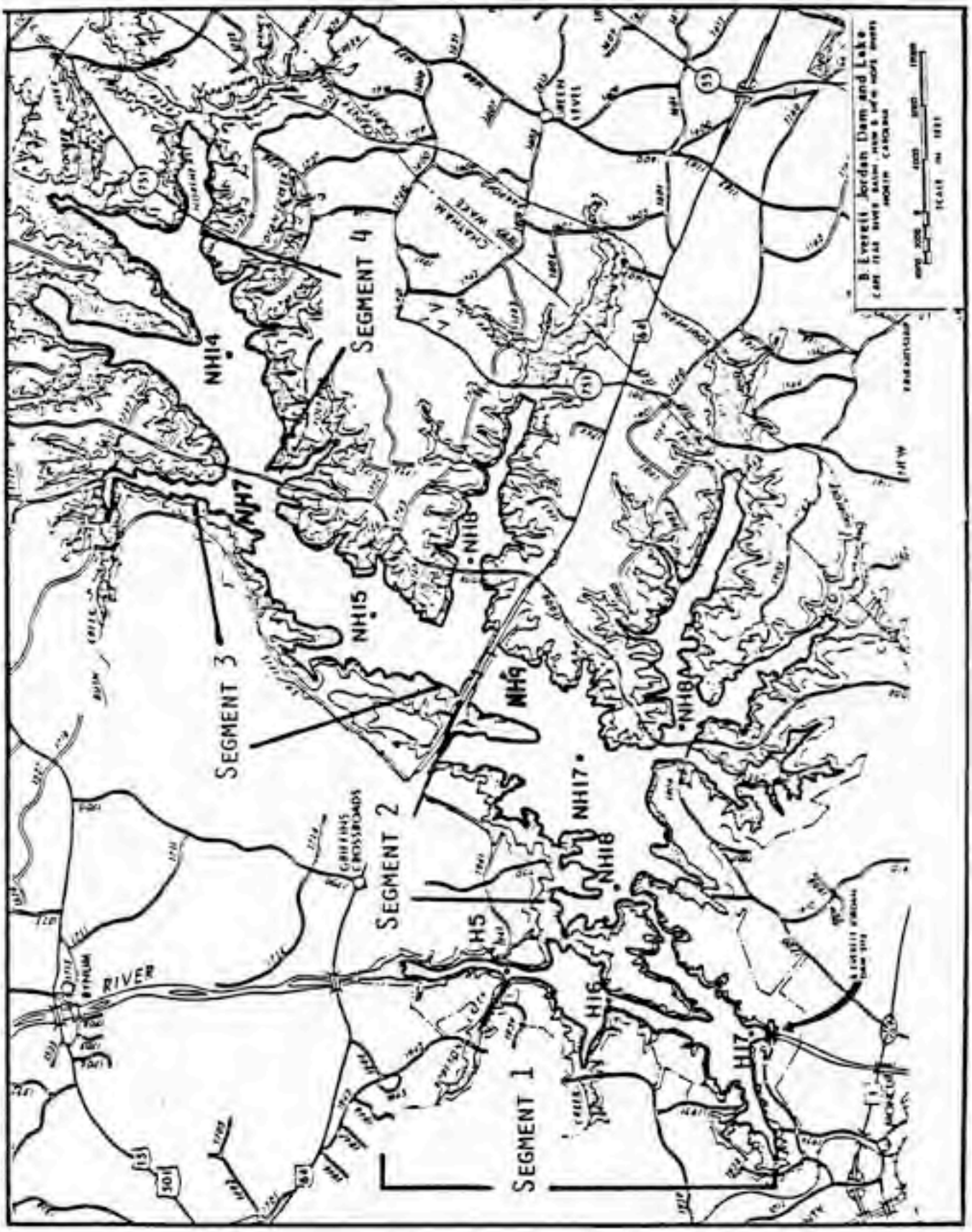


Fig 1. Map of Jordan Lake, N.C., showing sampling station locations (adapted from Weiss et. al., 1985).

similar method was used to extract atrazine from various real-water samples at concentrations less than one part per trillion (Richard et al. 1975). A modification of this method has been described by Pfaender et al. (1977) as well as others (Schnare 1979; Dietrich, Millington, and Christman 1983). Several aspects of these methods were combined for use in the present study.

Amberlite polymeric adsorbent XAD-2 (Rohm and Haas) was used to extract atrazine from lake water. This choice was made in anticipation of atrazine levels below the liquid-liquid extraction limit. However, XAD-2 resin requires significant cleanup prior to use. A multi-solvent soxhlet extraction procedure was chosen to accomplish this task similar to that reported by Junk et al. (1974). The resin was cleaned in batches of approximately 150 mL using the following solvents in sequence, each one used twice for 48 hours such that methanol was the first and final solvent: methanol, acetonitrile, methylene chloride, and diethyl ether. Resin batches cleaned in this manner were stored under fresh methanol until needed.

Lake water for atrazine analysis was spiked with simetryne, another s-triazine, prior to resin adsorption for quantification purposes. Simetryne was chosen as the surrogate standard because it is structurally similar to atrazine and is not used in the watershed of Jordan Lake (Turner, Digiano, and DeRosa 1984). It was assumed that simetryne was subject to the same sorption and equilibrium

reactions as atrazine. Spiking solutions were made by dissolving 100 mg simetryne in an appropriate volume of methanol to give solutions in the range of suspected atrazine levels. Spikes of these solutions were made directly into the sampling carboys one hour prior to the start of resin adsorption.

During that time period, the adsorption column (Biorad 100 mL.) was loaded with 10 mL resin (approximately 9 g) and the methanol was flushed out with 3-L of distilled dionized water. Flushing was interrupted after 1-L had passed through the column to disrupt air bubbles trapped in the resin bed. The spiked carboy containing the lake sample was then attached to the Biorad column and allowed to flow via gravity through the resin bed. All attachments were of Teflon and the flow rate was maintained at approximately 100 mL/min. Water flowing through the resin bed was collected and recorded to calculate the volume extracted. Occasionally, high algal biomass and detritus would slow the flow which was then maintained by a positive pressure of clean, dry nitrogen on the carboy. After the sample had passed onto the resin bed (approximately three hours), two 1-L rinses of the carboy with distilled dionized water were allowed to pass through the column. The remaining water was expelled and the resin dried by connecting the column directly to the nitrogen source. Resin containing adsorbed organics was placed in 25-mL Erlenmeyer flasks, sealed with Teflon tape and stored frozen prior to the elution step.

Elution Of Adsorbed Organics

Micro-soxhlet (25-mL) elution usually took place within the same week of sampling. The sample resin containing adsorbed compounds was placed on a bed of glass wool (previously extracted with methylene chloride) in the soxhlet. Methylene chloride (15 mL) was added to the round bottom flask of the soxhlet apparatus and allowed to cycle through the resin for 24 hours. At this time, methylene chloride containing the eluted organics was transferred back to the original Erlenmeyer flask and anhydrous sodium sulfate was added to remove any remaining water. Finally, the sample was transferred back to the round bottom flask, a 3-ball Snyder distillation column attached and the solvent was allowed to evaporate until only 1 mL of sample remained. The samples were stored in 2-mL vials equipped with Teflon/silicon septum screw caps. Prior to gas chromatographic analysis, samples were further concentrated with a gentle stream of clean, dry nitrogen to 25-50 ul, which represents a concentration factor of approximately 500,000.

Gas Chromatography And Mass Spectrometry Confirmation

Gas chromatographic determination of the presence and concentration of atrazine was usually completed during the week following sampling. A Carlo Erba HRGC 5160 mega series

gas chromatograph equipped with a Shimadzu integrator was used for all atrazine quantifications. Conditions were:

COLUMN:	30 meter DB-5, 1 um film thickness, J & W Scientific
FLOWRATE:	Helium, 1 mL/min @ 16 PSIG
INJECTOR:	Split-splitless injector, split ratio 10:1-20:1, septum purge 4 mL/min. March and April samples analyzed in splitless mode due to low concentrations
PROGRAM:	180°C (10 min), 6°/min, 260°C (5 min)
INJECTION:	1-2 ul
DETECTOR:	Flame ionization detector (FID), 280°C

Identification of atrazine and its surrogate, simetryne was routinely based on a pair of injections. The first injection contained only sample while the second injection included a standard solution containing both compounds in addition to sample. This second injection produced two peaks of greater amplitude (atrazine and simetryne) than all others, which allowed correct selection of the two compounds from the first chromatogram and subsequent quantification. Typically, three sample injections were made and averaged for reported concentrations. Peak identifications utilizing this method were confirmed for the June 19th sample from Station H16 by GC/MS. The GC/MS system utilized was a Hewlett-Packard 5710-A gas chromatograph interfaced with a VG-Micromass Model 7070F double focusing mass spectrometer. Mass spectra were enhanced using computer assisted subtraction routines. Chromatographic conditions were the same as above except that injections were made with an OCI-2 on-column injector

(SGE Corp.). Electron ionization conditions were:

IONIZING ELECTRON ENERGY: 70 eV
ACCELERATING VOLTAGE: 4 KV
TRAP CURRENT: 200 uA
MASS RANGE: 40-400
SOURCE TEMPERATURE: 200°C
SOURCE PRESSURE: 5 x 10^{-6} torr
CYCLE TIME: 0.7 sec/decade
RESOLUTION: 1000 @ 10% valley

Representative gas chromatograms for resin blanks, standards, and lake samples and the GC/MS confirmation spectra are presented Figures 2-6. Several organic compounds (Figure 6) found to be present in the June 19th sample from Station H16 have been previously identified in Haw River water (Dietrich, Millington, and Christman 1983).

The linear response range of the the Carlo Erba FID for atrazine and simetryne, and the response factor of both of these compounds relative to simetone was determined in a single experiment. Equal amounts of each compound were dissolved in methylene chloride and accurate dilutions of the resulting solutions were made such that a concentration range of two orders in magnitude was obtained for atrazine and simetryne (10 - 1000 ng/ul). Three solutions covering this range were made containing the analyte and surrogate standard at equal concentrations and a constant amount (100 ng/ul) of the internal standard, simetone. Each of these solutions was injected three times and the average peak count ratio of atrazine/simetone and simetryne/simetone was plotted against the known mass ratio of the analyte/internal standards added to the solution (Figures 7 and 8). The ratio of the

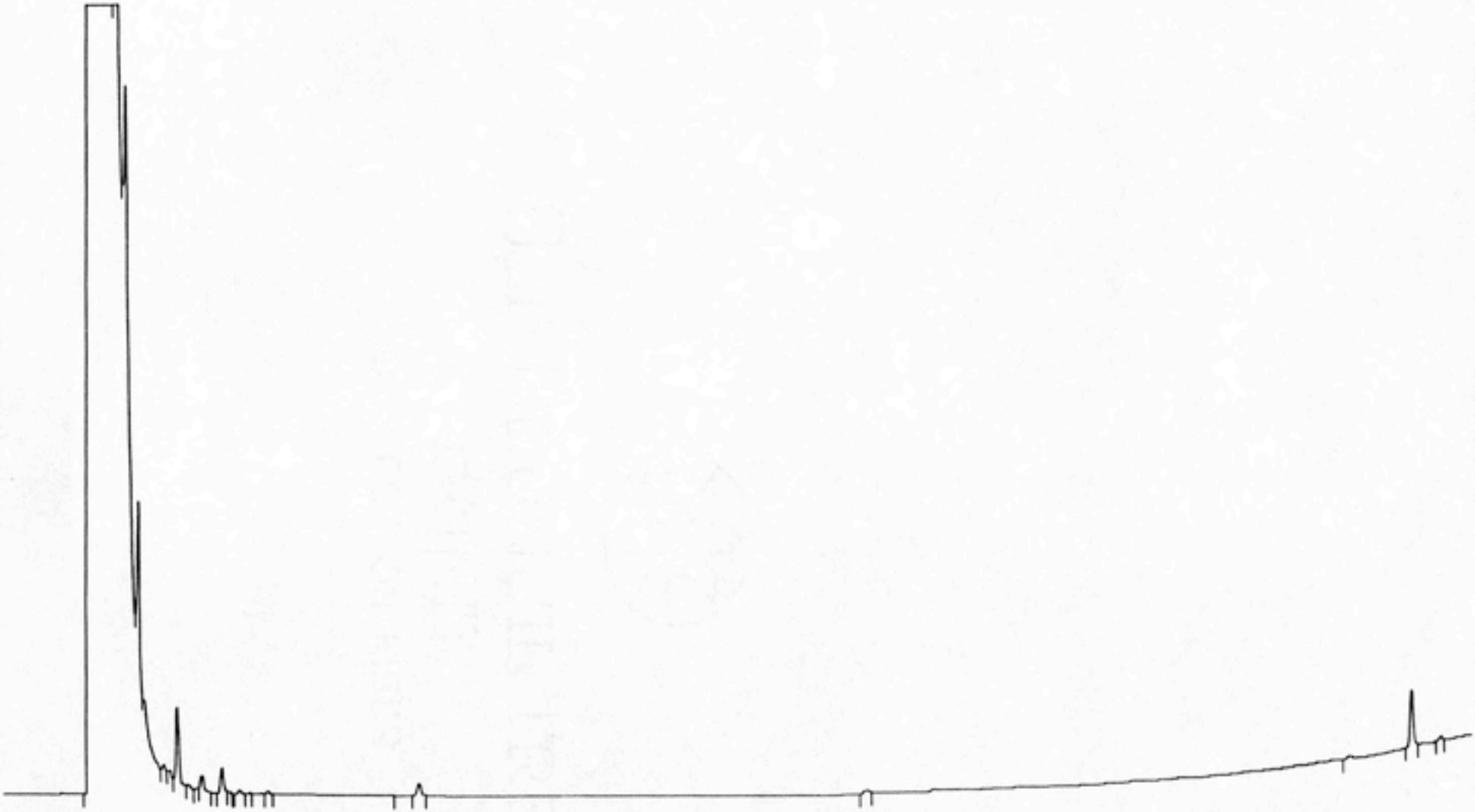


Fig 2. Gas chromatogram of XAD-resin blank eluate following extensive solvent clean-up.

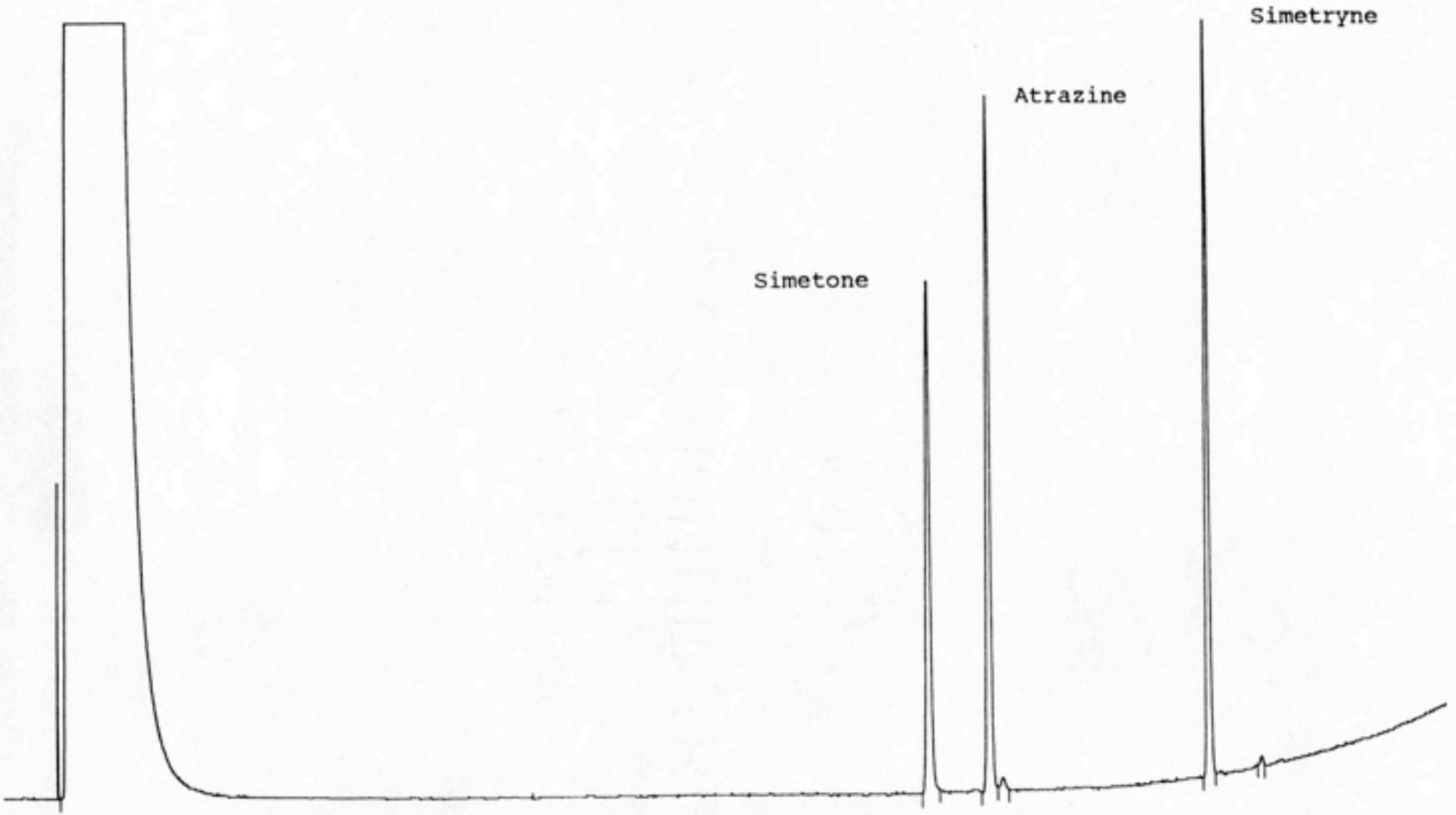


Fig 3. Gas chromatogram of a standard solution of simetone, atrazine, and simetryne.

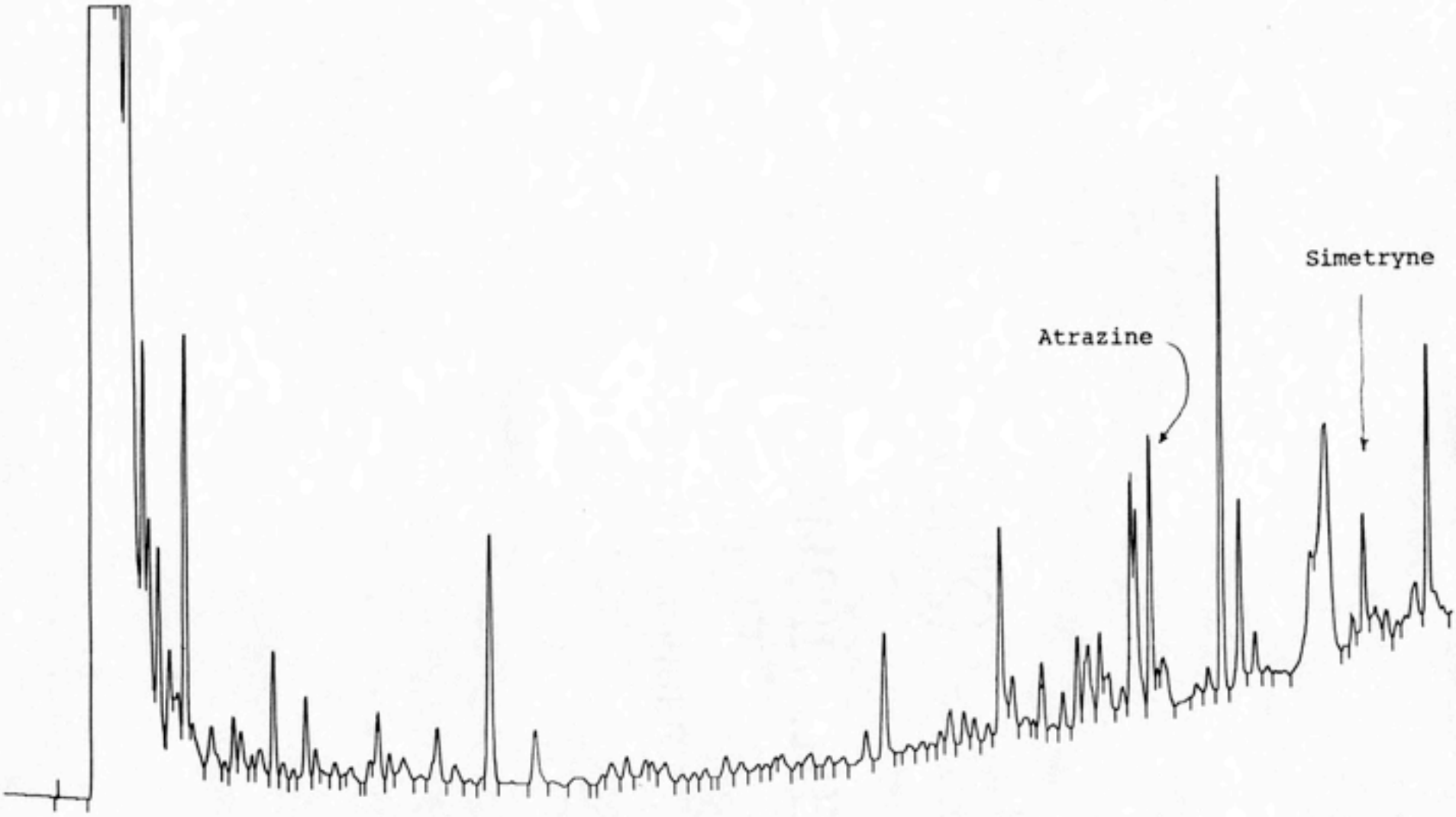


Fig 4. Gas chromatogram of organic compounds present at Station H16 on June 19th, showing endogenous atrazine and surrogate standard, simetryne.

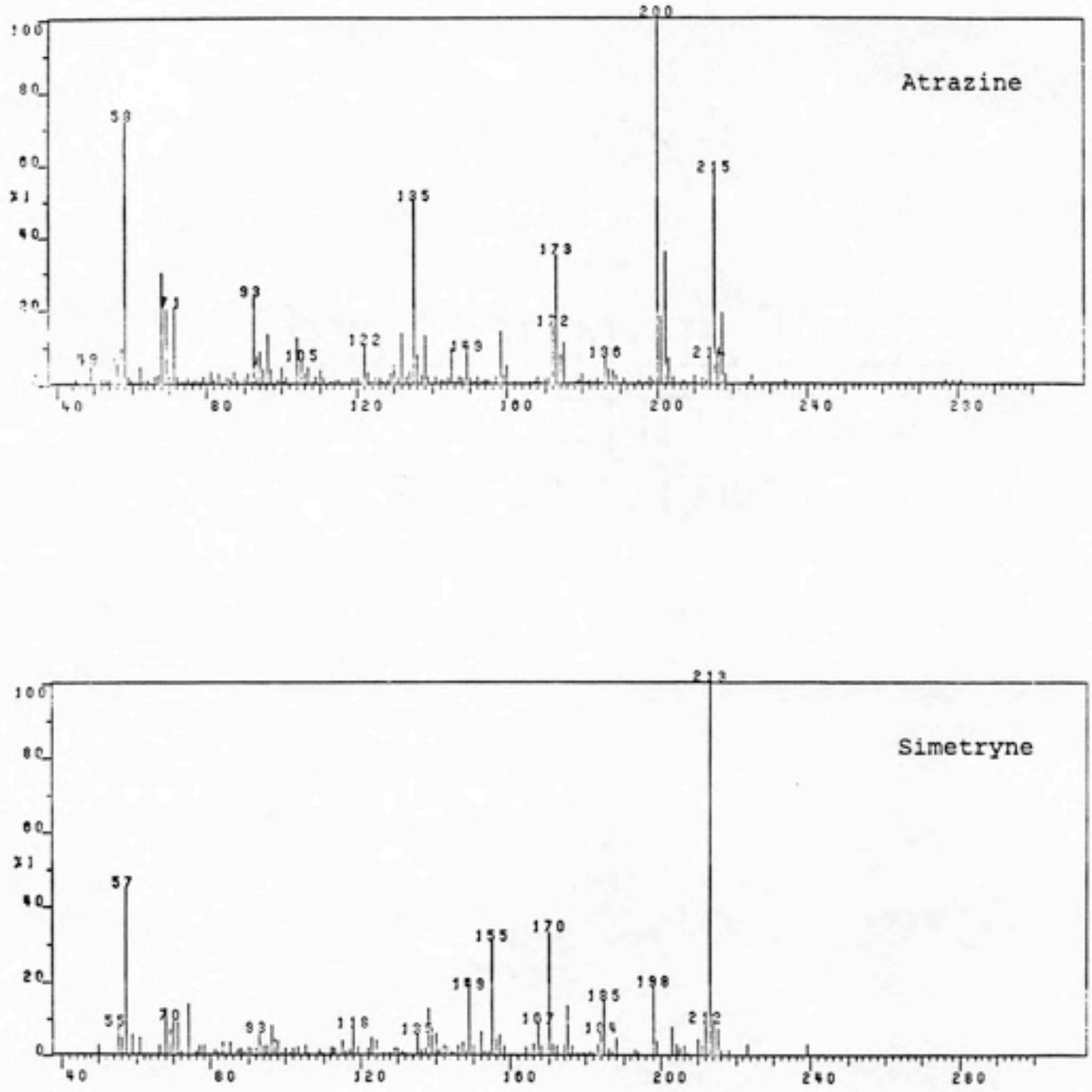


Fig 5. Mass spectra of atrazine and its surrogate, simetryne extracted from water sampled at Station H16 on June 19th.

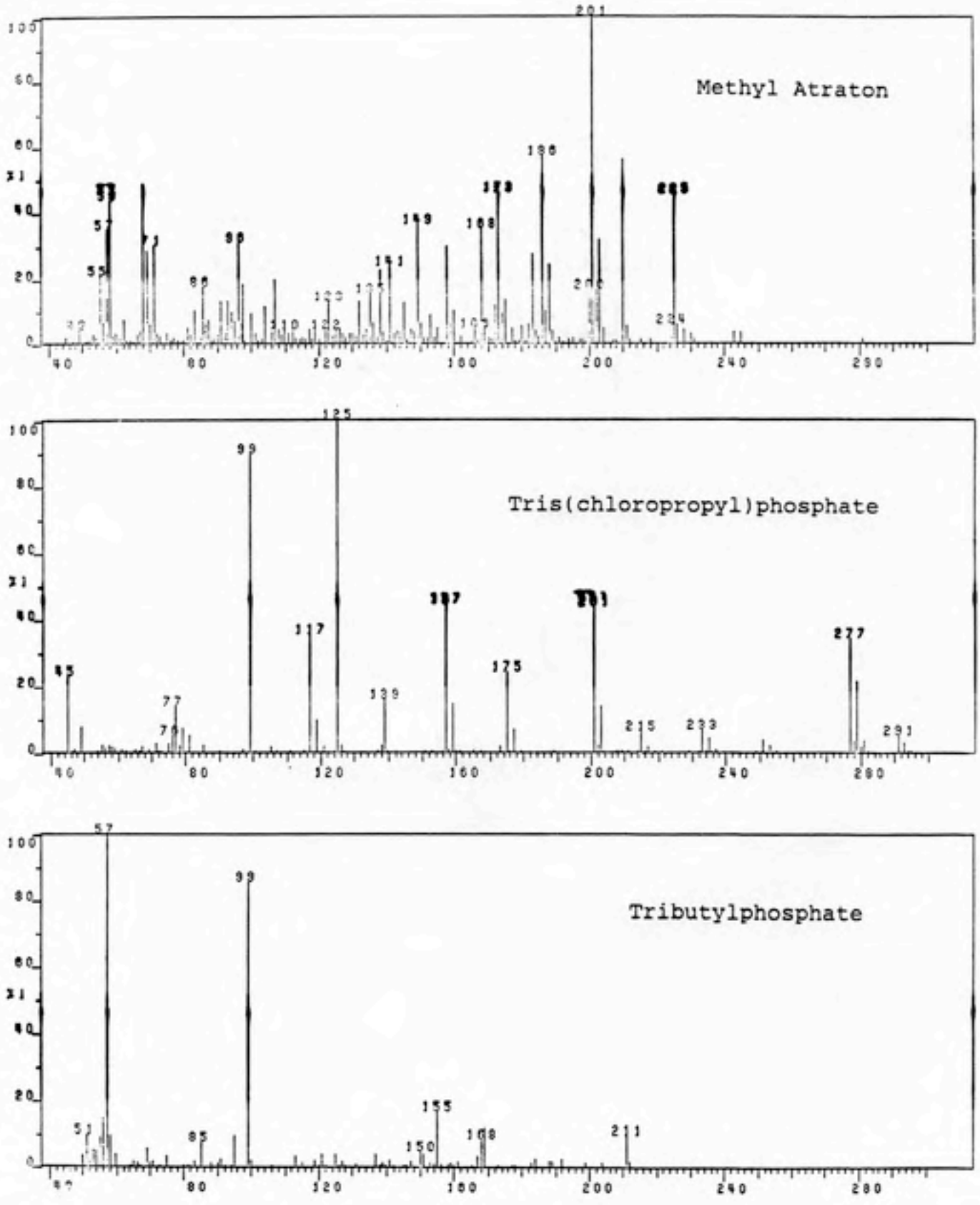


Fig 6. Mass spectra of organic pollutants present in water sampled at Station H16 on June 19th previously determined to be present in the Haw River.

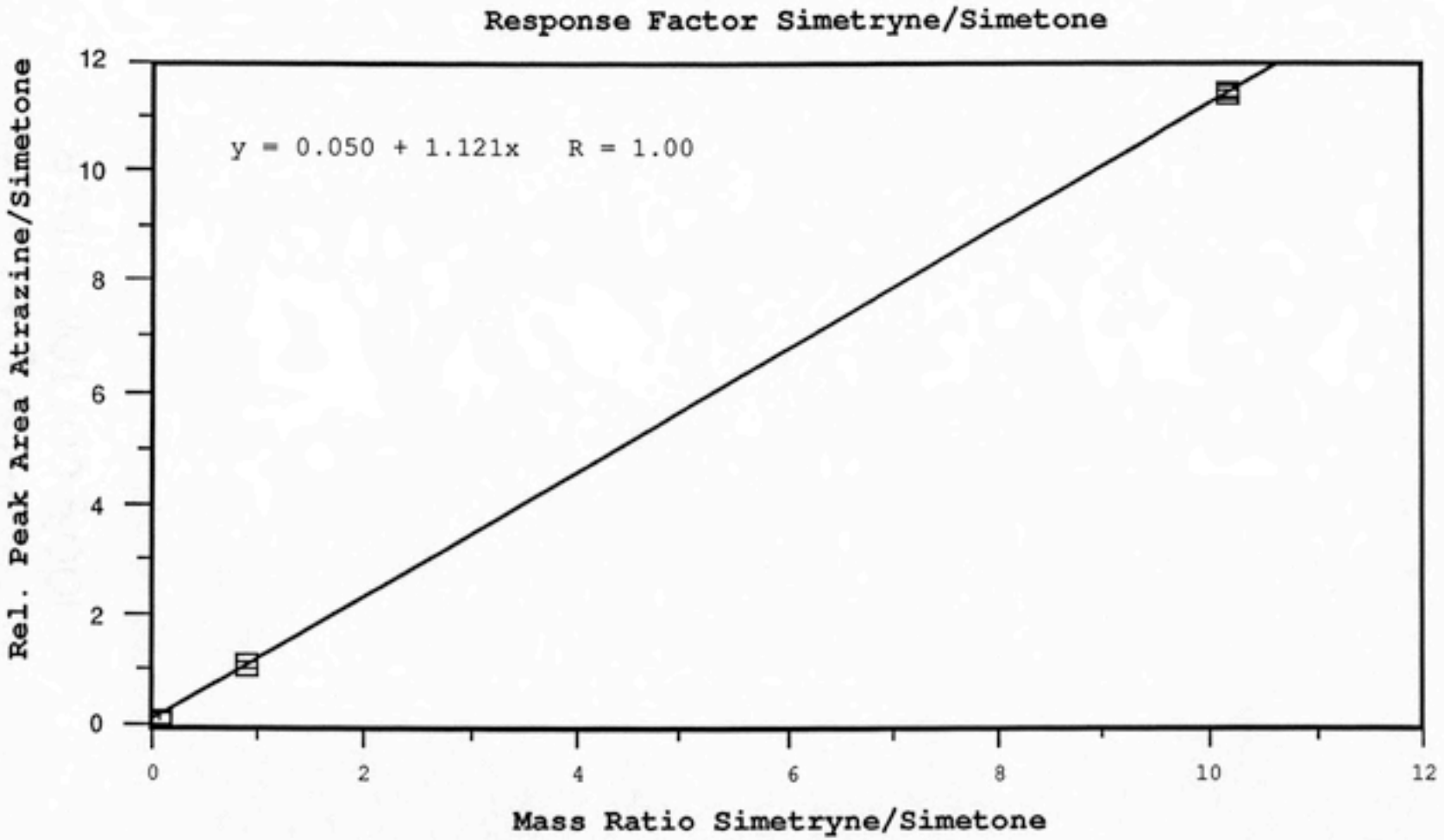


Fig.7. Flame ionization detector linear range, response factor of analyte, atrazine/internal standard simetone (three data points at each mass ratio).

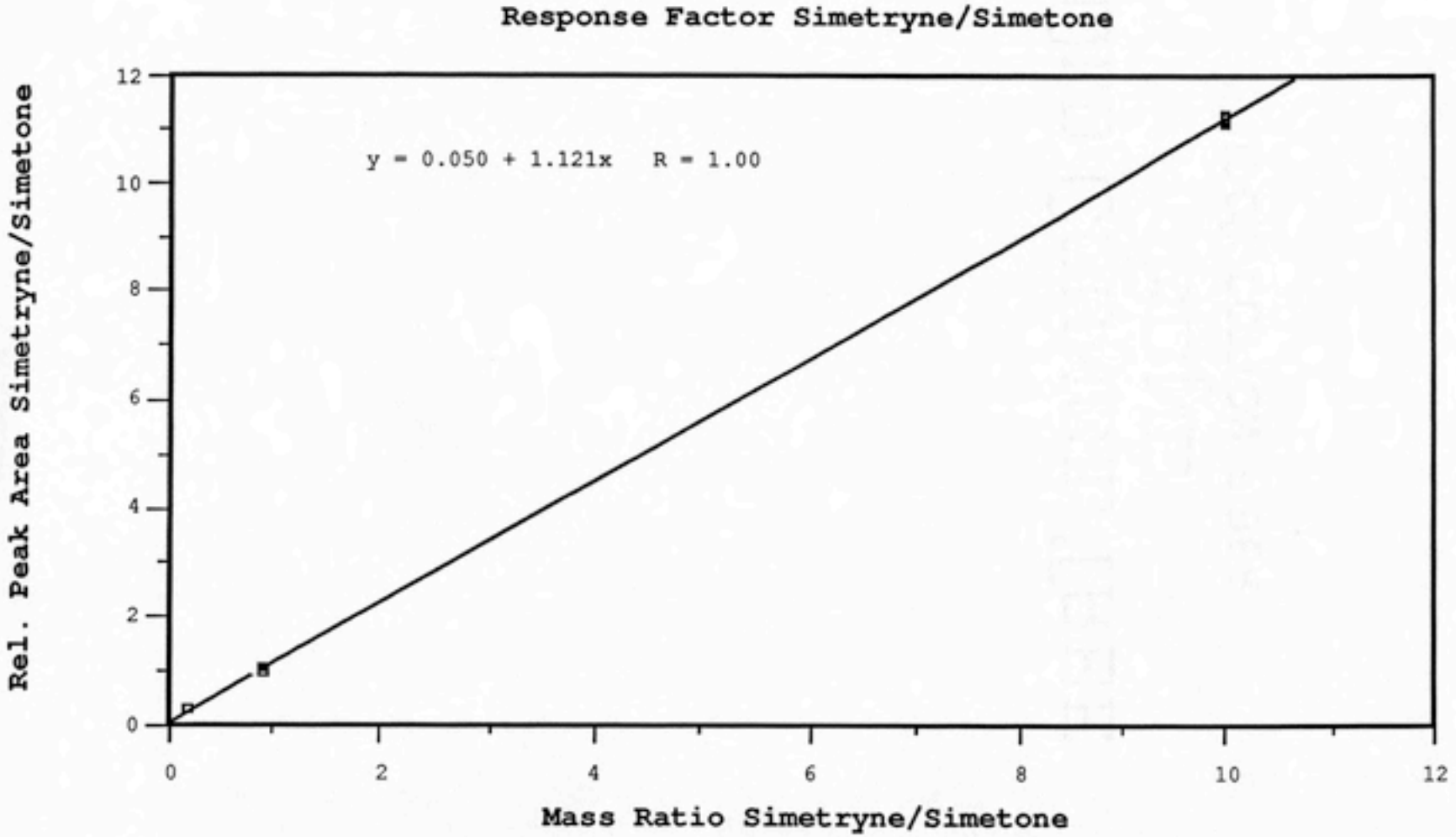


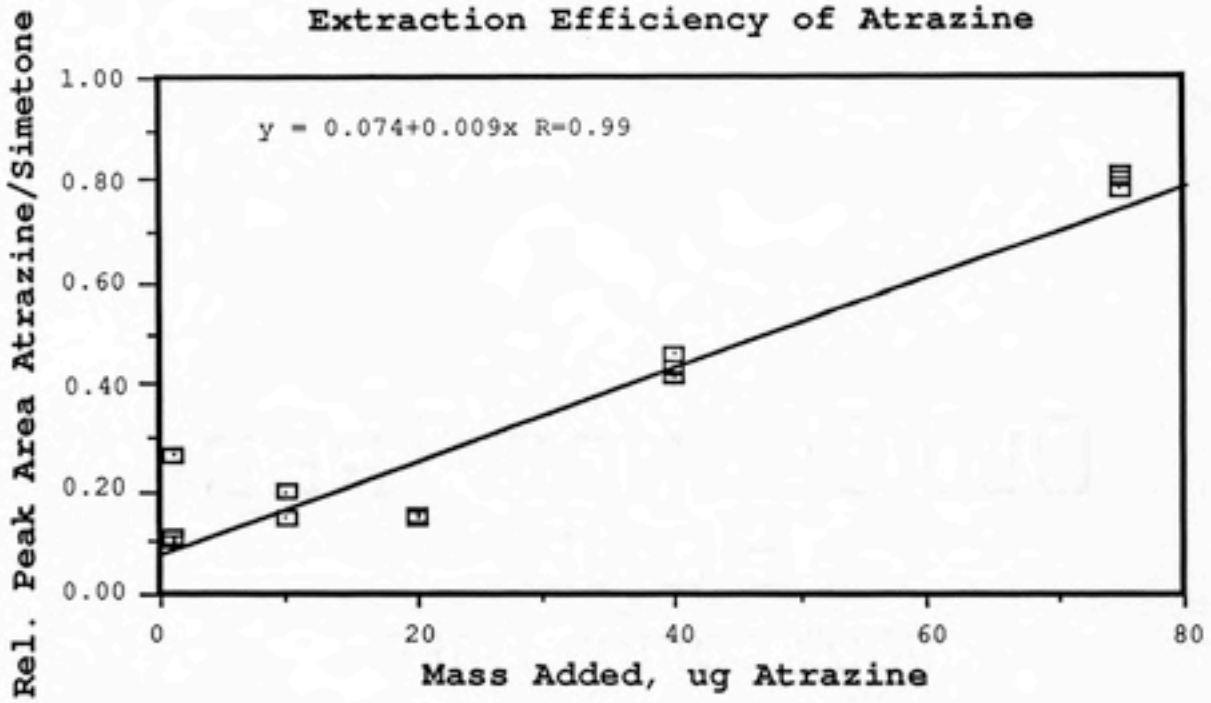
Fig. 8. Flame ionization detector linear range, response factor of surrogate, simetryne/internal standard simetone (three data points at each mass ratio).

resulting slopes (response factor) was used to calculate the atrazine concentration in lake samples as follows:

$$\text{Conc (ug/L)} = \frac{\text{STD} * \text{Ra/Rs} * \text{RF}}{\text{V}}$$

where: STD is the mass of simetryne added in ug
Ra is the integrator count for atrazine
Rs is the integrator count for simetryne
RF is the response factor (0.963), for (simetryne/atrazine)
V is the volume of water adsorbed in liters.

An experiment was designed to demonstrate that recoveries of the surrogate standard simetryne and analyte atrazine were comparable even under worst-case conditions (high turbidity). Five 20-L water samples were obtained as described above from Station NH14 (Figure 1) and spiked with a range of equal amounts of atrazine and simetryne (0.5 - 3.5 ug/L). This range was selected as being representative of suspected real-water concentrations. Extraction and concentration took place as described previously. A known and constant amount of internal standard, simetone, was added to the final extract prior to gas chromatographic analysis. Relative integrator count ratios (response factors taken into account) of surrogate standard and analyte to internal standard, were plotted against the mass of atrazine and simetryne added to the lake water (Figure 9). The slope of each line represents recovery for that compound. The slight upward deflection of the atrazine plot represents the presence of endogenous atrazine. Atrazine and simetryne were recovered with comparable efficiency: the slopes were 0.009 and 0.011 respectively.



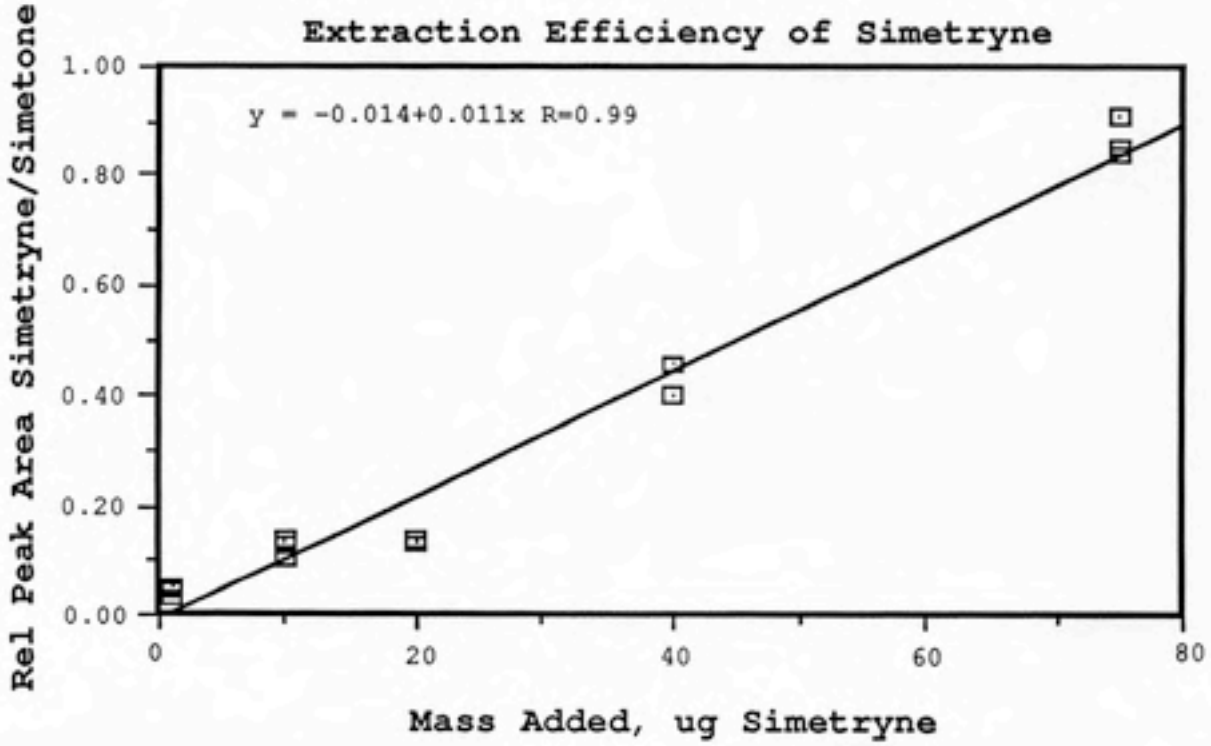


Fig. 9. Extraction efficiency of atrazine compared with simetryne under worst-case conditions (high turbidity).

Bioassay Media and Enumeration Techniques

Species specific phytoplankton responses to atrazine were determined using a modified version of the Selenastrum capricornutum Printz Algal Assay Bottle Test (Miller et al. 1978). In an attempt to provide a medium suitable for the most diverse population of algae, and in view of reports that some species may not use NO_3^- at all (Moss 1973), soil water (prepared as described by Pringsheim, 1946) was added to the medium (40 mL/L) and ammonium nitrate (12.75 mg/L) was substituted for sodium nitrate.

Atrazine used as dosing reagent, was made up prior to the bioassays by dissolving 100 mg of atrazine in 100 mL of acetone. A working stock solution was prepared by adding 10 mL of the acetone solution to stirred, near-boiling water, and the acetone was allowed to evaporate overnight. Dilutions of this solution were made such that 1 mL would give the appropriate atrazine concentration in the bioassay flasks. These concentrations were 0.25, 1.0, and 50 ug/L and 1.0, 10, and 50 ug/L atrazine for bioassay I and II respectively.

Water used to seed the bioassay flasks was obtained with a Kemmerer sampler from the SR1008 bridge close to Station NH14 on Jordan Lake. A composite sample representing the euphotic zone was transferred immediately to the laboratory where a subsample was concentrated by centrifugation. The supernatant was removed and cells resuspended in a known

volume of bioassay medium so that 1 mL of this seed, added to the bioassay flasks, resulted in an initial population of approximately 3000 cells/mL. Water was collected on June 19th and on October 9, 1985 for the innoculum used in bioassays I and II respectively.

Triplicate flasks of the control and three atrazine dosages containing 60 mL of media were incubated in 250 mL Pyrex Erlenmeyer flasks. The temperature was maintained at 25°C under continuous fluorescent lighting of approximately 400 footcandles. The flasks were rotated daily on a shaker table to ensure equal light exposure. Cotton was used to stopper the flasks which were shaken at 110 oscillations per minute to facilitate gas exchange.

Two methods of cell enumeration were investigated in this study. The "Vaspar" method is similar to the method of Campbell (1973). A known volume (15 ul) of the sample is placed under a cover glass of known surface area. A paraffin-petroleum jelly mixture is used to seal the cover glass and prevent drying of the sample. The number of cells/mL is calculated from the known area of the cover glass, the area of the transects counted, and the volume of water placed under the cover glass. This method has the advantage of allowing oil immersion (1250x magnification) examination for careful cell identification. A major difficulty with this method is that cells are quite often distributed unevenly over the slide, causing variation between transect counts.

The second method investigated utilized a hemacytometer which is commonly used for algal cell enumeration. A grid embedded in the hemacytometer allows calculation of cells/mL from the known volume of sample contained by the counting chamber. Even distribution of cells is enhanced by the design of the loading channel. The disadvantage encountered with this device is a result of its thickness which precludes the use of oil immersion lenses. However, adequate resolution is provided at 500x magnification. A comparison of counts obtained from these two methods is presented in Table 2.

It was determined that the "Vaspar" method, while yielding much greater resolution, was too variable for the purposes of this experiment. It was found that a combination of these two methods proved to be quite successful. Quantification was accomplished with a hemacytometer, and species identification was verified with the "Vaspar" method.

All cell counts were made with a Zeiss GLF compound microscope equipped with phase contrast. On each counting day, a 1-mL subsample was removed from each flask and cell clumps broken up by gentle grinding with a tissue grinder. A loop-full of this sample was transferred to a hemacytometer for quantification. At least 300 cells from each flask were counted with the hemacytometer at 500x magnification.

Counts were begun on Day 3 of bioassay I (BI) and continued every two days through Day 9, when it was certain that maximum biomass had been achieved at all dosage levels.

Table 2. A comparison of cell counts (cells/mL) of Oscillatoria limnetica made with a hemacytometer and by the "Vaspar" method.

METHOD	Hemacytometer	Vaspar
	49	48
	30	50
	45	60
	31	69
	50	32
	25	23
	16	100
	23	35
	33	10
	34	58
	**********	**********
Mean	34	48
Std. Dev.	11	26
Coeff. of Variance	0.34	0.53

Examination of the results from this experiment indicated that additional information could be obtained by daily cell counts, which were begun on Day 2 of bioassay II (BII) and continued through Day 8. A blind counting technique was utilized for the second experiment which assured that cell counts were made without knowledge of the atrazine concentration associated with that count.

Statistical Treatment Of Algal Responses To Atrazine

During BII, it was noted that certain replicates did not show good growth comparable to replicates of the same atrazine dose for any species. No clear reason can be determined for this occurrence. These replicates, one each from doses 1, 10, and 50 ug/L atrazine were not included in the statistical analysis or the graphic illustrations. A complete listing of the statistical results from these experiments is presented in Appendix A.

Four statistical models were developed to assess the species specific responses of algae to different doses of atrazine. In each model the natural logarithm of cell count/mL was used as the dependant variable. The independent variable was atrazine concentration used as a flask dose.

Cell counts for the predominant species or groupings were analyzed using ANOVA and least squared means methods of SAS (Statistical Analysis System). Specifically, analysis of variance (GLM procedure) with the least squared means (LSM) option was used to detect dose-related differences in cell

counts on a given day (Model A) or dose related differences in growth rate over a period of days (Model C). The CORR procedure with the Spearman option was invoked to detect the correlation between cell count and atrazine dose on a given day (Model B) or growth rate and atrazine dose over a period of days (Model D).

It was found that the natural logarithm transformation of cell count stabilized the variance of the dataset and enhanced compatibility with the normal distribution. The specific transformation was $\log_e$ (cells/mL+1), which allowed for cell counts of zero. The model specification for the analysis of variance was:

Response = Constant + Treatment effect.

In this specification, the "constant" can be interpreted as the mean for the control group, and the treatment effects can be interpreted as differences from control for all atrazine doses.

Least square mean values were calculated for each day/atrazine treatment combination for data found to be statistically significant ($P \leq 0.1000$, F test) by analysis of variance Models A and C. Pairwise comparisons for these least square mean values were tested using a two sided T test. Rejection of this hypothesis ($P \leq 0.1000$, T test) was taken as evidence that the two atrazine doses in question were statistically different. The computing formula used in the least square models was:

t = (pairwise difference)/(estimated standard error).

Spearman rank correlation coefficients were calculated in Models B and D to determine the linear dependence of cell count on atrazine dose. The hypothesis that the correlation coefficient r equaled zero was tested ($P \leq 0.1000$, T test) with rejection indicating that the dose/response inhibition or stimulation was statistically significant. The formula for the Spearman correlation was:

$$r = \frac{\Sigma(a_i - a)\,(c_i - c)}{\sqrt{\Sigma\,(a_i - c)^2\;\Sigma(c_i - c)^2)}}$$

where: a_i is the rank of the i^{th} atrazine value
c_i is the rank of the i^{th} cell count value
a and c are the means of the a_i and c_i values respectively.

RESULTS AND DISCUSSION

Atrazine Flow Dynamics In Jordan Watershed

B. Everett Jordan Lake has been described as having four distinct segments. These are defined by the causeways carrying traffic across the New Hope arm and the "narrows", a constricted portion upstream from the confluence of the Haw and New Hope arms on the New Hope side (Figure 1). Both rivers carry point and non-point discharges which, during low flow conditions, make up a large percentage of the total flow into the lake. However, the causeways on the New Hope side cause a great deal of nutrient sedimentation, and the resulting water quality has supported extensive recreational usage.

The Haw River supplies the lake with much more water than the New Hope flow (Figures 10 and 11). The 20-year average Haw to New Hope flow ratio is 4:1; the range is due to greater winter runoff from the larger watershed of the Haw River (Weiss 1986). During extreme hydrological events, water from the Haw River flows up the New Hope arm of the lake. Muddy water from the Haw was observed at the NC-64 causeway following one occurence when the lake level rose eight feet in three days during February, 1985.

Inter-segment volume fluxes in Jordan Lake have been described for the water year October 1982 - September 1983 (Moreau and Challa 1985). They suggested that for this

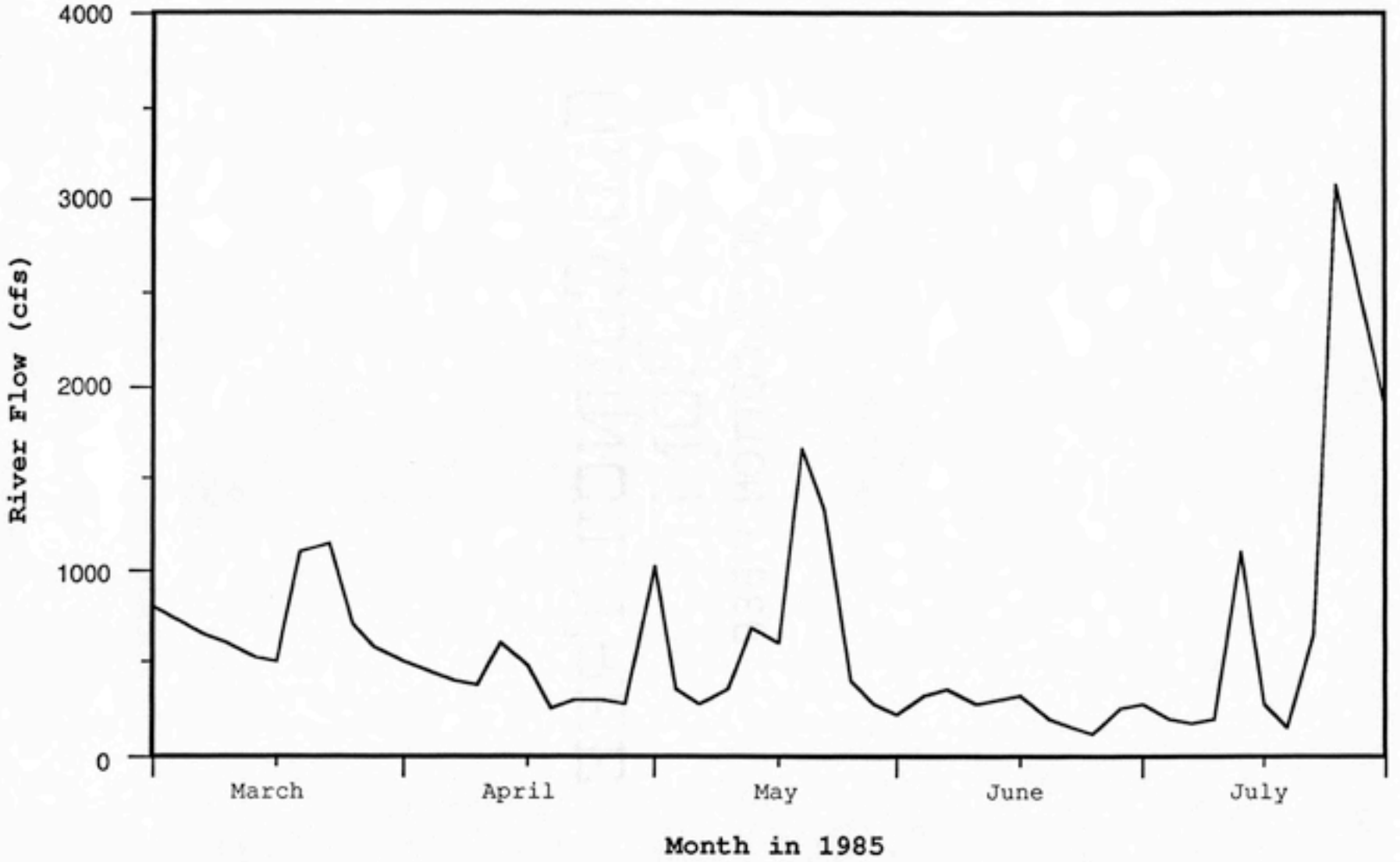


Fig. 10 Three-day mean flow into Jordan Lake at Segment I, from the Haw River Watershed: measured at Bynum, NC, during March-July, 1985. (USGS, provisional data)

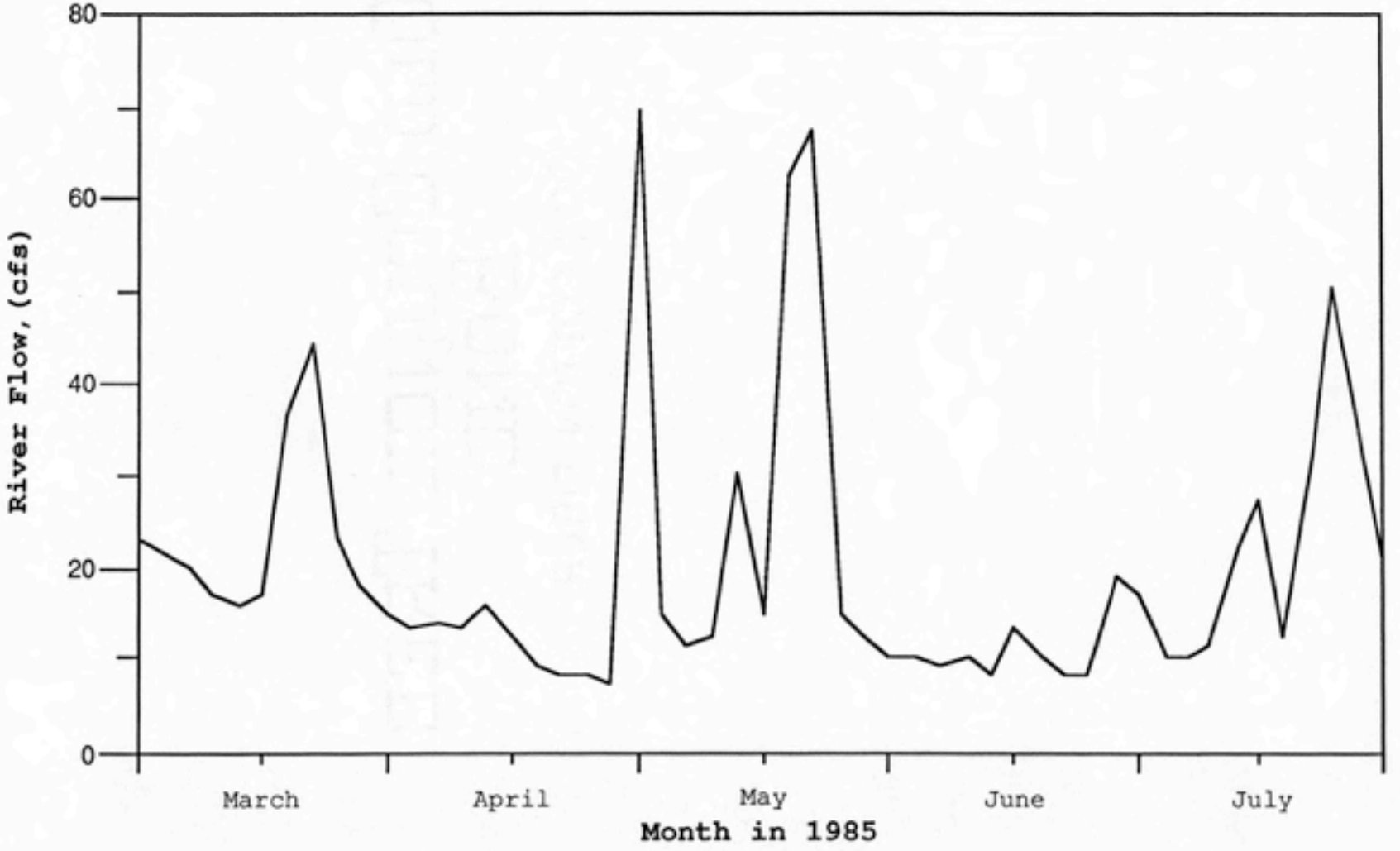


Fig. 11. Combined, three-day mean flow of Morgan, New Hope, and Northeast creeks into Segment 4 during March-July, 1985. (USGS, provisional data).

period, approximately 26 percent of the time there was back flow from Segment 1 to Segment 2; 23 percent of the time there was back flow from Segment 2 to 3; and 19 percent of the time there was back flow from Segment 3 to 4. It should be noted that these estimates assume complete mixing of each segment and treat the back flow as a mass of water. It is conceivable that vertical temperature differentials existing within a water mass cause back flow to occur in layers. This would allow even greater spatial back flow to take place. Segments 1 and 2 behave as rivers during and following large runoff events due to water backup and draw-down of stored water. Retention time can be as short as several days under these conditions (Weiss, Francisco, and Campbell 1985).

Previous research on synthetic organics in the Haw River has documented the presence of atrazine (Pfaender, et al. 1977; and Dietrich, Millington, and Christman 1983). Therefore the rationale for atrazine sampling station location in the lake was based on the agricultural activities known to take place in the watershed (Figure 12) and the flow and segmentation characteristics mentioned above. Land use patterns have been determined for the Jordan Lake watershed based on 1983 data (USSCS, 1985). Nearly 55,000 acres of corn are grown in the ten counties comprising the watershed, 50,000 acres in the Haw River basin alone. Approximately 5000 acres of corn are grown in the New Hope watershed (Figure 12, dotted line). Atrazine is also applied to

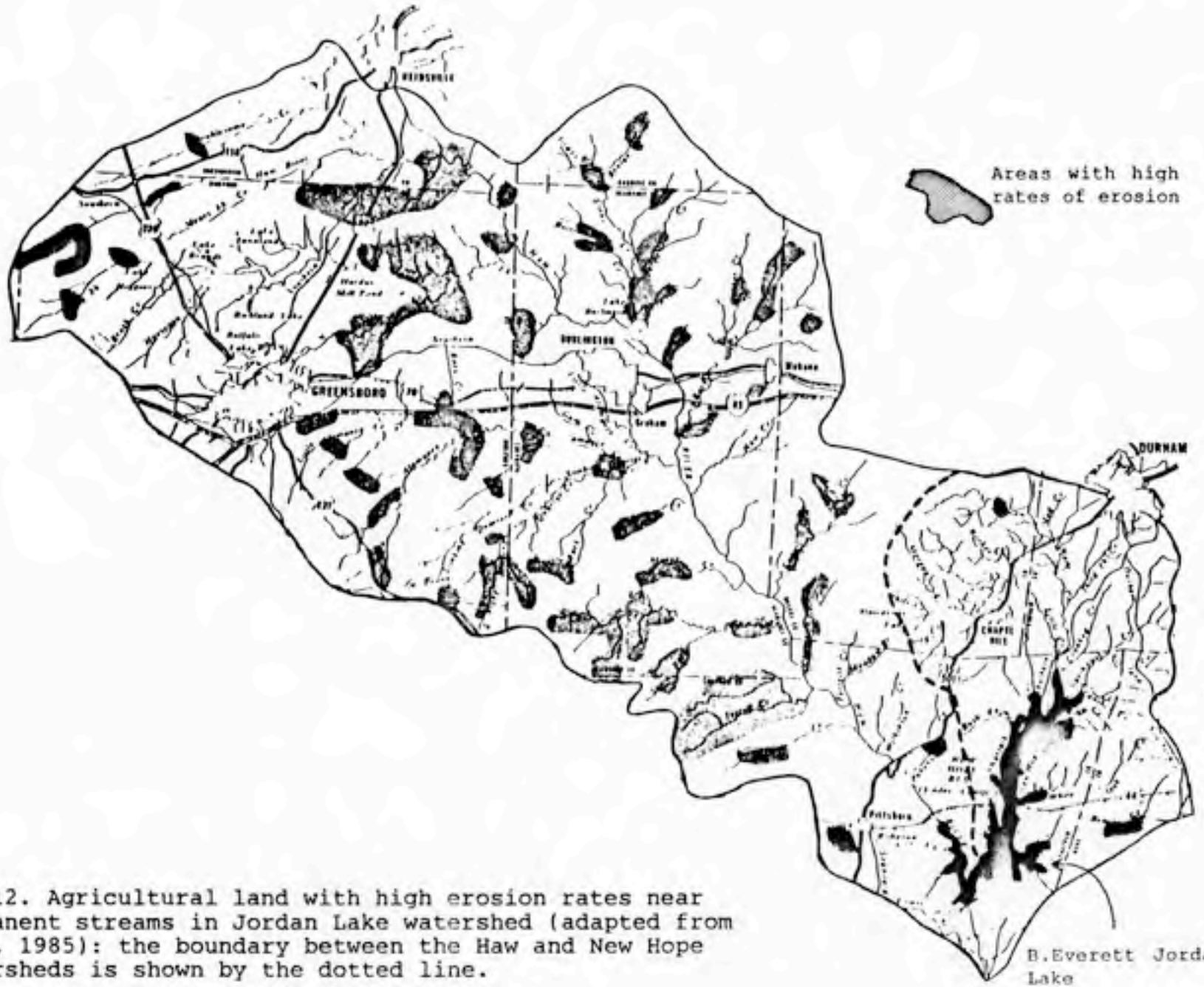


Fig 12. Agricultural land with high erosion rates near permanent streams in Jordan Lake watershed (adapted from NRCD, 1985): the boundary between the Haw and New Hope watersheds is shown by the dotted line.

sorghum crops which account for 7,800 and 200 acres of the Haw and New Hope watersheds respectively.

Atrazine residues in runoff waters will be transported in solution or sorbed to colloids and sediments. The spatial and temporal distribution of residues in a lake will not simply reflect the amount input. In Jordan Lake, the operation of the dam, biological and chemical interactions, as well as physical properties of the lake (flow characteristics and mixing depth) will determine the euphotic zone concentration. The lake is operated as a flood control reservoir, holding back runoff water until the downstream flow has decreased, at which time runoff water is released. Therefore, the lake level may fluctuate as much as 38 feet in response to extreme inputs. During periods of low-flow, dam output can exceed Haw River input. Often solar inputs will accompany these low flows such that the surface of the Haw arm of the lake is warmer than inflowing waters. If the dam discharge takes place at the bottom of the structure, cooler inflowing water will flow through the lake on the bottom resulting in essentially no allocthonous input to Segment 1. Much of the upper New Hope arm is shallow and subject to wind mixing which will deposit bottom residues in the water column. The deeper sections of the lake are less susceptible to this mechanism but during high flows or cold periods with high winds, mixing has been observed to take place at other than the classical limnological turnover times (Weiss, Francisco, and Campbell 1985). Phytoplankton populations

will respond to the conditions outlined above. During warm, low-flow periods, large biomass has been observed, although no "blooms" of the surface dwelling type have taken place.

Relationships Between the Storage Of Input Flows And Conductivity To Atrazine Residues In Jordan Reservoir

The concentrations of atrazine reported in this study were obtained from single samples of the water column. Although experiments were conducted to demonstrate comparable extraction efficiency for the analyte and surrogate standard, time and labor constraints did not allow field duplicate measurements to be made. Therefore, the uncertainty associated with single "grab" samples is recognized to be present for the data reported in Figures 13-15.

Atrazine application in North Carolina usually takes place during the middle of April; but in 1985, dry weather enabled crop planting and herbicide treatment during early April. Baseline atrazine residue data were obtained in March and bi-weekly samples were taken from early April through July.

April was characterized by low flows and hence, no significant storage. The dry weather following atrazine application delayed the expected atrazine pulse until May 22nd (Figures 13-15). The lake rose more than one foot in May. Storage of runoff water occurred in early and middle May prior to the May 22nd atrazine sampling. The storage of the two relatively small flows in May resulted in the highest

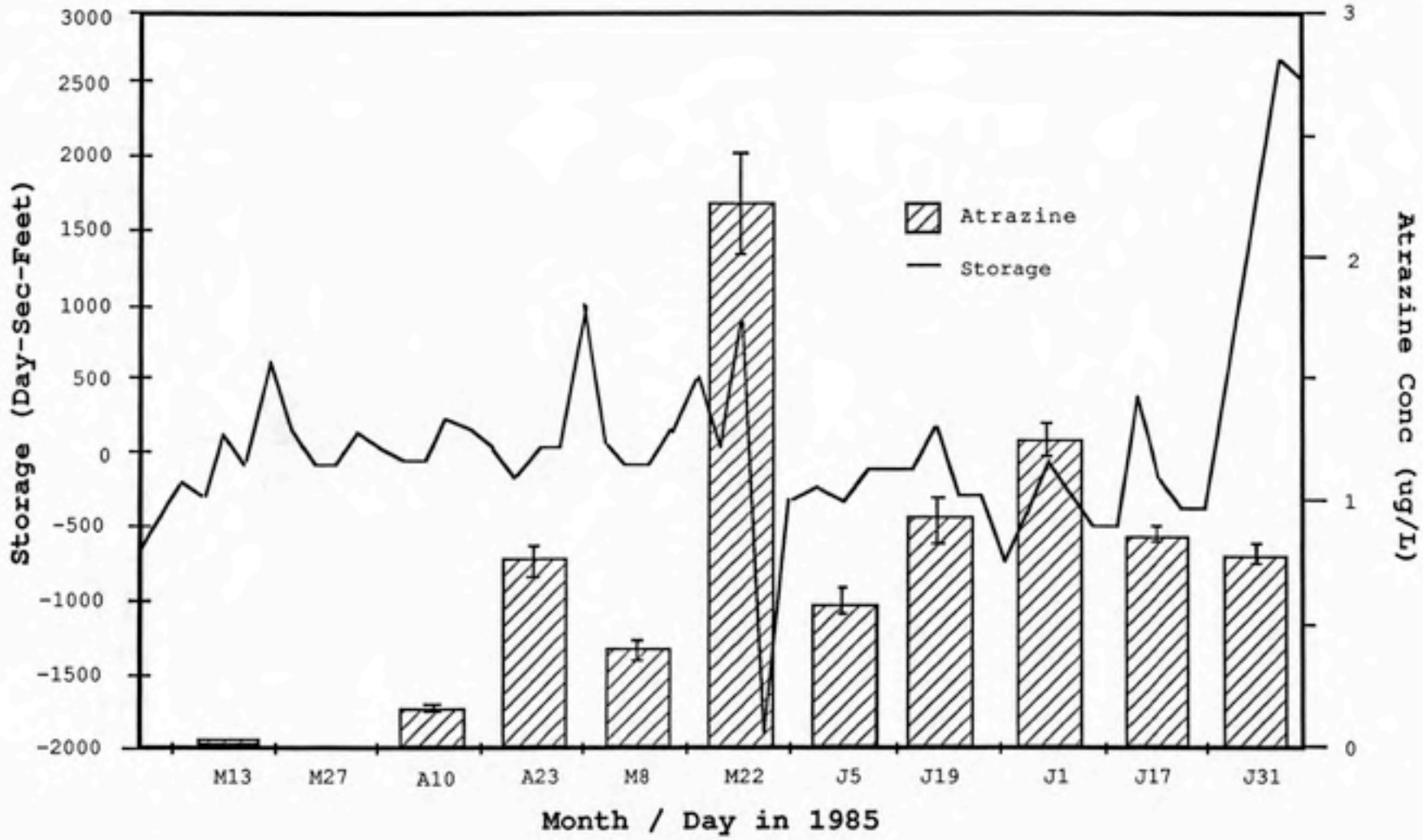


Fig. 13. The relationship between lake storage of input flows and atrazine concentration at Station H16. (Storage data from US Army Corps of Engineers)

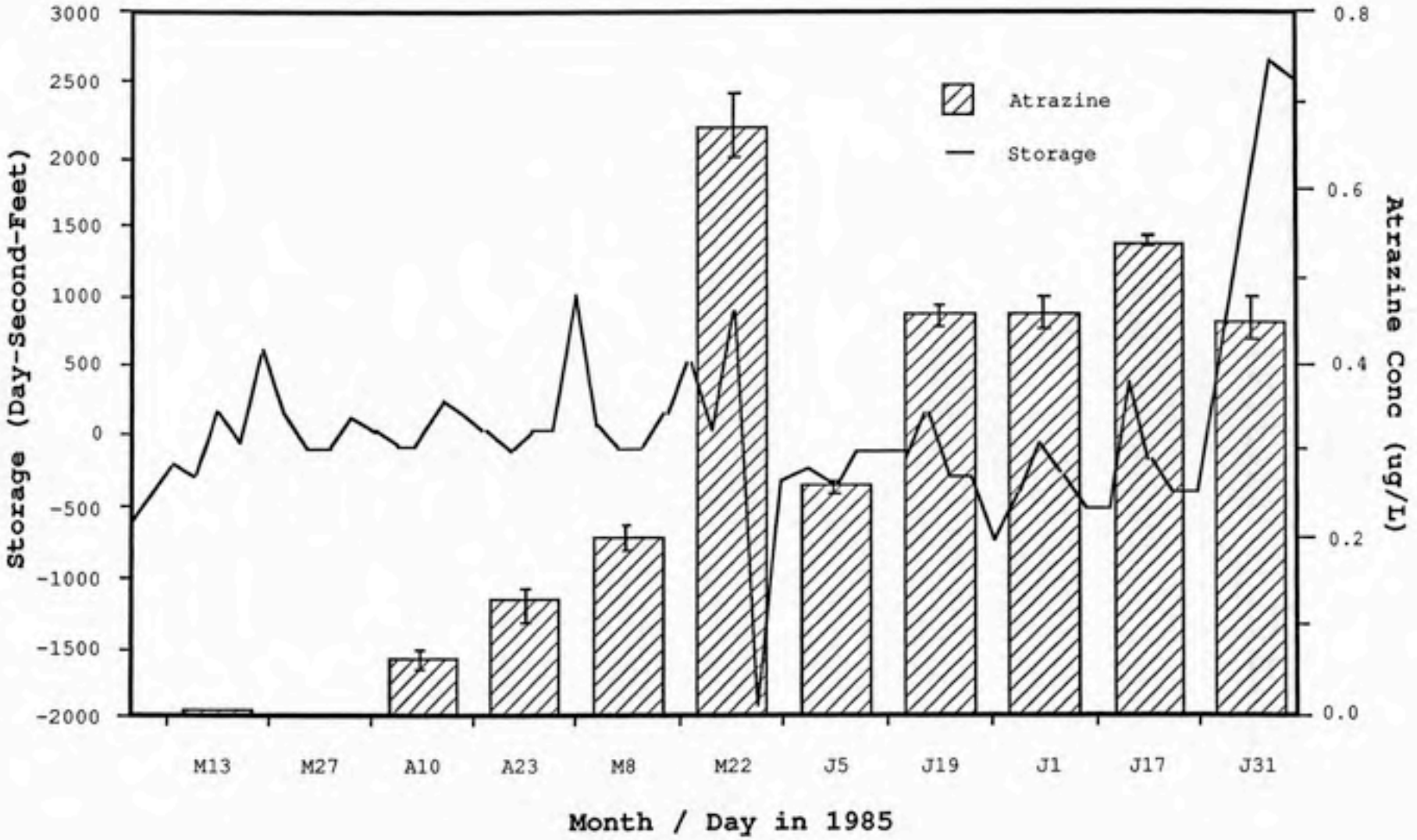


Figure 14. The relationship between lake storage of input flows and atrazine concentration at Station NH17. (Storage data from US Army Corps of Engineers).

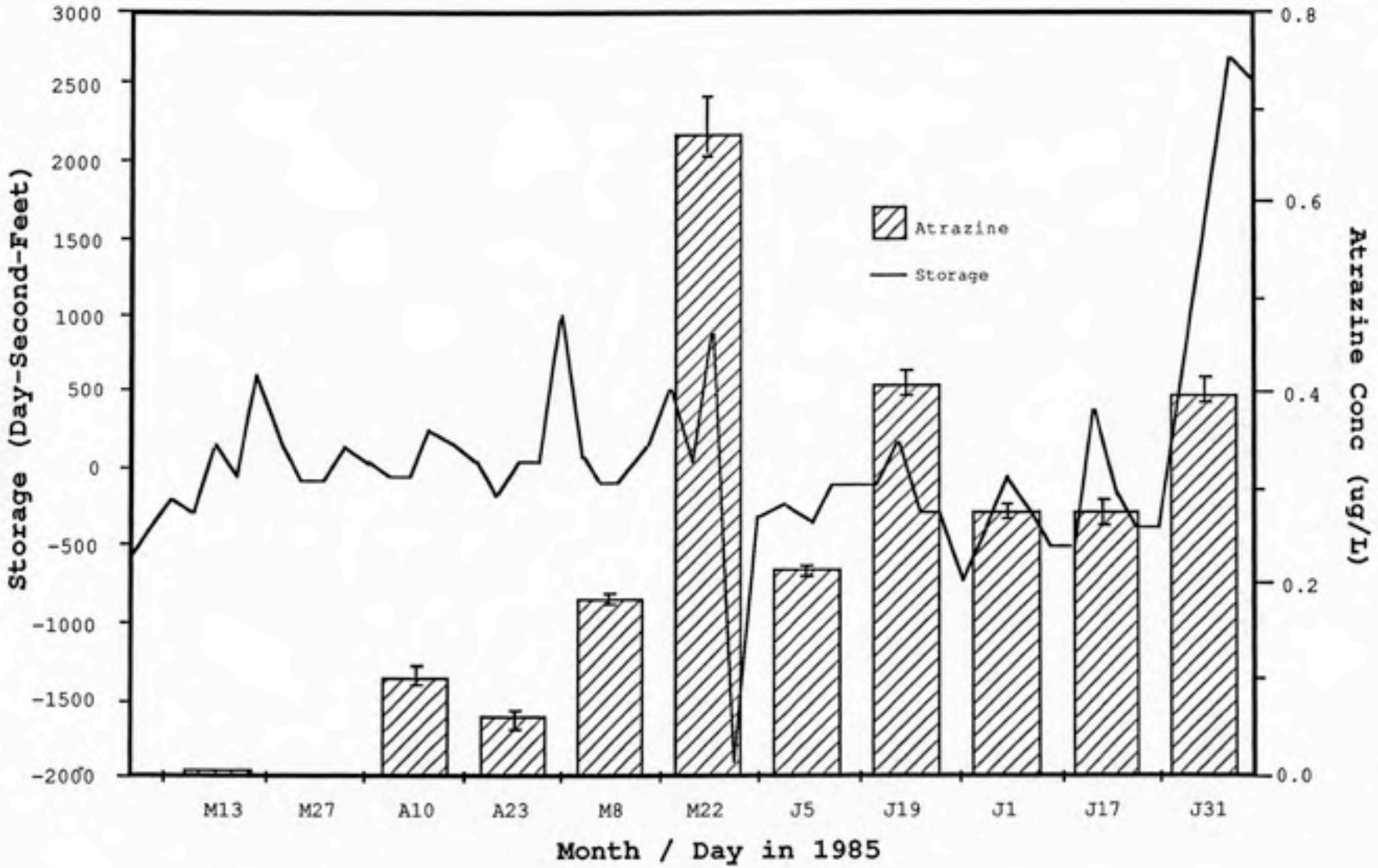


Fig. 15. The relationship between lake storage of input flows and atrazine concentration at Station NH15. (Storage data from US Army Corps of Engineers).

atrazine residue value recorded at Station H16 (Figure 13) during the study.

During periods of storage, flow out of segments 2, 3, and 4 will be retarded by downstream inflow of Segment 1 water. Therefore, implied retention times will be longer under low-flow conditions and shorter during post-storm draw-down.

High flows from source streams in the New Hope watershed will not have an immediate impact on the New Hope arm of Jordan Lake due to the long retention time of these flows. Direct runoff from fields adjacent to the lake was most likely responsible for the May 22nd pulse observed at NH17 (Figure 14) and NH15 (Figure 15). The higher concentration detected at Station NH17 was probably due to a combination of direct runoff and back-up of Haw water into Segment 2 during storage periods following atrazine application.

Indirect evidence for this hypothesis is provided by conductivity measurements. Conductivity is a measure of cation and anion activity and is related to dissolved material present in the water column. Segments 1 and 4 will generally contain the highest conductivity values due to erosional runoff and sewage effluent contained in source streams. There are no other direct sources of conductivity in the lake basin so the conductivity will decrease downstream as inorganic soluble ions become encorporated as organic and inorganic particulates and settle to the bottom. This action is enhanced by the segmenting features mentioned

above. Conductivity measurements at nine locations throughout the study show a regular downstream decrease in the Haw arm and also in the New Hope arm to NC-64 (Table 3). Conductivity downstream from this causeway increases, which is taken as evidence that Haw river water makes its way into segment 2.

The fact that conductivity in Segment 2 remained higher than in Segments 3 or 4 throughout the this year suggests that water from Segment 1 remains in Segment 2 long after back flow events have taken place. Flow events that took place in February of 1985 most likely resulted in large scale back flow displacement of Segment 1 water into Segment 2. The only large scale displacement taking place during the atrazine sampling period occurred July 25-31. Conductivity measurements in Segment 2 on July 31st indicate that Segment 1 water from this flow was present at Station NH17.

These observations bring up an important point concerning the relationship between flow and conductivity. The initial surge of a high flow event will have high conductivity due to its flushing effect on river beds and runoff of salts from fields. As runoff continues, temporal freshening of the flow will take place. Conductivity measurements taken at the end of the July flow event illustrate this effect (Table 3). The initial surge containing high conductivity flowed into Segment 2 whereas later flow, measured on the same day but at the top of Segment 1 (Station H5) resulted in lower conductivity.

Table 3. Water-column mean conductivity (umho/cm) for sites in Jordan Lake measured during 1985.

Segment →	1			2			3		4
Station →	H5	H16	H17	NH18	NH17	NH9	NH15	NH7	NH14
Date ↓									
2/27	84	84	85	95	94	96	99	95	86
3/13	149	131	115	111	105	102	98	98	99
3/27	151	150	165	110	108	105	101	102	108
4/10	159	153	140	116	110	106	102	104	112
4/23	189	172	146	122	115	111	102	107	110
5/8	207	188	169	120	119	113	108	112	125
5/22	215	207	172	124	119	112	110	116	135
6/5	169	160	154	124	126	116	115	118	139
6/19	224	196	165	140	122	119	118	124	136
7/1	226	204	168	130	128	127	131	130	144
7/17	220	249	203	136	134	128	129	134	153
7/31	141	136	150	156	160	134	124	127	143

June 5th samples revealed atrazine concentrations much lower than on the previous sampling date. Outflow, as illustrated by the negative storage values (Figure 13) following May 22nd, is suggested as the reason for this five-fold atrazine decline at Station H16. However, the retention time of water in Segments 2 and 3 is too great for this to result in the lower concentrations observed in the New Hope arm (Figures 14 and 15). It is more reasonable that the pulse on May 22nd in the New Hope segments was a result of fortuitous sampling of direct runoff which was diluted by June 5th or otherwise lost from the euphotic zone.

Low flow characterized the end of May, all of June and the first week of July (Figures 10 and 11). River inputs were similar to dam discharges for this period and very little storage took place. However, atrazine concentrations at all stations were greater than those present after the initial pulse in May. Station H16 shows a particularly regular increase through this period which can best be explained in terms of the biological sequence of events that took place (Figure 16) as follows.

Biological Accumulation Of Atrazine

The pulse of atrazine observed on May 22nd was accompanied by a large amount of algal nutrients. An opportunistic Cyanophyta species, Microcystis aeruginosa, was present in low densities on May 22nd but increased to 37% of the total density by June 5th (Campbell 1985). This species

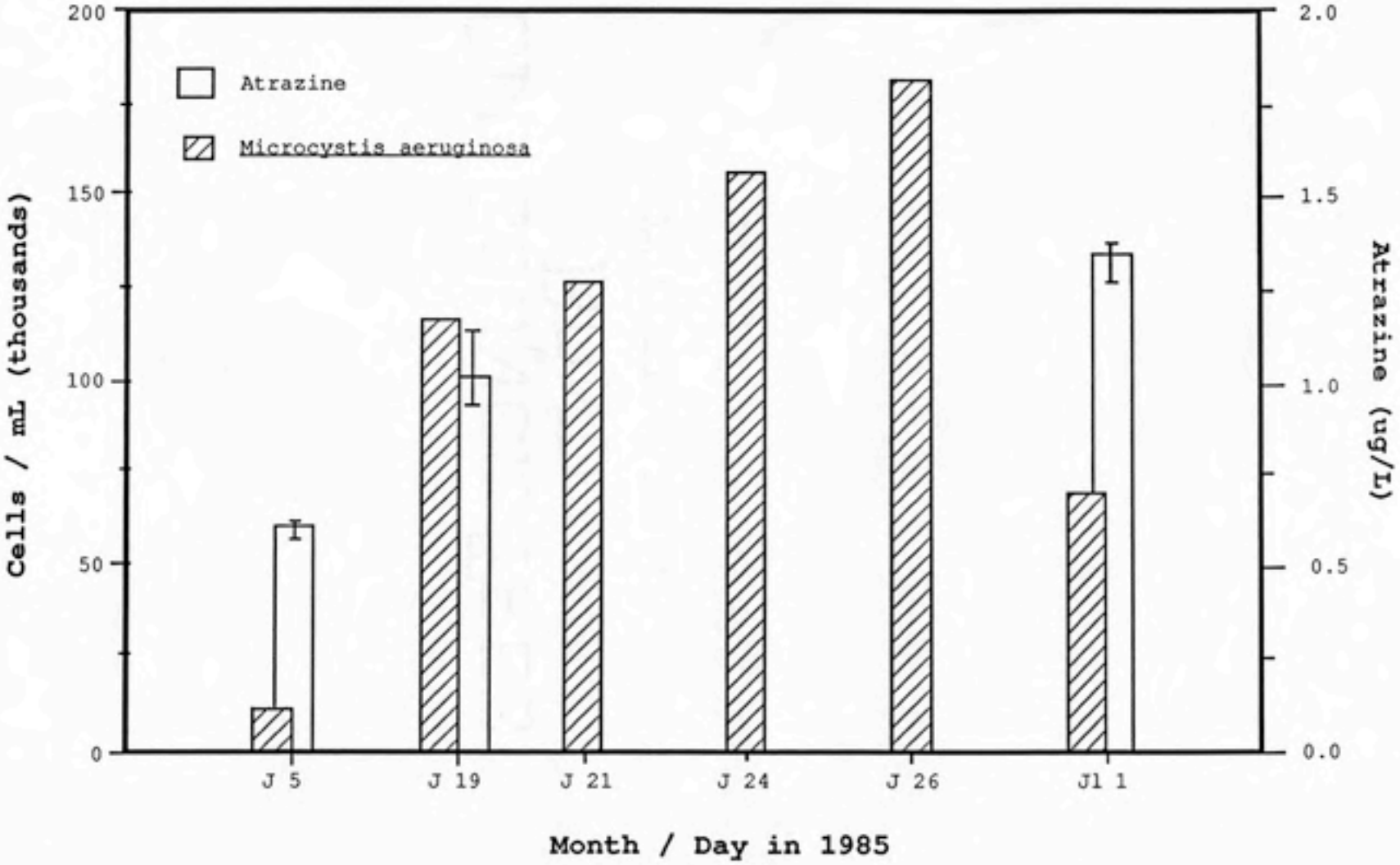


Fig. 16. The relationship between atrazine concentration and the presence of Microcystis aeruginosa in Segment I, during the period, June 5-July 1, 1985.

possesses high surface area, gas vacuoles which allow flotation, and has been shown to reduce competition via toxic excretions (Ingram and Prescott 1954). By June 26th, M. aeruginosa was 96% of the total biomass at Station H16 and was present at a density of 181,000 cells/mL (Campbell 1985). Veber et al. (1981) found that live and dead cell surfaces of Chlamydomonas vulgaris grown in the presence of ^{14}C atrazine adsorbed the herbicide from the media. Atrazine residues may have accumulated on the cell surface of M. aeruginosa during its explosive growth period. Lake mixing and nutrient depletion caused a decline in density by July 1st, although atrazine accumulation apparently reached a peak on this date. Cellular uptake as well as physio-chemical adsorption may have been taking place such that dead cells could also serve as a sink for atrazine. Atrazine concentrations declined after July 1st at Station H16, probably due to a combination of cell sinking, outflow from the lake, and chemical degradation. The results of in vitro bioassays presented below in this paper suggest that the success of Microcystis aeruginosa may be due in part to tolerance of atrazine.

Summary

Throughout the study, the highest concentrations of atrazine were found in Segment 1. Concentrations in the New Hope segments (Figures 14 and 15) were roughly one-third those observed in Segment 1 (Figure 13), and atrazine at NH17 was consistently higher than at NH15. The higher values at

NH17 most likely reflect a combination of hydrological inputs from Segment 1 and downstream algal accumulation discussed above. The atrazine values in Segment 2 and 3 remained lower than the May 22nd value but generally increased from the low concentration determined on June 5th. Possible explanations include greater mixing depths of June (this would tend to include atrazine from depths previously excluded from samples), direct runoff in late July, and algal bioaccumulation in June and July.

It would appear that the majority of atrazine entering Jordan Lake occurred as a single pulse following field application, and then dissipated rapidly. Additional inputs were relatively low by comparison, but concentrations at all stations remained higher than those measured prior to field application in April. It is suspected that, in the absence of extreme hydrological flows following initial input, the concentration of atrazine is determined by the biological and chemical interactions taking place within the lake. The bioassay data presented later in this paper indicate that atrazine concentrations determined for Jordan Lake in this study are not of a level which would have adverse effects on the phytoplankton population taken as a unit. However, statistically significant stimulation of Chlorella spp. was found to take place at atrazine concentrations present on several sampling dates in the Haw arm of the lake.

Species Specific Responses To Atrazine - Bioassays I And II

The biological assay system employed in this study utilized a natural phytoplankton population from Jordan Lake which allowed the best *in vitro* estimate of the effects of atrazine on phytoplankton. An ecosystem is a very complex, interdependent system which makes modeling challenging and subject to pitfalls if certain limitations are not taken into account.

Two *in vitro* experiments were conducted to assess the species specific responses of a natural phytoplankton population to the herbicide atrazine. Bioassay I (BI) was conducted in June and bioassay II (BII) in October of 1985. Results from these experiments are presented graphically in Figures 17-40. Each figure represents cell counts for an algal species or group of species by atrazine dose on the day indicated. The average of these counts is presented in the right-most set of bars and can be interpreted as the "growth potential" for that species or group at the given atrazine dose. The term "treatment" as used in this study includes all atrazine doses and the control unless indicated otherwise. Results for species or algal groupings found in both experiments are presented together.

Between 25 and 30 species were recognized throughout both experiments although microscopic examination at 1250x power suggested even greater diversity. The larger number of species presented for BII does not represent greater

diversity than for BI but rather, greater counting effort in the second experiment.

Clearly recognized limitations were present in the counting method (Table 2). Counting variability was a constant source of error in the sense that all phytoplankton species were counted using the same method. However, cell counts of abundant species represented the population more closely than counts of sparsely populated species. Therefore, greater confidence was placed in these results.

Careful microscopic examination at 1250x magnification minimized problems associated with species identification. Species not recognized were classified as unidentified. If doubt or confusion over the identification of a species or group arose, it has been discussed in the text.

The effect of atrazine was considered statistically significant in models producing P values less than 0.1000. This might be considered a conservative approach in view of the inherent variability associated with the assay. For this reason, results which did not produce P values less than or equal to 0.1000 but which suggested some trend or other effect of atrazine were included and clearly identified as appearing "graphically". Caution has been used in the interpretation of these graphically-apparent results and the reader is cautioned to pay close attention to the confidence expressed in the individual species results. Unless indicated otherwise, missing data can be interpreted as indicating that the species in question was below the

detection limit of the counting method which was 1 cell per slide or approximately 1000 cells per mL.

Total Count

(BI and BII, Figures 17A and 17B).

Both experiments resulted in similar trends for the total count (the sum of all individual species including unidentified members). Maximum biomass was achieved in control flasks by Day 5 (BI) or Day 6 (BII) with the lower atrazine doses outgrowing the controls. This was true even of the 10 ug/L dose in BII. However, cells at 10 ug/L lagged behind the control and 1 ug/L dose, and rapidly decreased after Day 7 such that the "growth potential" achieved was slightly less than control, 0.25 or 1.0 ug/L atrazine. There were no statistical differences detected between the two lower doses and the control on any day, or for the "growth potential" in either experiment.

Severe inhibition characterized 50 ug/L atrazine at all times during both BI and BII. The characteristic logarithmic phase of growth never took place at this dosage. Strong statistical differences between 50 ug/L and all other treatments were established after Day 3. During the logarithmic growth phase, Days 3-5 for BI and Days 3-6 for BII, the growth rate at 50 ug/L was statistically lower than that at 0.25 ug/L (BI), and 1 and 10 ug/L (BII). A statistically significant negative correlation between

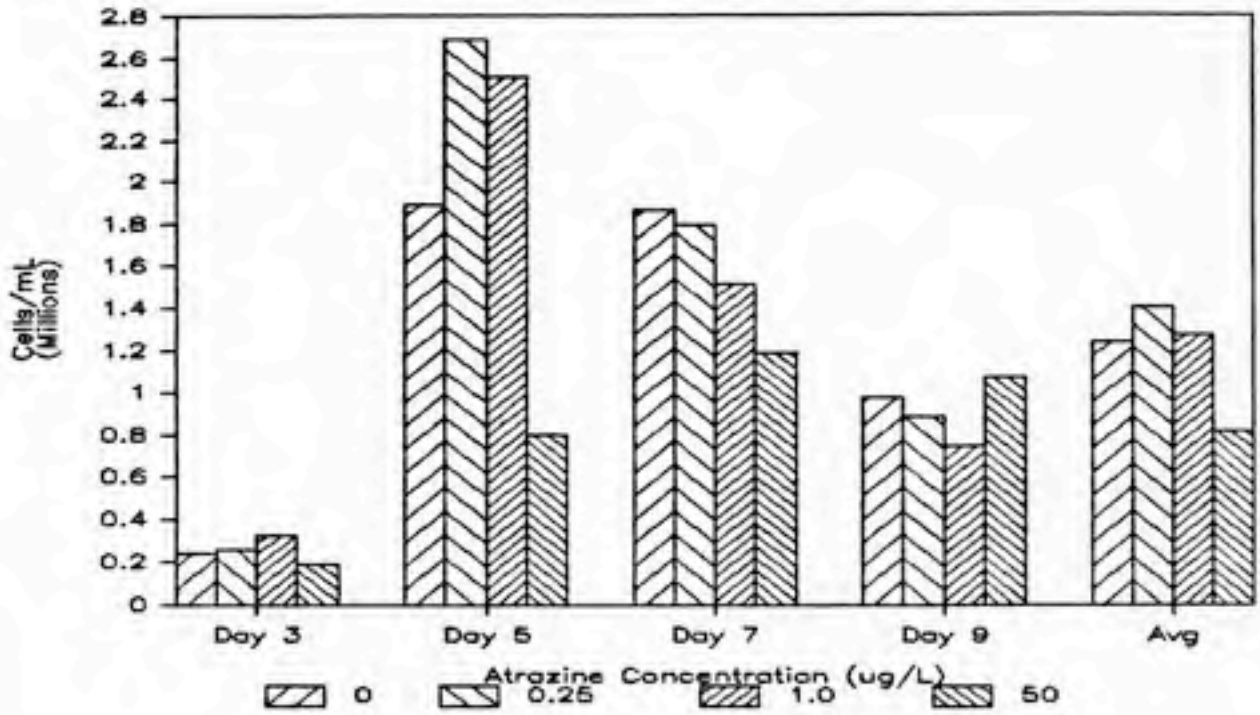


Fig 17A. Combined cell counts for all species present in BI, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

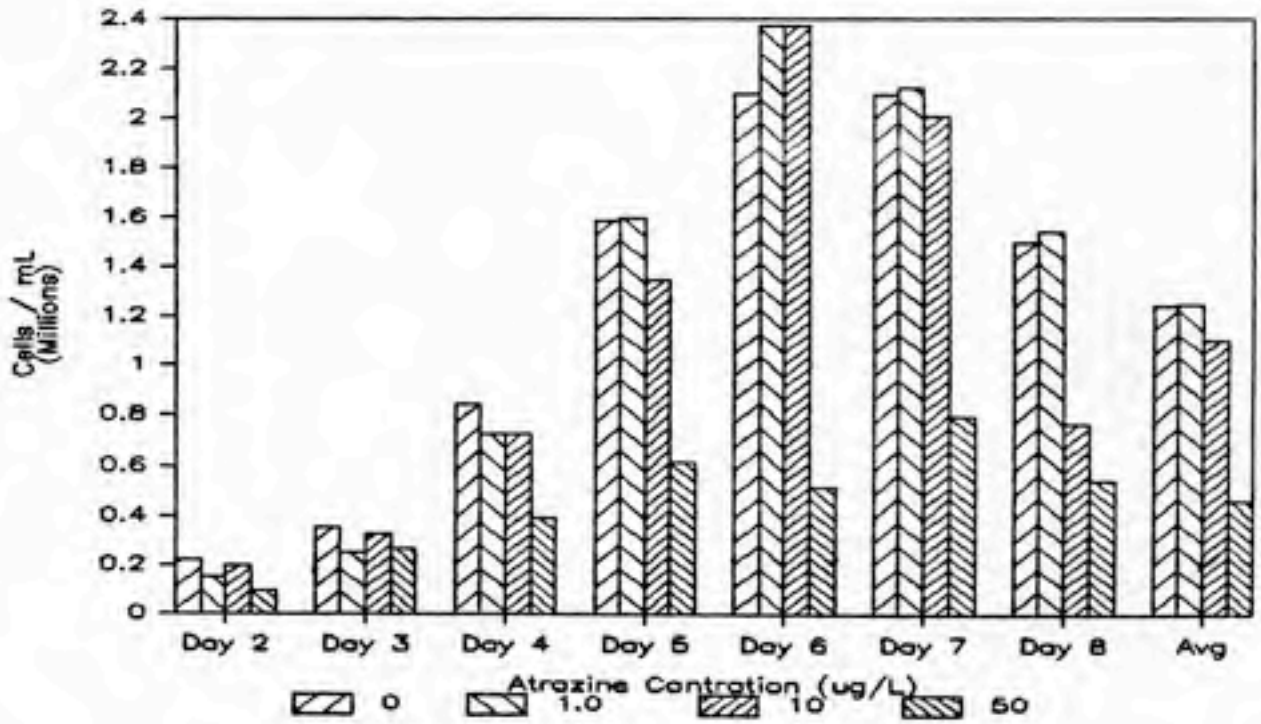


Fig 17B. Combined cell counts for all species present in BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

atrazine dose and cell count existed throughout both experiments.

It would appear then, that for a natural phytoplankton population taken as a whole, the in vitro "action level" for atrazine (that level below which no statistically significant differences from controls can be established) is between 10 and 50 ug/L. Although they were not found to be significantly different than the controls, low doses of atrazine (0.25-10 ug/L) did produce higher growth rates and biomass than controls. The literature provides evidence to support this type of effect in algae (Boger 1976) and (Torres and O'Flakerty 1976) and also in higher plants (Vance and Smith 1969).

Oscillatoria limnetica

(BI and BII, Figures 18A and 18B).

Oscillatoria limnetica, a member of the Cyanophyta, was the dominant species in both bioassays. Severe inhibition at 50 ug/L characterized both experiments. Strong statistical differences between 50 ug/L and other treatments were established in BI and BII after Day 3. Maximum biomass at this concentration was reached on Day 7 compared with Day 5 and Day 6 for the lower doses and controls of BI and BII respectively. The lower doses of atrazine in both experiments produced a maximum biomass that was greater than the controls, but this low-dose stimulation was not shown to be statistically significant. However, during the

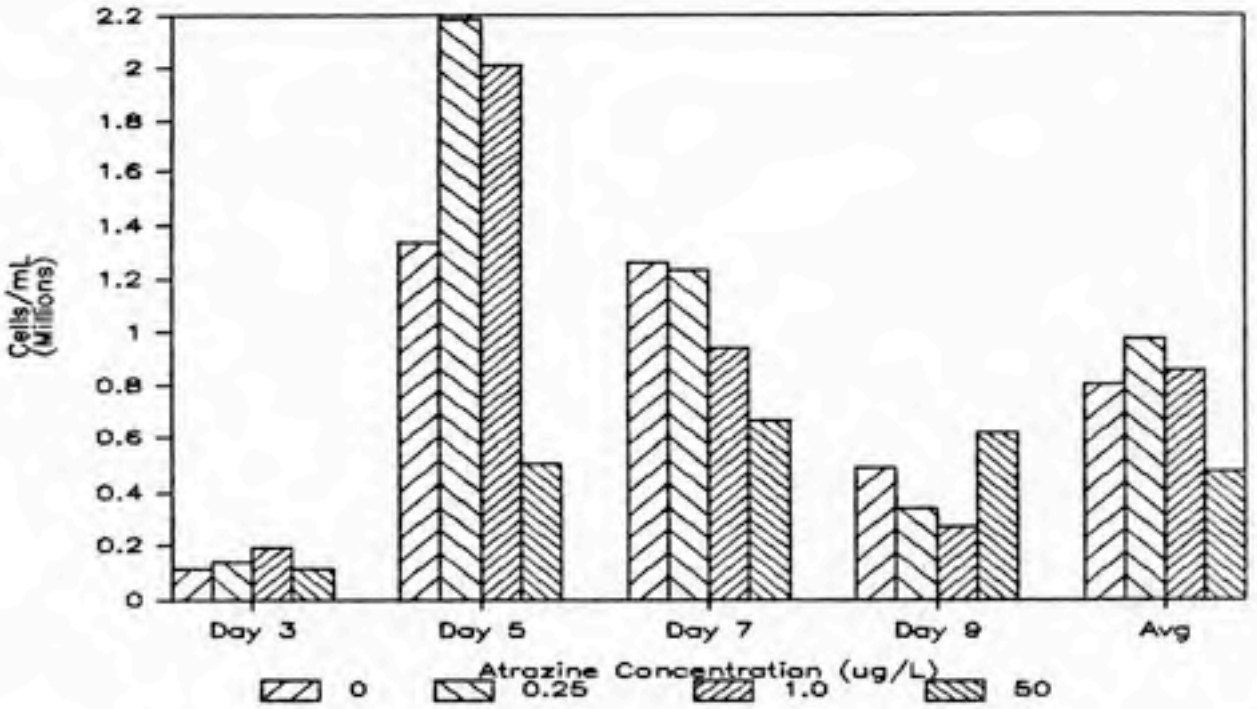


Fig 18B. Cell counts for Oscillatoria limnetica from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

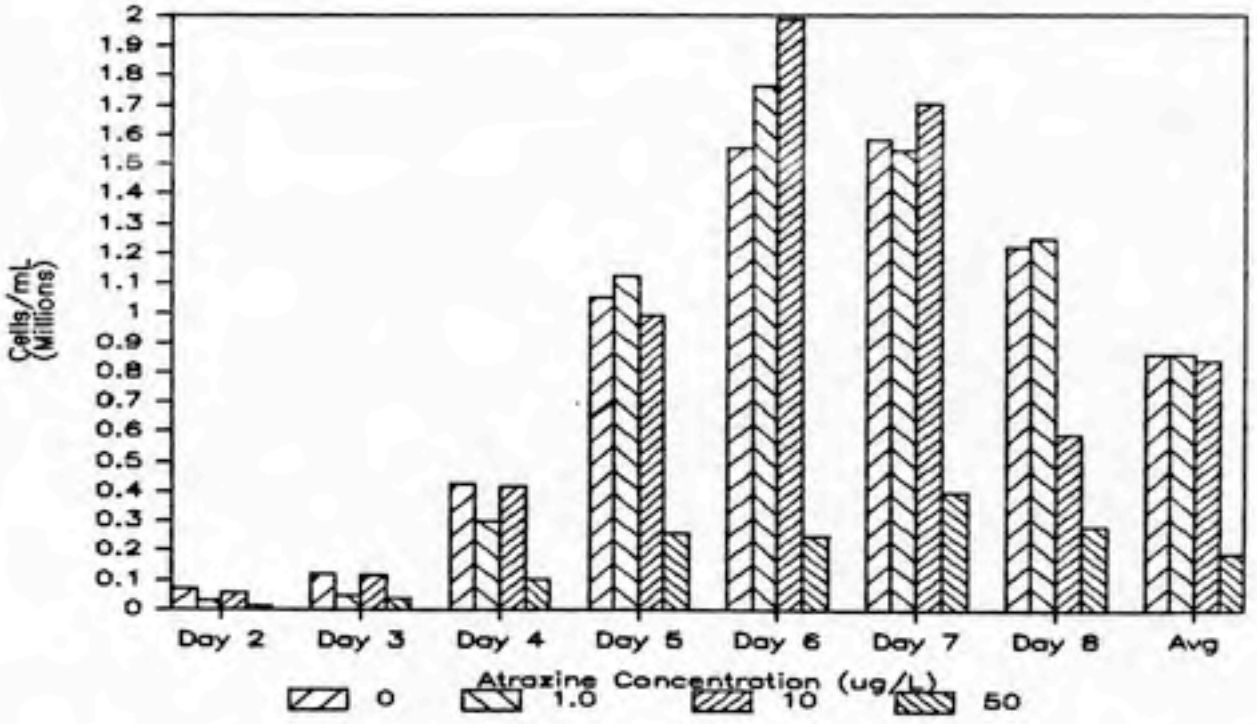


Fig 18A. Cell counts for Oscillatoria limnetica from BI, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

logarithmic growth phase (Days 3-6) of experiment II, the growth rate of cells in 1 ug/L atrazine was statistically greater than the control and also higher than for cells in 50 ug/L atrazine. During the logarithmic growth phase (Day 3-5) of BI, only cells in 0.25 ug/L atrazine had higher growth rates than those at 50 ug/L.

The resulting "growth potential" of this species from both experiments indicates that 50 ug/L atrazine produced a biomass that was statistically smaller than any other treatment. Doses of 1 and 10 ug/L produced essentially the same biomass as the control in BII while 0.25 ug/L atrazine allowed growth slightly greater than the control in BI. It may be concluded that Oscillatoria limnetica is resistant to concentrations of atrazine common in streams receiving field runoff. However, the higher concentrations of atrazine reported adjacent to atrazine treated fields may have potentially severe negative effects on this alga, while the much lower concentrations reported for reservoirs in this study may stimulate growth of this species.

An additional note relating to the taxonomy of this species; during the logarithmic phase of growth for BI and BII, this species was observed to have short (<1 um) spaces between each cell within a filament, and its appearance was not unlike that of another species, O. geminata. It may be that under ideal growth conditions, dividing cells of O. limnetica take on the appearance of another species, O.

geminata, and that these species are one and the same, namely O. limnetica.

Merismopedia tenuissima

(BII, Figure 19).

This species, another member of the Cyanophyta, was present in both bioassays but only quantified for BII. Maximum biomass for the experiment was obtained on Day 6 at 1 ug/L atrazine. In all other treatments, M. tenuissima peaked on Day 7. Cells in 1 and 10 ug/L atrazine outgrew the control on Days 5 and 6 and had slightly greater "growth potential" as well. At 50 ug/L, this species lagged behind other treatments following Day 5, but inhibition at this dosage level was not severe. There were no statistically significant differences found between any of the atrazine doses during the experiment. Slight inhibition may occur at atrazine doses above 10 ug/L, as illustrated by the "growth potential". However, this effect cannot be shown to be statistically significant.

Gleocapsa punctata

(BII, Figure 20).

Another member of the Cyanophyta, Gleocapsa punctata was not found in BI. However, this species was a subdominant in the water used to seed the second experiment and was present in relatively low numbers throughout that bioassay. Cells of this species were aggregated in colonies of 5 to 20 cells per

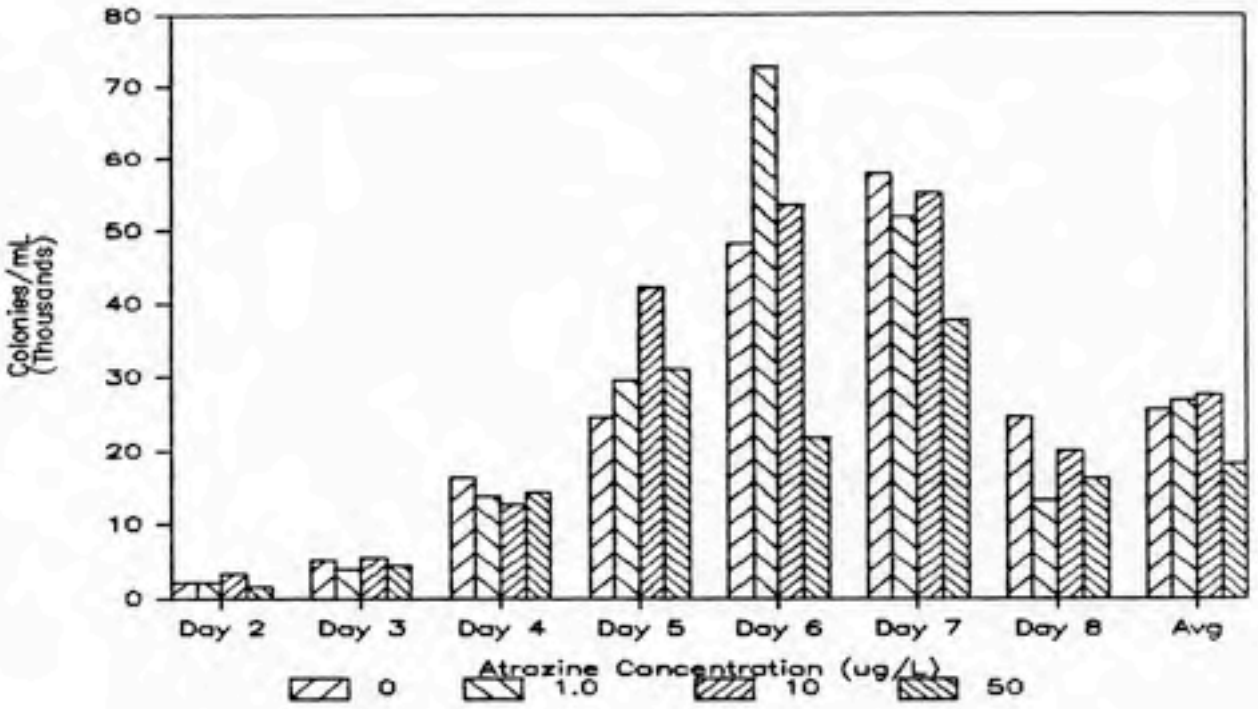


Fig 19. Cell counts for Merismopedia tenuissima from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

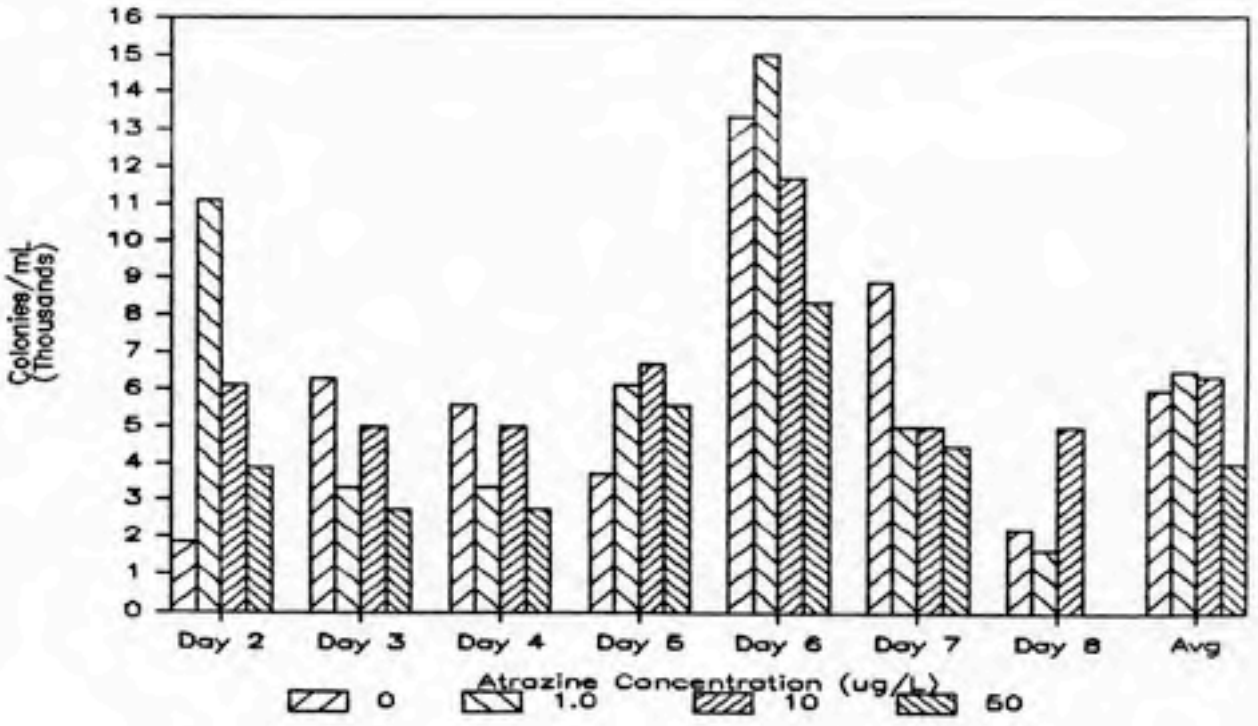


Fig 20. Cell counts for Gleocapsa punctata from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

unit and were quantified as units. This may have caused variation in biomass estimation due to variation in the number of cells per colony because colonies were not converted into cells/mL. The only statistical difference between treatments was found on Day 2 when 1 and 10 ug/L atrazine produced higher cell counts than the control. In terms of "growth potential", cells at 50 ug/L experienced slight atrazine inhibition relative to other treatments, much like that reported here for Merismopedia tenuissima. A comparison with the early growth (Days 2-4) of other species indicates rather poor or static growth. In view of its relatively large initial biomass, it would seem that this species could not compete well under these bioassay conditions.

Polycystis firma

(BII, Figure 21).

The final member of the Cyanophyta to be discussed, P. firma, was present in low numbers during both experiments although its quantification was only undertaken during BII. Cells of this alga are united in small, firmly packed clusters of 20 to 30 cells which were counted as units.

The maximum biomass for this species was achieved by cells in 1 ug/L atrazine on Day 6 which coincided with the control growth peak. Treatment with 10 ug/L atrazine produced essentially the same maximum biomass on Days 3, 4, and 5 while cells in 50 ug/L peaked on Day 5. The "growth

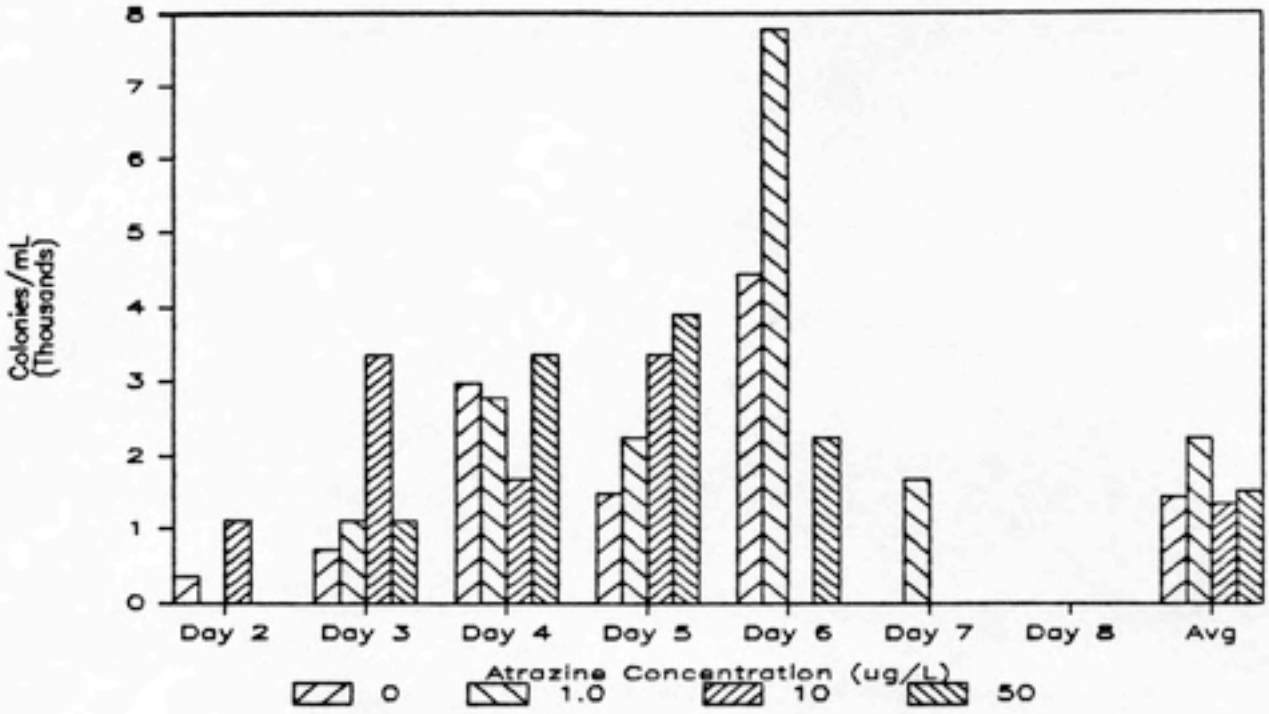


Fig 21. Cell counts for Polycystis firma from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

potential" for this species suggests a stimulation by 1 ug/L atrazine. However, statistical models indicate that atrazine has no effect on the "growth potential" of this species at any treatment. The apparent stimulation at 1 ug/L was probably due to the single pulse on Day 6. In view of the relatively good growth occurring prior to Day 6, the poor performance in the latter stages by this species would not seem to be due to atrazine inhibition. Nutrient depletion by more competitive species seems a more likely explanation.

Nephroselmis discoidea

(BI and BII, Figures 22A and 22B).

This biflagellate member of the Cryptophyta is easily recognized by its shape and motion and was present in both bioassays. In BI, maximum biomass was observed on Day 9 for the control, Day 5 for 50 ug/L atrazine, and Day 7 for 1 and 0.25 ug/L atrazine. In contrast, cells of this species were not observed after Day 5 of experiment II. During BII, maximum biomass for the control and 1 ug/L atrazine was observed on Day 4 and on Day 3 for 10 and 50 ug/L atrazine.

The "growth potential" illustrated by this species in both experiments suggests atrazine inhibition at all doses, although this inhibition cannot be shown to be statistically significant. The only significant differences between any treatments occurred in BI. Cell counts for the control and 1 ug/L dose on Day 3 were both statistically higher than at 50 ug/L atrazine. During both experiments, a negative

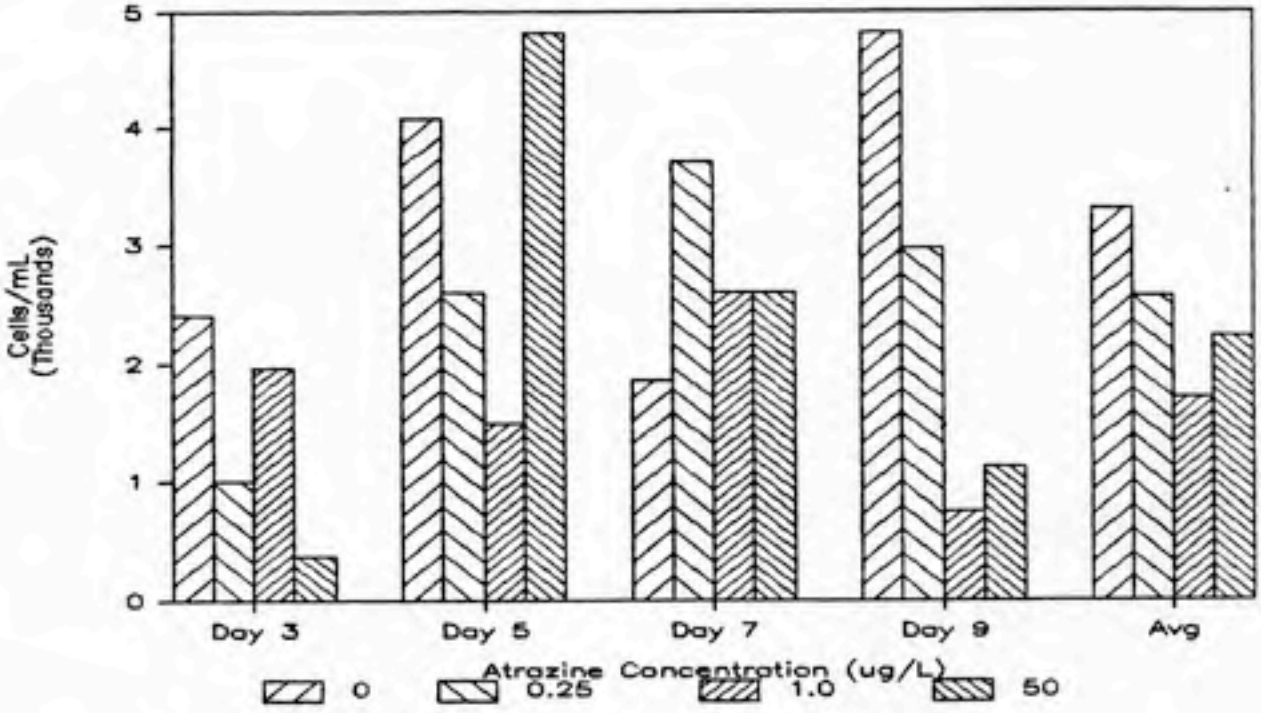


Fig 22A. Cell counts for Nephroselmis discoidea from BI, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

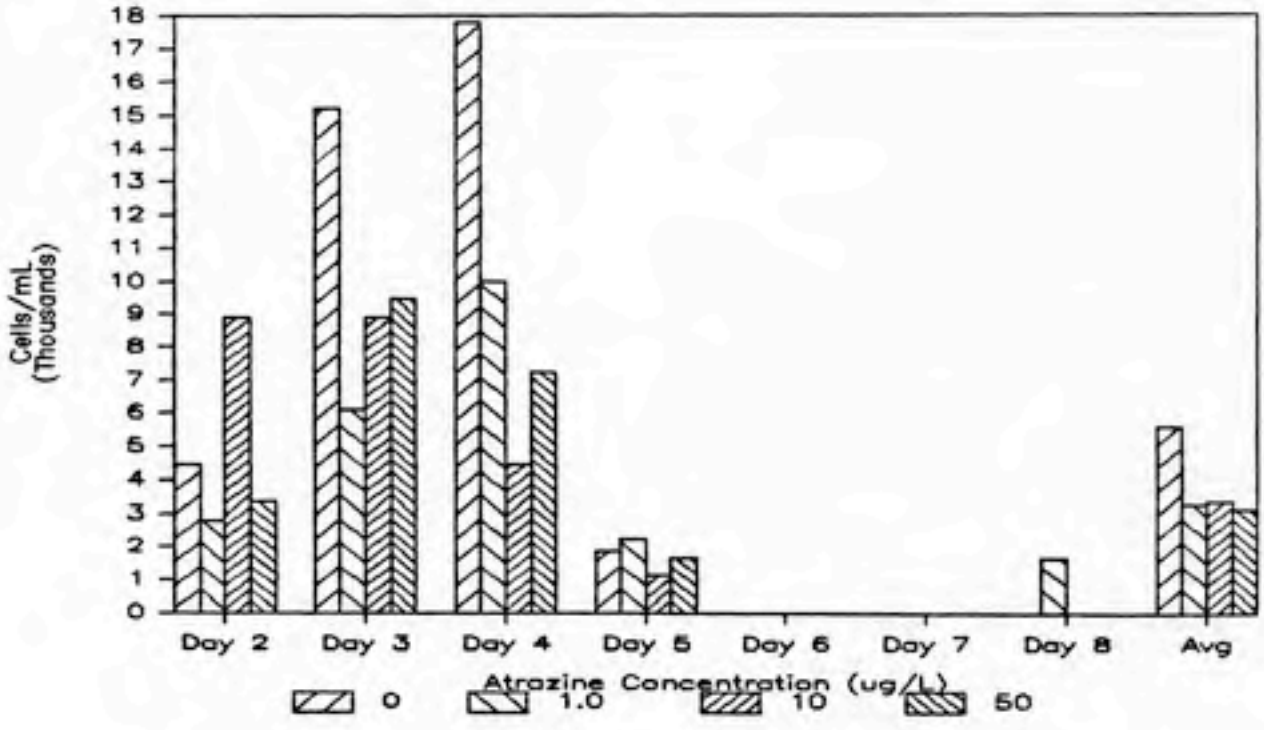


Fig 22B. Cell counts for Nephroselmis discoidea from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

association was observed between atrazine dosage level and the resulting cell count. This relationship was statistically significant on Days 3 and 9 of BI and on Day 4 of experiment BII.

Competition seems a likely explanation for the absence of Nephroselmis discoidea following Day 5 of BII. Prior to this time it was noted that cells of this species were sluggish and many cells had dropped their flagella. It was at this point that O. limnetica was in logarithmic phase of growth and established dominance. Many species of the Cyanophyta have been reported to produce toxins thought to give them an advantage in interspecific competition (Ingram and Prescott 1954). It may be that Nephroselmis discoidea was inhibited by one or more of the Cyanophyta during BII.

Cryptomonas spp.

(BII, Figure 23).

These biflagellated species, members of the division Cryptophyta, are common members of the phytoplankton found in Jordan Reservoir. In BI this group was either below the detection limit or not recognized. Cryptomonas spp. were present in moderate numbers in the water used as seed for BII, but were detected only briefly during the experiment. Cells in 1 ug/L reached maximum biomass on Day 6 and on Day 4 in all other treatments. No cells were detected at any treatment on Days 2, 7, or 8.

In view of the low counts observed, it is not surprising that statistical models did not detect any difference between doses or other associations of cell count with atrazine level. However, the lack of atrazine effect is supported by essentially equal "growth potential" at each treatment level so it would seem that this group of species is relatively unaffected by atrazine levels used in this experiment. This conclusion has been reached at even higher doses by others (DeNoyelles, Kettle, and Sinn 1982).

Cyclotella spp.

(BII, Figure 24).

These members of the Chrysophyta were subdominants in seed samples for both experiments but only counts from BII were analyzed statistically. This genus, primarily composed of Cyclotella pseudostelligera, did not experience good growth after Day 4. This is illustrated by the early peak and subsequent decline for cells at all treatment levels. Maximum counts for the control and 10 ug/L atrazine were on Day 3 while 1 and 50 ug/L peaked on Day 4. The peak on Day 4 at 50 ug/L atrazine was the maximum biomass of any treatment observed during the experiment. There were no statistically significant differences between the "growth potential" of the control, 1 or 50 ug/L, however 10 ug/L never reached maximum biomass comparable to other treatments and therefore demonstrates a lower "growth potential" than other treatments.

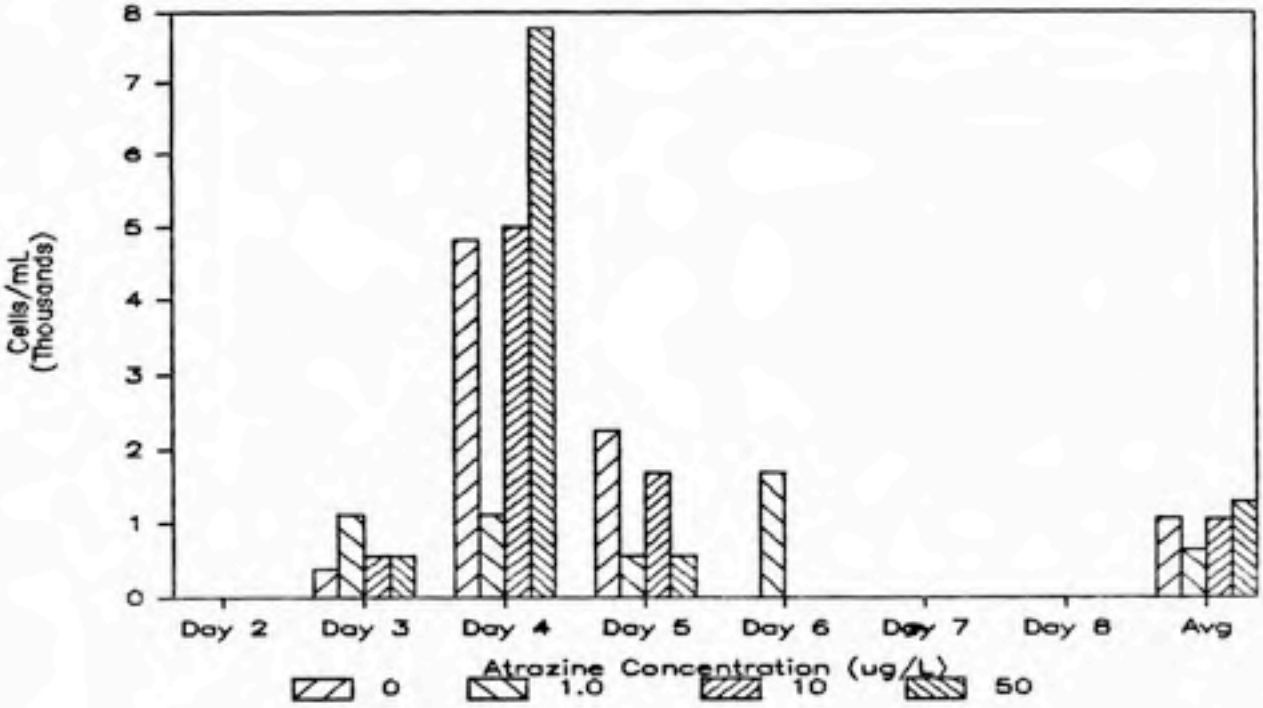


Fig 23. Cell counts for Cryptomonas spp. from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

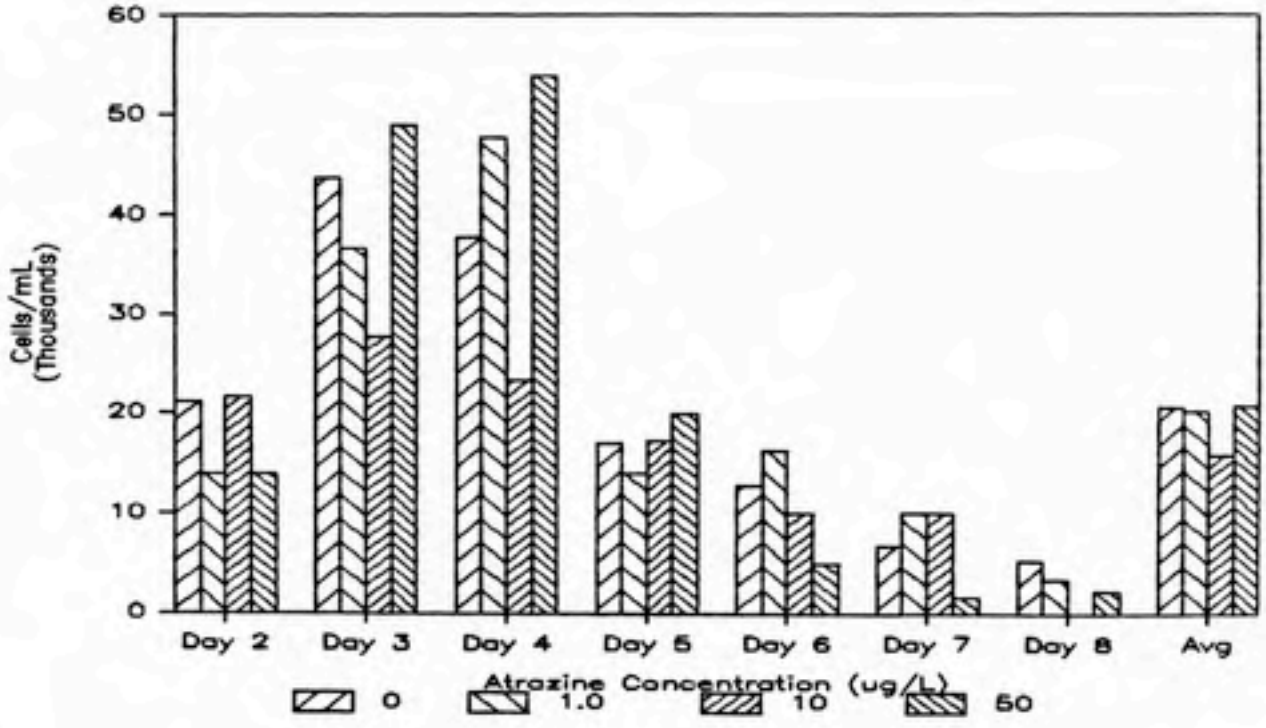


Fig 24. Cell counts for Cyclotella spp. rrom BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

Strong statistical evidence is provided for dose related differences on Day 2. The control cell counts as well as those at 10 ug/L atrazine were statistically larger than at 1 and 50 ug/L. However, by Day 4 growth in the control and 10 ug/L treatments was declining, whereas 1 and 50 ug/L atrazine produced biomass greater than the control and were statistically greater than the 10 ug/L treatment. Statistical models designed to detect differences in growth rates illustrate this effect. Doses 1 and 50 ug/L have statistically higher growth rates than 10 ug/L atrazine during the period Day 2-3. Growth of cells in 1 and 50 ug/L continued through Day 4 at a rate that is statistically higher than both the control and 10 ug/L atrazine. There were no statistically significant differences after Day 4.

The results for these species indicate a growth lag at low and high doses of atrazine, followed by stimulation at these doses. The intermediate concentration of 10 ug/L atrazine never approached the biomass obtained in other treatments. However, the "growth potential" shows only slight inhibition at 10 ug/L atrazine and no statistical differences between treatments were found. While it would appear that there is an absence of dose-related atrazine effects, it is suggested that the observed growth patterns are more a result of interspecific competition than an effect of atrazine.

Dictyosphaerium ehrenbergianum

(BII, Figure 25).

This species, a member of the Chlorophyta, was present in both experiments but only treated statistically for BII. D. ehrenbergianum grows in colonies with thin thread-like connections between each cell. Cell counts presented here represent cells per mL rather than colony units. D. ehrenbergianum was a subdominant in the seed used for BII and reached appreciable biomass in this experiment. However, there may have been some confusion between this species and the genus Chlorella when the thread-like connections were not clearly visible.

Maximum biomass for the control and 10 ug/L was observed on Day 5. This control biomass was the largest obtained for any treatment during the experiment. Cells in 1 and 50 ug/L atrazine peaked two days later. The control and 10 ug/L treatments showed regular increases followed by decline, while the 10 and 50 ug/L doses had two distinct phases of growth, possibly a result of varying competition levels.

The "growth potential" indicates that biomass decreased with increasing atrazine dosage level except for the anomaly at 10 ug/L which was shown to be statistically different than the control. Growth at all treatments proceeded at a rather low rate until the control peak on Day 5. This biomass was statistically larger than that observed at 1 ug/L atrazine. By Day 6 the control and 1 ug/L atrazine flasks contained significantly larger biomass than the 50 ug/L dose. All

treatments were statistically different than the low count for the 10 ug/L dose on Day 7.

Cell counts for Dictyosphaerium ehrenbergianum resulted in a negative dose/response association throughout the experiment. This effect was statistically significant on Days 5 and 6 as well as for the "growth potential".

In general, these results suggest the absence of an "action level" for this species. While all doses of atrazine produced appreciable biomass (with the exception of 10 ug/L), an extended lag time was observed for all doses when growth curves are compared with controls. In addition, none of the atrazine-treated populations approached the control level of "growth potential".

Chlorella spp.

(BII, Figure 26).

This genus was composed primarily of C. vulgaris and C. ellipsoidea. It was a minor component of the original seed sample for both experiments but was only quantified for BII.

Maximum biomass was achieved on Day 5 in all the atrazine dosed flasks but not until Day 6 for the control. Chlorella spp. in 1 ug/L atrazine outgrew the control throughout the experiment and resulted in the largest biomass observed during BII on Day 5. The "growth potential" of cells at this dose was found to be statistically larger than that at either 10 or 50 ug/L atrazine. Neither of the higher doses of atrazine produced populations that approached the

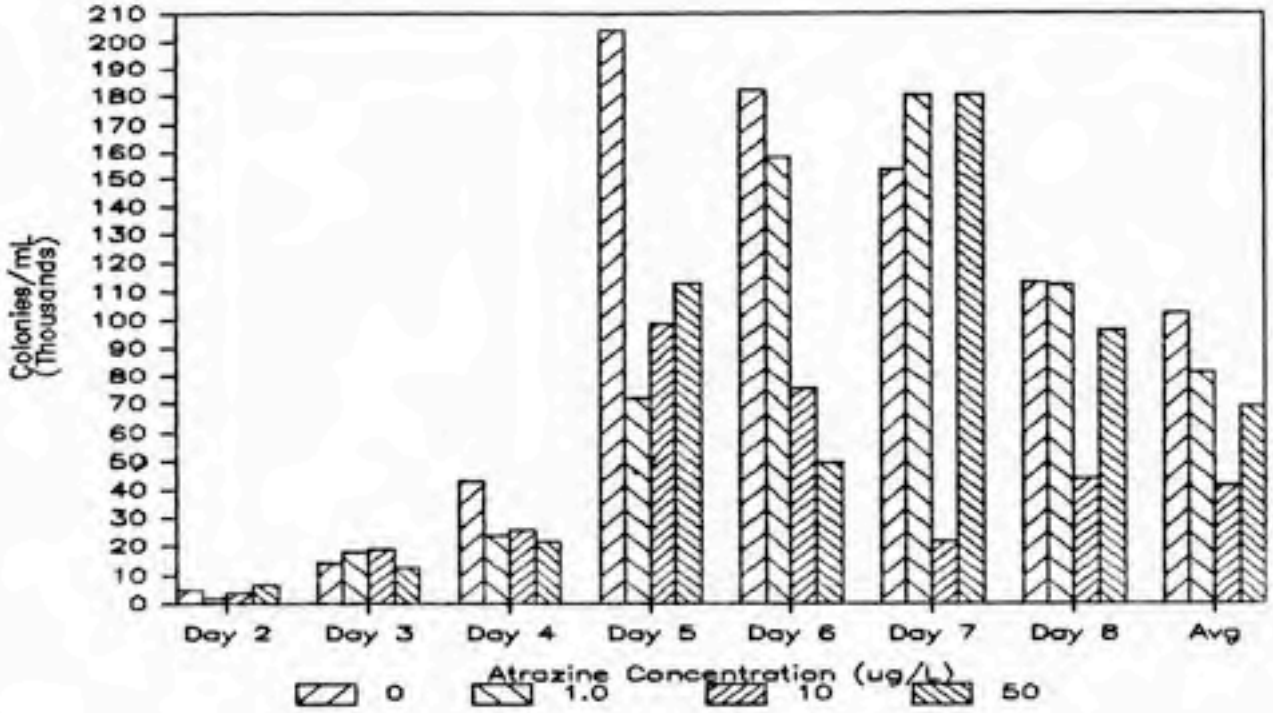


Fig 25. Cell counts for Dictyosphaerium ehrenbergianum from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

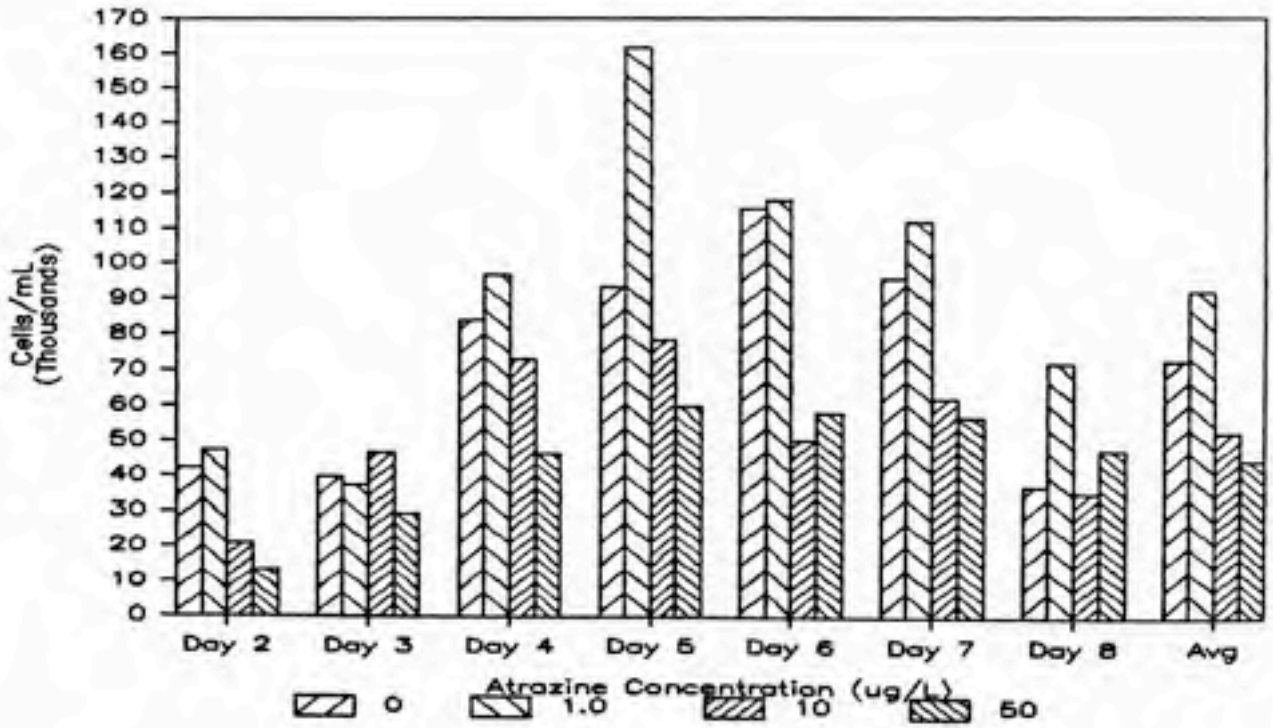


Fig 26. Cell counts for Chlorella spp. from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

biomass observed at the lower dose or control. Growth at these atrazine levels lagged behind other treatments, particularly the 1 ug/L dose.

The control and 1 ug/L treatments produced biomass that was statistically greater than that dosed with 50 ug/L atrazine on Day 2. Treatment with 1 ug/L continued to produce statistically greater biomass than 50 ug/L atrazine on Days 4 and 5. A negative association between atrazine dosage level and cell count was established throughout the experiment. Statistical evidence for this effect is provided on Days 2, 4, 6, and 7 as well as by the "growth potential". This evidence suggests that members of this genus were stimulated at low doses and inhibited at high doses of atrazine.

Ankistrodesmus spp.

This genus is a common fresh water member of the Chlorophyta and was present in moderate numbers in the seed samples used for both experiments. The two experiments are not strictly comparable because Ankistrodesmus falcatus var. spirilliformis was distinguished from the other members of this genus in BII but not during the first experiment. Therefore, results are discussed by experiment.

Ankistrodesmus spp.

(BI, Figure 27).

Maximum biomass was achieved at 50 ug/L atrazine on Day 7 and by all other treatments on Day 5. There were no significant differences between the "growth potential" of any two treatments. There was, however, a growth lag produced during the first five days by the 50 ug/L atrazine dose. During logarithmic growth (Day 3-5), the growth rate at 50 ug/L atrazine was statistically lower than any other treatment. A dose/response inhibition during this period was also shown to be statistically significant. The effects of atrazine appear to be temporary, as cells in flasks treated with 50 ug/L atrazine produced essentially the same maximum biomass as other treatments two days later.

Ankistrodesmus spp.

(BII, Figure 28).

Maximum biomass for this genus was observed in all flasks on Day 5. Treatment with 50 ug/L atrazine stimulated growth, producing cell counts that were equal to or greater than the control after Day 3. The stimulation at 50 ug/L atrazine is illustrated by the "growth potential" for this species although it is only significantly different from that obtained at 10 ug/L. As has been illustrated by other species or groups, the 10 ug/L atrazine dose inhibited growth to the greatest extent for this genus, most likely an indirect result of greater growth by O. limnetica. Cells in

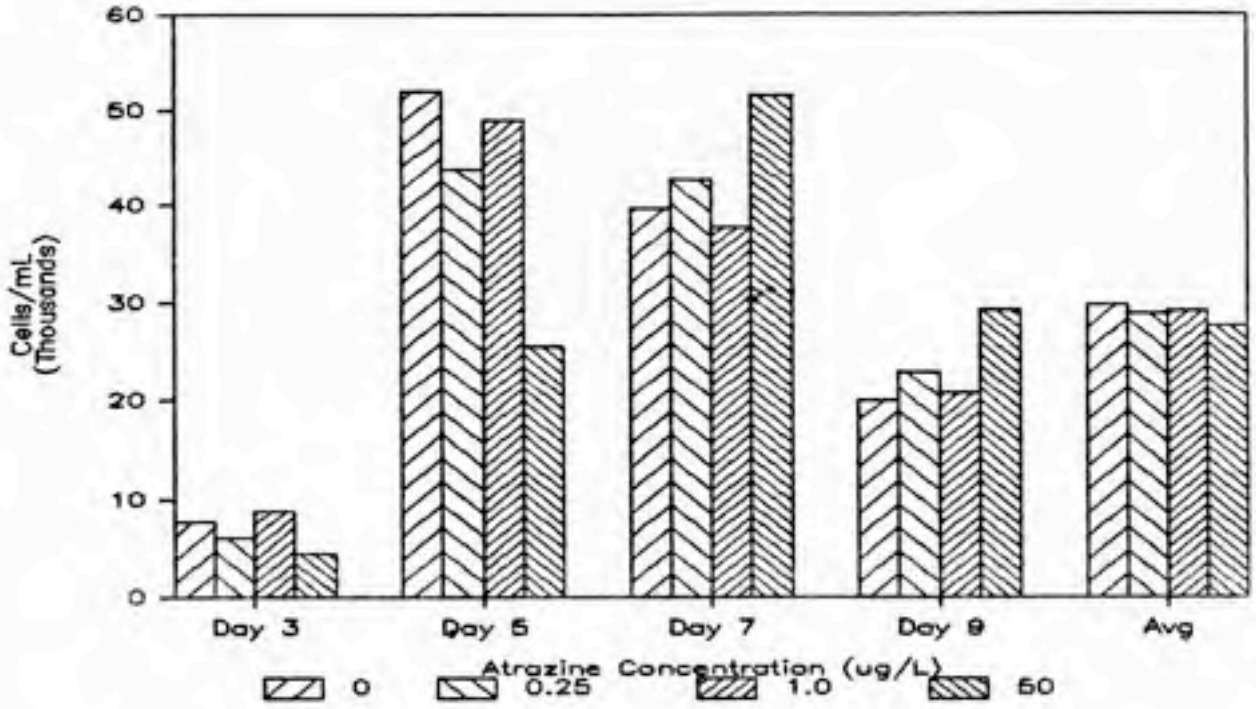


Fig 27. Cell counts for Ankistrodesmus spp. from BI, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

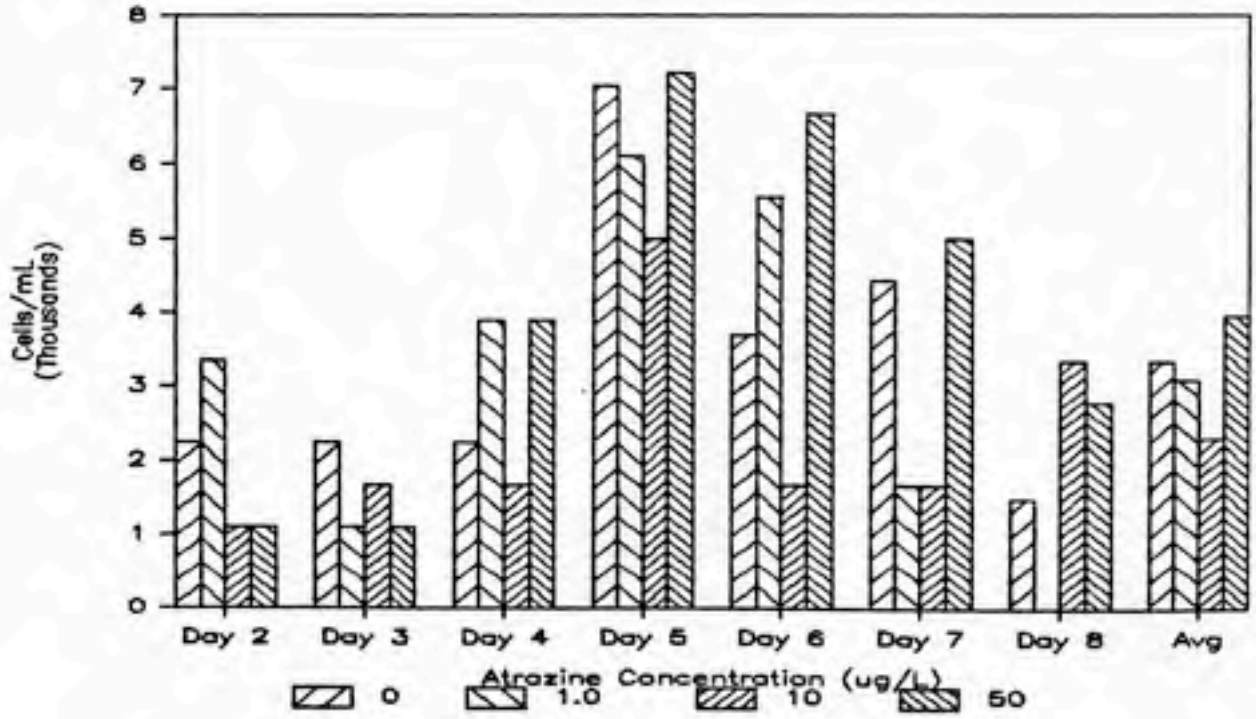


Fig 28. Cell counts for Ankistrodesmus spp. from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

10 ug/L atrazine approached the level of growth observed at other treatments only on Day 5 and were generally much lower. Although these observations appear graphically, statistical models fail to detect any dose-related differences.

Ankistrodesmus falcatus var. spirilliformis

(BII, Figure 29).

As discussed previously, this species was present in both experiments but only distinguished from other members of the genus during the second bioassay. Cells at 1 ug/L atrazine generally outgrew the control throughout the experiment. This treatment produced the maximum biomass observed for the species on Day 5. Maximum biomass at 10 ug/L occurred on Day 6 followed by the control and 50 ug/L on Day 7. Inhibition at 50 ug/L atrazine was somewhat severe, never allowing the population to establish the typical logarithmic phase of growth. On Day 5 this dose produced a biomass statistically lower than 1 and 10 ug/L atrazine. The apparent inhibition at 50 ug/L was not statistically significant. These results suggest that atrazine produces low-dose stimulation and high-dose inhibition in this species, but the results are insufficient for this conclusion.

It should be noted that if counts for Ankistrodesmus spp. and A. falcatus var. spirilliformis are combined from BII, they are similar to counts for Ankistrodesmus spp. from BI.

Chlamydomonas

(BI and BII)

This flagellated genus of the Chlorophyta is regularly present in low numbers in Jordan Lake and was present in water used to seed both bioassay experiments. Interesting results for this genus from BI led to a more focused effort on individual species of this genus during BII and hence the larger number of species reported. Two rather distinct species were quantified in both experiments

Chlamydomonas (Total Count)

(BII, Figure 30).

Eight Chlamydomonas species were observed during BII. Six species were quantified individually, treated statistically, and are discussed below. In addition, all observations were combined and are the subject of the immediate discussion.

Statistically significant dose-related differences observed for this group occurred on Day 2 when the control had significantly higher biomass than 1 and 50 ug/L atrazine. Maximum biomass at 10 ug/L atrazine occurred on Day 3, followed by all other populations on Day 4. The largest biomass observed during the experiment was present on Day 4 in the 50 ug/L flasks. Continued growth at this level was most likely prevented by the logarithmic growth of the dominant Oscillatoria limnetica following Day 4.

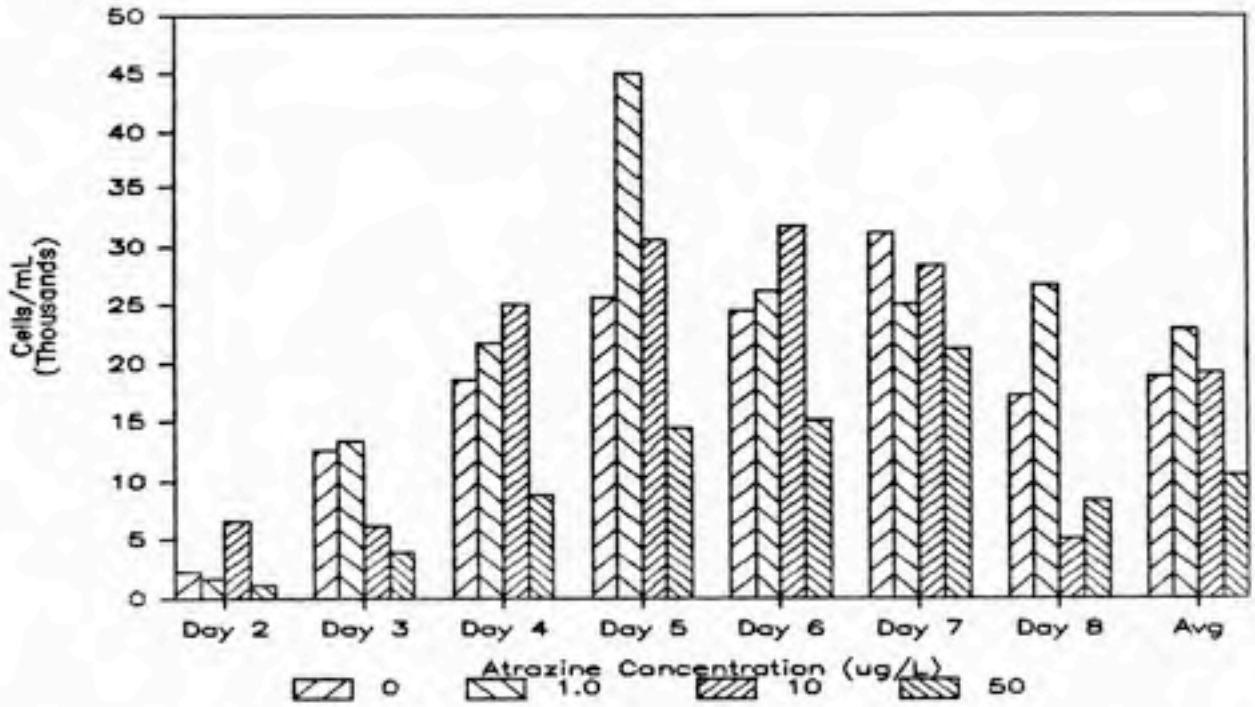


Fig 29. Cell counts for Ankistrodesmus falcatus var. spirilliformis from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

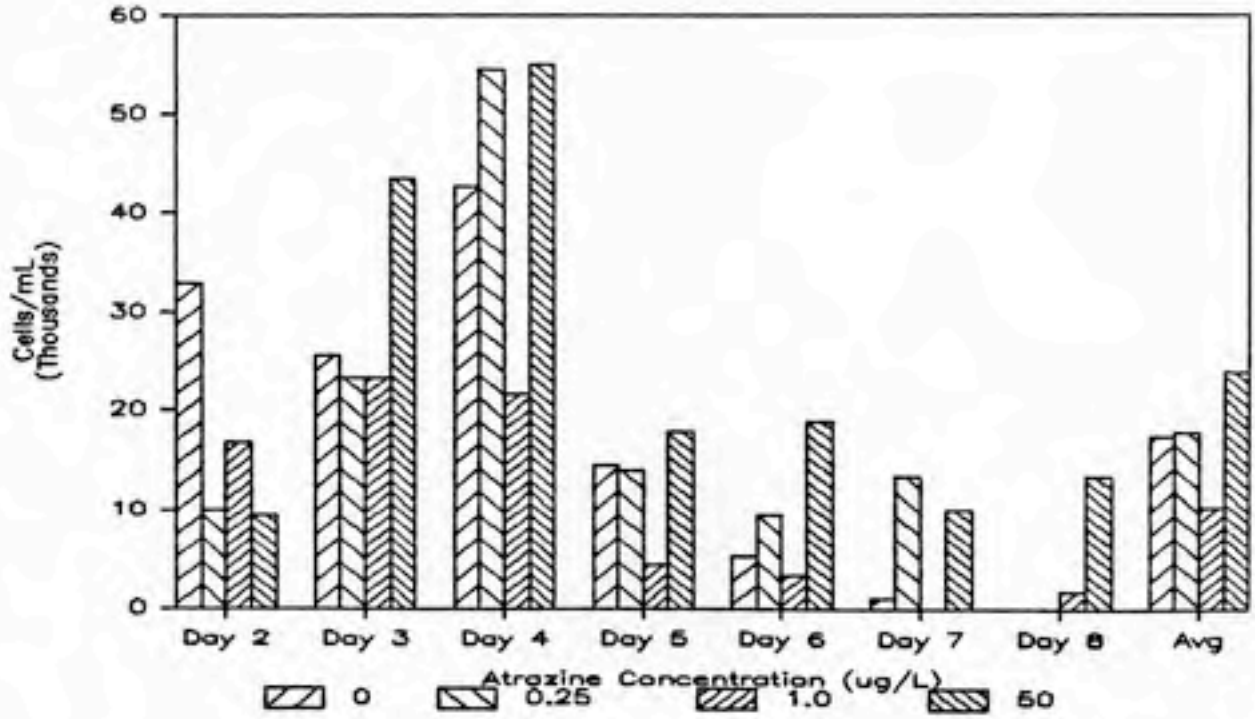


Fig 30. Combined cell counts for all Chlamydomonas spp. present in BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

Growth during the early stages of the experiment was indicative of resistance to the effects of atrazine. The "growth potential" for this group illustrates this point, being greatest at 50 ug/L atrazine. The depressed "growth potential" at 10 ug/L atrazine has been noted for other species but is perhaps, most pronounced for this genus.

Chlamydomonas debaryana

(BI and BII, Figures 31A and 31B).

This species was quantified and treated statistically in both experiments. However, it was absent after Day 5 of BII. This species yielded the largest biomass at 50 ug/L atrazine on Day 5 and 3, of bioassays I and II, respectively. No other treatment approached this biomass in either experiment. Cell counts for atrazine at 50 ug/L proved to be statistically larger than the control and 1 ug/L on Day 5, and 0.25 ug/L on Day 7 in BI. There were strong statistical differences between the greater growth rates for cells in the 50 ug/L atrazine flasks and all other treatments during the logarithmic phase of growth (Day 3-5) of BI. The resulting cell counts on Day 5 indicate that a statistically significant dose/response stimulation took place during this phase.

Statistical dose-related differences were absent for this species in the second experiment. However, examination of the early pulse at 50 ug/L atrazine, and the "growth potential" illustrates that the same general trends were

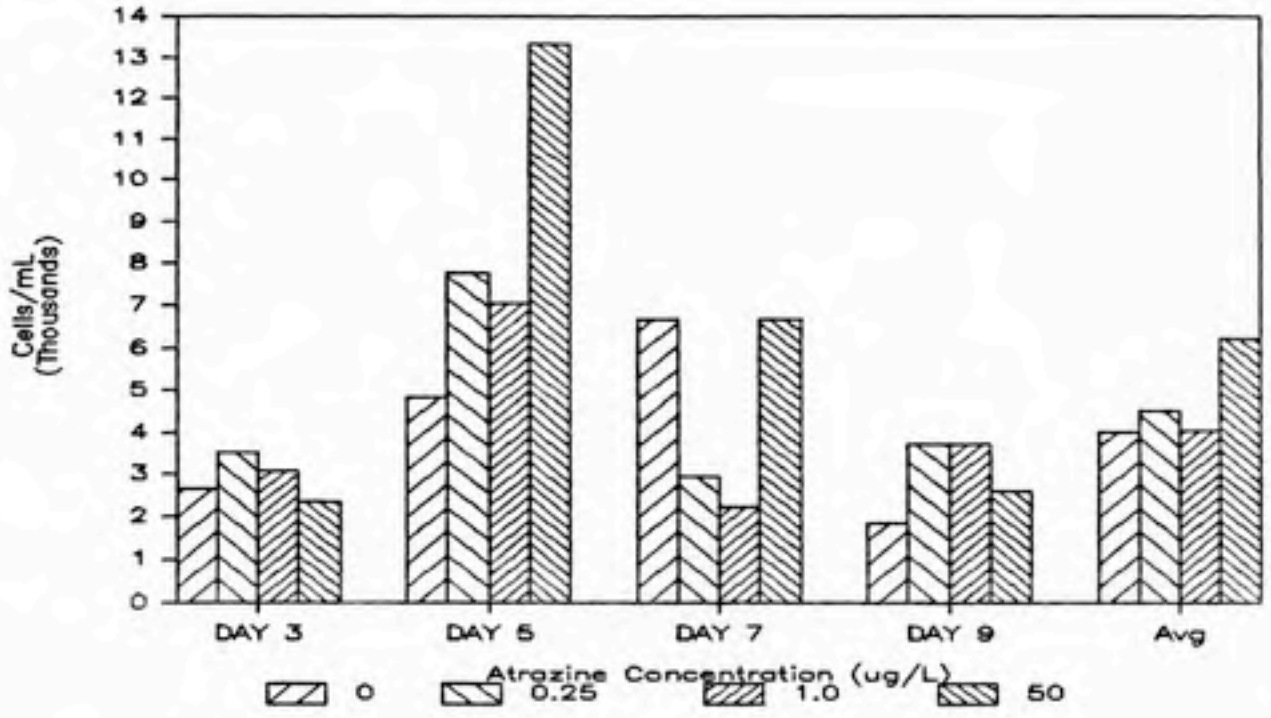


Fig 31A. Cell counts for Chlamydomonas debaryana from BI, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

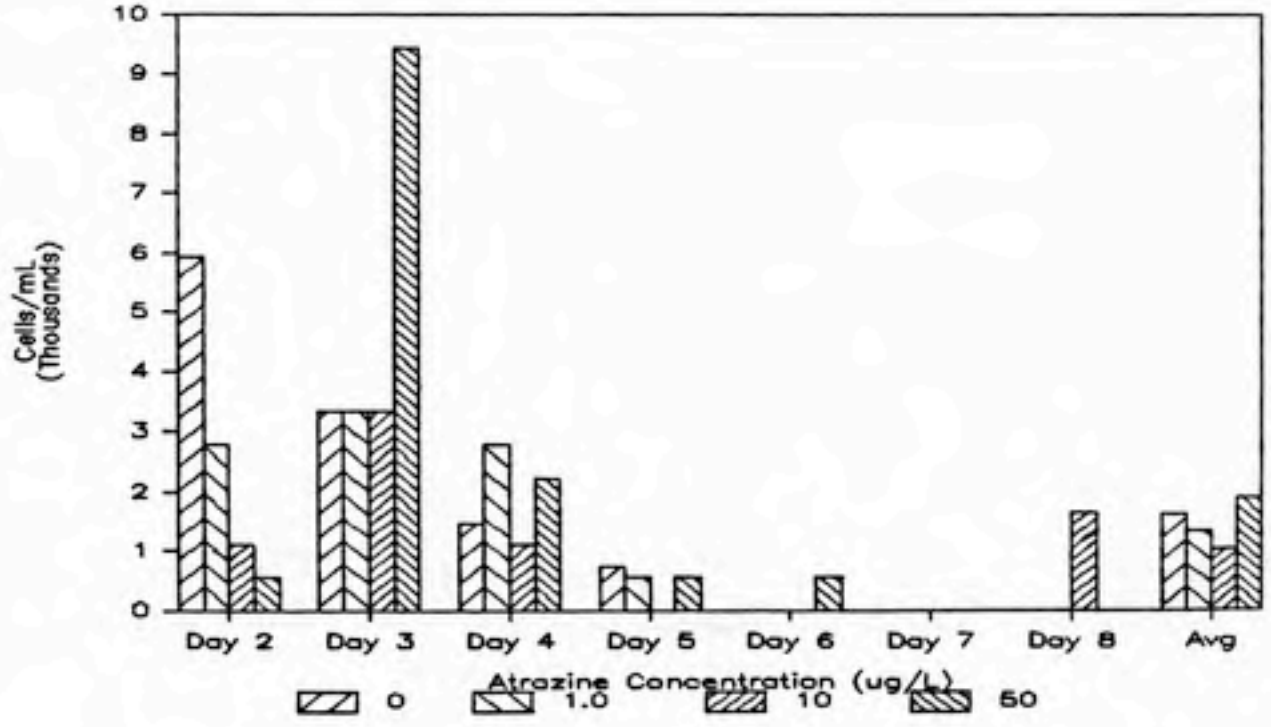


Fig 31B. Cell counts for Chlamydomonas debaryana from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

operating. It may be concluded that this species is relatively resistant to the effects of atrazine.

Chlamydomonas sp. "L"

(BI and BII, Figures 32A and 32B).

This yet-to-be described species has a distinctive elongated cell shape (Campbell 1985). It was present and statistically analyzed in both experiments.

Maximum biomass in the first experiment was achieved at the 50 ug/L dose on Day 5 and at the 1 ug/L treatment on Day 4 of BII. Only the 50 ug/L dose provided significant cell counts after Day 4 of BII, whereas cells were present in all treatments throughout BI. The lower biomass observed at 1 ug/L atrazine on Day 7 of BI was statistically different from all other treatments, while only 50 ug/L atrazine showed growth on Days 6 and 8 of BII. Although both experiments generally show a positive association between atrazine and cell count, this effect was only statistically significant on Days 6 and 8 of BII.

The general trend for this species in both bioassays is illustrated by the "growth potential". With the exception of the intermediate dosage decline, there was a regular increase in cell count with atrazine dose. This is taken as evidence for atrazine resistance and, most likely, a dose-related stimulation of this species.

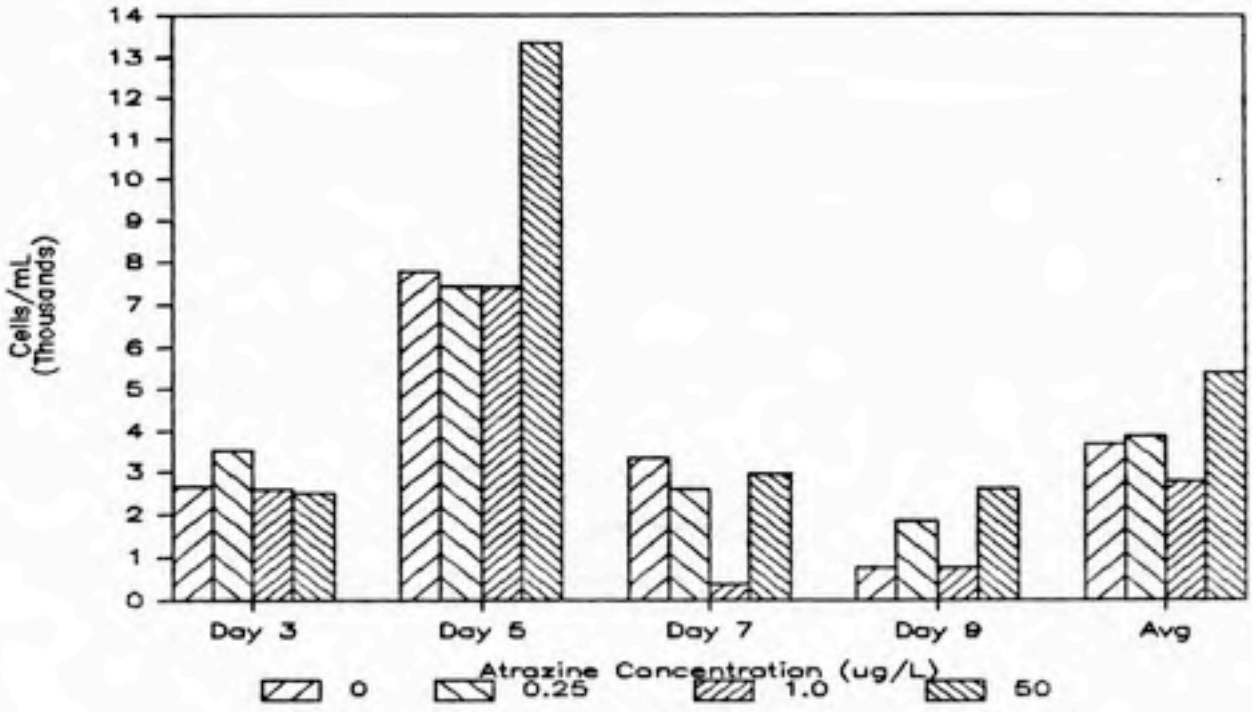


Fig 32A. Cell counts for Chlamydomonas sp."L" from BI, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

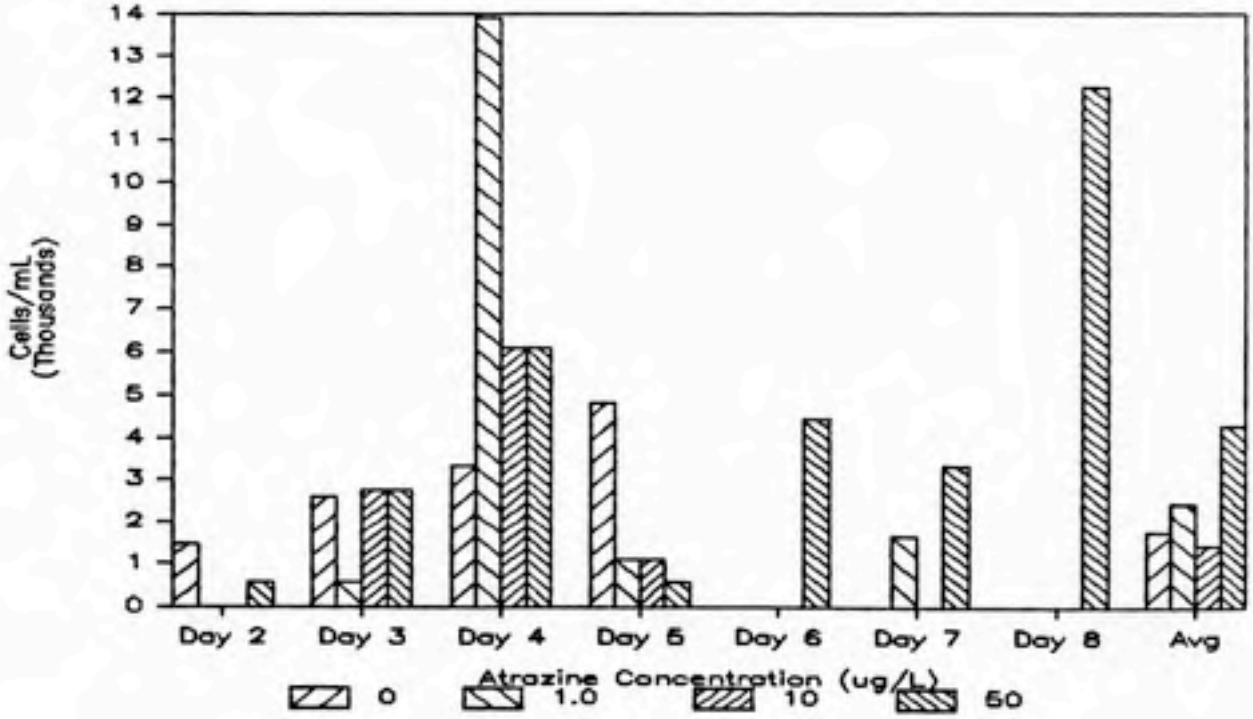


Fig 32B. Cell counts for Chlamydomonas sp."L" from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

Chlamydomonas altera

(BII, Figure 33).

Chlamydomonas altera was the dominant species of this genus. Maximum biomass for the experiment was achieved by cells in 50 ug/L atrazine on Day 4. The control and 1 ug/L doses also reached peak biomass on Day 4 while cells in the 10 ug/L dose did so one day earlier.

Growth in all flasks declined after Day 4 and cells were only detected consistently in the 50 ug/L flasks. This led to the large "growth potential" at 50 ug/L atrazine which was statistically greater than that observed at 10 ug/L. Cells in 10 ug/L atrazine declined after Day 3 and were either absent or at low levels through the end of the bioassay. Except for the poor performance at 10 ug/L, C. altera can be characterized as insensitive to the effects of atrazine and possibly stimulated at the highest atrazine dose.

Chlamydomonas mucicola

(BII, Figure 34).

Chlamydomonas mucicola is somewhat smaller than C. altera (3-4 x 6-7 um) but similar in shape. The growth curve for this species does not show as sharp a decline following peak biomass on Day 4 which has characterized other members of this genus. C. mucicola is the only Chlamydomonas species for which 50 ug/L atrazine did not produce the largest biomass of all treatments. The control and 1 ug/L treatments contained considerably larger biomass than the higher-dosed

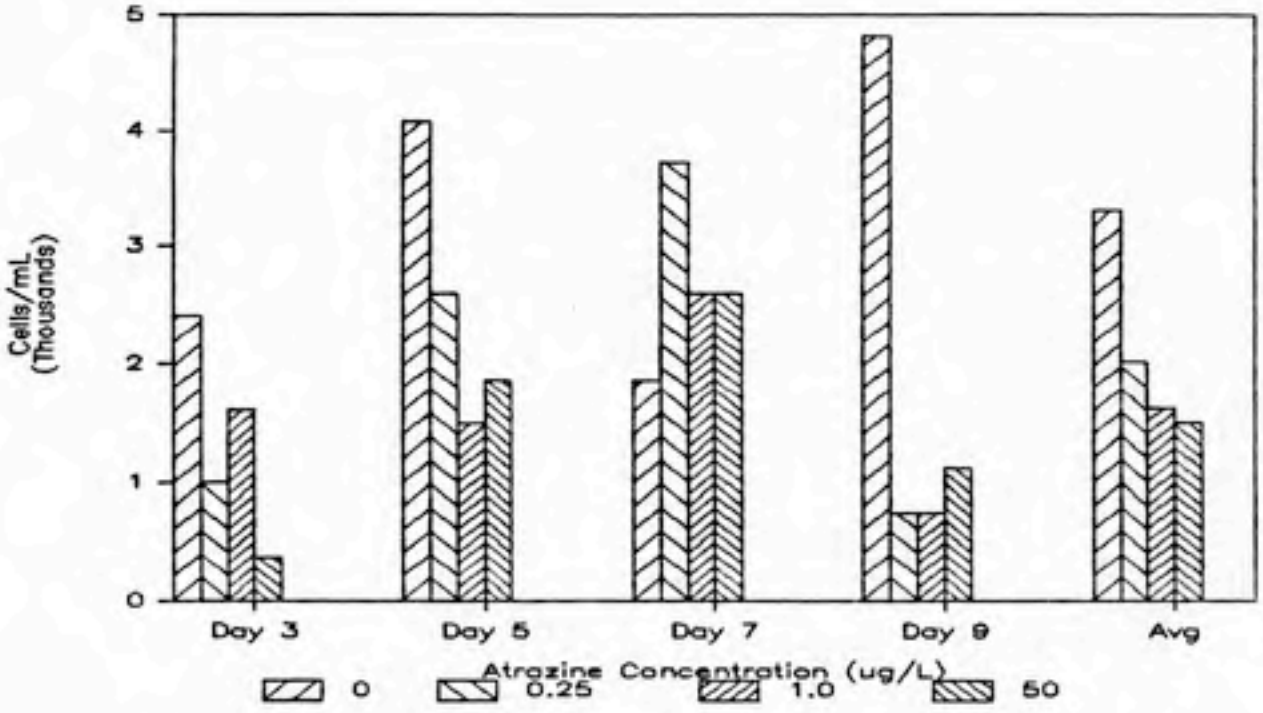


Fig 33. Cell counts for Chlamydomonas altera from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

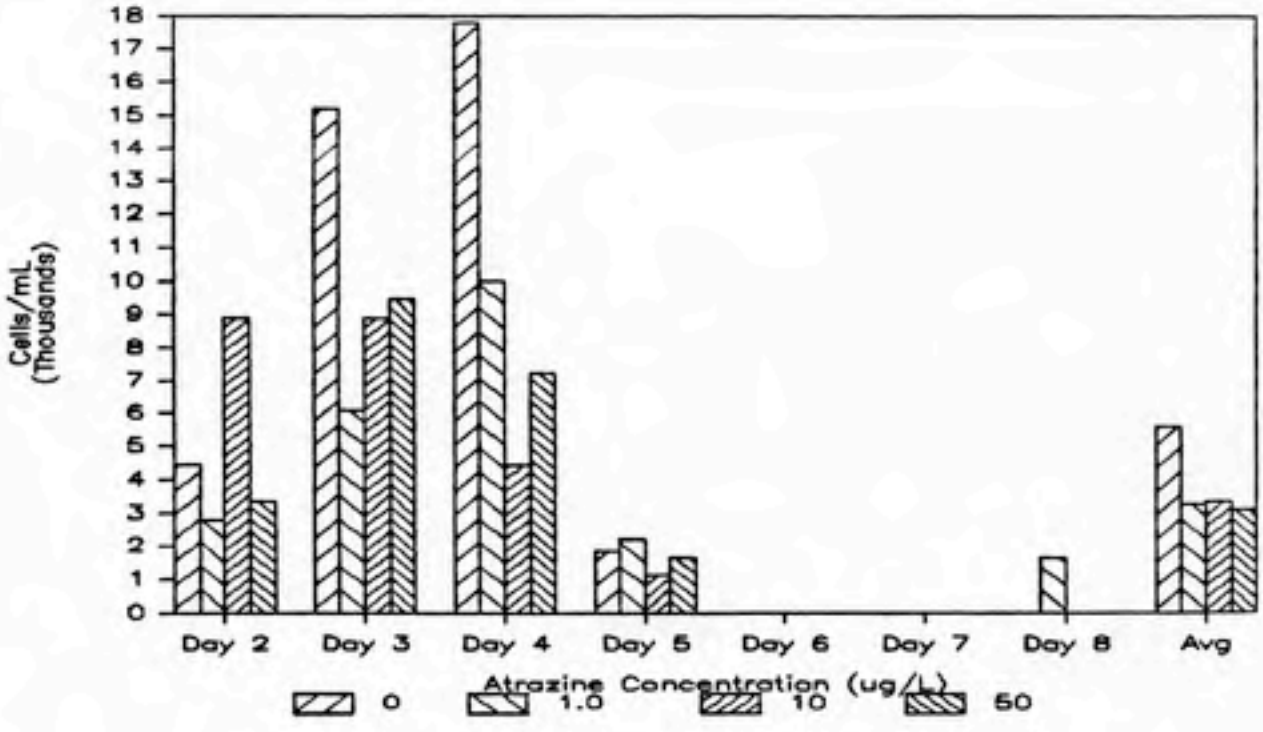


Fig 34. Cell counts for Chlamydomonas mucicola from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

populations on Day 4, from which statistically different cell counts were obtained for 1 and 10 ug/L atrazine. The growth rate observed at 1 ug/L during Day 2-4 was statistically higher than all treatments. It would appear that low-dose stimulation took place in the early phase of the experiment and tapered off rather rapidly, so that the "growth potential" shows a slight decrease at 1 ug/L atrazine. Counts for populations dosed with 50 ug/L atrazine decreased more gradually after Day 4 than other treatments. This produced a "growth potential" comparable to the control. At 10 ug/L atrazine, growth was inhibited throughout the experiment compared to other treatment levels, and cells were generally absent in this treatment after Day 4.

Chlamydomonas mucicola did not show the dose-related stimulation illustrated by other species of this genus. Except for the 10 ug/L decrease in "growth potential", this species was generally insensitive to the effects of atrazine.

Chlamydomonas globosa

(BII, Figure 35).

Counts for this almost spherical species were low relative to other members of this genus. No cells were detected in any flask after Day 5. Atrazine at 50 ug/L produced the maximum biomass observed for this species on Day 3 followed by peaks for the control and 1 ug/L treatments on Day 4. Cells in the 10 ug/L doses were absent after Day 2.

The only results shown to be statistically significant arise from the absence of growth in the 10 ug/L flasks. However, the lack of growth at this dose is noted as being characteristic of the genus. Additional conclusions do not appear warranted in view of the low and variable counts.

Scenedesmus spp.

(BII, Figure 36).

The genus Scenedesmus was well represented in both bioassays. At least eight species of this genus were recognized during these experiments but were only quantified during the second bioassay. Due to low individual species counts, it was decided that grouping these species would be the most efficient method to pursue. Statistical analysis of the cell counts was only performed for the results from BII.

Cell counts from the control flasks increased regularly to a peak biomass on Day 7. The 1 ug/L atrazine dose produced a population that followed a somewhat regular increasing growth curve. This produced the largest biomass observed for the species on Day 6. The growth curve for cells dosed with 10 ug/L atrazine was relatively flat, but reached a peak on Day 4. Cells treated with 50 ug/L atrazine were noticeably inhibited relative to other treatments and reached a peak biomass on Day 7.

Statistical differences were found between all treatments and 50 ug/L atrazine on Day 4 (no cells found at this dose) and on Day 8 when there was a negative association

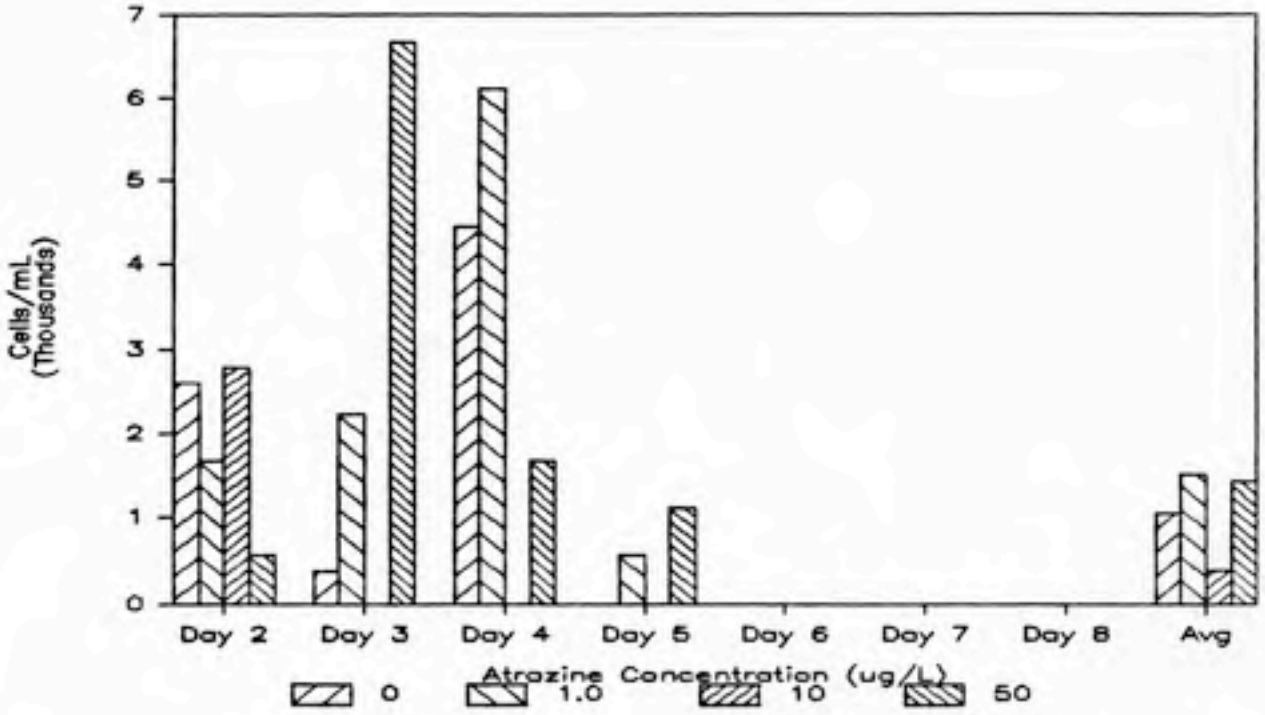


Fig 35. Cell counts for Chlamydomonas globosa from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

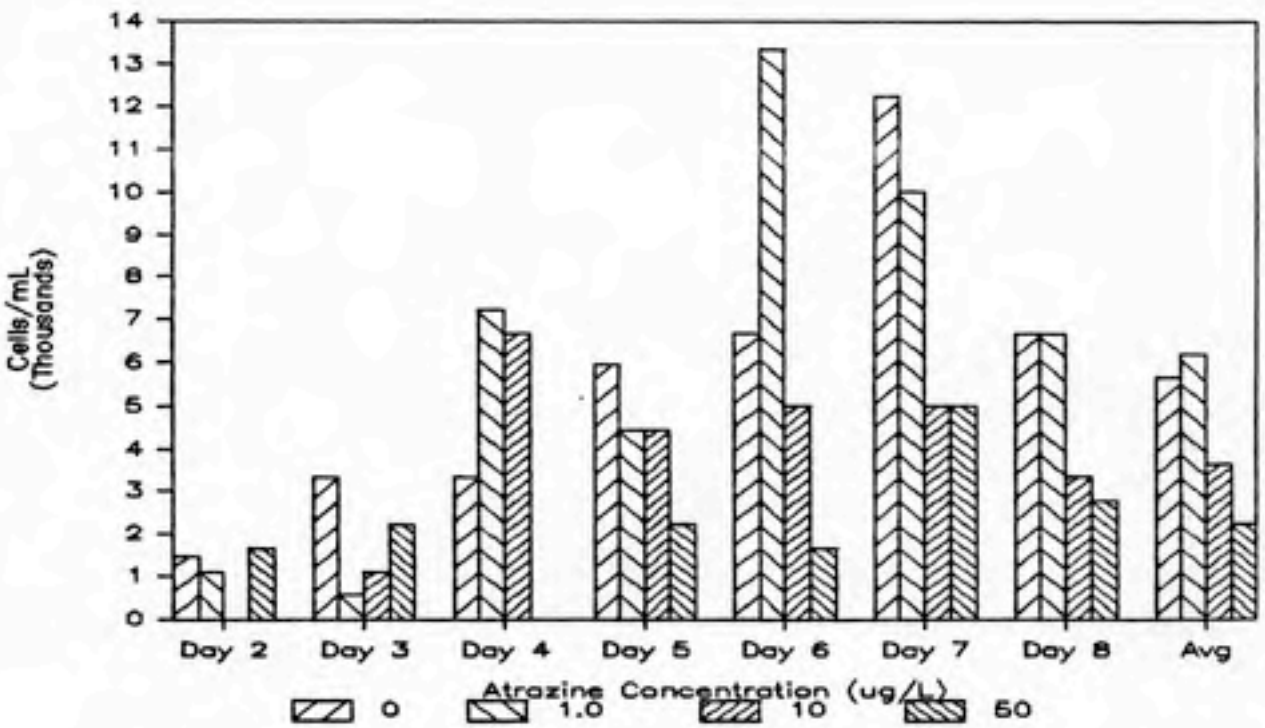


Fig 36. Cell counts for Scenedesmus spp. from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

between cell count and atrazine dosage level. This relationship was negative throughout the bioassay. Examination of the "growth potential" for this genus indicates a slight low-dose stimulation at 1 ug/L atrazine and severe inhibition at higher doses. Of interest is the late peak biomass observed for the control and 1 ug/L treatments. It would appear that after heavy competition during the early phases of the experiment from the more dominant species, notably Oscillatoria limnetica, this genus was able to recover at the control and 1 ug/L treatments and utilize nutrients unavailable to it earlier. However, the effect of atrazine was too great for this renewed growth to take place at the higher doses. Therefore, this genus may be characterized as being sensitive to doses of atrazine higher than 10 ug/L. Similar results have been presented by DeNoylles, Kettle, and Sinn (1982).

Kirchneriella lunaris

(BII, Figure 37).

Kirchneriella lunaris was present only in low numbers in the seed sample used for bioassay II. Cells of this species in the control, 10, and 50 ug/L treatments showed a regular increase to peak biomass on Day 5 followed by gradual decline. All doses of atrazine produced larger maximum biomass than the control. Atrazine at 1 ug/L produced a cycle of rising and declining biomass with peaks on Days 4, 6, and 8. On Day 6, the biomass produced at this dose was

the maximum observed for this species. In view of the regular increase and decline observed at other treatments, the pattern of rise and decline at 1 ug/L appears to have no clear explanation.

Statistically different cell counts occurred on Day 7, when the control cell count was larger than that at 10 ug/L atrazine, and on Day 8 when the control was statistically lower than that at 1 ug/L atrazine. The "growth potential" illustrates a dose-related stimulation by atrazine for this species as well as exceptional biomass at 1 ug/L. Although this effect is not statistically significant in this investigation, similar results have been presented by DeNoyelles, Kettle, and Sinn (1982).

Merotrichia capilata

(BII, Figure 38).

This species of the Chloromonadophyta was not detected until Day 4 and only sporadically, in low numbers thereafter. Cells were not detected in control or 1 ug/L treatments after Day 6. Only 50 ug/L atrazine produced appreciable biomass after this time.

No statistically significant differences could be detected between any treatments. There was, however, a positive association between atrazine dosage level and cell count. This fact is supported by the general increase in "growth potential" with dose. It is difficult to make conclusive statements about the effect of atrazine on this

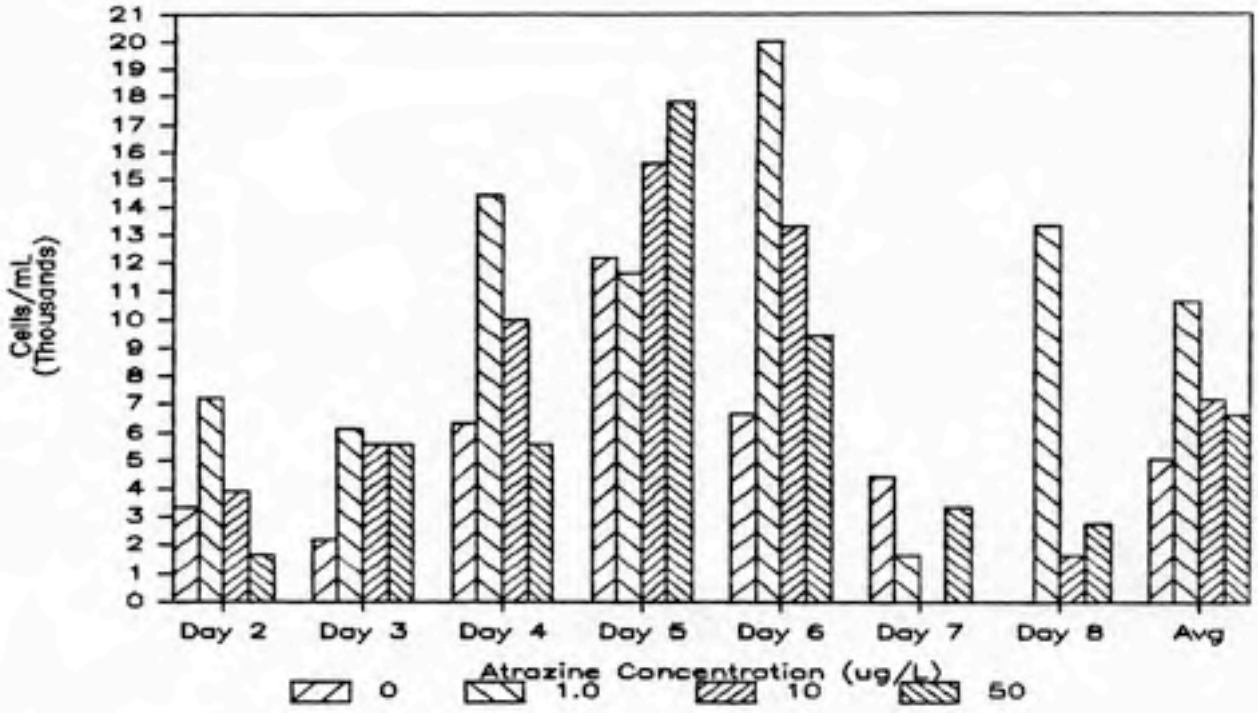


Fig 37. Cell counts for Kirchneriella lunaris from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

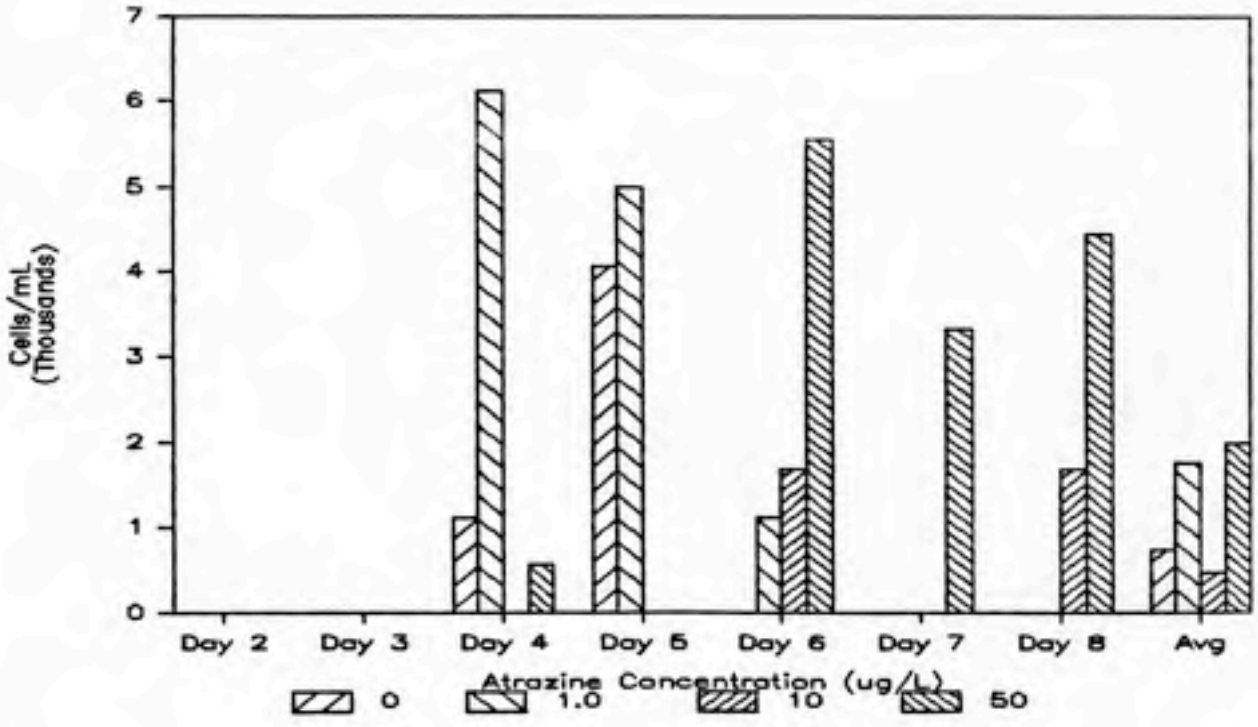


Fig 38. Cell counts for Merotrichia capilata from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

species due its sporadic performance which probably hindered statistical modeling of the resulting cell counts.

Colorless Flagellates

(BII, Figure 39).

Cells of this group of colorless algae were noted in BI but only quantified and treated statistically from BII. Maximum biomass for the experiment was observed in control flasks on Day 4. This was the only treatment which illustrated a regular temporal increase and decrease. Treatments 1 and 50 ug/L peaked on Day 3 whereas cells treated with 10 ug/L atrazine did not reach maximum cell count until Day 6.

The only statistically significant result occurred on Day 4 when the control biomass was significantly larger than that at 1 or 50 ug/L atrazine. The "growth potential" for this group indicates a lack of effect by atrazine although the slight increase at 10 ug/L is of interest in view of the numerous species showing a decrease at this dose. Colorless species have long been recognized as natural members of the phytoplankton (Smith 1950). However, atrazine has been reported to cause "bleaching", or loss of chlorophyll in species of algae grown in its presence (Ashton and Crafts 1981). It is conceivable that species which experienced a decline at 10 ug/L atrazine (notably Chlamydomonas) had been "bleached" and therefore identified as colorless flagellates.

Unidentified Phytoplankton

(BII, Figure 40).

This group of species was made up of phytoplankton that were either present in such low numbers that time did not allow their quantification, or not recognized. It was noted during the experiment that the majority of this group were members of the Chlorophyta.

The control, 1, and 10 ug/L treatments all illustrated regular increases in biomass through Day 7 when maximum biomass was achieved except for a decline on Day 5. The 1 ug/L atrazine dose contained the largest biomass observed during the bioassay on Day 7. Cells in 50 ug/L atrazine produced a flat growth curve with maximum biomass occurring on Day 7. The control and 1 ug/L atrazine produced cell biomass statistically larger than that of 50 ug/L on this day.

The "growth potential" indicates a slight stimulation at 1 ug/L atrazine and inhibition at 10 and 50 ug/L although this effect is not statistically significant. This result is not unlike that observed for other members of the Chlorophyta, namely Scenedesmus spp. and Ankistrodesmus falcatus var. spirilliformis. The "growth potential" achieved at 50 ug/L atrazine was statistically lower than that of any other treatment. The correlation between atrazine dose and cell count remained negative throughout the

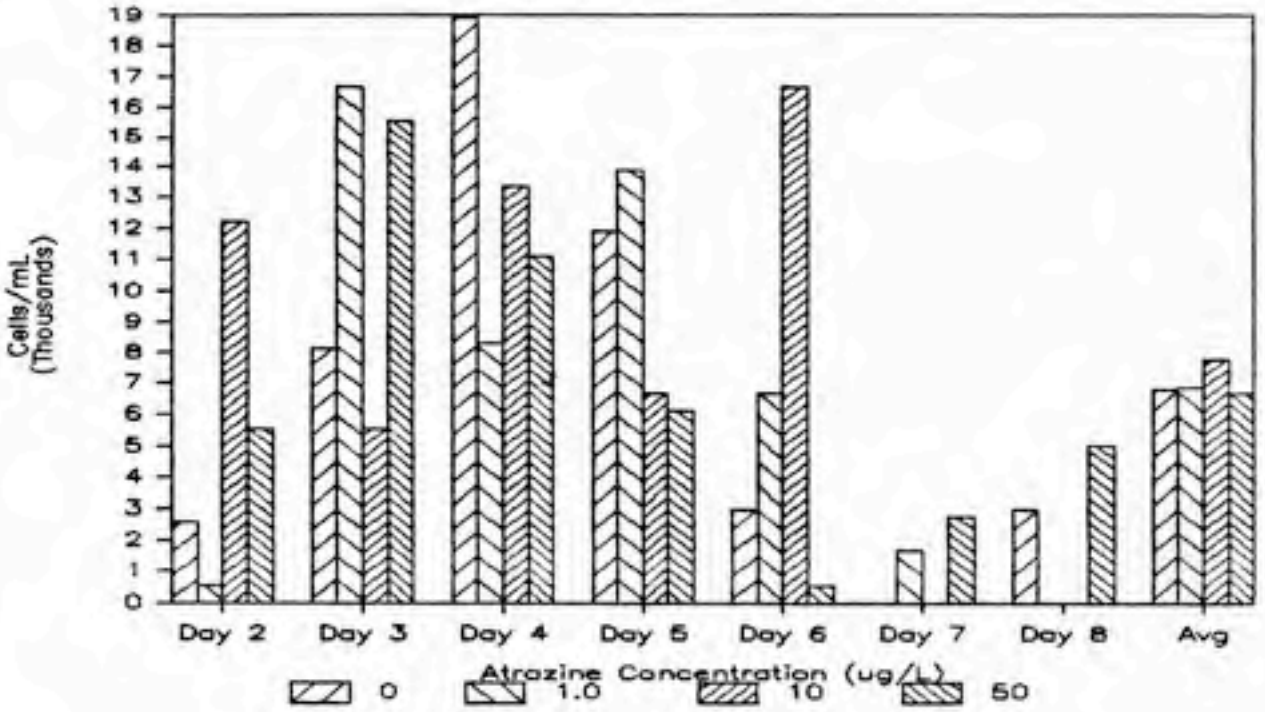


Fig 39. Combined cell counts for Colorless Flagellates from BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

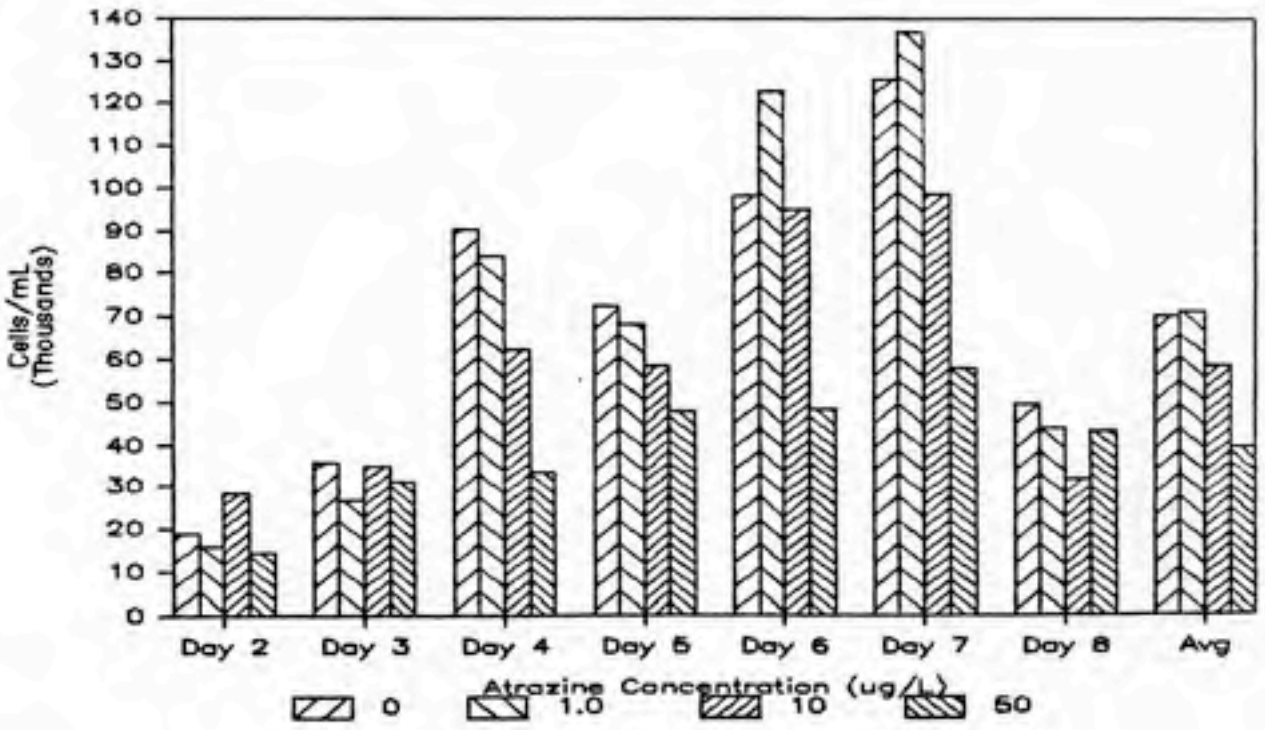


Fig 40. Combined cell counts for all unidentified species present in BII, illustrating the effects of atrazine on cell count for the days and at the doses indicated.

experiment and was found to be statistically significant on Days 4, 6, and 7 as well as for the "growth potential".

It seems clear that the effects of atrazine on this group, composed mainly of members of the Chlorophyta is one of increasing inhibition at doses higher than 1 ug/L.

Phylogenetic Trends Associated With The Effects Of Atrazine

Several observations have been made concerning the species specific level of atrazine effects that may be the result of interspecific competition or inhibition. The individual species results presented above are summarized by the type of atrazine effect observed (inhibition, stimulation ect.) in Table 4.

The effect of atrazine on the procaryotic Cyanophyta, was characterized by low-dose stimulation and high-dose inhibition. The Cryptophyta illustrated the extremes of atrazine effects: either being unaffected by atrazine or inhibited at all doses. The single member of the Chrysophyta appears to be unaffected by atrazine. Members of the Chlorophyta illustrated a wide range of responses to atrazine, possibly due to the diversity within this division. Merotrichia capilata, placed in the Chloromonadophyta, showed no definitive response to atrazine.

Table 4. Summary of species specific responses of phytoplankton to the effects of atrazine - BI and BII.

Species	Division	Expt. #	No effect	Inhib at low doses	Inhib. at high doses	Stim. at low doses	Stim. at high doses	Inhib. at 10 ug/l	Lag in growth	Order
Total Count	--	1			*	*				--
"	--	2			*	*				--
O. limnetica	Cyanophyta	1			*	*				Oscillatoriales
"	"	2			*	*				"
M. Tenuissima	"	2			*	*			*	Chroococcales
G. punctata	"	2			*	*				"
P. firma	"	2				*?				"
N. discoidea	Cryptophyta	1	*?	*						Cryptomonadales
"	"	2		*						"
Cryptomonas spp.	"	2								"
Cyclotella spp.	Chrysophyta	2	*				*?	*	*?	Centrales
D. ehrenbergianum	Chlorophyta	2	*		*	*?		*	*	"
Chlorella spp	"	2			*				*	Chlorococcales
Andistrodesmus spp.	"	1		*					*	"
"	"	2	*				*	*		"
A. spirilliformis	"	2			*				*	"
Total Chlamydomonas	"	2					*	*		Volvocales
C. debaryana	"	1					*			"
"	"	2						*		"
C. sp. "L"	"	1					*			"
"	"	2					*	*		"
C. altera	"	2					*?	*		"
C. mucicola	"	2					*	*	*?	"
C. globosa	"	2					*?	*	*?	"
Scenedesmus spp.	"	2			*	*?	*?			Chlorococcales
K. lunaris	"	2				*?	*?			"
M. capilata	Chloromonadophyta	2						*	*	Chloromonadales
Colorless spp.	--	2								--
Unidentified spp.	--	2		*	*					--

Interspecific Relationships And The Effects Of Atrazine

The Chlamydomonas species all illustrated stimulation at the highest atrazine dose. Sexual reproduction involving chloroplast gene recombination has been observed for C. reinhardtii (Gillham 1978). This species has been shown to undergo a genetic mutation in the presence of atrazine which confers resistance to the effects of the herbicide (Erickson et al. 1984). In view of these facts, as well as the ubiquitous distribution of atrazine in aquatic environments, genetic resistance to the effects of atrazine seems the most likely explanation for the ability of this genus to grow under high doses in the present study.

Many species experienced good growth only during the first four Days of BII. This effect was particularly pronounced for the Nephroselmis discoidea and Chlamydomonas species (Figures 22A, 22B, and 30-35). Poor growth during the middle and latter stages of BII may have been a result of competition with the dominant species for available nutrients. The stimulated growth of Chlamydomonas species at 50 ug/L, may have been due to a combination of genetic resistance to atrazine and the lack of interspecific competition at this treatment.

The rather curious decline in "growth potential" illustrated by many species during BII at 10 ug/L atrazine was most likely another indirect result of the herbicide. Examination of the cell counts by dose for O. limnetica during the period (Day 3-7) (Figure 18B), reveals that cells

of this species at 10 ug/L atrazine generally outgrew all other doses, which possibly resulted in depressed growth (resulting from nutrient competition and/or toxin production) at 10 ug/L for other species.

The success of the Cyanophyta species present in both experiments at all but the highest doses of atrazine may have been due to a combination of competitive ability and tolerance to the effects of lower doses. The extreme inhibition at 50 ug/L and lack of sexual reproduction would down-play the role of genetic resistance to atrazine. An interesting possibility might be related to the photosynthetic differences between the procaryotic Cyanophyta and the eucaryotic members of the phytoplankton. However, this question is beyond the scope of this research.

Another, more easily envisioned possibility involves the physio-chemical adsorption of atrazine to cell surfaces. This would take place at all doses, reducing the solution concentration of atrazine but at some dose between 10 and 50 ug/L, an equilibrium is reached such that an inhibiting concentration remains in solution.

Several species illustrated a growth lag at the higher atrazine doses, particularly the Chlorococcales (Table 4), but go on to produce maximum biomass comparable to controls. It is suggested that cellular uptake of atrazine, distinguished from, but in addition to physio-chemical adsorption, reduced the available atrazine concentration in solution. Atrazine that was taken into the cell and bound to

a protein of the electron transport chain would not be available even after those inhibited cells had died. This would allow later good growth at high atrazine doses. If this was indeed the case, the decrease in solution atrazine may also help explain the poor growth of atrazine-resistant Chlamydomonas species after Day 4. That is, if genetically conferred atrazine resistance requires atrazine for electron transport, and hence growth, a decrease in atrazine concentration should be followed by a decrease in growth.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

I. Atrazine was present at detectable levels at all sampling stations throughout the study period. Atrazine residues in Segment 1 were roughly three times those found in the New Hope segments but considerably lower than levels found to produce severe inhibition in the phytoplankton populations tested *in vitro*. Lowest levels were found prior to agricultural field application. Highest concentrations were detected following field application, which may have been due to storage of two relatively small flow events. The rapid decline of residues in Segment 1 by early June was most likely associated with outflow from the lake, organic and inorganic particulate settling, deeper mixing depths and biological degradation.

II. Atrazine, methyl atraton, tribubutylphosphate, and tris(chloropropyl)phosphate (previously identified in the Haw River) were confirmed by GC/MS analysis to be present in Segment 1 of Jordan Lake.

III. Biological accumulation of atrazine by *Microcystis aeruginosa* may have taken place in Segment 1 during late May through June of 1985. It is likely that tolerance of the effects of atrazine contributed to the growth success of this species.

IV. Phytoplankton populations tested *in vitro* illustrated species specific inhibitions, stimulations, or no dose-related responses to the effects of atrazine.

A. Combined cell counts for all species, indicated severe inhibition at 50 ug/L for the population although lower doses produced maximum biomass larger than controls.

B. Members of the Cyanophyta were unaffected or stimulated by atrazine concentrations less than or equal to 10 ug/L, but severely inhibited at 50 ug/L atrazine.

1. *Oscillatoria limnetica* was severely inhibited by 50 ug/L, but lower doses produced greater maximum biomass and had greater logarithmic growth rates than controls.

2. Moderate growth lag and depression at 50 ug/L atrazine characterized *Merismopedia tenuissima* and *Gleocapsa punctata*.

C. *Nephroselmis discoidea*, a member of the Cryptophyta was inhibited by all concentrations of atrazine.

D. The Chrysophyta, represented by *Cyclotella* sp., did not appear to be affected by atrazine.

E. The Chlorophyta illustrated several different types of responses to atrazine. Many members illustrated growth depression at 10 ug/L.

1. Dictyosphaerium ehrenbergianum did not grow well at 10 ug/L and all atrazine doses produced growth lags compared to controls.
2. Chlorella spp. were stimulated by 1 ug/L atrazine but inhibited at higher doses.
3. Ankistrodesmus falcatus var. spirilliformis was affected in the same manner as Chlorella spp. but to a lesser extent.
4. Species of Chlamydomonas, including C. debaryana, C. sp. "L", C. altera, C. mucicola, and C. globosa were all inhibited most strongly at 10 ug/L atrazine, most likely a result of competitive exclusion by Oscillatoria limnetica. In 50 ug/L atrazine, these species grew as well as, or better than in control flasks. The simulation response is interpreted as evidence for genetic resistance to the effects of atrazine.
5. Cell counts for Scenedesmus spp. suggest that a low-dose stimulation and a high-dose inhibition occurred.
6. Kirchneriella lunaris may be stimulated by increasing doses of atrazine.

Recommendations For Lake Management And Future Research

I. Watershed management practices designed to decrease farmland erosion would result in lower atrazine residues in Jordan Lake and crops would likely require lower rates of herbicide application. This would also be true for other farm products subject to loss via runoff.

II. Additional research should be done in the following areas:

A. *In situ* phytoplankton toxicity experiments involving enclosures, will provide conditions for more closely approximating natural growth conditions.

B. *In vitro* toxicity experiments as described in the present study should be conducted in conjunction with experiments using single species of the population to provide better comparisons of direct and indirect phytoplankton responses to atrazine.

C. Segments of Jordan Lake which are designated for future drinking water intake sites and subject to Haw River water incursion should be analyzed for the presence of synthetic organic compounds due to known discharges of these compounds in the Haw River watershed.

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Appendix A.

Results of Statistical Analysis Using Model A.

Results of Model A showing the effect of atrazine on daily and average ("growth potential") cell counts. The analysis of variance (F test for the overall model) is given above the least square mean (T test) result for differences between atrazine dose (ug/l). *=P<0.1000; **=P<0.0500; ***=P<0.0100; ****=P<0.0010.

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Ankistrodesmus	1	3	model	0.6	0.5700		
spp.		5	model	32.1	0.0001		****
			dose 0,50			0.0006	****
			dose .25,50			0.0006	****
			dose 1,50			0.0006	****
		7	model	1.4	0.3227		
		9	model	0.9	0.4771		
	Expt Avg.		model	1.0	0.4360		
	2	2	model	1.3	0.3830		
		3	model	0.8	0.5342		
		4	model	1.1	0.4180		
		5	model	1.2	0.3904		
		6	model	0.6	0.6275		
		7	model	1.0	0.4478		
		8	model	3.5	0.1063		
	Expt. Avg.		model	4.8	0.0616		
			dose 10,50			0.0840	*
Ankisrodesmus	2	2	model	0.5	0.6959		
falcatus var.		3	model	1.6	0.4108		
spirilliformis		4	model	9.3	0.0175		**
			dose 1,50			0.0600	*
			dose 10,50			0.0270	**
		5	model	3.0	0.1371		
		6	model	0.9	0.5076		
		7	model	0.3	0.8125		
		8	model	2.9	0.1384		
	Expt. Avg.		model	4.4	0.0719		
Chlamydomonas	2	2	model	3.2	0.1239		
altera		3	model	0.1	0.9761		
		4	model	1.8	0.2665		
		5	model	0.7	0.5699		
		6	model	1.5	0.3156		
		7	model	1.8	0.2725		
		8	model	1.3	0.3721		
	Expt. Avg.		model	4.9	0.0597		*
			dose 10,50			0.0738	*

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Chlamydomonas debaryana	1	3	model	1.4	0.3017		
		5	model	11.7	0.0027		*
			dose 0,50			0.0024	*
			dose 1,50			0.0414	*
		7	model	4.6	0.0368		*
			dose .25,50			0.0708	*
		9	model	1.2	0.3845		
	Expt. Avg.		model	8.8	0.0066		***
			dose 0,50			0.0126	**
			dose 1,50			0.0138	**
	2	2	model	1.7	0.2756		
		3	model	5.3	0.0513		*
		4	model	1.1	0.4346		
		5	model	0.6	0.6667		
		6	model	1.3	0.3721		
		7	no cells found				
		8	model	1.3	0.3721		
	Expt. Avg.		model	0.8	0.5315		
Chlamydomonas globosa	2	2	model	1.8	0.2727		
		3	model	5.6	0.0468		**
		4	model	201.1	0.0001		****
			dose 0,10			0.0006	****
			dose 1,10			0.0006	****
			dose 50,10			0.0006	****
		5	model	5.0	0.0577		*
		6	no cells found				
		7	no cells found				
		8	no cells found				
	Expt. Avg.		model	3.7	0.0974		*
Chlamydomonas mucicola	2	2	model	3.1	0.1286		
		3	model	0.1	0.9608		
		4	model	6.1	0.0405		**
			dose 1,10			0.0642	*
		5	model	3.3	0.1135		
		6	model	0.4	0.7428		
		7	model	0.9	0.4926		
		8	cells not found				
	Expt. Avg.		model	4.7	0.0635		*
Chlamydomonas spp. (total count)	2	2	model	6.9	0.0319		**
			dose 0,50			0.0696	*
			dose 0,1			0.0858	*
		3	model	0.8	0.5332		
		4	model	4.6	0.0682		*
		5	model	1.1	0.4453		
		6	model	0.7	0.5877		
		7	model	1.8	0.2604		
		8	model	4.6	0.0668		*
	Expt. Avg.		model	3.4	0.1114		

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Chlamydomonas sp. "L"	1	3	model	0.7	0.5970		
		5	model	2.7	0.1175		
		7	model	5.6	0.0224		*
			dose 0,1			0.0534	*
			dose .25,1			0.0654	*
			dose 1,50			0.0600	*
		9	model	0.3	0.8464		
	Expt. Avg.		model	3.5	0.0698		*
	2	2	model	1.2	0.3932		
		3	model	0.5	0.7210		
		4	model	0.7	0.5698		
		5	model	0.6	0.6248		
		6	model	293.0	0.0001		****
			dose 0,50			0.0006	****
			dose 1,50			0.0006	****
			dose 10,50			0.0006	****
		7	model	0.9	0.4921		
		8	model	40.8	0.0006		****
			dose 0,50			0.0012	***
			dose 1,50			0.0018	***
			dose 10,50			0.0018	***
	Expt. Avg.		model	0.3	0.8393		
Chlorella spp.	2	2	model	7.1	0.0302		**
			dose 0,50			0.0660	*
			dose 1,50			0.0732	*
		3	model	2.5	0.1745		
		4	model	5.8	0.0439		**
			dose 1,50			0.0678	*
		5	model	6.6	0.0348		**
			dose 1,50			0.0468	**
		6	model	3.5	0.1062		
		7	model	1.0	0.4647		
		8	model	0.6	0.6723		
	Expt. Avg.		model	8.9	0.0191		**
			dose 1,10			0.0948	*
			dose 1,50			0.0336	**
Colorless Flagellates	2	2	model	2.8	0.1471		
		3	model	2.2	0.2118		
		4	model	12.2	0.0098		**
			dose 0,1			0.0132	*
			dose 0,50			0.0702	*
		5	model	1.9	0.2530		
		6	model	1.2	0.4156		
		7	model	4.8	0.0627		*
		8	model	2.5	0.1717		
	Expt. Avg.		model	0.8	0.5394		

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Cryptomonas	2	2	no cells found				
spp.		3	model	0.05	0.9828		
		4	model	1.8	0.2714		
		5	model	0.4	0.7805		
		6	model	1.3	0.3721		
		7	no cells found				
		8	no cells found				
	Expt.	Avg.	model	1.3	0.3709		
Cyclotella	2	2	model	15.2	0.0060		***
pseudostelligera			dose 0,1			0.0294	**
			dose 0,50			0.0294	**
			dose 1,10			0.0312	**
			dose 10,50			0.0312	**
		3	model	4.6	0.0679		*
		4	model	6.9	0.0315		**
			dose 1,10			0.0894	*
			dose 10,50			0.0552	*
		5	model	0.2	0.9245		
		6	model	2.3	0.1932		
		7	model	1.8	0.2663		
		8	model	1.2	0.4060		
	Expt.	Avg.	model	1.0	0.4498		
Dictyosphaerium	2	2	model	0.04	0.9865		
ehrenberhianum		3	model	0.2	0.8637		
		4	model	0.4	0.7720		
		5	model	5.0	0.0568		*
			dose 0,1			0.0894	*
		6	model	11.3	0.0115		*
			dose 0,50			0.0210	**
			dose 1,50			0.0480	**
		7	model	27.9	0.0015		***
			dose 0,10			0.0036	***
			dose 1,10			0.0042	***
			dose 10,50			0.0036	***
		8	model	0.5	0.7082		
	Expt.	Avg.	model	9.6	0.0161		**
			dose 0,10			0.0192	**
Gleocapsa	2	2	model	15.8	0.0055		***
punctata			dose 0,1			0.0126	**
			dose 0,10			0.0396	**
		3	model	1.0	0.4811		
		4	model	0.6	0.6155		
		5	model	0.2	0.9114		
		6	model	0.7	0.5971		
		7	model	1.0	0.4504		
		8	model	1.5	0.3297		
	Expt.	Avg.	model	1.8	0.2713		

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Kirchneriella lunaris	2	2	model	1.2	0.4074		
		3	model	1.8	0.2622		
		4	model	2.0	0.2281		
		5	model	0.8	0.5509		
		6	model	0.7	0.6073		
		7	model	5.2	0.0548		*
			dose 0,10			0.0990	*
		8	model	6.0	0.0409		**
			dose 0,1			0.0780	*
	Expt. Avg.		model	2.9	0.1394		
Merismopedia tenuissima	2	2	model	0.5	0.6958		
		3	model	0.5	0.6746		
		4	model	0.4	0.7816		
		5	model	0.7	0.5926		
		6	model	3.8	0.0942		*
		7	model	0.2	0.9228		
		8	model	0.6	0.6380		
	Expt. Avg.		model	1.1	0.4246		
Merotrichia capilata	2	2	no cells found				
		3	no cells found				
		4	model	0.5	0.6823		
		5	model	3.8	0.0927		*
		6	model	2.4	0.1862		
		7	model	999.9	0.0001		****
			all doses,50			0.0006	****
		8	model	0.9	0.4917		
	Expt. Avg.		model	0.7	0.5749		
Nephroselmis discoidea	1	3	model	4.4	0.0420		**
			dose 0,50			0.0870	*
			dose 1,50			0.0990	*
		5	model	2.1	0.1827		
		7	model	0.6	0.6643		
		9	model	1.1	0.4157		
	Expt. Avg.		model	1.0	0.4268		
	2	2	model	1.1	0.4296		
		3	model	0.9	0.5077		
		4	model	4.5	0.0690		*
		5	model	0.3	0.8220		
		6	no cells present				
		7	no cells present				
		8	model	1.3	0.3721		
	Expt. Avg.		model	1.0	0.4498		

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Oscillatoria	1	3	model	1.2	0.3721		
limnetica		5	model	9.8	0.0046		***
			dose 0,50			0.0816	*
			dose .25,50			0.0066	***
			dose 1,50			0.0144	**
		7	model	6.0	0.0190		**
			dose 0,50			0.0324	**
			dose .25,50			0.0456	**
		9	model	3.1	0.0884		*
	Expt. Avg.		model	7.2	0.0114		**
			dose 0,50			0.0822	*
			dose .25,50			0.0138	**
			dose 1,50			0.0540	*
	2	2	model	8.8	0.0194		**
			dose 0,50			0.0276	**
			dose 10,50			0.0738	*
		3	model	11.6	0.0110		**
			dose 0,50			0.0348	**
			dose 1,10			0.0882	*
			dose 10,50			0.0480	**
		4	model	15.3	0.0059		**
			dose 0,50			0.0090	***
			dose 1,50			0.0468	**
			dose 10,50			0.0150	**
		5	model	11.9	0.0103		**
			dose 0,50			0.0204	**
			dose 1,50			0.0246	**
			dose 10,50			0.0348	**
		6	model	11.7	0.0106		**
			dose 0,50			0.0264	**
			dose 1,50			0.0030	***
			dose 10,50			0.0234	**
		7	model	29.3	0.0013		***
			dose 0,50			0.0030	***
			dose 1,50			0.0048	***
			dose 10,50			0.0030	***
		8	model	14.2	0.0071		***
			dose 0,50			0.0126	**
			dose 1,50			0.0174	**
	Expt. Avg.		model	88.4	0.0001		****
			dose 0,50			0.0006	****
			dose 1,50			0.0006	****
			dose 10,50			0.0006	****
Polycystis	2	2	model	0.6	0.6493		
firma		3	model	0.6	0.6228		
		4	model	0.3	0.8034		
		5	model	0.7	0.5738		

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Polycystis	2	6	model	276.4	0.0001		****
firma (cont'd)			dose 0,10			0.0006	****
			dose 1,10			0.0006	****
			dose 50,10			0.0006	****
		7	model	1.3	0.3720		
		8	no cells present				
	Expt. Avg.		model	0.6	0.6713		
Scenedesmus	2	2	model	1.7	0.2855		
spp.		3	model	1.2	0.3959		
		4	model	123.0	0.0001		****
			dose 0,50			0.0006	****
			dose 1,50			0.0006	****
			dose 10,50			0.0006	****
		5	model	1.4	0.3557		
		6	model	1.1	0.4209		
		7	model	1.1	0.4442		
		8	model	2.6	0.1672		
	Expt. Avg.		model	2.5	0.1720		
Total Count	1	3	model	1.6	0.2638		
		5	model	12.3	0.0023		***
			dose 0,50			0.0312	**
			dose .25,50			0.0066	***
			dose 1,50			0.0036	***
		7	model	6.6	0.0151		**
			dose 0,50			0.0234	**
			dose .25,50			0.0408	**
		9	model	2.6	0.1240		
	Expt. Avg.		model	8.6	0.0069		***
			dose 0,50			0.0408	**
			dose .25,50			0.0336	**
			dose 1,50			0.0090	**
	2	2	model	15.2	0.0060		***
			dose 0,10			0.0240	**
			dose 0,50			0.0078	***
		3	model	2.8	0.1451		
		4	model	13.1	0.0083		***
			dose 0,50			0.0108	**
			dose 1,50			0.0396	**
			dose 10,50			0.0408	**
		5	model	11.5	0.0111		**
			dose 0,50			0.0192	**
			dose 1,50			0.0270	**
			dose 10,50			0.0516	*

Algal Species	Expt.	Day	Source	F	Pr>F	Pr>T	P
Total Count (cont'd)	2	6	model	13.8	0.0075		***
			dose 0,50			0.0186	**
			dose 1,50			0.0186	**
			dose 10,50			0.0186	**
		7	model	14.2	0.0070		***
			dose 0,50			0.0132	**
			dose 1,50			0.0186	**
			dose 10,50			0.0228	**
		8	model	4.5	0.0705		*
	Expt.	Avg.	model	98.7	0.0001		****
			dose 0,50			0.0006	****
			dose 1,50			0.0006	****
			dose 10,50			0.0006	****
Unidentified spp.	2	2	model	1.6	0.3079		
		3	model	0.8	0.5306		
		4	model	4.7	0.0651		*
		5	model	0.3	0.8507		
		6	model	3.3	0.1149		
		7	model	9.0	0.0187		**
			dose 0,50			0.0384	**
			dose 1,50			0.0354	**
		8	model	0.2	0.9233		
	Expt.	Avg.	model	14.4	0.0068		***
			dose 0,50			0.0114	**
			dose 1,50			0.0156	**
			dose 10,50			0.0876	*

Appendix A.(Cont'd)

Results of Statistical Analysis Using Models B and D.

Results of Model B and Model D showing the association of cell count with atrazine dose. Spearman correlation coefficients are given with associated probabilities (T test.) *=P<0.1000; **=P<0.0500; ***=P<0.0100; ****=P<0.0010.

			Model B.			Diff. Betwn	Model D		
Algal Species	**Expt**	**Day**	**r**	**Pr≥r**	**P**	**Day**	**r**	**Pr≥r**	**P**
Ankistrodesmus spp.	1	3	-0.28	0.3768		3 and 5	-0.24	0.4573	
		5	-0.65	0.0224	**	3 and 7	0.34	0.2714	
		7	0.39	0.2083		3 and 9	0.41	0.1853	
		9	0.46	0.1364					
	Expt. Avg.		-0.38	0.2250					
	2	2	-0.48	0.1934		2 and 3	-0.06	0.8758	
		3	-0.32	0.3921		2 and 4	0.31	0.4178	
		4	0.11	0.7702		2 and 5	0.27	0.4900	
		5	-0.18	0.6511		2 and 6	0.36	0.3400	
		6	0.19	0.6270		2 and 7	0.32	0.3960	
		7	0.03	0.9323		2 and 8	0.39	0.3000	
		8	0.34	0.3736					
	Expt. Avg.		0.08	0.8261					
Ankistrodesmus falcatus var. spirilliformis	2	2	0.12	0.7529		2 and 3	-0.40	0.2814	
		3	-0.66	0.0525	*	2 and 4	-0.40	0.2814	
		4	-0.31	0.4182		2 and 5	-0.40	0.2927	
		5	-0.30	0.4319		2 and 6	-0.22	0.5636	
		6	-0.32	0.4047		2 and 7	-0.13	0.7412	
		7	-0.19	0.6264		2 and 8	-0.30	0.4319	
		8	-0.36	0.3443					
	Expt Avg.		-0.39	0.3021					
Chlamydomonas altera	2	2	-0.51	0.1577		2 and 3	0.50	0.1725	
		3	0.11	0.7749		2 and 4	0.57	0.1115	
		4	0.28	0.4600		2 and 5	0.06	0.8774	
		5	-0.10	0.7962		2 and 6	0.61	0.0813	*
		6	0.48	0.1862		2 and 7	0.28	0.4651	
		7	0.06	0.8663		2 and 8	0.51	0.1577	
		8	0.49	0.1766					
	Expt. Avg.		0.28	0.4599					
Chlamydomonas debaryana	1	3	-0.26	0.4092		3 and 5	0.79	0.0023	***
		5	0.84	0.0006	****	3 and 7	0.06	0.8404	
		7	-0.06	0.8398		3 and 9	0.20	0.5217	
		9	0.04	0.8905					
	Expt. Avg.		0.55	0.0630	*				

Algal Species	Expt	Day	Model B. r	Pr>r	P	Diff. Betwn Day	Model D r	Pr>r	P
Chlamydomonas debarayna (cont'd)	2	2	-0.68	0.0418	**	2 and 3	0.71	0.0321	**
		3	0.56	0.1208		2 and 4	0.45	0.2238	
		4	0.08	0.8267		2 and 5	0.12	0.7615	
		5	-0.27	0.4861		2 and 6	0.68	0.0418	**
		6	0.49	0.1766		2 and 7	0.68	0.0418	*
		7	no cells found			2 and 8	0.65	0.0563	**
		8	0.21	0.5846					
	Expt. Avg.		0.23	0.5500					
Chlamydomonas globosa	2	2	-0.58	0.1032		2 and 3	0.52	0.1478	
		3	0.55	0.1253		2 and 4	-0.07	0.8594	
		4	-0.59	0.0935	*	2 and 5	0.57	0.1089	
		5	0.61	0.0802	*	2 and 6	0.58	0.1032	
		6	no cells found			2 and 7	0.58	0.1032	
		7	no cells found			2 and 8	0.58	0.1032	
		8	no cells found						
	Expt. Avg.		0.08	0.8326					
Chlamydomonas mucicola	2	2	-0.28	0.4709		2 and 3	0.14	0.7233	
		3	-0.18	0.6477		2 and 4	0.27	0.4795	
		4	-0.50	0.1725		2 and 5	0.19	0.6264	
		5	0.06	0.8842		2 and 6	0.19	0.6264	
		6	-0.11	0.7692		2 and 7	0.43	0.2474	
		7	0.32	0.4012		2 and 8	0.28	0.4709	
		8	no cells found						
	Expt. Avg.		-0.32	0.4047					
Chlamydomonas sp. "L"	1	3	-0.18	0.5645		3 and 5	0.24	0.4573	
		5	0.44	0.1536		3 and 7	-0.19	0.5451	
		7	-0.29	0.3559		3 and 9	0.21	0.5187	
		9	0.24	0.4458					
	Expt. Avg.		0.29	0.3562					
	2	2	-0.30	0.4366		2 and 3	-0.17	0.6599	
		3	-0.06	0.8837		2 and 4	0.12	0.7580	
		4	0.15	0.6983		2 and 5	-0.05	0.9037	
		5	-0.41	0.2721		2 and 6	0.86	0.0032	***
		6	0.74	0.0244	**	2 and 7	0.56	0.1209	
		7	0.36	0.3348		2 and 8	0.86	0.0032	***
		8	0.74	0.0224	**				
	Expt. Avg.		0.01	0.9825					
Chlamydomonas spp. (Total Count)	2	2	-0.75	0.0191	**	2 and 3	0.53	0.1381	
		3	0.30	0.4258		2 and 4	0.46	0.2086	
		4	-0.03	0.9301		2 and 5	0.21	0.5947	
		5	-0.18	0.6329		2 and 6	0.61	0.0813	*
		6				2 and 7	0.40	0.2927	
		7	0.14	0.7173		2 and 8	0.87	0.0025	***
		8	0.78	0.0130	**				
	Expt. Avg.		-0.08	0.8433					

Algal Species	Expt	Day	Model B. r	Pr≥r	P	Diff. Betwn Day	Model D r	Pr≥r	P
Chlorella	2	2	-0.84	0.0044	***	2 and 3	0.73	0.0256	**
spp.		3	-0.26	0.4941		2 and 4	0.64	0.0659	*
		4	-0.61	0.0824	*	2 and 5	0.57	0.1115	
		5	-0.56	0.1183		2 and 6	0.32	0.4047	
		7	-0.62	0.0744	*	2 and 7	0.70	0.0342	**
		6	-0.68	0.0452	**	2 and 8	0.39	0.3043	
		8	-0.02	0.9650					
	Expt. Avg.		-0.76	0.0177	**				
Colorless	2	2	0.58	0.1040		2 and 3	-0.35	0.3528	
Flagellates		3	0.34	0.3654		2 and 4	-0.66	0.0525	*
		4	-0.57	0.1089		2 and 5	-0.58	0.1050	
		5	-0.68	0.0459	**	2 and 6	-0.33	0.3783	
		6	-0.01	0.9823		2 and 7	0.23	0.5466	
		7	0.56	0.1173		2 and 8	-0.01	0.9825	
		8	0.30	0.4284					
	Expt. Avg.		-0.01	0.9824					
Cryptomonas	2	2	no cells found			2 and 3	0.10	0.8059	
spp.		3	0.10	0.8059		2 and 4	0.34	0.3676	
		4	0.34	0.3676		2 and 5	-0.12	0.7519	
		5	-0.12	0.7519		2 and 6	-0.07	0.8569	
		6	-0.07	0.8569		2 and 7	no cells found		
		7	no cells found			2 and 8	no cells found		
		8	no cells found						
	Expt. Avg.		0.23	0.5454					
Cyclotella	2	2	-0.39	0.2998		2 and 3	0.33	0.3914	
spp.		3	-0.09	0.8168		2 and 4	0.30	0.4319	
		4	0.28	0.4631		2 and 5	0.33	0.3783	
		5	0.24	0.5238		2 and 6	-0.33	0.3783	
		6	-0.54	0.1321		2 and 7	-0.19	0.6264	
		7	-0.26	0.5011		2 and 8	-0.23	0.5483	
		8	-0.15	0.7040					
	Expt. Avg.		-0.19	0.6264					
Dictyosphaerium	2	2	0.02	0.9543		2 and 3	0.01	0.9737	
ehrenbergianum		3	-0.02	0.9561		2 and 4	-0.18	0.6424	
		4	-0.35	0.3528		2 and 5	-0.33	0.3783	
		5	-0.60	0.0910	*	2 and 6	-0.28	0.4599	
		6	-0.88	0.0020	***	2 and 7	-0.03	0.9301	
		7	-0.12	0.7580		2 and 8	-0.07	0.8606	
		8	-0.34	0.3654					
	Expt. Avg.		-0.68	0.0446	**				

Algal Species	Expt	Day	Model B. r	Pr>r	P	Diff. Betwn Day	Model D r	Pr>r	P
Gleocapsa punctata	2	2	0.42	0.2572		2 and 3	0.33	0.3783	
		3	-0.55	0.1216		2 and 4	0.11	0.7749	
		4	-0.27	0.4810		2 and 5	0.42	0.2594	
		5	0.14	0.7150		2 and 6	0.21	0.5791	
		6	-0.50	0.1705		2 and 7	-0.08	0.8420	
		7	-0.41	0.2701					
		8	-0.09	0.8173					
	Expt. Avg.		-0.44	0.2340					
Kirchneriella lunaris	2	2	-0.21	0.5787		2 and 3	0.33	0.3783	
		3	0.36	0.3462		2 and 4	0.11	0.7747	
		4	-0.04	0.9267		2 and 5	0.42	0.2594	
		5	0.50	0.1703		2 and 6	0.21	0.5791	
		6	0.19	0.6264		2 and 7	-0.08	0.8420	
		7	-0.36	0.3343		2 and 8	0.45	0.2260	
		8	0.47	0.2011					
	Expt. Avg.		0.34	0.3697					
Merismopedia tenuissima	2	2	0.06	0.8821		2 and 3	-0.04	0.9112	
		3	-0.08	0.8305		2 and 4	-0.15	0.6913	
		4	-0.23	0.5448		2 and 5	0.05	0.9036	
		5	0.33	0.3874		2 and 6	-0.10	0.7919	
		6	-0.40	0.2814		2 and 7	-0.18	0.6424	
		7	-0.33	0.3914		2 and 8	-0.21	0.5947	
		8	-0.17	0.6572					
	Expt. Avg.		-0.53	0.1458					
Merotrichia capilata	2	2	no cells found			2 and 3	no cells found		
		3	no cells found			2 and 4	-0.31	0.4212	
		4	-0.31	0.4212		2 and 5	-0.59	0.0926	*
		5	-0.59	0.0926	*	2 and 6	0.81	0.0083	***
		6	0.81	0.0083	***	2 and 7	0.75	0.0208	**
		7	0.75	0.0208	**	2 and 8	0.55	0.1228	
		8	0.55	0.1228					
	Expt. Avg		0.28	0.4560					
Nephroselmis discoidea	1	3	-0.52	0.0860	*	3 and 5	0.37	0.2370	
		5	-0.14	0.6578		3 and 7	0.43	0.1593	
		7	0.20	0.5339		3 and 9	-0.02	0.9467	
		9	-0.51	0.0913	*				
	Expt. Avg.		-0.39	0.2106					
	2	2	0.19	0.6315		2 and 3	-0.13	0.7412	
		3	-0.47	0.1971		2 and 4	-0.21	0.5853	
		4	-0.82	0.0070	***	2 and 5	-0.02	0.9475	
		5	-0.07	0.8557		2 and 6	-0.19	0.6315	
		6	no cells found			2 and 7	-0.19	0.6315	
		7	no cells found			2 and 8	-0.26	0.5070	
		8	-0.07	0.8569					
	Expt. Avg.		-0.51	0.1619					

Algal Species	Expt	Day	Model B. r	Pr>r	P	Diff. Betwn Day	Model D r	Pr>r	P
Oscillatoria	1	3	0.13	0.6850		3 and 5	-0.65	0.0228	**
limnetica		5	-0.39	0.2118		3 and 7	-0.63	0.0294	**
		7	-0.88	0.0001	****	3 and 9	0.00	1.0000	
		9	0.22	0.5003					
	Expt. Avg.		-0.52	0.0844	*				
	2	2	-0.76	0.0185	**	2 and 3	0.48	0.1900	
		3	-0.46	0.2183		2 and 4	0.16	0.6749	
		4	-0.67	0.0484	**	2 and 5	0.03	0.9301	
		5	-0.58	0.1050		2 and 6	-0.14	0.7245	
		6	-0.20	0.6105		2 and 7	0.27	0.4887	
		7	-0.51	0.1639		2 and 8	-0.17	0.6586	
		8	-0.88	0.0015	***				
	Expt. Avg.		-0.54	0.1325					
Polycystis	2	2	-0.10	0.7864		2 and 3	0.29	0.4526	
firma		3	0.30	0.4388		2 and 4	0.13	0.7396	
		4	-0.14	0.7175		2 and 5	0.57	0.1089	
		5	-0.65	0.0593	*	2 and 6	-0.38	0.3176	
		6	-0.50	0.1727		2 and 7	0.04	0.9170	
		7	-0.07	0.8569		2 and 8	0.10	0.7864	
		8	no cells found						
	Expt. Avg.		0.03	0.9464					
Scenedesmus	2	2	-0.23	0.5501		2 and 3	0.05	0.8939	
spp.		3	-0.39	0.2944		2 and 4	-0.15	0.6913	
		4	-0.26	0.4939		2 and 5	0.16	0.6749	
		5	-0.58	0.1046		2 and 6	-0.42	0.2590	
		6	-0.44	0.2353		2 and 7	0.08	0.8261	
		7	-0.30	0.4258		2 and 8	0.12	0.7645	
		8	-0.74	0.0239	**				
	Expt. Avg.		-0.52	0.1478					
Total Count	1	3	-0.17	0.5914		3 and 5	-0.58	0.0467	**
(all spp.)		5	-0.39	0.2118		3 and 7	-0.32	0.3044	
		7	-0.88	0.0001	****	3 and 9	0.09	0.7896	
		9	0.12	0.7384					
	Expt. Avg.		-0.54	0.0701	*				
Total Count	2	2	-0.83	0.0053	***	2 and 3	0.40	0.2814	
		3	-0.58	0.0987	*	2 and 4	0.02	0.9475	
		4	-0.70	0.0342	**	2 and 5	-0.26	0.5033	
		5	-0.69	0.0409	**	2 and 6	-0.33	0.3914	
		6				2 and 7	0.02	0.9475	
		7	-0.57	0.1050		2 and 8	-0.24	0.5531	
		8	-0.73	0.0256	**				
	Expt. Avg.		-0.84	0.0044	***				

Algal Species	Expt	Day	Model B. r	Pr>r	P	Diff. Betwn Day	Model D r	Pr>r	P
Unidentified	2	2	-0.03	0.9298		2 and 3	-0.17	0.6586	
spp.		3	-0.34	0.3762		2 and 4	-0.73	0.0256	**
		4	-0.85	0.0037	***	2 and 5	-0.11	0.7749	
		5	-0.32	0.3960		2 and 6	-0.69	0.0409	**
		6	-0.60	0.0869	*	2 and 7	-0.51	0.1639	
		7	-0.72	0.0283	**	2 and 8	-0.20	0.6105	
		8	-0.27	0.4887					
	Expt. Avg.		-0.81	0.0085	***				

Appendix A. (cont'd)

Results of Statistical Analysis Using Model C.

Results of Model C, showing the effect of atrazine on growth rate. The analysis of variance (F test for the overall model) is given above the least square mean (T test) result for differences between atrazine dose (ug/l). *=P<0.1000; **=P<0.0500; ***=P<0.0010; ****=P<0.00010.

Algal Species	Expt.	Diff. Betwn Days	Source	F	Pr>F	Pr>T	P
Ankistrodesmus spp.	1	3 and 5	model	0.2	0.8620		
		3 and 7	model	0.8	0.5246		
		3 and 9	model	1.2	0.3596		
	2	2 and 3	model	1.3	0.3701		
		2 and 4	model	0.6	0.6208		
		2 and 5	model	1.0	0.4567		
		2 and 6	model	0.5	0.7132		
		2 and 7	model	0.4	0.7597		
		2 and 8	model	3.7	0.0975		*
Ankistrodesmus falcatus var. *spirilliformis*	2	2 and 3	model	0.7	0.5889		
		2 and 4	model	0.4	0.7563		
		2 and 5	model	0.5	0.7182		
		2 and 6	model	0.4	0.7672		
		2 and 7	model	0.3	0.8012		
		2 and 8	model	0.8	0.5390		
Chlamydomonas altera	2	2 and 3	model	1.4	0.3525		
		2 and 4	model	2.0	0.2410		
		2 and 5	model	0.4	0.7650		
		2 and 6	model	2.9	0.1383		
		2 and 7	model	2.0	0.2264		
		2 and 8	model	1.6	0.3045		
Chlamydomonas debaryana	1	3 and 5	model	16.6	0.0008		****
			dose 0,50			0.0012	***
			dose .25,50			0.0036	***
			dose 1,50			0.0076	***
		3 and 7	model	4.2	0.0454		**
		3 and 9	model	1.0	0.4386		
	2	2 and 3	model	2.7	0.1589		
		2 and 4	model	1.4	0.3423		
		2 and 5	model	0.5	0.7220		
		2 and 6	model	1.6	0.3123		
		2 and 7	model	1.7	0.2756		
		2 and 8	model	1.4	0.3453		

Algal Species	Expt.	Diff. Betwn Days	Source	F	Pr>F	Pr>T	P
Chlamydomonas globosa	2	2 and 3	model	6.5	0.0358		**
			dose 10, 50			0.0708	*
		2 and 4	model	8.0	0.0234		**
			dose 0,10			0.0966	*
			dose 1,10			0.0918	*
			dose 50,10			0.0348	**
		2 and 5	model	5.5	0.0483		**
			dose 0,50			0.0810	*
		2 and 6	model	1.8	0.2727		
		2 and 7	model	1.8	0.2727		
		2 and 8	model	1.8	0.2727		
Chlamydomonas mucicola	2	2 and 3	model	0.1	0.9412		
		2 and 4	model	11.3	0.0114		**
			dose 0,1			0.0192	**
			dose 10,1			0.0288	**
			dose 50,1			0.0924	*
		2 and 5	model	2.9	0.1419		
		2 and 6	model	0.5	0.7190		
		2 and 7	model	1.1	0.4252		
		2 and 8	model	3.1	0.1286		
Chlamydomonas sp. "L"	1	3 and 5	model	1.6	0.2553		
		3 and 7	model	5.1	0.0296		**
			dose 0,1			0.0684	*
			dose 0,50			0.0678	*
		3 and 9	model	0.3	0.8268		
	2	2 and 3	model	0.2	0.8601		
		2 and 4	model	1.5	0.3302		
		2 and 5	model	0.3	0.8498		
		2 and 6	model	3.5	0.1059		
		2 and 7	model	2.4	0.1786		
		2 and 8	model	4.4	0.0713		*
			dose 0,50			0.0918	*
Chlamydomonas spp. (Total Count)	2	2 and 3	model	2.4	0.1809		
		2 and 4	model	5.4	0.0510		*
		2 and 5	model	1.0	0.4590		
		2 and 6	model	1.4	0.3486		
		2 and 7	model	2.1	0.2181		
		2 and 8	model	4.4	0.725		*
Chlorella spp.	2	2 and 3	model	3.1	0.1253		
		2 and 4	model	1.4	0.3376		
		2 and 5	model	1.3	0.3793		
		2 and 6	model	1.0	0.4541		
		2 and 7	model	0.7	0.5936		
		2 and 8	model	0.8	0.5683		

Algal Species	Expt.	Diff. Betwn Days	Source	F	Pr>F	Pr>T	P
Colorless Flagellates	2	2 and 3	model	4.7	0.0638		*
			dose 1,10			0.0942	*
		2 and 4	model	2.5	0.1783		
		2 and 5	model	2.6	0.1606		
		2 and 6	model	1.8	0.2651		
		2 and 7	model	2.3	0.1909		
		2 and 8	model	1.6	0.3015		
Cryptomonas spp.	2	2 and 3	model	0.05	0.9828		
		2 and 4	model	1.8	0.2714		
		2 and 5	model	0.4	0.7805		
		2 and 6	model	1.3	0.3721		
		2 and 7	no cells found				
		2 and 8	no cells found				
Cyclotella spp.	2	2 and 3	model	11.2	0.0118		**
			dose 1,10			0.0588	*
			dose 10,50			0.0156	**
		2 and 4	model	15.8	0.0055		***
			dose 0,50			0.0726	*
			dose 10,50			0.0114	**
			dose 1,10			0.0168	**
		2 and 5	model	0.5	0.6970		
		2 and 6	model	1.9	0.2515		
		2 and 7	model	1.6	0.2958		
		2 and 8	model	1.3	0.3729		
Dictyosphaerium ehrenbergianum	2	2 and 3	model	0.06	0.9776		
		2 and 4	model	0.02	0.9964		
		2 and 5	model	0.01	0.9989		
		2 and 6	model	0.05	0.9855		
		2 and 7	model	0.08	0.9688		
		2 and 8	model	0.04	0.9882		
Gleocapsa punctata	2	2 and 3	model	4.6	0.0673		*
		2 and 4	model	4.6	0.0674		*
		2 and 5	model	0.6	0.6625		
		2 and 6	model	7.9	0.0239		**
			dose 0,1			0.0450	**
			dose 0,10			0.0924	*
		2 and 7	model	1.2	0.4078		
		2 and 8	model	1.1	0.4218		
Kirchneriella lunaris	2	2 and 3	model	1.3	0.3636		
		2 and 4	model	0.3	0.8258		
		2 and 5	model	1.5	0.3145		
		2 and 6	model	0.5	0.7120		
		2 and 7	model	4.8	0.0615		*
		2 and 8	model	3.7	0.0973		*

Algal Species	Expt.	Diff. Betwn Days	Source	F	Pr>F	Pr>T	P
Merismopedia tenuissima	2	2 and 3	model	0.5	0.7223		
		2 and 4	model	0.6	0.6717		
		2 and 5	model	0.4	0.7431		
		2 and 6	model	0.3	0.8031		
		2 and 7	model	0.4	0.7894		
		2 and 8	model	0.6	0.6251		
Meritrichia capilata	2	2 and 3	no cells found				
		2 and 4	model	0.5	0.6823		
		2 and 5	model	3.8	0.0927		*
		2 and 6	model	2.4	0.1862		
		2 and 7	no cells found				
		2 and 8	model	0.9	0.4917		
Nephroselmis discoidea	1	3 and 5	model	5.6	0.0225		**
			dose 0,50			0.0798	*
			dose 1,50			0.0348	**
		3 and 7	model	2.1	0.1813		
		3 and 9	model	0.7	0.5693		
	2	2 and 3	model	1.3	0.3761		
		2 and 4	model	1.3	0.3728		
		2 and 5	model	0.9	0.4971		
		2 and 6	model	1.1	0.4296		
		2 and 7	model	1.1	0.4296		
		2 and 8	model	1.0	0.4786		
Oscillatoria limnetica	1	3 and 5	model	4.4	0.0414		**
			dose .25,50			0.0504	*
		3 and 7	model	1.8	0.2225		
		3 and 9	model	2.4	0.1432		
	2	2 and 3	model	0.7	0.5740		
		2 and 4	model	0.5	0.7083		
		2 and 5	model	3.9	0.0877		*
		2 and 6	model	7.5	0.0266		**
			dose 0,1			0.0996	*
			dose 1,50			0.0438	**
		2 and 7	model	3.8	0.0908		*
		2 and 8	model	1.5	0.3146		
Polycystis firma	2	2 and 3	model	0.2	0.8628		
		2 and 4	model	1.0	0.4636		
		2 and 5	model	1.2	0.3953		
		2 and 6	model	4.8	0.0625		*
		2 and 7	model	1.3	0.3724		
		2 and 8	model	0.6	0.6493		
Scenedesmus spp.	2	2 and 3	model	0.2	0.8628		
		2 and 4	model	1.0	0.4636		
		2 and 5	model	1.2	0.3953		
		2 and 6	model	4.8	0.0625		*
		2 and 7	model	1.3	0.3724		
		2 and 8	model	0.6	0.6493		

Algal Species	Expt.	Diff. Betwn Days	Source	F	Pr>F	Pr>T	P
Total Count (all spp.)	1	3 and 5	model	4.0	0.522		*
			dose .25,50			0.0606	*
		3 and 7	model	1.2	0.3673		
		3 and 9	model	2.6	0.1291		
	2	2 and 3	model	1.6	0.3017		
		2 and 4	model	0.6	0.6163		
		2 and 5	model	3.6	0.1025		
		2 and 6	model	10.3	0.0139		**
			dose 1,50			0.0192	**
			dose 10,50			0.0576	*
		2 and 7	model	1.5	0.3253		
		2 and 8	model	1.2	0.3982		
Unidentified spp.	2	2 and 3	model	0.7	0.5772		
		2 and 4	model	3.8	0.0922		*
		2 and 5	model	0.4	0.7674		
		2 and 6	model	3.1	0.1280		
		2 and 7	model	1.6	0.2976		
		2 and 8	model	0.3	0.8171		