THE EFFECTS OF NUTRIENT, pH AND HERBICIDE LEVELS ON ALGAL GROWTH

By

BASHIR IBRAHIM TUBEA

Bachelor of Science
University of Wyoming
Laramie, Wyoming
1972

Master of Science
University of Connecticut
Storrs, Connecticut
1974

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LEVELS ON ALGAL GROWTH

Thesis Approved:

Lester W. Reed
Thesis Adviser

Billy L. Tucker

Eddie Basler

Don S. Murray

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Some waters from Oklahoma lakes and streams may contain high concentration of dissolved nutrient elements such as P, K, Ca, Mg, N and high pH, while other waters in Oklahoma are lower in the same nutrient elements and the pH is less basic.

The nutrient element input in Oklahoma waters probably originates from the soils and geological formations around the lakes and streams. The fertilizers which are applied on the soils which are close to these water systems contribute to an increase in the level of these elements in water. When these nutrient elements drain into water bodies, they strongly encourage the eutrophication process. Eventually eutrophication processes could lead to changes in the aquatic ecosystem by encouraging and stimulating the growth rates of certain species of algae while discouraging others depending on the type and levels of the nutrient elements present in the water. Thus the algae population is often markedly affected by the increases or decreases of the essential nutrient elements. When herbicides are directly applied as aquatic herbicides or indirectly transported by runoff and/or irrigation waters, the herbicides may change the naturally occurring aquatic environment. The change may come from the interaction between algae and nutrient element levels, or between herbicides, nutrient elements and pH levels of the water. Thus, information is needed on the interaction of these factors as they affect algae growth.

The principal objectives of this research were to investigate the interactions of nutrient elements, pH levels, and herbicide concentrations on the growth of algae under laboratory culture conditions.

CHAPTER II

LITERATURE REVIEW

Herbicides are widely used in modern agriculture. It is important to know the herbicides impact on the environment, particularly the effects of herbicides in the aquatic ecosystem. There are some indications that some quantities of herbicides which are applied to agricultural lands are ultimately carried by runoff water, erosion or leachate (6). Picloram is considered to be one of the most promising herbicides presently used for brush control in agricultural and non-agricultural areas. Because picloram may not rapidly decompose in soil, depending on the field conditions, it may drain into non-flowing water (10). Runoff samples obtained after 10 days from irrigated plots which had been treated with picloram revealed the presence of 17 parts per billion (ppb) of picloram and the concentrations decreased with time and with increased water quantity. Runoff water obtained from untreated plots, but adjacent to plots treated with picloram, contained 89.7 ppb of picloram. Samples obtained from impounded water on the watershed approximately 100m away from the treated area was found to contain about 70 ppb of picloram after 27 days of treatment. Eight days after picloram was applied to field plots, water samples obtained 1.7km away from the treated area showed the presence of picloram at a concentration of less than 1 ppb (9). White, et al (45), found atrazine (see Table I) in runoff water when applied to field plots (6.5 percent slope) at the rate of 3.36kg/ha. They showed that, under normal conditions, 0.1 kg/ha or lesser amounts of the herbicide were commonly encountered in the runoff. Even though the concentration of the herbicide runoff was not too high, the first stage of runoff could carry higher concentration and be of importance in the environment, and in the long run may cause damages to non-target plants in fields and in the aquatic ecosystem.

TABLE I

COMMON AND CHEMICAL NAMES OF HERBICIDES

Common Name	Chemical Name
1. Amitrol	3-amino-s-triazole
2. Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine
3. Barban	4-chloro-2-butynyl m-chlorocarbanilate
4. Dacthal	dimethyl tetrachloroterephthalate
5. Dinoseb	2-sec-butyl-4,6-dinitrophenol
6. Diphenamid	N,N-dimethyl-2,2-diphenylacetamide
7. Diquat	6,7-dihydrodipyridol [1,2-α:2',1'-c] pyrazinediium ion
8. Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
9. Fluometuron	1,1-dimethyl-3-(α,α,α-trifluoro-m-toly) urea
10. M & B 8882	(methyl 4-nitrobenzenesulphonylcarbamate)
11. Metribuzin	4-amino-6-(1,1-dimethylethyl)-3-(methylthio) 1,2,4-triazine-5(4H)-one
12. Monuron	3-(P-chlorophenyl)-1,1-dimethylurea
13. Picloram	4-amino-3,5,6-trichloropicolinic acid
14. Prometryn	2,4-bis(isopropylamino)-6-methylthio)-s-triazine
15. Simazine	2-chloro-4,6-bis(ethylamino)-s-triazine
16. Trifluralin	α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-P-toluidine
17. 2,4-D	(2,4-dichlorophenoxy) acetic acid
18. 2,4,5-T	(2,4,5-trichlorophenoxy) acetic acid

Another atrazine study on runoff in a corn (Zea mays L.) field was a preemergence application. The runoff collected was found to contain an average of 24 percent of the total atrazine applied. When the recommended rate (2.2 kg/ha) was applied to Pennsylvania soils, the atrazine losses with runoff water as well as the soil sediments was found to be close to 0.05 kg/ha (23). Baldwin (5) studied herbicide movement in soil. He applied prometryn and fluometuron and found that when these two herbicides are applied on a dry soil the general losses for both herbicides by runoff were about 0.5 percent or less of that initially applied to the soil. In actual field conditions, the herbicide transportation to aquatic systems depends upon many factors. The greatest herbicide transportations are generally associated with irrigation or heavy rains immediately after herbicidal application (45). Runoff shortly after atrazine application showed significant amounts of the herbicide, as high as 15 percent or 1.17 to 4.91 ppm of the total applications, were detected in water from a surface-contoured watershed (35). Similar concentrations but with a different herbicide was bioassayed by Barnett et al (6). He applied 2,4-D concentrations ranging from 0.005 parts per million (ppm) to 5.0 ppm as a standard curve and then measured the effect of washoff from cultivated Cecil sandy loam soil on cucumber (Cucumis satives L.) roots. The results showed a decrease of root length as the concentration of 2,4-D increased. A field bioassay involving cucumbers, sugar beets (Beta vulgaris L.), oats (Avena sativa L.), soybeans (Glycine max L.), and Chlorella to monitor their order of sensitivity to atrazine revealed that Chlorella was more sensitive than soybean, less sensitive than sugar beet and about the same as cucumber and oat (27). Another experiment conducted by Trichell, et al (42), confirmed the effects of herbicides with runoff on plant growth. They wrote, "There were sufficient amounts of all herbicides in 100 ml of runoff water obtained 24 hours after herbicidal application to kill or seriously alter the growth of black valentine beans (Phaseolus vulgaris L.)." Picloram was one of the herbicides they used at the rate of 1.12 to 2.24 kg/ha in an Irving clay loam.

Most of the studies involving herbicides and algae interactions were mainly intended for screening herbicide activity and for studies on their mode of action; thus, very few if any studies were designed to study the algae as a non-target recipient of the herbicides which drain into water systems as a runoff or leachate from agricultural lands. Some workers who utilize algae for their bioassay in screening purposes have evaluated the phytotoxicity of certain herbicides. The results indicated that as a group the substituted-urea and s-triazine herbicides have good algaecide properties (25). Screening with algae as an assay showed Chlorella vulgaris to have a highly significant response to low concentrations of monuron, diuron, and prometryn (2).

It became common knowledge, after many studies, that herbicides are effective and selective on higher plants. The effectiveness and selectivity of herbicides have not been dealt with extensively with algae which are also an important part of the ecological link. Wright (46) investigated the effects of some herbicides using the algae, Chlorella pyrenoidosa as an assay organism and showed that growth was inhibited by some herbicides more than other herbicides. As an example of differences in effectiveness, barban at a concentration of 1 ppm controlled 90 percent of the growth of Chlorella, whereas M & B 8882 inhibited 50 percent of the growth at 25 ppm. A concentration of 200 mg/ml of dacthal, simazine, 2,4-D and 2,4,5-T did not show algaecide effects (19), whereas atrazine at lower concentration, 0.22 ppm, stopped the growth of Chlorella pyrenoidosa and Chlorella vulgaris was markedly stimulated. Differences in the susceptibility of algae species to herbicides should be an anticipated phenomena. It was shown that 0.5 μg/ml of atrazine completely inhibited the growth of Chlamydomonas reiunhardi while its growth was stimulated by diphenamid (28). The normal growth of five out of nine algae species tested was controlled by 0.004 ppm of monuron (29). Experimental results for five species of soil algae, Chlorella being one, showed metribuzin and two analogs drastically reduced algae population as the concentrations of the herbicides increased (3). It was pointed out that fluometuron had the effect of suppressing the growth of unicellular green algae such as Chlorella pyrenoidosa. Moreover, fluometuron was noted to reduce the chlorophyll content of algae (40).

The enrichment of water of lakes and streams with nutrients can originate from the soils around and under the lakes and streams, drainage of domestic fertilizers, from agricultural lands and urban sewage (24, 21). Thus, the nutrient budget of lakes and streams depends upon the input of nutrients from the above source, the influence of rainfall and the difference between incoming and outgoing volumes of water (21). Earlier researchers found that increased levels of dissolved nutrients can change the aquatic ecosystem such as algae growth which is stimulated by the increased dissolved nutrients in water (36).

Algae populations are often markedly affected by increases or decreases of certain elements, such as N and P. Most algae are good consumers of N, suggesting that large quantity of algae will be associated with waters high in N content. A deficiency of N is one of the most limiting factors in algae growth. Also, P was indicated to exert a marked influence on algae populations (21, 40).

In modern agriculture where the use of fertilizers has increased drastically, one would anticipate an environmental impact due to fertilizers, particularly in nearby lakes and streams, because there are indications that fertilizers from agricultural lands have been a good source of nutrients which nourish heavy blooms of algae in water systems (21). Prescott (33) in a review article indicated that when fertilizers such as N, P, K in the ratio of 12-24-12 are added into waters (streams or lakes), the growth of algae increased. Normally, algae respond to nutrients like higher plants and when agricultural lands are fertilized, a change of algae blooms downstream should be anticipated. An effluent obtained from irrigated and fertilized agricultural land for crop production was shown to supply enough nutrients to cause higher growth of algae in downstream waters (26).

More work is needed in the area of algae growth as affected by the herbicides drained from agricultural lands into waters which in turn have different salinity and nutrient levels. With higher plants, herbicide effects were noted to change nutrient element uptake by producing changes in metabolic activity of the cell or by a change in permeability of the cell membrane (32). Relatively high salt concentrations in water or in growth cultures have an inverse effect on chlorophyll synthesis and high salinity had a marked effect on respiration, cell division and growth of unicellular marine algae (31). Work with a marine unicellular algae showed a herbicide salinity interaction. Substituted urea herbicides depressed carbohydrate concentrations in all tested species of algae and the carbohydrate decreased as the salinity increased (43). From the ongoing information it appears that there are enough indications to cause a concern about the runoff water from agricultural lands treated with herbicides as they may relate to algae growth. Because there is a need for additional information in this area, dealing with herbicides and algae interaction in aquatic systems where different nutrient levels prevail, a research project was initiated to investigate the interaction of algae with nutrients, and pH levels.

CHAPTER III

MATERIALS AND METHODS

Two levels of pH and several nutrient elements which occur in natural waters of rivers and lakes in Oklahoma were selected as variables for the growth of algae. The nutrient elements selected were Ca, Mg, K, P, and N. The concentration levels of these nutrient elements and pH were obtained from "Appraisal of the Water and Related Land Resources of Oklahoma."* Concentrations of the solutions used in these experiments are shown in Table II.

TABLE II

LEVELS OF THE NUTRIENT ELEMENTS AND THE pH

	pH	P	N	K	Ca	Mg
		mg/l				
High	10.0	0.23	130.0	14.0	170.0	58.0
Low	4.0	0.003	0.1	0.8	5.0	1.0

*By Oklahoma Water Resources Board.

The solutions without the herbicides were autoclaved for 30 minutes at 6.8 kg/cm^2 and 121 C and allowed to cool to room temperature.

The two levels of pH and nutrient elements represent normal extremes of naturally occurring waters. The respective pH levels were obtained by the addition of HCL or NaOH to Bold's Basal Growth Media, also known as Bristol's Solution. The N source was $NaNO_3$. To avoid the contaminated levels, the selected levels for N were representative of areas not close to feed lots or sewage disposal areas. P was supplied in the form of $NaH_2PO_4 \cdot H_2O$ and the selection of P levels were representative of areas where possible contaminations from urban effluent such as detergents or fertilizers from nearby fields were least suspected. K selection was based on the same criteria as P. Ca and Mg selections were based upon the size of the lake. The larger lakes were preferred. In the case of rivers, selections were based upon the distance away from the source.

The concentrations of other nutrient elements in the growth media were those from Bold's Basal Media or Bristol solution and are shown below:

(a) Macronutrients--used 10 ml each/940 ml

$NaNo_3$	10g/400 ml or 25g/1000 ml
$CaCL_2$ $2H_2$	1g/400 ml
$MgSO_4$ $7H_2O$	3g/400 ml
K_2HPO_4	3g/400 ml
KH_2PO_4	7g/400 ml
NaCl	1g/400 ml

(b) EDTA--used 1 ml/1

EDTA	50g/1
KOH	31g/1

(c) Iron--used 1 ml/1

$FeSO_4$ $7H_2O$	4.98g/1
H_2SO_4	1.0 ml/1

(d) Boron--used 1 ml/1

H_3BO_4	11.42g/1

(e) Micronutrients--used 1 ml/1

$ZnSO_4$ $7H_2O$	8.82g/1
$MnCl_2$ $4H_2O$	1.44g/1
MoO_3	0.71g/1
$CuSO_4$ $5H_2O$	1.57g/1
$Co(NO_3)_2$ $6H_2O$	0.49g/1

Chlorella pyrenoidosa Chick and Lyngbya birgei Dyar were obtained from the Starr Culture of Indiana University. These organisms are easy to culture and are common to Oklahoma lakes and streams. The algae were first cultured in Bristol's medium until they reached their respective logarithmic phase of growth. Aliquots were obtained and then centrifuged for about 5 minutes at 12,000 rpm. The supernatant was then decanted and the cells were washed twice in distilled water, with strong agitation or vigorous shaking each time. The supernatant was decanted and discarded after centrifugation. Aliquots of algae were mixed with distilled water, so optical densities of the experimental solutions were 1.0 to 0.8. The washed cells were added to their respective experimental solutions containing the appropriate low or high concentrations of the nutrient elements as well as the other essential elements. The four herbicides used as treatments were added at concentrations of 0.1, 1.0 and 10.0 μM.

The herbicides used in these experiments were selected to represent particular groups or families of herbicides. Reasonable comparisons of their effects on algae and the presence of nutrient element levels could thus be made. The herbicides used were picloram, prometryn, dinoseb and fluometuron. After treatment, the initial optical density (O.D.) of 10 ml aliquots were measured. They were as follows: Chlorella, 0.110; and Lyngbya, 0.099. The pH of the solutions other than that of pH treatments was about 6.8 and increased slightly with time and with growth rates. The treatment cultures in 50 ml Erlenmeyer flasks were stoppered with cotton and placed on a shaker in a randomized design. They were shaken at 80 cycles/min. under continuous illumination of 3500 lux. Different times were allocated for the growth of each algae which was selected to correspond to their logarithmic growth period. The growth periods for the two algae were as follows: Lyngbya, 48 hrs.; and Chlorella, 72 hrs. The temperature was maintained between 25 and 30 C. Whenever the algae stuck on the walls of the flask, they were removed from walls with a rubber policeman. A homogenizer was applied to break up the aggregates of Lyngbya and uniform cultures were obtained. Water lost due to evaporation was replaced with distilled water. Growth rates were determined by measuring the optical density at harvest with a Bausch and Lomb Spectronic 20 spectrophotometer at a wave length of 678 nm.

The results for the four replicates were reported as adjusted K values. K was designated to represent the growth rates in doublings per day, and was calculated according to the method of McCarthy and Patterson (31). The formula used is as follows: $K = 79.7/\Delta t \log_{10}(FOD/IOD)$

Where K = growth rate in number of doublings per day;

FOD = final optical density; IOD - initial optical density;

Δt = growth period in hours

K adjustment was made by converting all negative K values to zero and then averaging the four replications. Hereafter, K will be shown as K adjusted, particularly in the graphs.

CHAPTER IV

RESULTS

In preliminary work, picloram at 0.1, 1.0, 10.0 μM did not cause any effect on growth as shown by spectrophotometric density measurements of the two algae used in this research. Therefore, the data for this herbicide is now being used as control. The other herbicides showed algaecide effects. The results of the treatments using three herbicides and high and low levels of P as they affect the growth of Chlorella nnd Lyngbya are shown in Figures 1 and 2. Prometryn was very active against Chlorella and Lyngbya. The growth rates of both algae were sharply reduced. Statistical analysis showed significant inhibition of growth rates due to prometryn even at the lowest concentration of 0.1 μM on both Chlorella (Figure 1) and Lyngbya (Figure 2). P levels did not alter the prometryn effectiveness and caused no significant difference on growth rates. Apparently, there were no interactions between prometryn and the P levels used. At the two levels of P, fluometuron was effective in reducing growth rates of both algae but less effective than prometryn. The growth rates were inhibited at 1.0 μM or higher, whereas 0.1 μM prometryn was effective. Fluometuron toxicity was the same at high or low levels of P and, again, there was no significant difference of growth due to P. The growth rates at high and low P levels were significantly different only in dinoseb treatments of Chlorella. Dinoseb did not significantly alter growth rates at either level of P in Chlorella but growth of Lyngbya was

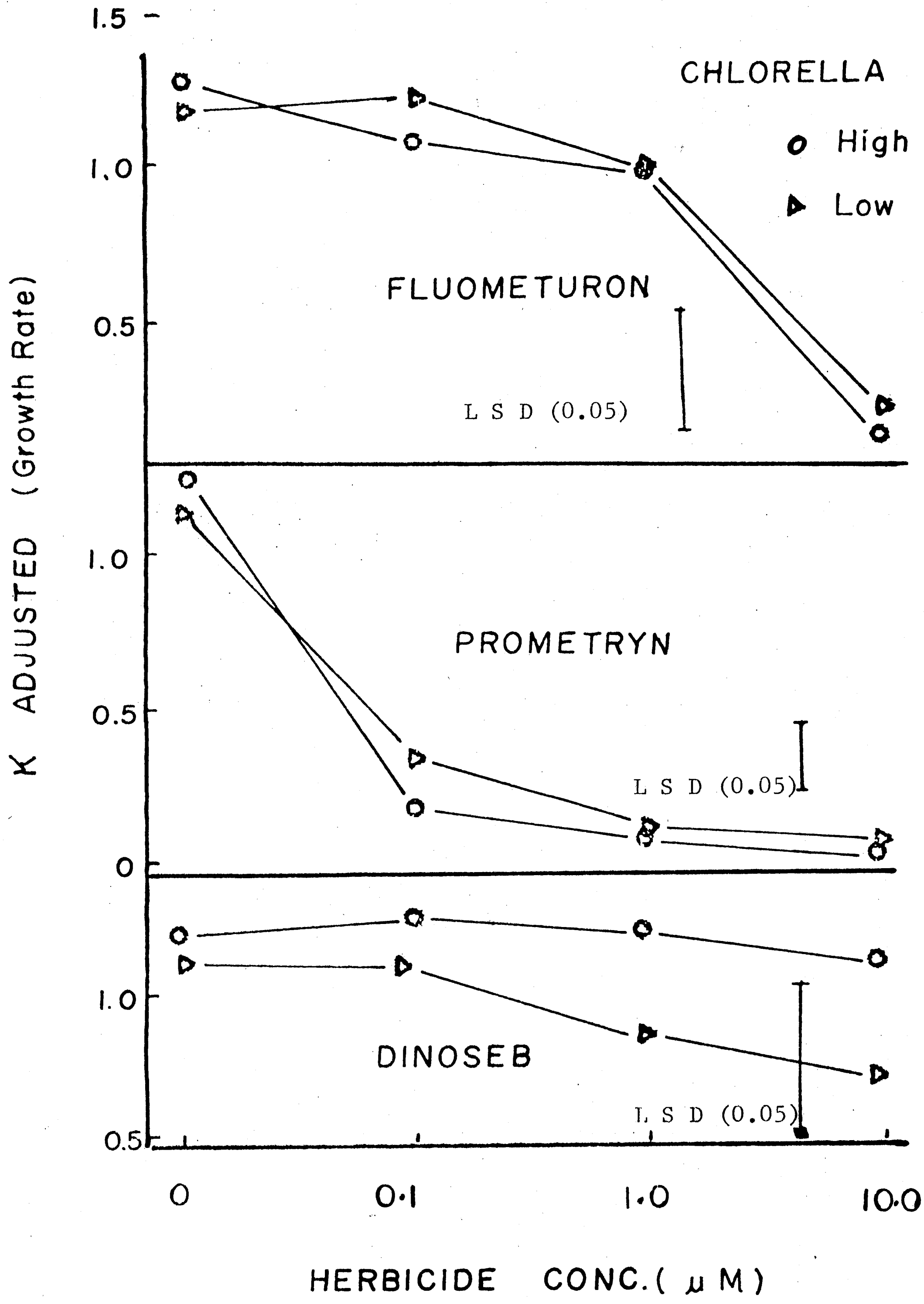

Figure 1. The Influence of P and Herbicides on Growth Rate of Chlorella

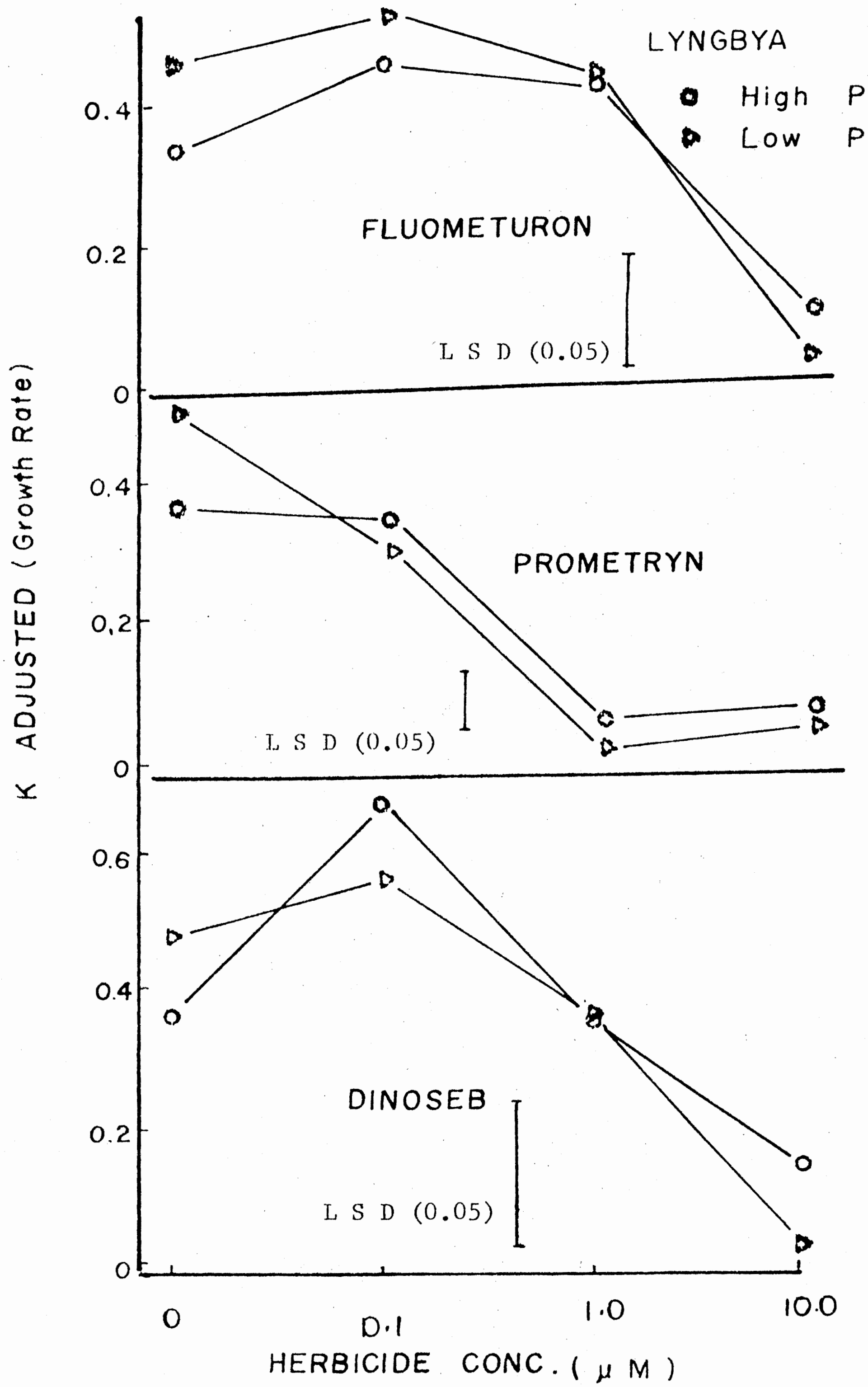

Figure 2. The Influence of P and Herbicides on Growth Rate of Lyngbya

inhibited by higher concentrations (10 μM) of dinoseb.

The effects of treatments with three herbicides at low and high N levels on the growth of Chlorella and Lyngbya (Figures 3 and 4) showed that prometryn was statistically effective in lowering the growth rates of both algae at low concentrations (0.1 μM). The statistical analysis revealed significant prometryn N levels interactions to Chlorella and Lyngbya; this interaction may reflect the fact that growth of Lyngbya was very low at low N and thus there was no growth to be decreased by the herbicide. Fluometuron and dinoseb were relatively less effective. Concentrations of 1 to 10 μM were required for the development of sufficient toxicities to reduce Lyngbya growth rates significantly. Dinoseb was not toxic to Chlorella at any concentration but was inhibitory to Lyngbya at 10 μM The growth rates were much higher for the high N treatments for both algae. The data revealed N-herbicide interaction for fluometuron and dinoseb in Lyngbya and for fluometuron in Chlorella but, like the data for prometryn, growth was very low for low N treatments and further growth inhibition by the herbicides was not possible; this effect probably was responsible for these significant interactions.

Results obtained from herbicides and K levels on algae growth are reported in Figures 5 and 6. Prometryn reduced growth rates of Chlorella at 0.1 μM and Lyngbya at 1.0 μM concentrations at high K levels. Prometryn may have been more effective at low K levels on growth of Lyngbya since growth was reduced at 0.1 μM prometryn with low K. The growth rates were statistically different due to prometryn concentrations and no significant difference in growth was caused by K level. Fluometuron, which appeared less effective than prometryn, caused significant decreases in growth especially at 10 μM concentration and like prometryn appeared to

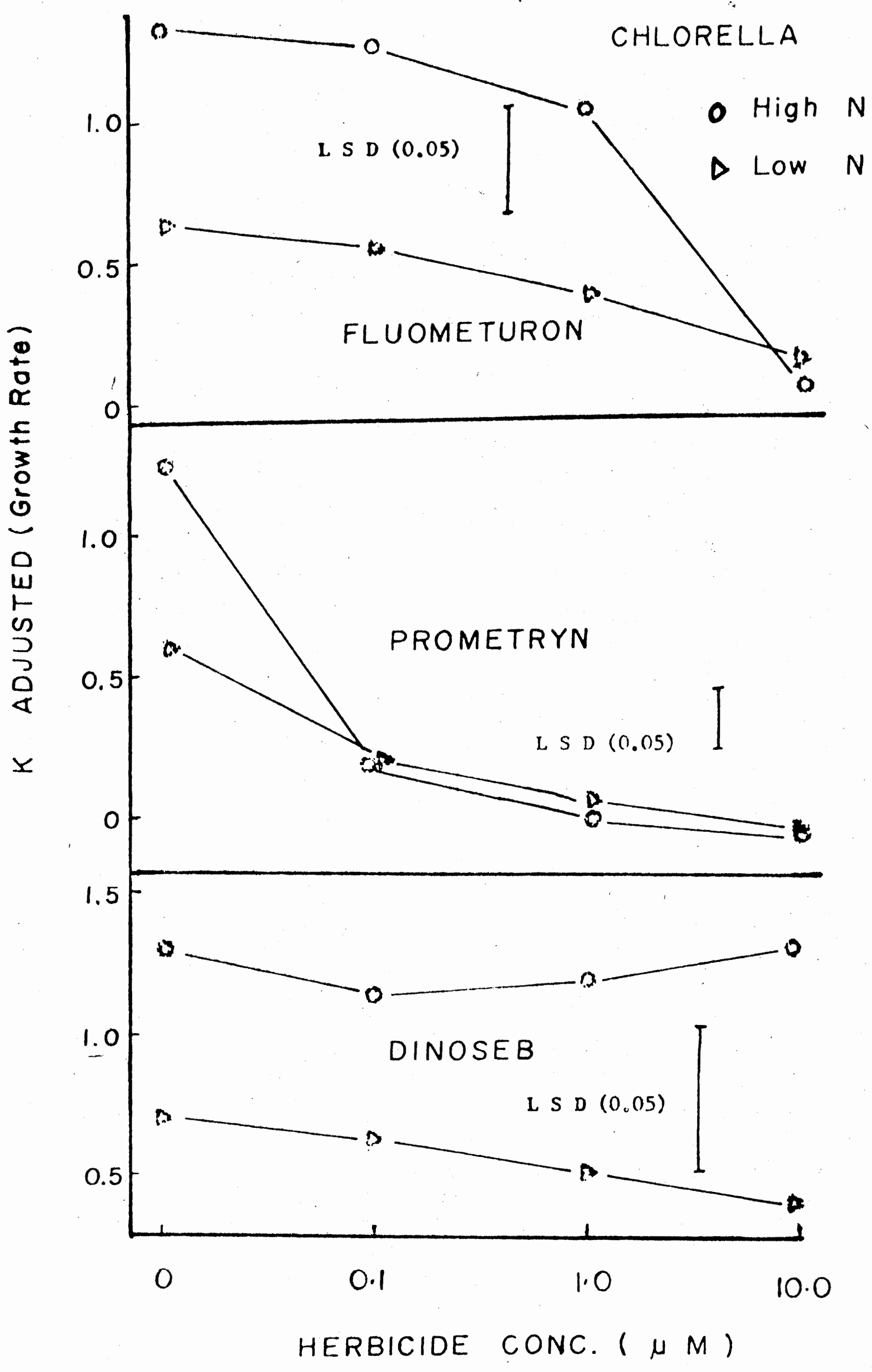

Figure 3. The Influence of N and Herbicides on Growth Rate of Chlorella

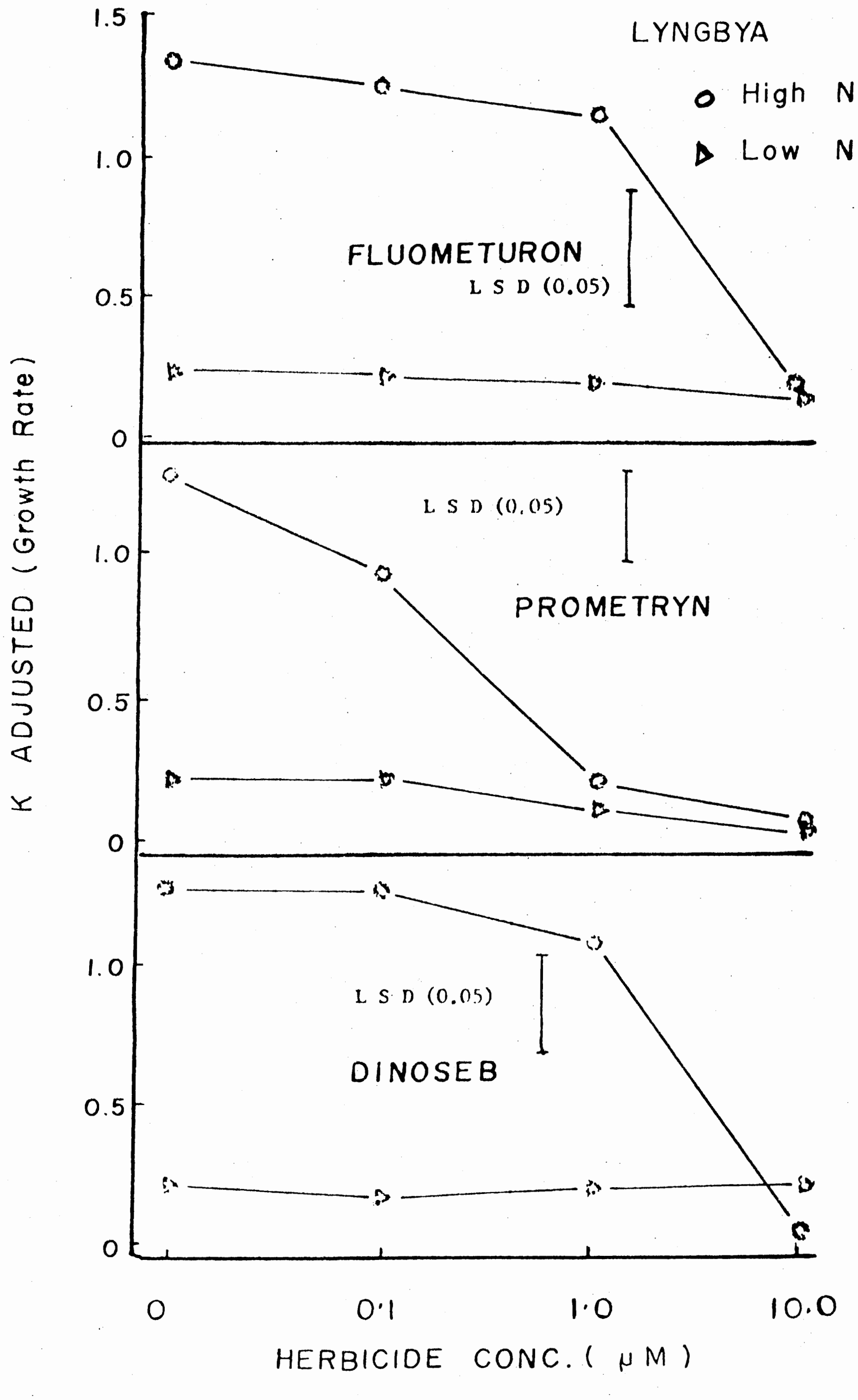

Figure 4. The Influence of N and Herbicides on Growth Rate of Lyngbya

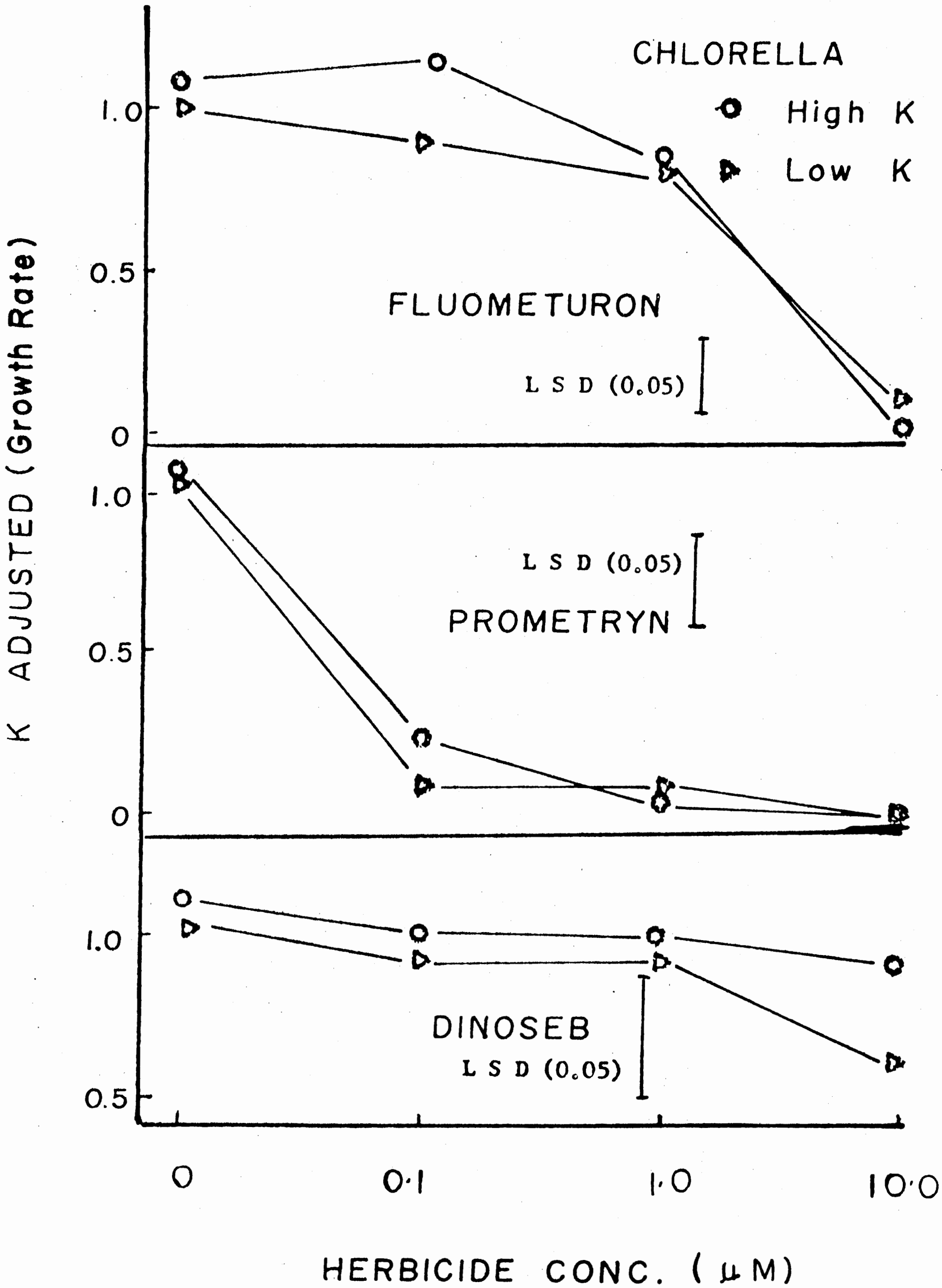

Figure 5. The Influence of K and Herbicides on Growth Rate of Chlorella

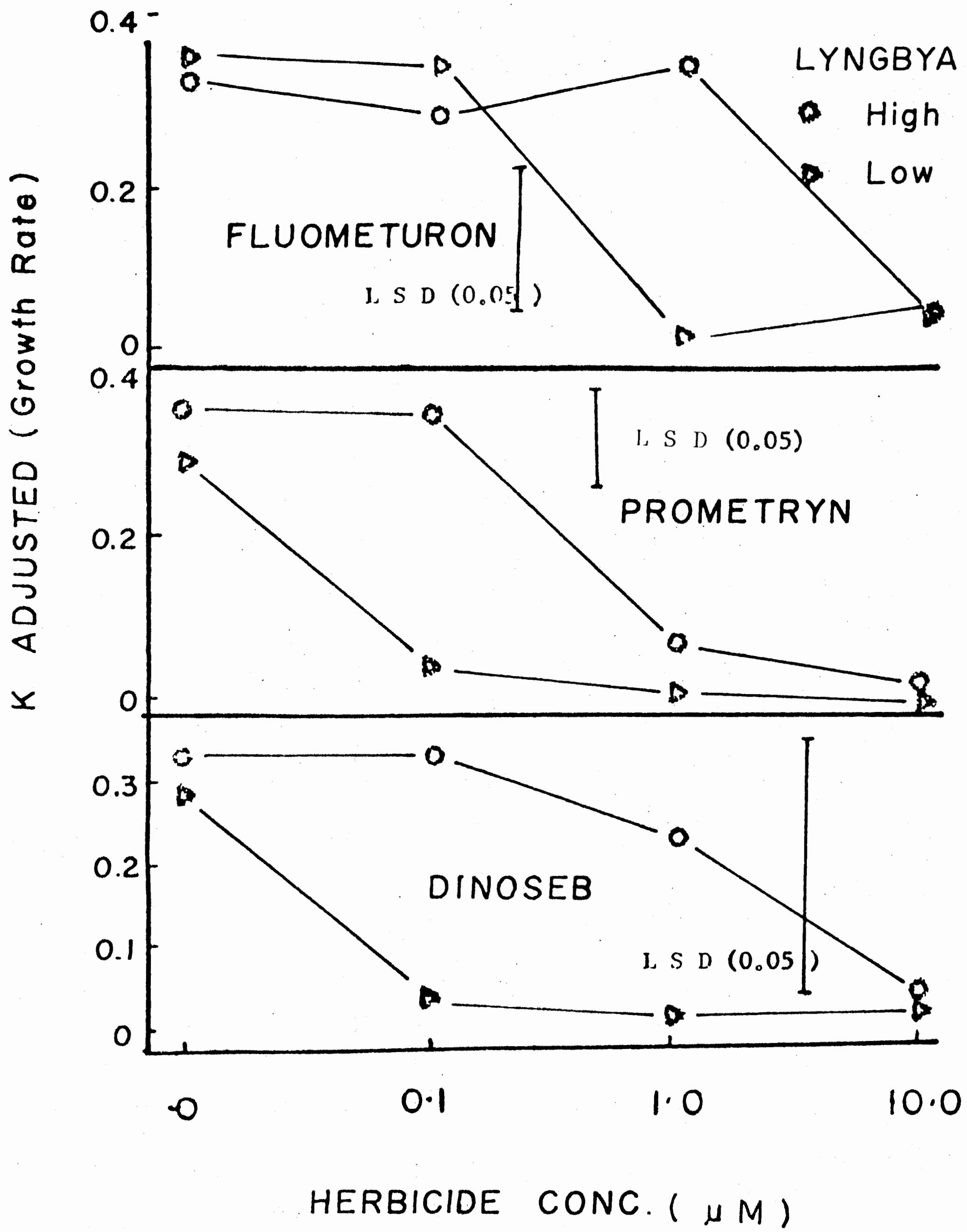

Figure 6. The Influence of K and Herbicides on Growth Rate of Lyngbya

be more effective on Lyngbya at low K. Dinoseb was toxic to Lyngbya at about the same level as fluometuron. Dinoseb and fluometuron showed significantly different effects on the growth of Chlorella where only fluometuron reduced growth. As was the case for prometryn and fluometuron, dinoseb also appeared to have been more active on Lyngbya at low K levels although there were no significant interactions for any of these herbicides and K levels.

The results of the herbicide activity with high and low Ca levels on the two algae showed reduction of growth with herbicide concentrations (Figures 7 and 8). As in previous results, prometryn inhibited the growth of Chlorella and Lyngbya at low concentration (0.1μM). The growth rates for the two algae were statistically different due to the prometryn. Between the two algae, Chlorella was more sensitive to prometryn than Lyngbya. Significant difference in growth due to Ca levels occurred only in Lyngbya. The higher levels of Ca caused a slightly higher growth response in Lyngbya. Fluometuron inhibited the growth of Chlorella and Lyngbya but at higher concentration (1μM) than prometryn. Fluometuron treatments caused significant differences in both algae while levels of Ca caused significant difference only with Lyngbya. Fluometuron as well as prometryn appeared to be about equally active at low or high Ca levels. Dinoseb was even less effective than fluometuron on the growth of Chlorella. Dinoseb significantly reduced only the growth of Lyngbya. All three herbicides were about equally inhibitory to algae at low and high Ca. There was a significant interaction between herbicides and Ca levels only for prometryn and Lyngbya whereas prometryn was apparently more toxic at the low Ca level.

The effects of the two Mg levels and herbicide concentrations on

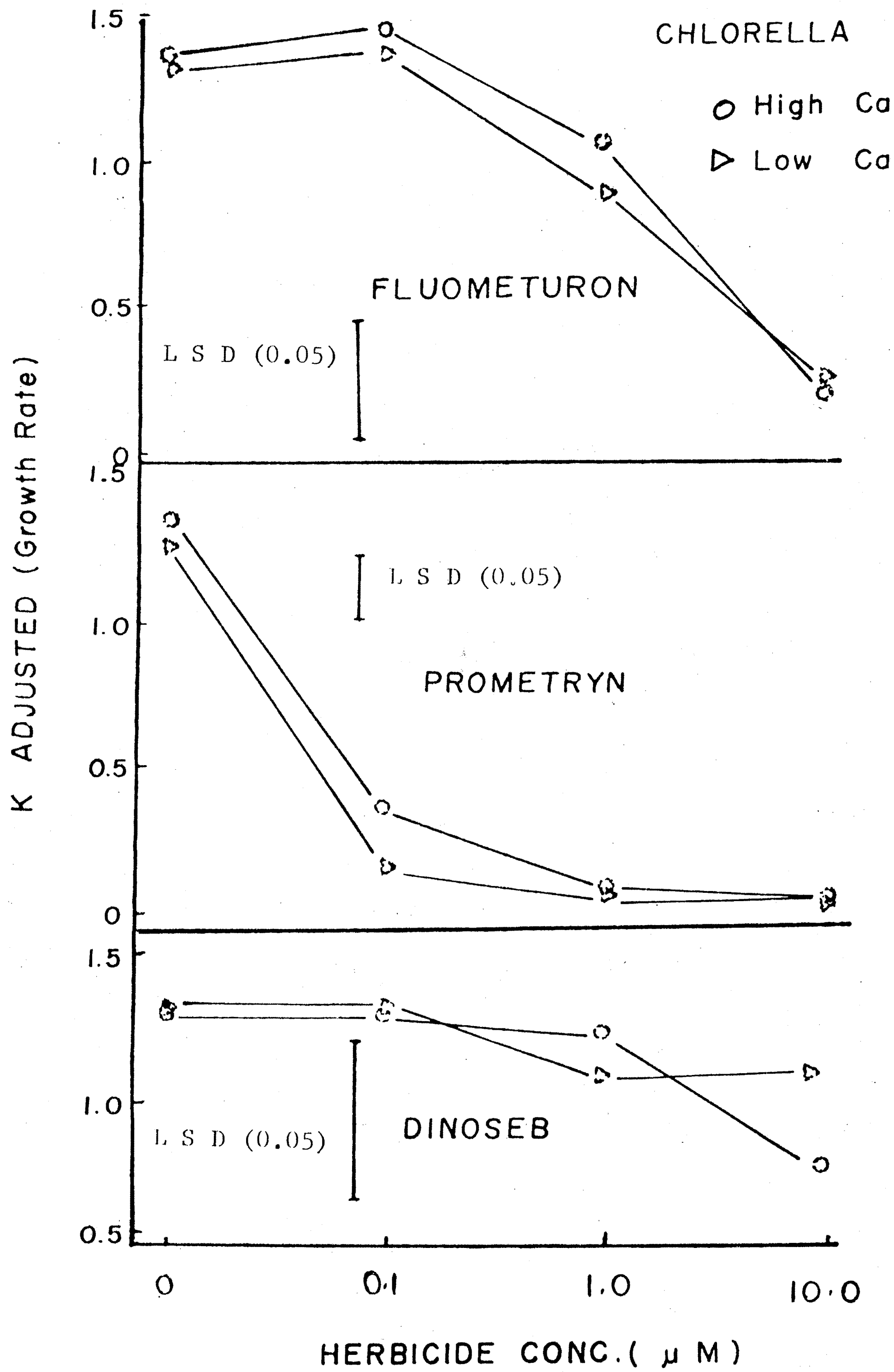

Figure 7. The Influence of Ca and Herbicides on Growth Rate of Chlorella

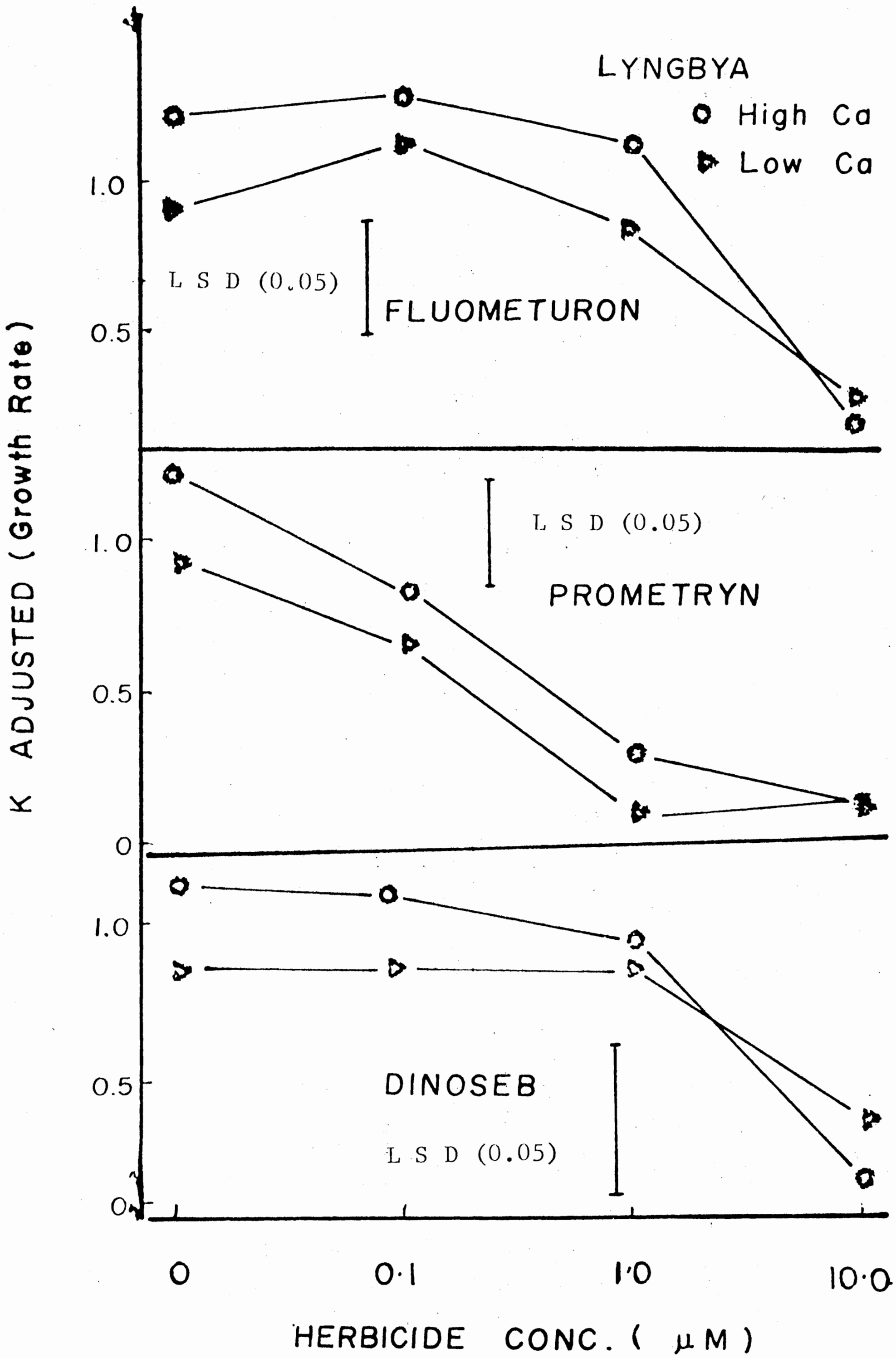

Figure 8. The Influence of Ca and Herbicides on Growth Rate of Lyngbya

Chlorella and Lyngbya growth (Figures 9 and 10) were very similar to those reported with Ca and herbicide treatments. Again prometryn showed more toxicity than fluometuron and was more active against Chlorella than Lyngbya. The required concentrations to produce inhibition were 0.1 μM for prometryn and 1.0 μM for fluometuron. Fluometuron also may have been more toxic to Chlorella than Lyngbya. There was statistical differences in growth rates due to herbicides only at the higher concentration of 10.0 μM. Dinoseb showed less herbicide activity in reducing growth rates of Chlorella than Lyngbya, even though dinoseb produced significant inhibition of both algae at 10.0 μM. The algae growth were not significantly different at the two Mg levels. The three herbicides appeared to be about equally inhibitory to algae at low and high Mg and showed no significant interactions.

The effects of herbicides at low and high pH are shown in Figures 11 and 12. Lyngbya appeared to be very sensitive to pH. Very little growth occurred at pH 4 while growth was rapid at high pH. Chlorella grew well at low and high pH. The data show prometryn to be the more active herbicide at both low and high pH and it was more active in Chlorella than Lyngbya. Prometryn and fluometuron were both about equally active at low and high pH in Chlorella. In Lyngbya very little growth occurred at low pH so that little inhibition by these herbicides was possible at this pH. An interesting feature of the data is the absence of dinoseb activity in Lyngbya at high pH and also at low pH although the growth rate of Lyngbya was very small at low pH. In Chlorella also there was a lack of activity of dinoseb at high pH. At low pH the herbicide appeared to cause a decrease in growth but the analysis of variance did not show significance at the 0.05 level. Dinoseb was not extremely active against Chlorella

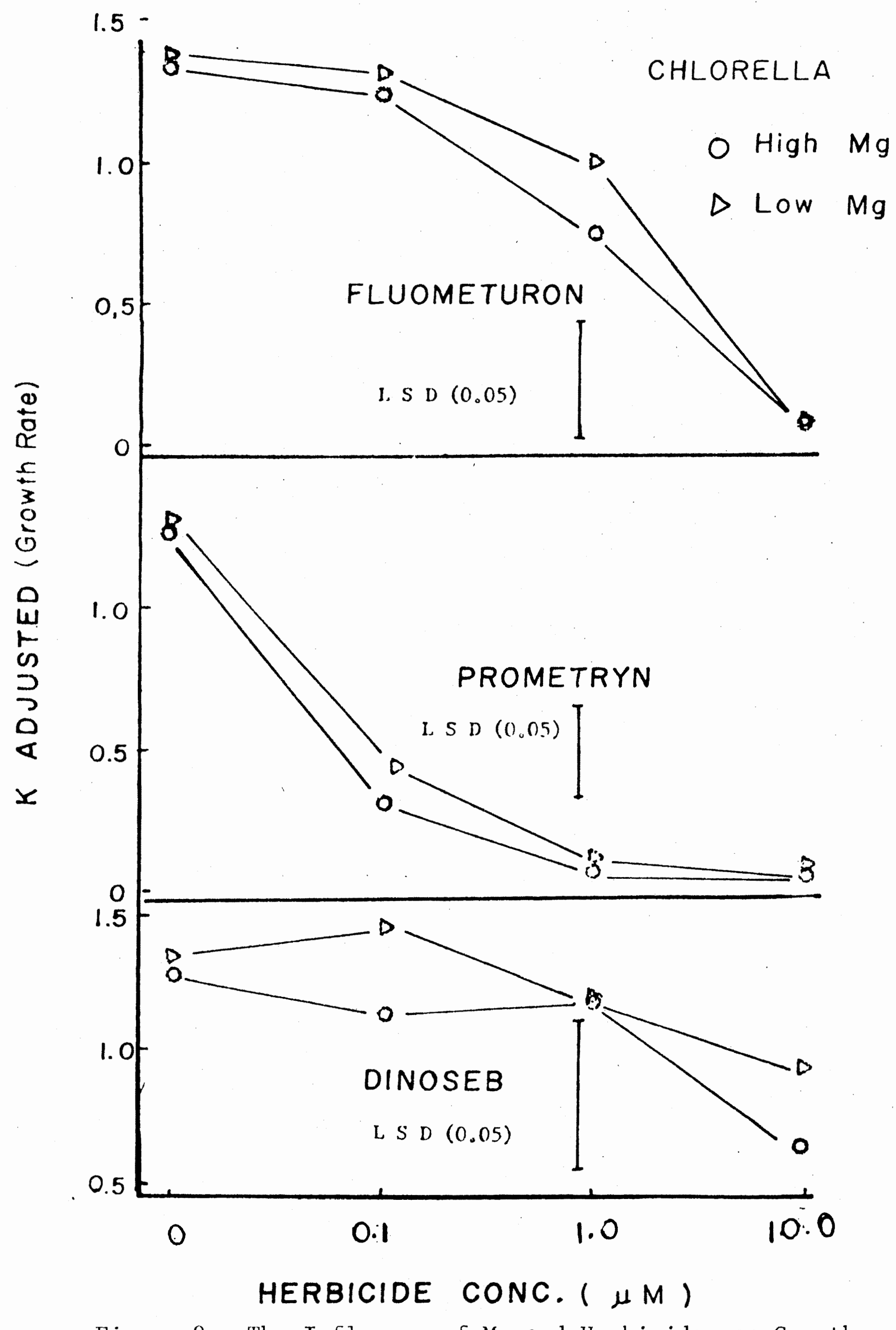

Figure 9. The Influence of Mg and Herbicides on Growth Rate of Chlorella

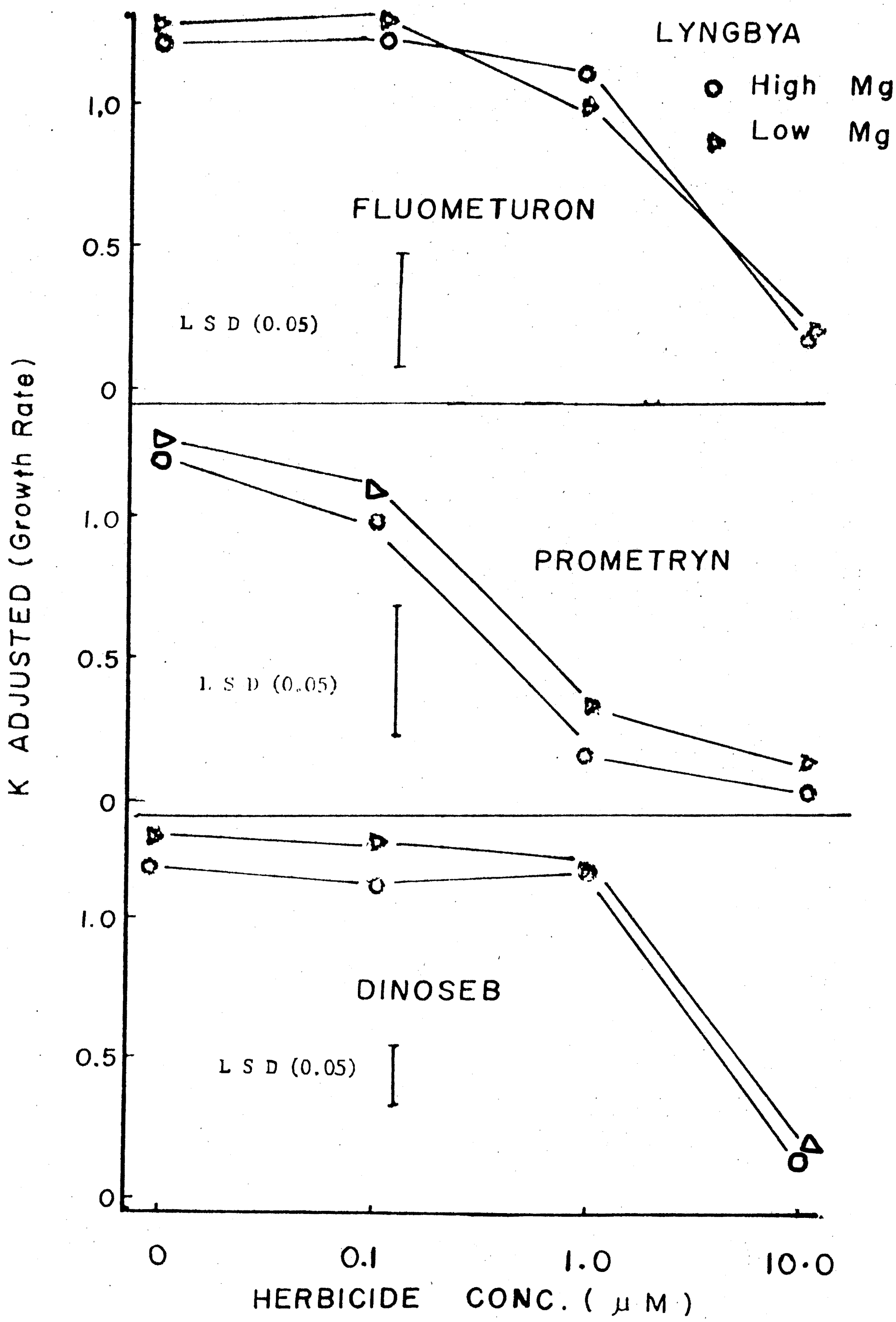

Figure 10. The Influence of Mg and Herbicides on Growth Rate of Lyngbya

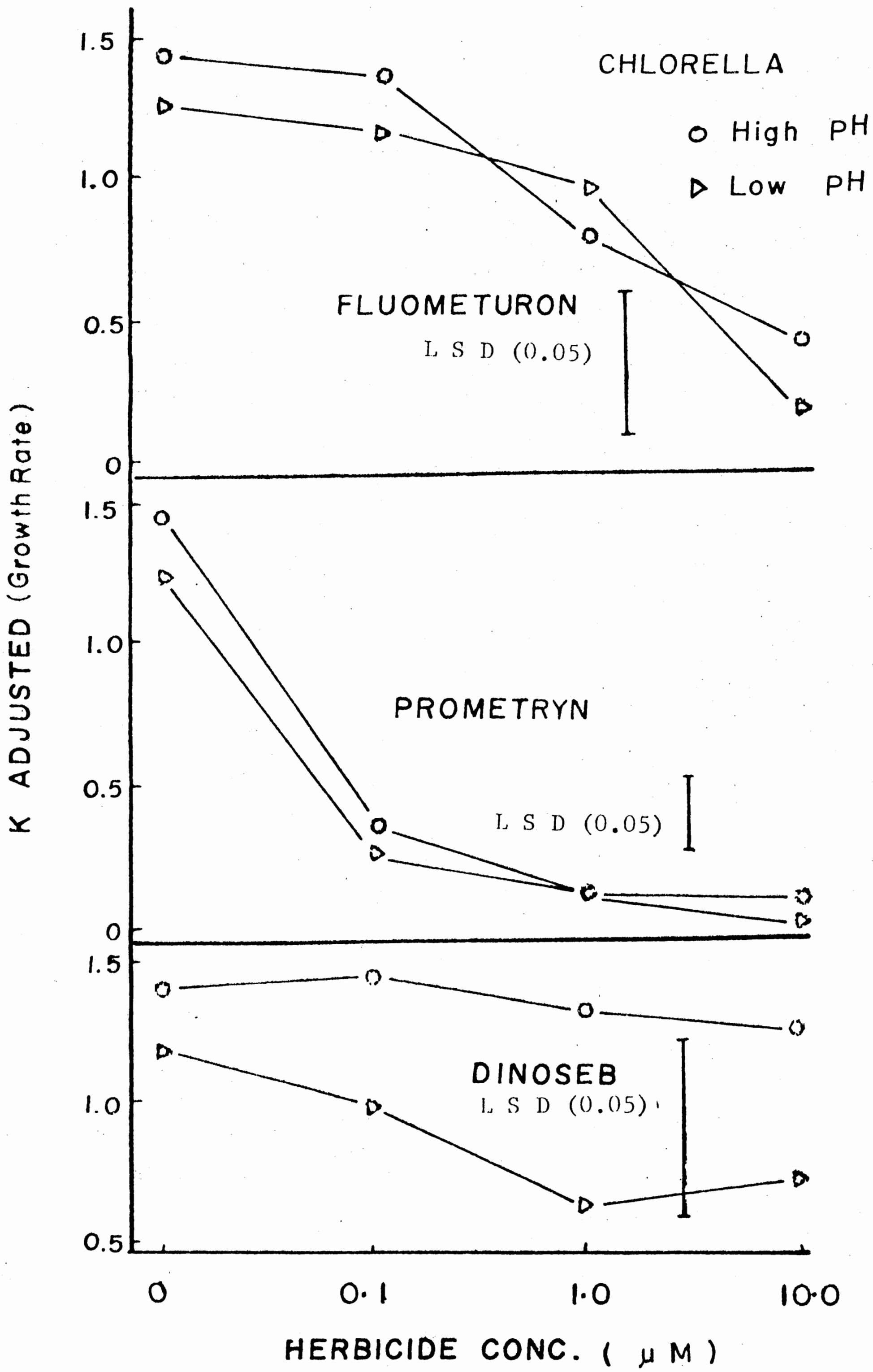

Figure 11. The Influence of pH and Herbicides on Growth Rate of Chlorella

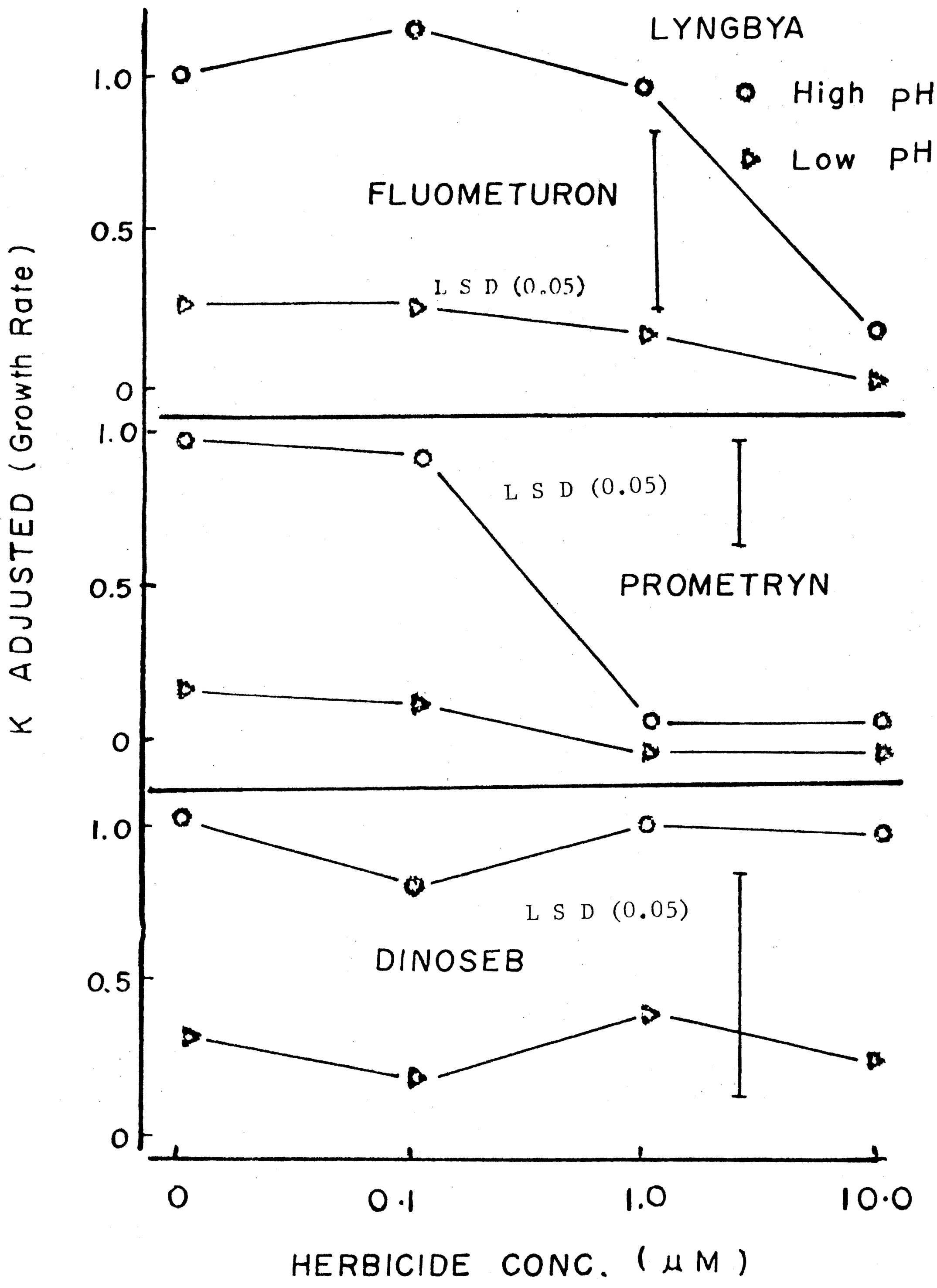

Figure 12. The Influence of pH and Herbicides on Growth Rate of Lyngbya

in some of the other mineral treatments and was completely inactive against Chlorella in high nitrogen. However, dinoseb was active in all of the other mineral treatments in Lyngbya and thus it is surprising that it is not active against Lyngbya at high pH.

The experiment with K and Lyngbya was repeated except that growth measurements were taken not only at 2 days after herbicide treatments but also at 4 days. The data are shown in Figures 13 and 14. The previous experiment (Figure 6) indicated that all three herbicides may have been more toxic at low K levels although there were no significant interactions revealed by the statistical analysis. In the present experiment (Figure 13), the same trends for growth inhibition at 2 days may have been evident for prometryn and fluometuron but again they were small and not significant, and no such trend was seen for dinoseb. At 4 days of growth (Figure 14), there were trends indicating that the herbicides were all more toxic to Lyngbya at high K levels.

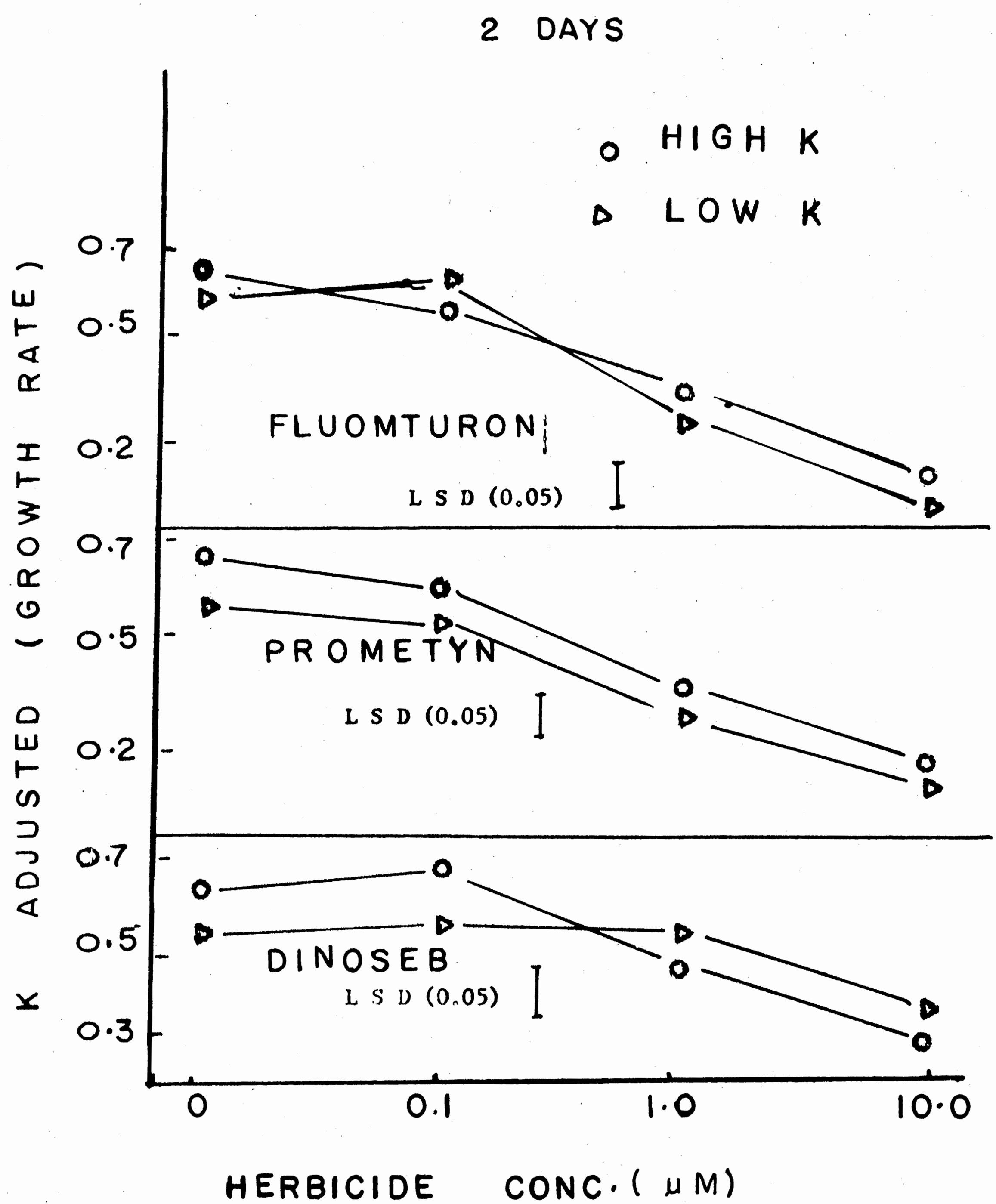

Figure 13. The Influence of K and Herbicides on Growth Rate of Lyngbya for 2 Days

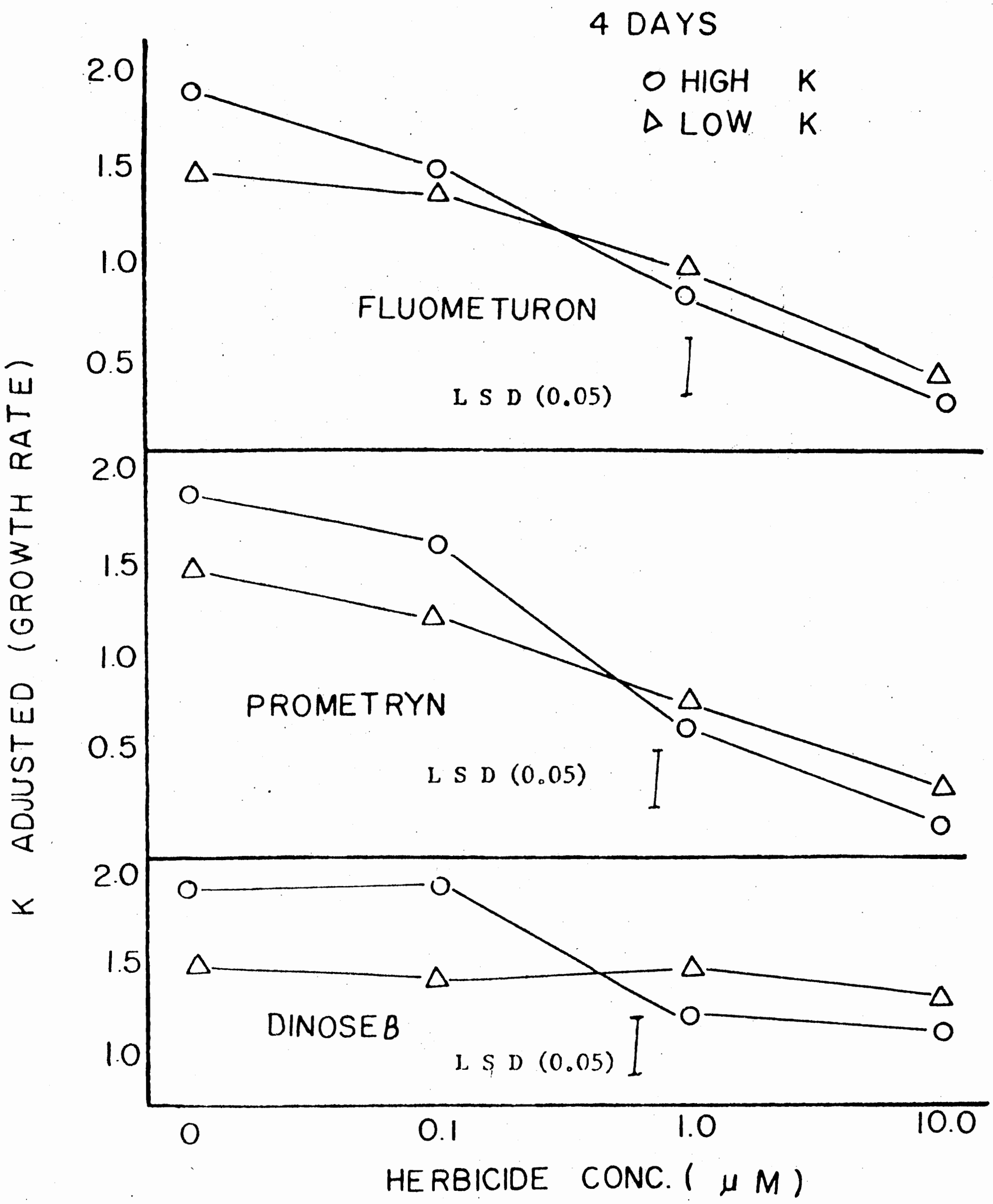

Figure 14. The Influence of K and Herbicides on Growth of Lyngbya for 4 Days

CHAPTER V

DISCUSSION

In comparing the data of all nutrients and pH levels it is apparent that prometryn was more toxic to Chlorella and Lyngbya than fluometuron and dinoseb, and was more toxic to Chlorella than to Lyngbya. Fluometuron appeared to be more toxic than dinoseb and was about equally toxic to both algae. In contrast, dinoseb was more toxic to Lyngbya than to Chlorella in all mineral treatments but was not toxic to Lyngbya or Chlorella at high pH. Picloram was not inhibitory to growth under the conditions used in these experiments.

One of the major objectives of the research was to determine whether herbicides may show extraordinarily high or low activity at unusual (high or low) nutrient or pH levels. The "Statistical Analysis System" (SAS) used in these experiments was designed by Barr and Goodnight (7) and determined by analysis of variance not only herbicide and nutrient effects on growth but interactions between herbicides and nutrient levels as well. Significant interactions should indicate either excessive or low toxicity due to mineral levels. The data revealed a number of significant interactions. This was particularly true of N levels where all three herbicides (prometryn, fluometuron and dinoseb) showed significant interactions in Lyngbya and for prometryn and fluometuron in Chlorella growth. This would indicate that the herbicides are more toxic at high N levels. One factor that tends to negate such a conclusion is that growth of Lyngbya was very low in low N and thus there was only a small possible growth inhibition by herbicides. This was not necessarily true for Chlorella where there was considerable growth at low N and it appears that toxicity of prometryn and fluometuron were higher at high N. This is a point which needs further work. A point of interest is that dinoseb did not show toxicity at all in the presence of high N. This herbicide does have a different mechanism of action than prometryn and fluometuron, and this may account for its difference in response to high N. It is possible that the amount of N applied was so low that the N was immediately depleted from the medium and thus reduced growth. The most common N concentration in natural waters was estimated to be about 1.06 ppm and any additional N was found to increase growth rates of aquatic plants while levels at 0.11 ppm greatly reduces growth (16). A number of researchers have reported effects of N levels on the herbicide effects on growth of higher plants (4, 8, 13, 35). One possibility is that high N may enhance herbicide uptake and thus promote toxicity. Ammonium nitrate was shown to enhance uptake of 2,4,5-T in tree leaves (13) and other studies (4, 8, 13) have shown that N salts such as NH_4CL, $NH_4SO_4$and NH_4NO_3 enhanced the effectiveness of herbicides. Studies by Reis (34) showed that low levels of triazine herbicides often stimulate growth and protein content, especially in plants grown on low N. Low levels of prometryn were not noted to stimulate growth of algae at low N levels in the present experiments.

The only other nutrient condition which showed a significant interaction with herbicides was pH level in Lyngbya treated with prometryn and fluometuron was very close to a significant interaction (Figure 11) indicating that these herbicides may have been more toxic to Lyngbya at high pH. Others have shown pH to be a factor in the toxicity of triazine herbicides to higher plants. Prometryn was shown to be more toxic to cucumber seedlings when added to the nutrient solution at pH 7.5 to 6.5 than when the pH was 4.5 to 5.5. Such an effect may be caused by increased uptake of herbicides such as prometryn at high pH. The triazine herbicides have N atoms which should be capable of ionizing by taking on protons at low pH. Such an ionized herbicide would be less soluble in the lipids of plant membranes and thus would be transported into living cells less readily than the unionized molecule. In the present experiments, growth of Lyngbya was very low at low pH and it is not certain that prometryn was less toxic at this pH since growth was already curtailed by pH. In Chlorella where growth was fairly good at low pH, there was no indication that prometryn or fluometuron was less active at low pH. Chlorella tolerates acidity down to pH 3.4 (12) and this may explain its fairly good growth rate at pH 4 in the present experiments. Lyngbya may have a higher pH requirement and this may explain why it has a lower growth rate than Chlorella at the low pH in the present experiments.

One of the interesting pH effects was the lack of dinoseb activity in Chlorella or Lyngbya at high pH. Dinoseb is similar to the well-known phosphorylation uncoupler, 2,4-dinitrophenol (DNP) which is thought to act by allowing pH gradients to collapse (4). Perhaps high pH in the nutrient solution may counteract such a pH effect of dinoseb and thus lower its activity against growth of Chlorella and Lyngbya. However, dinoseb was not very active against Chlorella even at low pH.

Some herbicides such as 2,4-D contains carboxyl groups which ionize at high pH. After ionization these molecules are less permeable in the lipid membranes and thus are taken up less at high pH values. Wedding and Erickson (44) showed 2,4-D to be taken up in Chlorella more at low pH.

Even though statistically not significant, each herbicide appeared to be more effective in reducing growth rates of Lyngbya at low K (Figure 6). Upon repeating this experiment (Figure 13) similar trends were noted for prometryn and fluometuron although the effects were small and no such trends were noted for Chlorella. Bingham and Upchurch (12) reported that high K rates reduced diuron effects more than lower rates of K. Possibly high K may reduce herbicide effects. At 4 days of culture, the herbicides appeared to be more toxic at high K (Figure 14). Perhaps high K reduces herbicide toxicity only during the early phases of algae growth. Generally, K levels alone in the growth media did not cause significant changes in growth rates of Chlorella or Lyngbya. It was noted that addition of K above the sufficiency level (0.1 mg/liter) did not cause any growth changes in cotton (*Gossypium hirsutum* L.) and ryegrass (*Lolium perenne* L.) (11). This sufficiency level was well above the low level of K which was used in this experiment. Therefore, changes would not be anticipated if algae are to be compared with cotton and ryegrass.

The lack of significant difference between the growth of the two Ca levels on Chlorella and also Lyngbya except in the prometryn treatment could be attributed to the generally accepted idea which is that Ca is not one of the major essential elements for algae growth, particularly for Chlorella. It was pointed out that, even if Ca is omitted from some media, it would have little or no effect on algae growth. Morever, it was indicated that Ca and Mg are widely interchangeable (20). Thus Ca levels tested in this research could be affected by the presence of Mg in the media and vice versa. Among the three herbicides used with Ca levels, prometryn showed the greatest algaecide effects followed by fluometuron and dinoseb which showed the least effect. There were no significant Ca level and herbicide interaction. The combination of 2,4-D with Ca increased 2,4-D toxicity on plants whereas Ca combination with diquat decreased the toxicity on plants (41). Depending upon the type of herbicide which was combined with Ca, it appeared that Ca could enhance or retard herbicide activity.

No significant differences were observed on the growth rates of Chlorella and Lyngbya when combined with Mg levels. As mentioned before, Mg and Ca are widely interchangeable in algae growth, thus the reduction of Mg level from the media could be supplemented or compensated by Ca and the effect due to Mg levels did not show up. Brenchley and Appleby (14) showed more atrazine toxicity on Mg deficient tomato (Solanum esculentum) plants treated with continuous light. It was speculated that Mg was active in preventing chlorophyll destruction and did not directly affect atrazine toxicity. Algae growth at high Mg generally showed lower growth rates than the low levels of Mg although they were not significantly different. Thus the high Mg levels could not be regarded as a protective mechanism against herbicides in the present work.

There were no significant interactions of P levels and herbicides (Figures 1 and 2). Dhillon (18) had studied the interaction of simazine and P and showed that low concentrations of 5 to 10 ppm of simazine with P fertilization increased the growth of red pine seedlings while higher concentrations of 15.0 to 20.0 ppm depressed growth. Prometryn, the triazine herbidide, used in the present work did not enhance growth of algae at the lowest concentration used. There were only inhibitions of growth of Lyngbya and Chlorella although dinoseb and possibly fluometuron may have slightly enhanced growth of Lyngbya at the 0.1 μM concentration.

Chu (16) postulated that neither the highest nor the lowest rates of P recorded in ordinary freshwater bring about changes in algae growth. On the other hand, the presence of herbicides might have induced a detrimental effect on the P uptake which in turn could reduce the growth rates. This was found to be so in higher plants. Herbicides such as amitrol, diuron, simazine, and trifluralin were found to interfere with P uptake (1, 11, 15, 39). This could be one reason why these herbicides and the ones used in this work became toxic and suppress plant growth and algae growth respectively.

The statistical results which are indicated in Table III are all at the 0.05 level.

TABLE III

RESULTS OF THE STATISTICAL ANALYSIS (AOV)

Algae	Herbicide	Factor	Ca F Value	Ca Prob.>F	K F Value	K Prob.>F	Mg F Value	Mg Prob.>F	N F Value	N Prob.>F	P F Value	P Prob.>F	pH F Value	pH Prob.>F
Lyngbya	Dino.	H-L	13.745	0.000	1.890	0.162	84.043	0.000	10.121	0.000	3.714	0.027	0.300	0.827
		M-L	0.349	0.567	2.662	0.114	0.458	0.513	42.217	0.000	0.990	0.667	21.988	0.000
		H*M	1.268	0.311	0.502	0.689	0.592	0.631	8.041	0.001	2.674	0.073	0.328	0.807
	Fluo.	H-L	17.112	0.000	0.719	0.555	36.173	0.000	12.014	0.000	2.202	0.117	3.736	0.027
		M-L	2.634	0.116	0.012	0.911	0.041	0.835	107.315	0.000	0.111	0.742	16.883	0.001
		H*M	0.451	0.723	1.221	0.327	0.255	0.859	10.384	0.000	0.010	0.998	1.250	0.317
	Prom.	H-L	14.137	0.000	1.624	0.213	40.720	0.000	14.476	0.000	2.150	0.123	8.801	0.001
		M-L	7.365	0.013	0.625	0.556	1.551	0.225	32.768	0.000	1.190	0.288	28.660	0.000
		H*M	2.991	0.053	0.627	0.609	0.101	0.958	8.541	0.001	0.654	0.592	6.525	0.003
Chlorella	Dino.	H-L	1.123	0.363	3.201	0.044	2.936	0.056	2.612	0.077	1.308	0.298	0.594	0.629
		M-L	0.014	0.905	3.167	0.086	1.524	0.229	25.390	0.000	6.066	0.021	7.170	0.014
		H*M	0.333	0.804	0.418	0.745	0.377	0.773	0.175	0.912	0.222	0.881	0.466	0.713
	Fluo.	H-L	34.632	0.000	19.396	0.000	34.377	0.000	21.505	0.000	25.073	0.000	12.570	0.000
		M-L	0.071	0.788	0.054	0.813	0.095	0.759	31.699	0.000	0.114	0.738	0.752	0.600
		H*M	0.407	0.752	1.532	0.234	0.011	0.998	5.286	0.007	0.097	0.960	0.414	0.748
	Prom.	H-L	93.204	0.000	63.237	0.000	54.895	0.000	44.704	0.000	57.736	0.000	80.158	0.000
		M-L	0.324	0.581	0.779	0.609	0.076	0.781	8.154	0.009	0.620	0.555	1.964	0.173
		H*M	0.673	0.581	0.547	0.659	0.268	0.849	6.534	0.003	0.331	0.805	0.439	0.731

Dino. = Dinoseb
Fluo. = Fluometuron
Prom. = Prometryn

H-L = Herbicide level
M-L = Mineral level
H*M = Herbicide-mineral interactions

CHAPTER VI

SUMMARY AND CONCLUSIONS

Among the four herbicides tested, prometryn concentrations at 0.1, 1.0, and 10.0 μM showed the most algaecide effects. The 10.0 μM concentrations were most effective, drastically reducing growth rates. Fluometuron showed less algaecide effects than prometryn. Fluometuron concentrations at 1.0 and 10.0 μM also strongly reduced growth rates of algae. Dinoseb concentrations showed much lesser effects than either fluometuron or prometryn with 10.0 μM concentration causing some growth reduction. Picloram produced no significant difference on growth rates of algae.

In general, N and pH levels showed the most significant differences due to the levels used. Higher growth rates were obtained with higher levels of N and pH. Ca and Mg showed lesser statistical difference on growth rates of algae than N and pH. Higher Ca levels produced relatively higher growth rates than the low levels of Ca which caused some significant difference on Lyngbya growth. Mg showed just the opposite of Ca, that is to say, higher growth rates were noted with low Mg levels even though the growth difference did not show any statistical difference. K levels used in this research did not show any significant difference on growth rates of algae. P behaved more or less the same as K except with dinoseb on Chlorella where significant difference due to P levels were observed. Of the two species of algae used in this research, Lyngbya was more susceptible to the herbicides.

Significant interactions among herbicides and nutrient or pH levels were found for N, pH and, to an extent, Ca with Lyngbya. The results of this research can help future studies concerning the interactions of herbicides and nutrient elements and their different levels in Oklahoma natural waters. For algaecide purposes, prometryn and fluometuron at high concentrations (10.0 μM) were most effective.

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VITA

Bashir Ibrahim Tubea

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECTS OF NUTRIENT, pH AND HERBICIDE LEVELS ON ALGAL GROWTH

Major Field: Soil Science

Biographical:

Personal Data: Born in Borama, Somalia, East Africa, in November, 1944, the son of Ibrahim Tubea and Saeda Mead.

Education: Amoud Secondary School, Borama, Somalia, 1962-1966, General Certificate of Education Degree; University of Wyoming, Laramie, Wyoming, 1968-1972, Bachelor of Science Degree in Soil Science; University of Connecticut, Storrs, Connecticut, 1972-1974, Master of Science in Soil Science; completed the requirements for the Doctor of Philosophy Degree at Oklahoma State University in July, 1979.

Professional Experience: Research associate at Langston University, working with the interaction of nutrient elements with herbicides on algae growth.