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FINAL REPORT

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"Biodegradation of rocket propellent waste, ammonium perchlorate"

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PRINCIPAL INVESTIGATOR — DR. SYED M.Z. NAQVI

CO-INVESTIGATOR — DR. ABDUL LATIF

TECHNICAL ADVISOR — MR. WILLIAM WOLVERTON
NASA N.S.T.L.
Bay St. Louis, Mississippi

RESEARCH ASSISTANT — Mr. Daniel Malone - Undergraduate
(September 1, 1975 to May 31, 1976)

PARTICIPATING INSTITUTION — Alcorn State University
Department of Biological Sciences
Lorman, Mississippi 39096

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During the year 1974-'75, we made an effort to study the short term effects of ammonium perchlorate on selected organisms. A long-term experiment was set up at the NASA National Space & Technological Laboratories, Bay St. Louis, Mississippi. This was designed to assess the changes incurred by ammonium perchlorate in nitrogen and chloride contents of soil within a period of 3 years. Another facet of our work slightly diverged from ammonium perchlorate biodegradation. An attempt was made to produce methane gas from anaerobic fermentation of aquatic weed, Alternanthera philoxeroides (Mart.) Griesb. This report consists of the following:

I. SHORT-TERM EFFECTS OF AMMONIUM PERCHLORATE

A. Percent germination and growth of wheat.

B. Percent germination and growth of cotton.

C. Percent germination and growth of rye grass.

D. Total biomass determination of rye grass.

E. Growth of Chlamydomonas sp. upto 1164 hours.

F. Growth of Escherichia freundii upto 192 hours.

G. Growth of Bacillus proteus upto 192 hours.

H. Growth of Azotobacter chroococcum upto 192 hours.

II. LONG-TERM EFFECTS OF AMMONIUM PERCHLORATE

Nitrogen and chlorine determinations of soil upto a period of 3 years.

pH determinations of soil samples upto 2 years.

III. BIOGAS PRODUCTION FROM ALLIGATOR WEED Alternanthera philoxeroides.

The fact that no research work is documented on effects of ammonium perchlorate, literature review did not yield any useful information. Work reported in this paper can virtually be considered as pioneer investigations on ammonium perchlorate bigodegradation and its short and long-term effects. Detailed data are represented by tables and graphs. Record of raw data are maintained in a bound note-book. Short description of the methodology used is also made. Results and conclusions are given for each sub-title. Manuscripts are under preparation which will be sent for publication in THE JOURNAL OF BACTERIOLOGY and CROP SCIENCE. A summary of our work will be sent for THE SPACE AND TECHNOLOGICAL REPORTS, in near future.

A. Percent germination and growth of wheat, Triticum vulgare

Seed germination and growth of wheat were tested by placing 20 seeds in sterilized petri-dish containing Kimpax (sterilized, non-nutritive absorbing material). The seeds were surface-sterilized by immersing them in 0.2% sodium hypochlorite solution for 10 minutes and by several subsequent washing in distilled water. All seeds were equally spaced in the petri-dish. A series of 50 such petri-dishes were divided into 6 groups (5 treatments and a control). Germination and growth of each group was recorded. Total of 966 seeds were thus tested.

Since ammonium perchlorate is highly soluble in water, stock solution was prepared in concentration of 1 percent. Further dilutions were made by serial dilution method. Germination of all seeds was done at room temperature. Percent mortality (or ungerminated seeds), and growth of seedlings was recorded after 120 hours. Data are presented in Tables 1 and 2 and Figures 1 and 2. Average height of seedling and the standard deviation were calculated on a computer. Similar methods were adopted for cotton and rye-grass with minor modifications which will be described with each context. All precautions were taken to provide identical conditions for treated and control seedlings.

Table 1---Average height (cm) and standard deviation of wheat seedlings measured after 120 hours.

Conc. of NH_4ClO_4 in test solutions	Petri dish No.	Ave. Ht. of seedlings in each petri-dish	Ave. Ht. of plants in all petri-dishes of each treat-ment	SD
Control	I	9.9		3.75
"	II	9.26		2.72
"	III	8.60		2.45
"	IV	9.82		3.87
"	V	8.41	9.2	2.32
1 ppb	VI	7.84		2.92
"	VII	9.85		3.69
"	VIII	11.53		4.18
"	IX	8.45		3.15
"	X	10.90		3.64
"	XI	9.13		4.54
"	XII	10.0		3.79
"	XIII	7.54	9.40	3.28
500 ppb	XIV	10.49		3.08
"	XV	9.15		3.48
"	XVI	10.70		3.90
"	XVII	8.72		2.33
"	XVIII	9.68		2.19
"	XIX	10.59		3.58
"	XX	10.20		3.38

Table 1--- Contd.

500 ppb	XXI	8.58	9.76	3.69
1ppm	XXII	9.79		3.21
"	XXIII	10.66		4.74
"	XXIV	9.45		2.23
"	XXV	9.10		4.404
"	XXVI	10.4		2.32
"	XXVII	9.99		1.30
"	XXVIII	8.39		3.37
"	XXIX	9.05	9.60	3.91
10 ppm	XXX	7.62		2.59
"	XXXI	8.14		3.12
"	XXXII	7.99		2.90
"	XXXIII	5.91		1.58
"	XXXIV	5.69		2.53
"	XXXV	7.94		3.48
"	XXXVI	6.82		2.74
"	XXXVII	7.55	7.21	3.28
500 ppm	XXXVIII	4.84		0.812
"	XXXIX	5.75		2.01
"	XL	5.43		0.88
"	XLI	5.59		1.71
"	XLII	5.50		1.69
"	XLIII	5.23		0.86
"	XLIV	5.36		1.00
"	XLV	5.72	6.41	2.18

Table 2--- Percentage of ungerminated wheat seeds grown in treated and untreated soil.

Conc. of NH_4ClO_4 in soil	Total No. of seeds tested	Percentage of ungerminated seeds
Control	160	42.0
1ppb	160	45.6
500 ppb	160	46.2
1ppm	160	40.0
10 ppm	160	38.1
500 ppm	160	37.5

Conclusions (Tables 1, 2; Figures 1, 2)

Interesting results were obtained which are presented in Tables 1 and 2 and Figures 1 and 2. In comparison to control, average seedling growth increased in 1 ppb, 500 ppb and 1 ppm , but it decreased significantly in 10 and 500 ppm treatments. However, contrary to expected results, germination success was greatest in highest treatment (500 ppm) and lowest in 500 ppb treatment. It could be explanied by the fact that the lowest number of seeds germinated in 500 ppb treatment provided more space and nutrients for the later growth of seedlings which resulted in highest average growth. On the other hand, in 500 ppm treatment, the growth of seedlings was inhibited by ammonium perchlorate but maximum number of seeds were able to germinate. Therefore, this compound seems to have its affect in later growth of wheat.

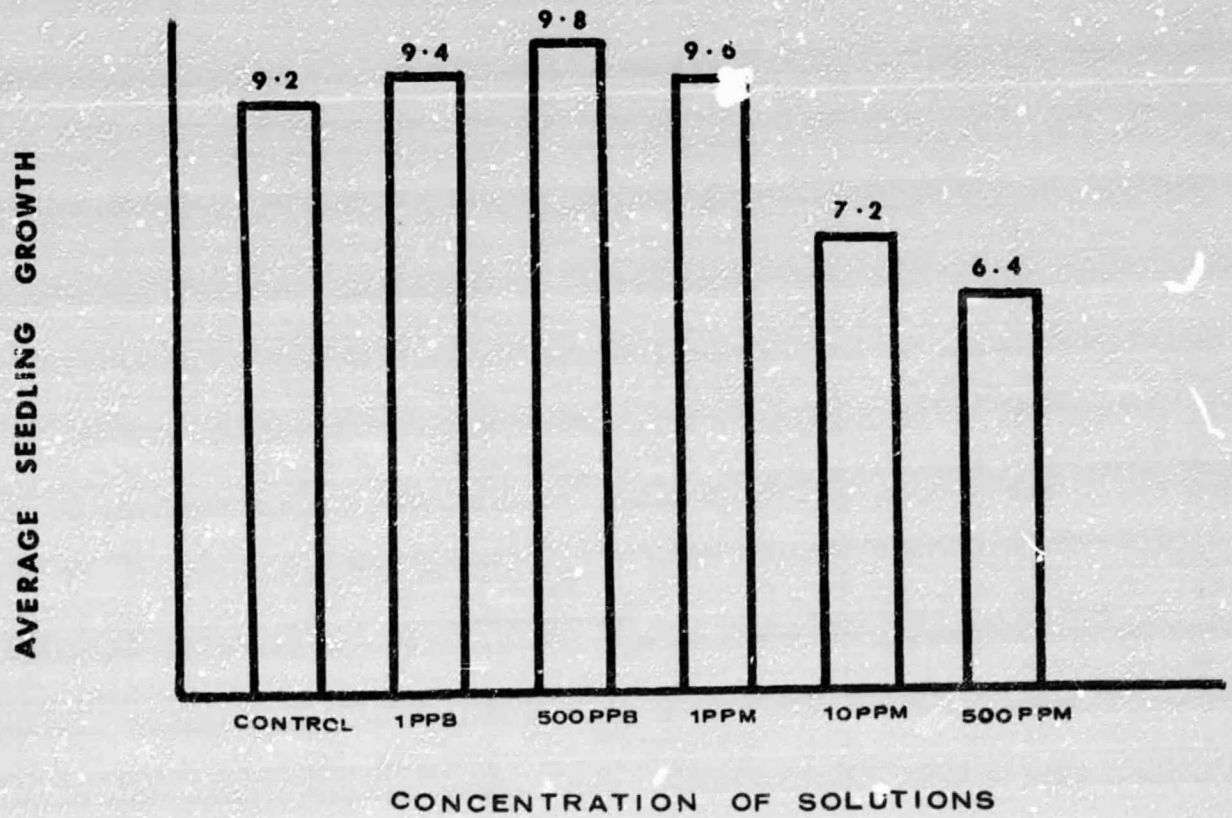


Fig. 1. Average seedling growth of wheat, Triticum vulgare (measured in cm) in various concentrations of ammonium perchlorate.

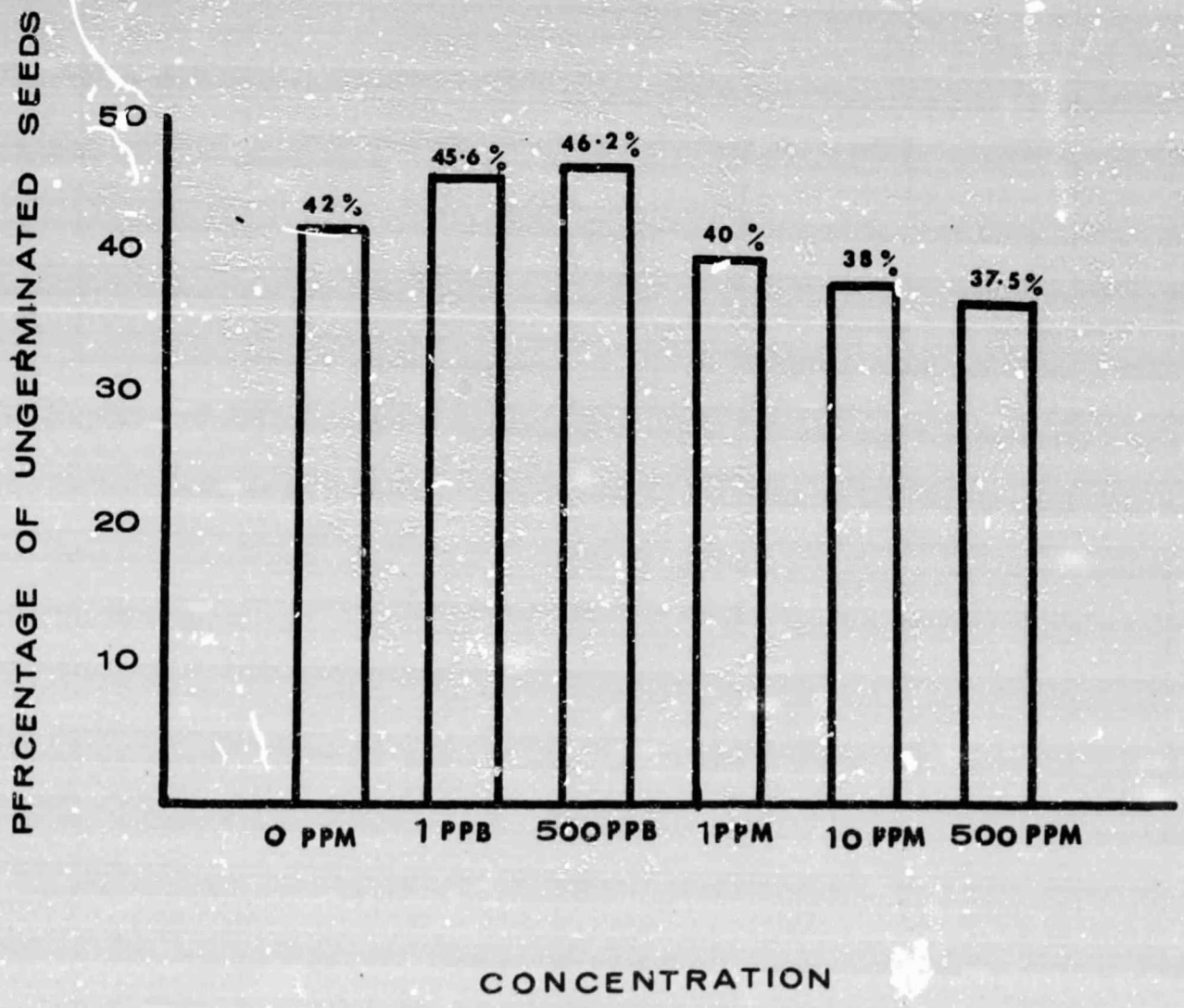


Fig. 2. Percentage of ungerminated seeds of wheat, Triticum vulgare, in various concentrations of ammonium perchlorate.

Cotton: The following table (Table No. 3) represents the percentage of seeds which were unable to germinate. In 55.0 gram treated soil the highest number of seeds were prevented to germinate due to the toxicity of ammonium perchlorate. The percentage of ungerminated seeds in 0.55 g treated soil was unexpectedly lower than control. Soil for germination was brought from field-plots, where initial treatment was made on June 24, 1974, @ 0.55, 5.5 and 55.0 grams of ammonium perchlorate homogeneously mixed with surface soil of 1^2 meter plots. Experimental and control plots were designated on the basis of Randomized Complete Block Design.

Table 3--- Percentage of non-germinated cotton seeds grown in ammonium perchlorate treated and control soil.

Conc. of ammonium perchlorate in soil (Grams/Meter^2)	No. of seeds tested	% of un-germinated seeds
Control	60	25.0
0.55	50	10.0
5.50	50	25.0
55.0	50	56.7

Conclusion: Even after approximately after 2 years of initial treatment, soil has retained its toxicity in those plots which were treated with 55.0 g of this compound. However, there seems to be insignificant difference between the

control and 0.55 and 5.50 gram treatments.

Rye-grass: The soil for germination of rye-grass was also obtained from the experimental plots which were established almost 2 years ago at NSTL, Bay St. Louis, Mississippi. To determine the effect of ammonium perchlorate, growth of seedlings was recorded upto 28 days. At the end of this period, all plants were dried at 80 C for 24 hours and weighed on a Mettler balance. Percentage of un-germinated seeds was also recorded.

Table 4---Percentage of un-germinated rye-grass seeds and total biomass of seedlings determined after 28 days.

Conc. of NH_4ClO_4 in soil/sq.meter	% of un-germinated seeds	No. of seeds tested	Biomass * dry weight in grams
Control	47.0	200	0.324
0.55	41.0	100	0.210
5.50	51.2	100	0.130
55.0	73.0	100	0.040

* Based on total dry weight of 16 individual plants after 6 weeks growth-period.

Conclusion: Basically similar results were obtained for germination of rye-grass. Highest number of seeds germinated in 0.55 treatment and the lowest in 55.0 g treated soil. However, there is a consistent decrease in biomass in direct proportion to increasing concentration of ammonium perchlorate. (Fig. 3, 4).

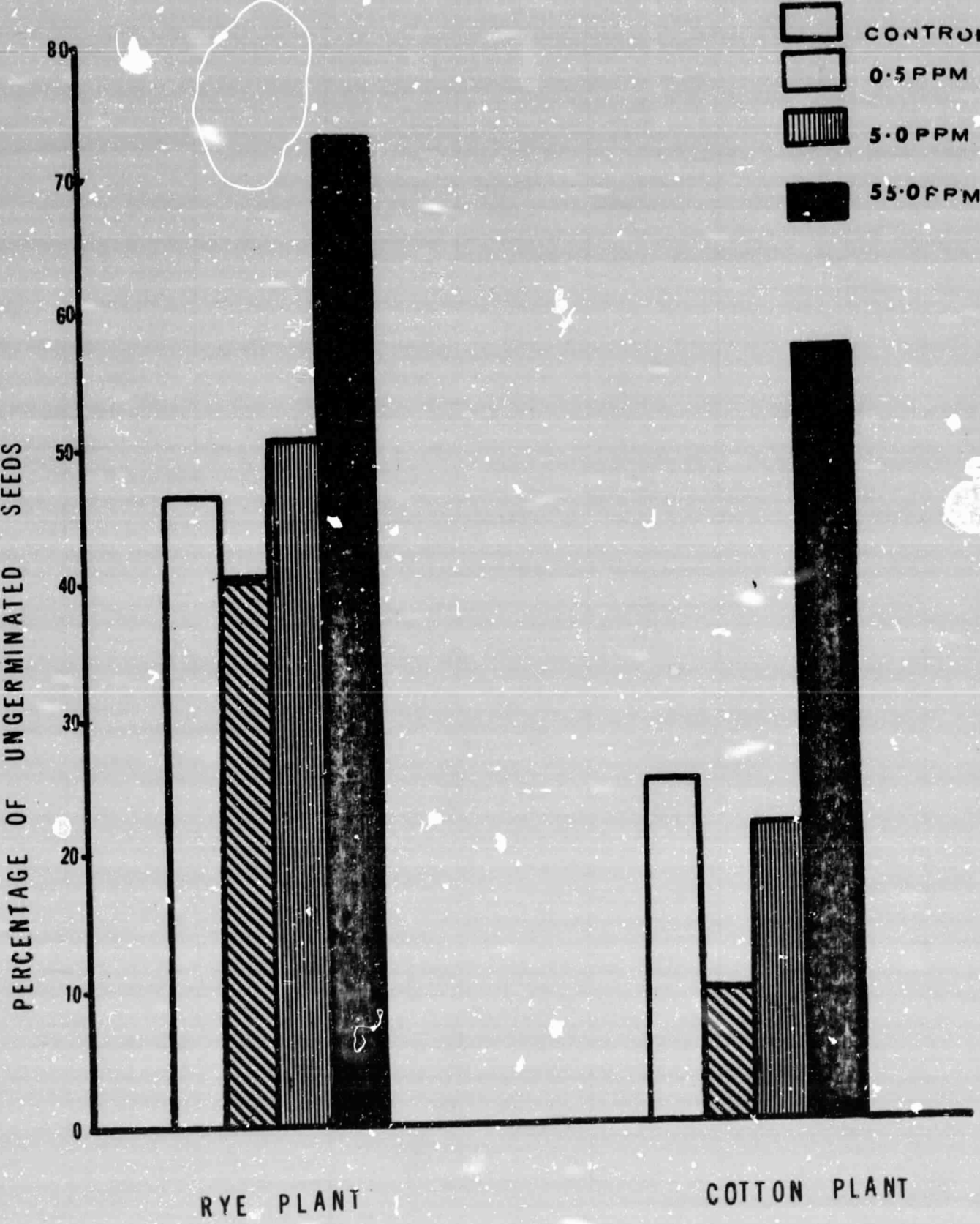


Fig. 3. Percentage of ungerminated seeds of rye-grass and cotton treated with various concentrations of ammonium perchlorate.

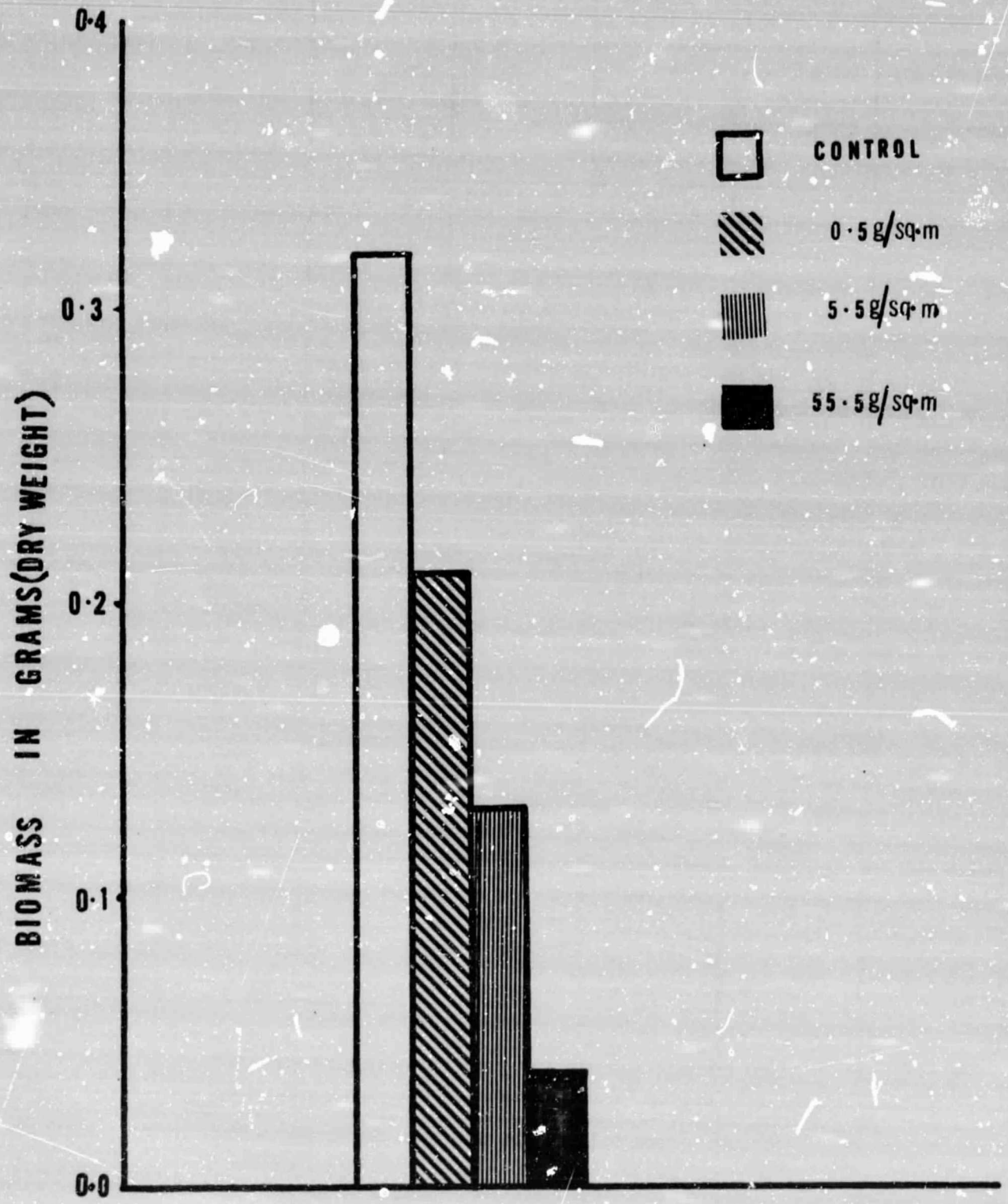


Fig. 4. Total biomass (dry) of rye-grass grown in various concentrations of ammonium perchlorate.

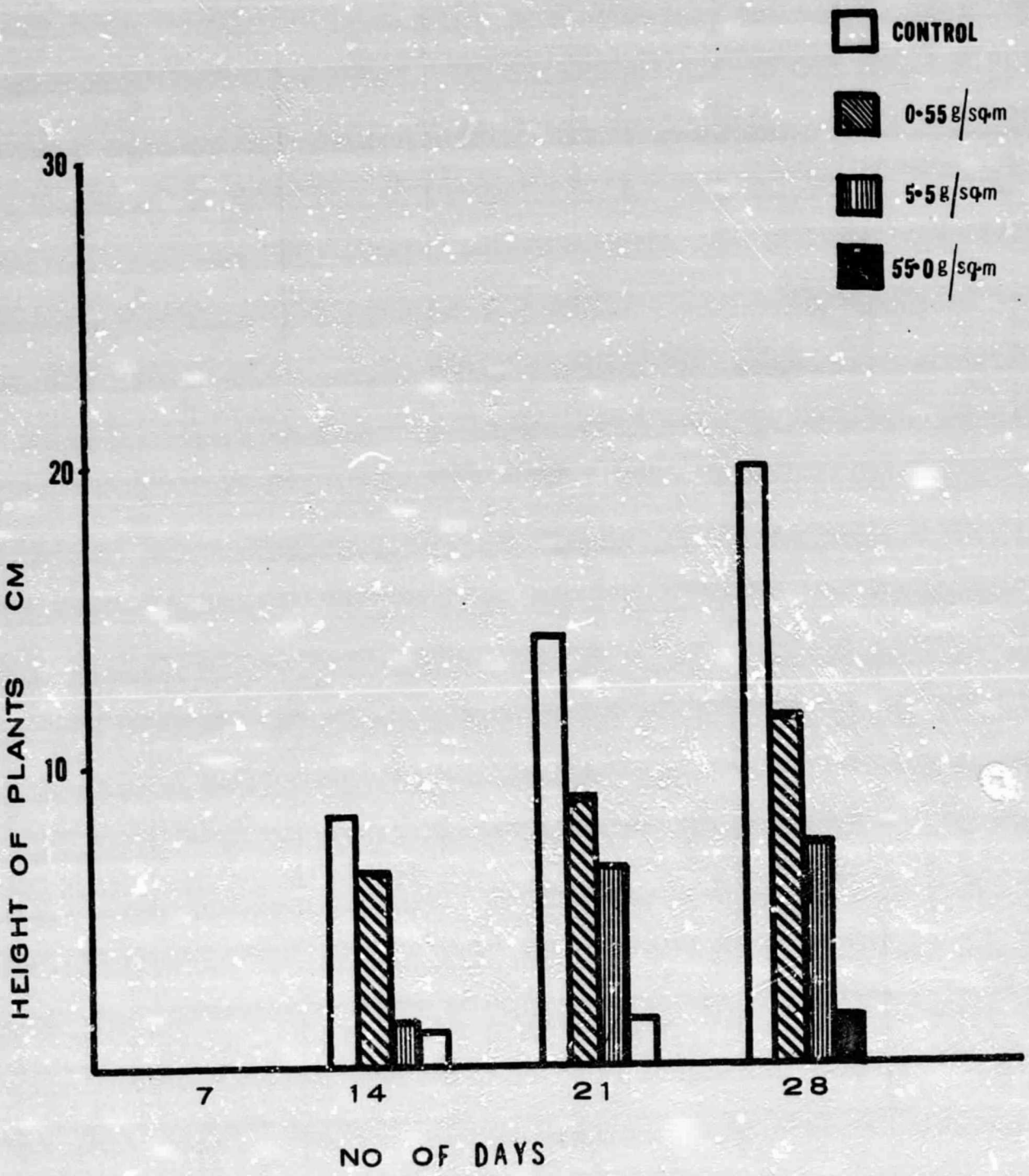


Fig. 5. Growth-rate of rye seedlings treated with various concentrations of ammonium perchlorate.

Table 5---Growth rate of rye-grass measured in centimeters upto a period of 28 days.

NH_4ClO_4 Conc. in soil	Seedling Ht. (Cm.) 14 days	Seedling Ht. (Cm.) 21 days	Seedling Ht. (Cm.) 28 days
Control	8.4	14.2	19.8
0.55	6.4	9.0	11.6
5.50	1.2	6.6	7.4
55.0	1.0	1.5	1.7

Conclusion: Data are fairly apparent in depicting the effect of ammonium perchlorate. There is a marked decrease in the highest concentration of this compound, exhibiting its toxicity retention after two years. (Fig. 5).

Growth of Chlamydomonas

Pure culture of Chlamydomonas was purchased from Turtox Co. Further culture was maintained in Knop's solution at room temperature. The constituents of the medium were mixed together according to Turtox Service Leaflet No. 6 (6-2). Test solution was prepared by diluting 1% stock solution of ammonium perchlorate. No problems were encountered in dissoloving this compound in distilled water since it is highly soluble in it. The cultures were grown in a dust-free atmosphere and the growth of Chlamydomonas was measured on a Bausch & Lomb Spectronic-20 Meter, at 600 nm.

We have reported the results of 96 hour growth in the final report of 1975; where the growth rate of this alga was greater in 1.0 and 10.0 ppb ammonium per-chlorate treatments than the control. We are reporting here, growth of this organism extending to 1164 hours, measured at several intervals. The concentration of ammonium perchlorate in growth medium was 1.0, 10.0, and 100.0 ppb and ppm. This provided a wide range of testing from a very low to a very high level. Although the raw data are maintained for all the above concentrations, 10.0 ppb and ppm have been omitted from Table 6 and Figures 6,7, for the sake of clarity.

Table 6---Growth of Chlamydomonas spp. measured upto 1164 hours in various concentrations of ammonium perchlorate at 600 nm.

Conc. of NH_4ClO_4	Treatment time (Hrs.)	O.D. X 100
Control	0.0	3.94
	143.0	4.43
	192.0	4.91
	236.0	6.39
	336.0	13.08
	357.0	4.58
	405.0	4.58
	432.0	12.50
	454.0	5.93
	831.0	6.58
	1128.0	4.58
	1164.0	4.26
1ppb	0.0	4.91
	143.0	5.24
	192.0	4.58
	236.0	6.57
	336.0	14.47
	357.0	5.38
	405.0	4.12
	432.0	12.69
	454.0	5.23
	831.0	6.58
	1128.0	4.12
	1164.0	4.76
10 ppb	0.0	3.15
	143.0	3.15
	192.0	3.62
	236.0	5.88
	336.0	14.87
	357.0	4.74
	405.0	2.69
	432.0	8.09
	454.0	3.62
	831.0	4.26
	1128.0	2.69
	1164.0	4.10

Table 6 contd---

100.0 ppb	0.0	4.10
	143.0	3.76
	192.0	3.94
	236.0	6.24
	336.0	12.49
	357.0	5.88
	405.0	3.81
	432.0	10.79
	454.0	5.38
	831.0	6.58
	1128.0	3.81
	1164.0	5.73
1.0 ppm	0.0	3.32
	143.0	3.32
	192.0	3.63
	236.0	5.72
	336.0	13.10
	357.0	4.75
	405.0	4.26
	432.0	9.88
	454.0	5.39
	831.0	5.07
	1128.0	4.26
	1164.0	4.76
10.0 ppm	0.0	3.16
	143.0	3.15
	192.0	2.69
	236.0	5.38
	336.0	15.49
	357.0	5.39
	405.0	2.69
	432.0	8.34
	454.0	4.89
	831.0	3.00
	1128.0	2.69
	1164.0	3.31
100.0 ppm	0.0	3.78
	143.0	3.78
	192.0	3.78
	336.0	14.88
	357.0	4.74
	405.0	3.14
	432.0	9.88
	454.0	5.23
	831.0	1.47
	1128.0	3.14
	1164.0	4.42

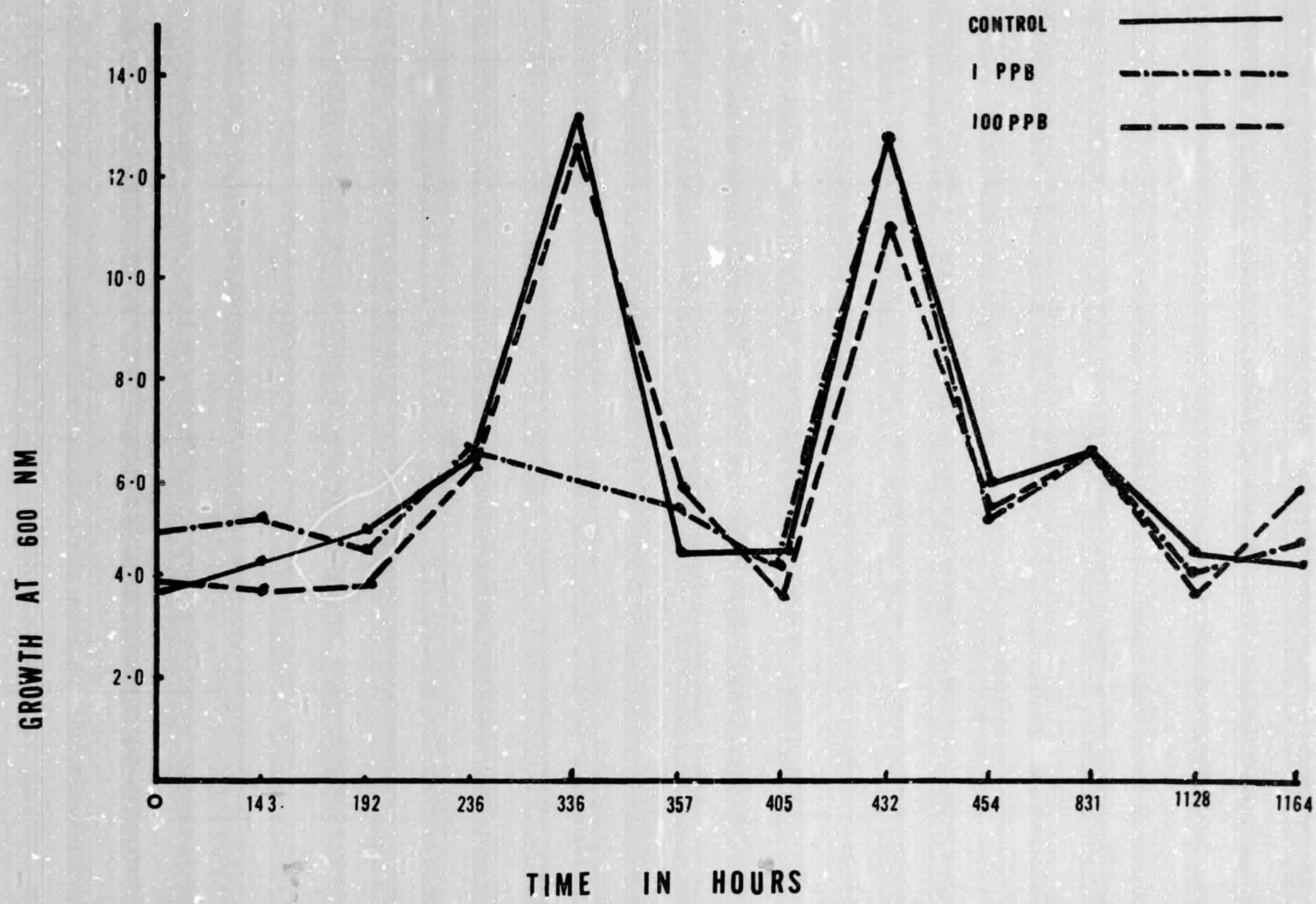


Fig. 6. Growth of Chlamydomonas spp. in various connentrations of ammonium perchlorate.

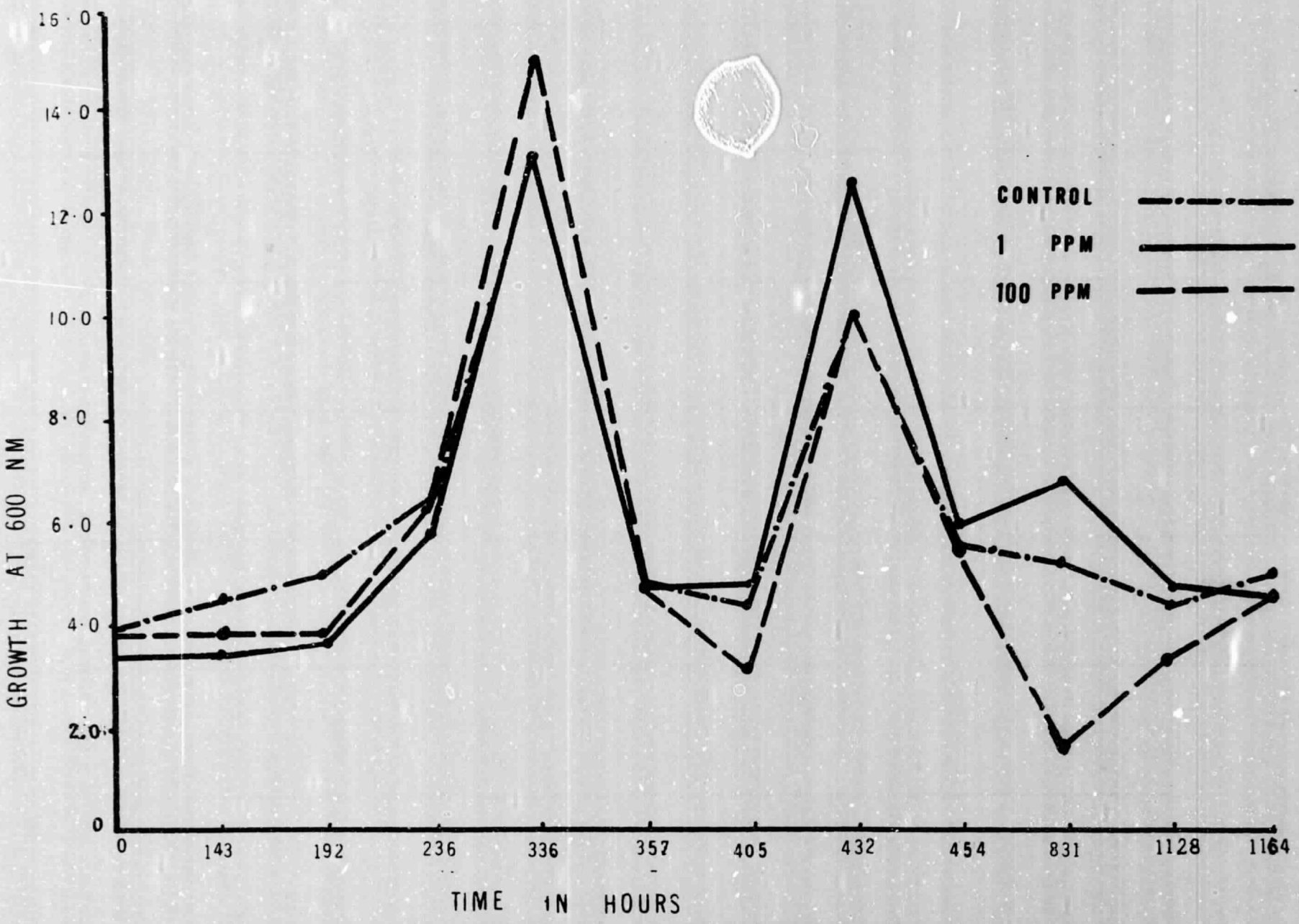


Fig. 7. Growth of Chlamydomonas spp. in various concentrations of ammonium perchlorate.

Conclusions: (Fig. 6,7, Table 6).

1. The growth of Chlamydomonas had 2 peaks at 336 and 432 hours after recording the initial growth.
2. No significant effect is noticed even in 100.0 ppm ammonium perchlorate treatments, except that in the above mentioned concentration, the growth of Chlamydomonas decreased very much in comparison to other treatments. However, at the end of 1164 hours, it was very close to the control.
3. At the end of 1164 hours growth of Chlamydomonas was approximately same in all the treatments, further exhibiting the fact that the compound had no marked toxicity in a long-term period.
4. Due to some unknown reason, growth of Chlamydomonas had only 1 peak in 1.0 ppb treatment, while there were two peaks in rest of the treatments including the control.

Escherichia freundii (Fig. 8, 9, Table 7)

Pure cultures of E. freundii and Bacillus proteus were purchased from Turtox Co. The colonies were transferred to autoclaved liquid media. Desired amounts of ammonium perchlorate stock solution were added subsequently to obtain the required concentrations. The cultures of E. freundii were kept at 38°C and of B. proteus at 24°C. The organisms were acclimatized at these temperatures 48 hours before ammonium perchlorate was added to culture media. The following consituents were

used to make the specified media:

	Amount/100 ml
Potassium phosphate monobasic 0.6%	4 ml
Potassium phosphate dibasic 0.6%	4 ml
Sodium chloride	0.5 g
Ammonium sulfate	0.5 g
Dextrose	0.2 g
Casein hydrolysate	0.2 g
Agar	1.0 g
Distilled water	92.0 ml

Table 7---Growth of Escherichia freundii meausred upto 192 hours at 600 nm (X 100).

No. of hours	Control	50 ppb	500 ppb	50 ppm	500 ppm
		Ammonium perchlorate concentration			
0.0	37.00	26.00	27.00	35.00	36.00
24.0	200.00	122.00	125.00	35.00	110.00
48.0	159.0	111.00	112.00	50.00	98.00
72.0	118.00	100.00	117.00	65.00	86.00
96.0	77.00	89.00	113.00	79.00	75.00
120.0	91.00	97.00	113.00	117.00	115.00
144.0	105.00	105.00	113.00	155.00	156.00
168.0	140.00	117.00	127.00	147.00	147.00
192.0	178.00	130.00	142.00	138.00	138.00

Figures 8 and 9 show growth of E. freundii in a graphical manner.

Conclusions:

1. The first peak of growth in control bacteria occurred at 24 hours; the growth declined upto 96 hours and then increased upto 192 hours.
2. Much shorter peak occurred in 50 and 500 ppb treated organisms at 24 hour. The growth did not show marked difference beyond this period, finishing slightly less than the control.
3. In 50 and 500 ppm treatments, bacterial growth was much reduced in the first 24 hours of incubation. Practically no growth occurred in 50 ppm. However, in both the treatments, another peak of growth occurred at 144 hours, which was not noticed in any other treatment or the control.
4. It is assumed that 50 and 500 ppm levels inhibited the initial growth but later this much amount of ammonium perchlorate increased the bacterial growth, probably by serving as an additional source of nitrogen.
5. The highest amount tested in this experiment (500 ppm) does not seem to be toxic for the growth of E. freundii, but on the contrary seems to benefit these microorganisms which is evident from their growth curve (Figure 9).

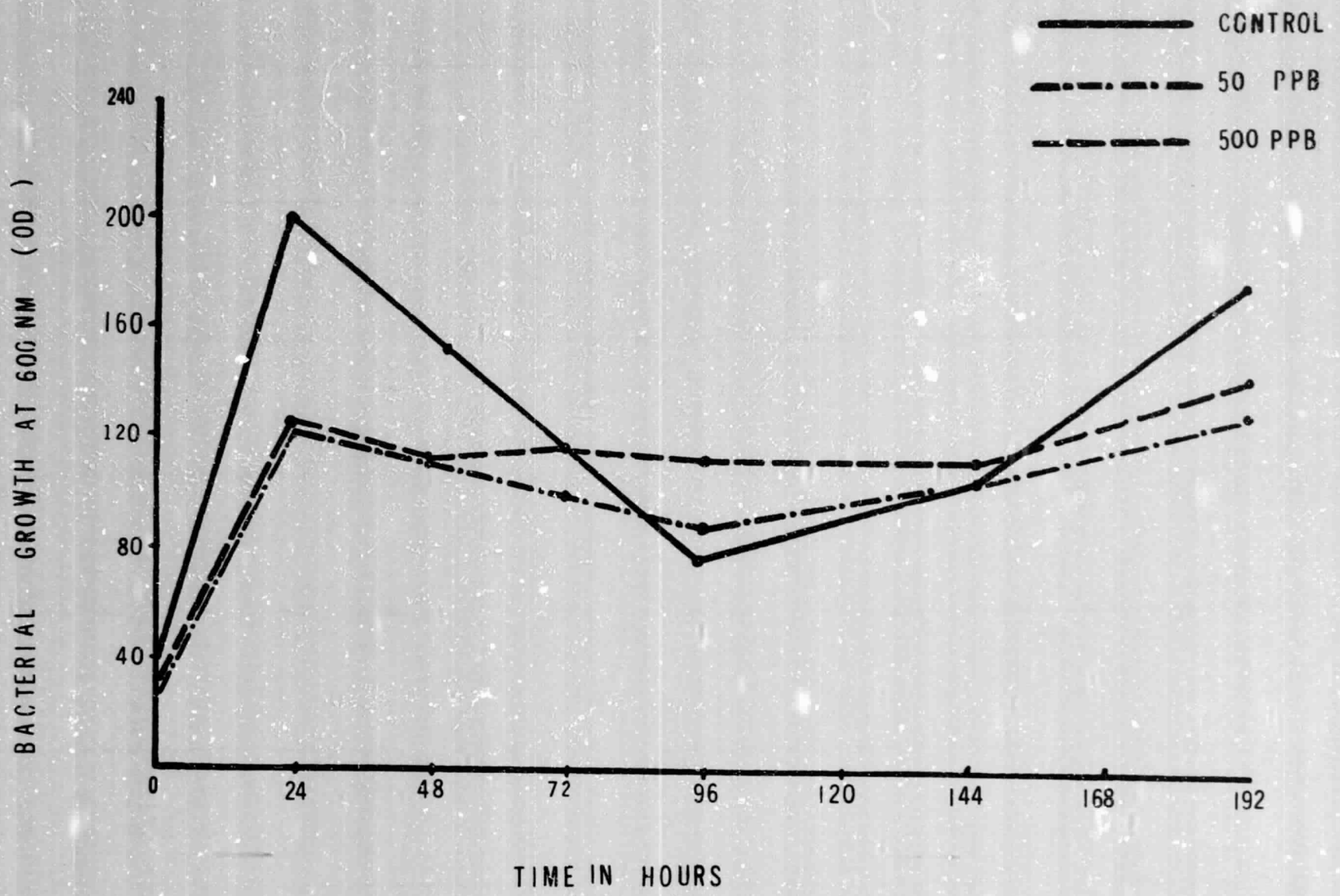


Fig. 8. Growth of Escherichia freundii in various concentrations of ammonium perchlorate.

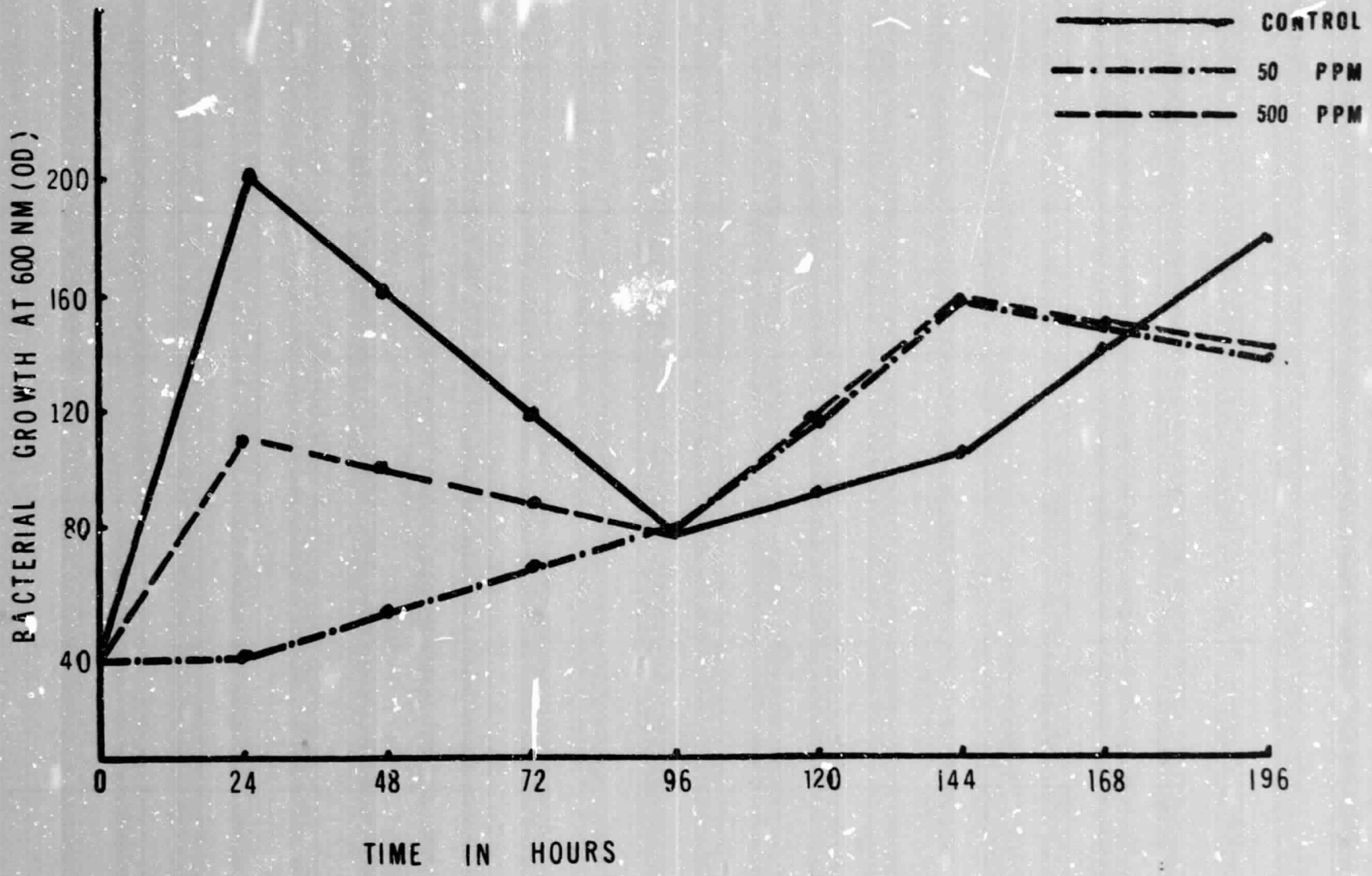


Fig. 9. Growth of Escherichia freundii in various concentrations of ammonium perchlorate.

Bacillus proteus:

Table 8---Growth of Bacillus proteus upto 192 hours, measured at 600 nm (X 10) on Spectronic 20.

No. of hours	Control	50 ppb	500 ppb	50 ppm	500 ppm
0.00	0.5	1.4	0.8	1.9	0.8
24.0	8.4	7.6	1.1	7.7	7.8
48.0	5.8	10.2	5.6	9.5	9.2
96.0	0.6	15.4	14.6	13.1	16.0
120.0	7.6	16.4	17.3	16.6	16.0
144.0	14.6	17.3	19.9	20.0	20.0
168.0	13.1	13.4	14.6	15.3	15.0
192.0	11.6	9.5	9.3	10.5	10.0

Conclusions: (Fig. 10, 11, Table 8)

1. In control group the growth declined very much at 96 hours and then reached its maximum at 144 hours.
2. No such reduction in growth was noticed in 50 and 500 ppm; there was a consistent increase in bacterial growth upto 144 hours reaching its full saturation, which was greater than control.
3. Similarly much better growth occurred in 50 and 500 ppb treatments upto 144 hours.
4. No significant difference in growth of treated and control bacteria was observed at the end of testing period (192 hours).

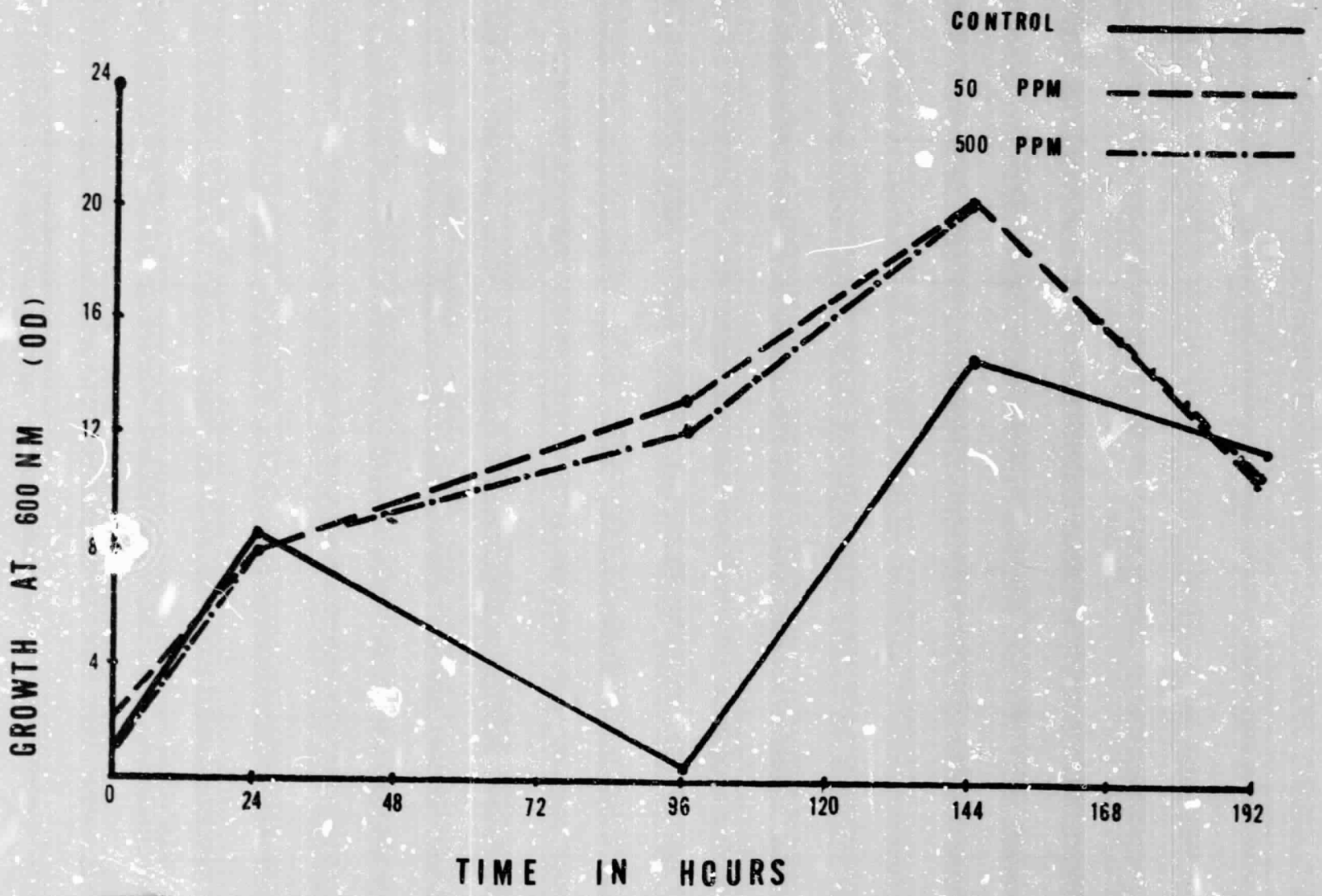


Fig. 11. Growth of Bacillus proteus in varicus concentrations of ammonium perchlorate.

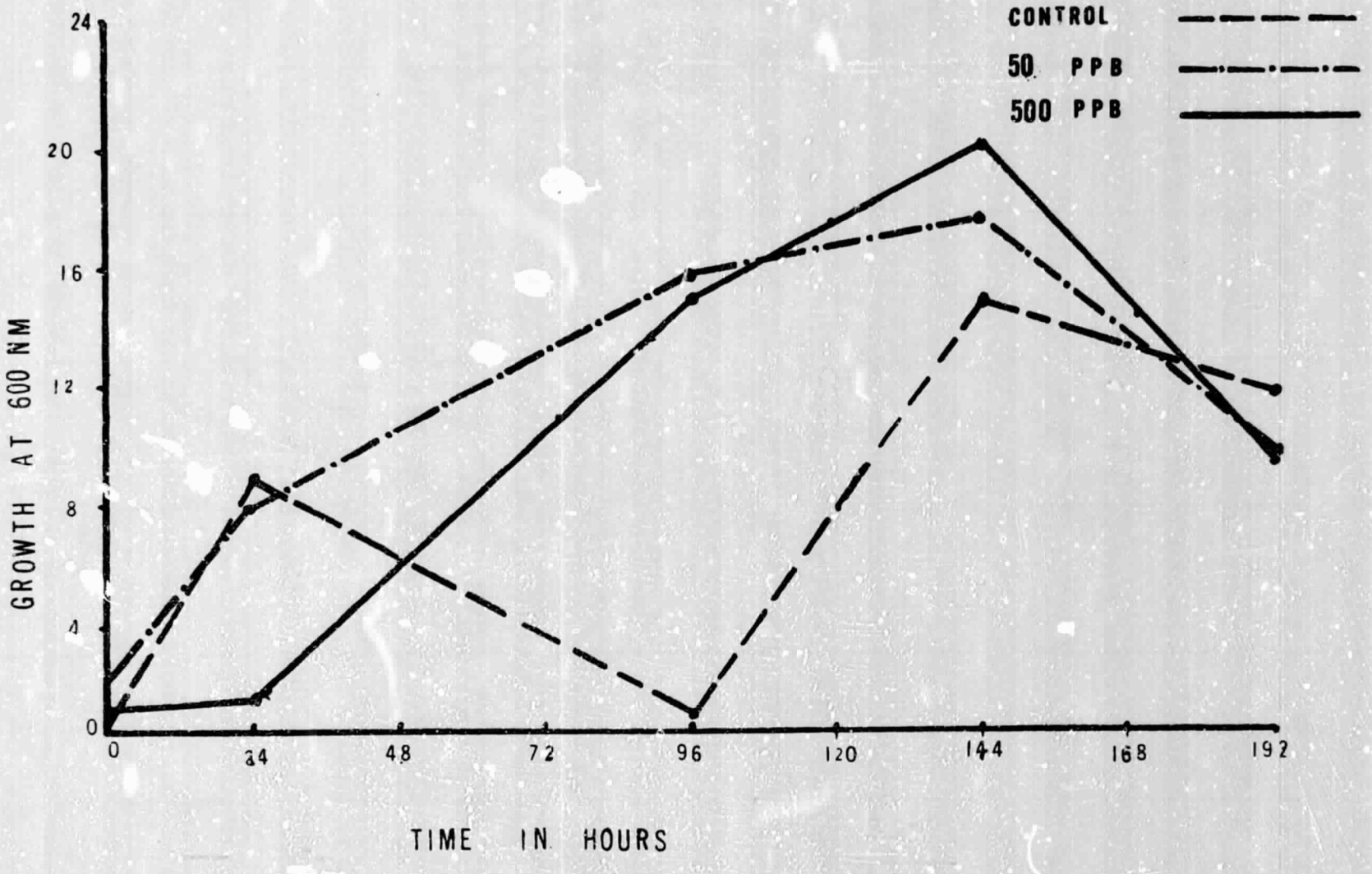


Fig. 10. Growth of Bacillus proteus in various concentrations of ammonium perchlorate.

5. In all the concentrations of ammonium perchlorate, growth of Bacillus proteus was better than control. This showed that this compound is non-toxic to B. proteus even in very high concentrations; and in addition somehow is responsible for better growth of these micro-organisms.

Azotobacter chroococcum: (Fig. 12, 13, Table 9)

Due to the fact that this species is one of the nitrogen-fixing bacteria, it prompted us to study in details. In natural environment these organisms fix atmospheric nitrogen, however, nitrates have been found to be lethal in higher concentrations for the growth of Azotobacter. Interesting results were obtained for these bacteria:

Table 9---Growth of Azotobacter chroococcum upto 192 hours in ammonium perchlorate (1 ppb-100ppm), measured on spectronic-20, at 600 nm. (OD X 10)

Number of Hours	Conc. of NH_4ClO_4 Control	1ppb	10ppb	100ppb	1ppm	10ppm	100ppm
0	0.655	0.458	0.809	1.135	1.135	0.915	0.862
24	1.135	0.969	1.249	1.457	1.487	1.177	1.549
48	1.805	1.534	2.093	2.182	2.024	**1.549**	1.549
72	2.076	2.024	2.460	2.347	2.460	2.129	1.956
96	2.596	2.596	2.819	2.164	2.518	2.460	2.076
192	3.690	4.690	5.380	4.850	3.770	3.670	2.950

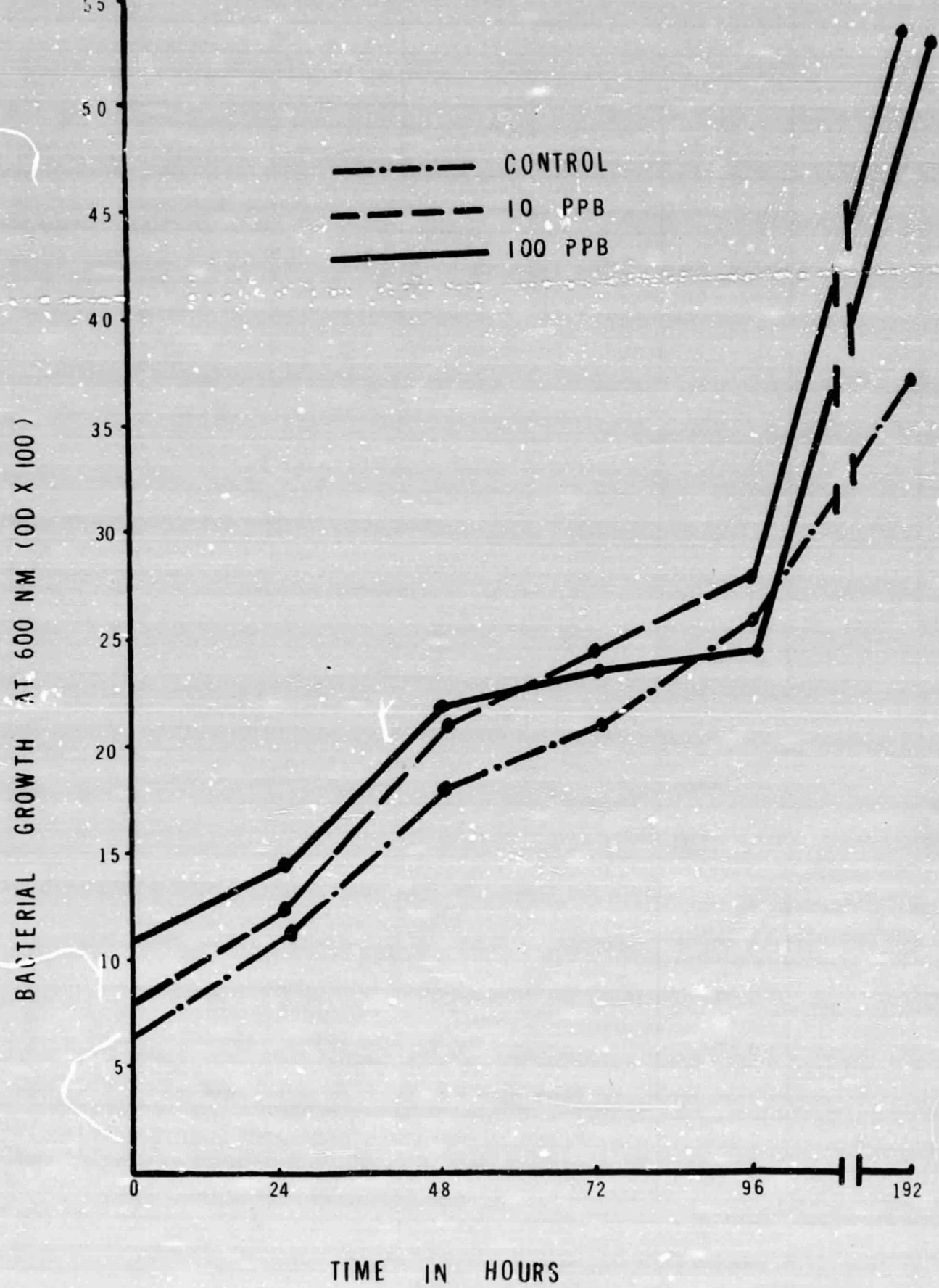


Fig. 12. Growth of Azotobacter chroococcum in various concentrations of ammonium perchlorate.

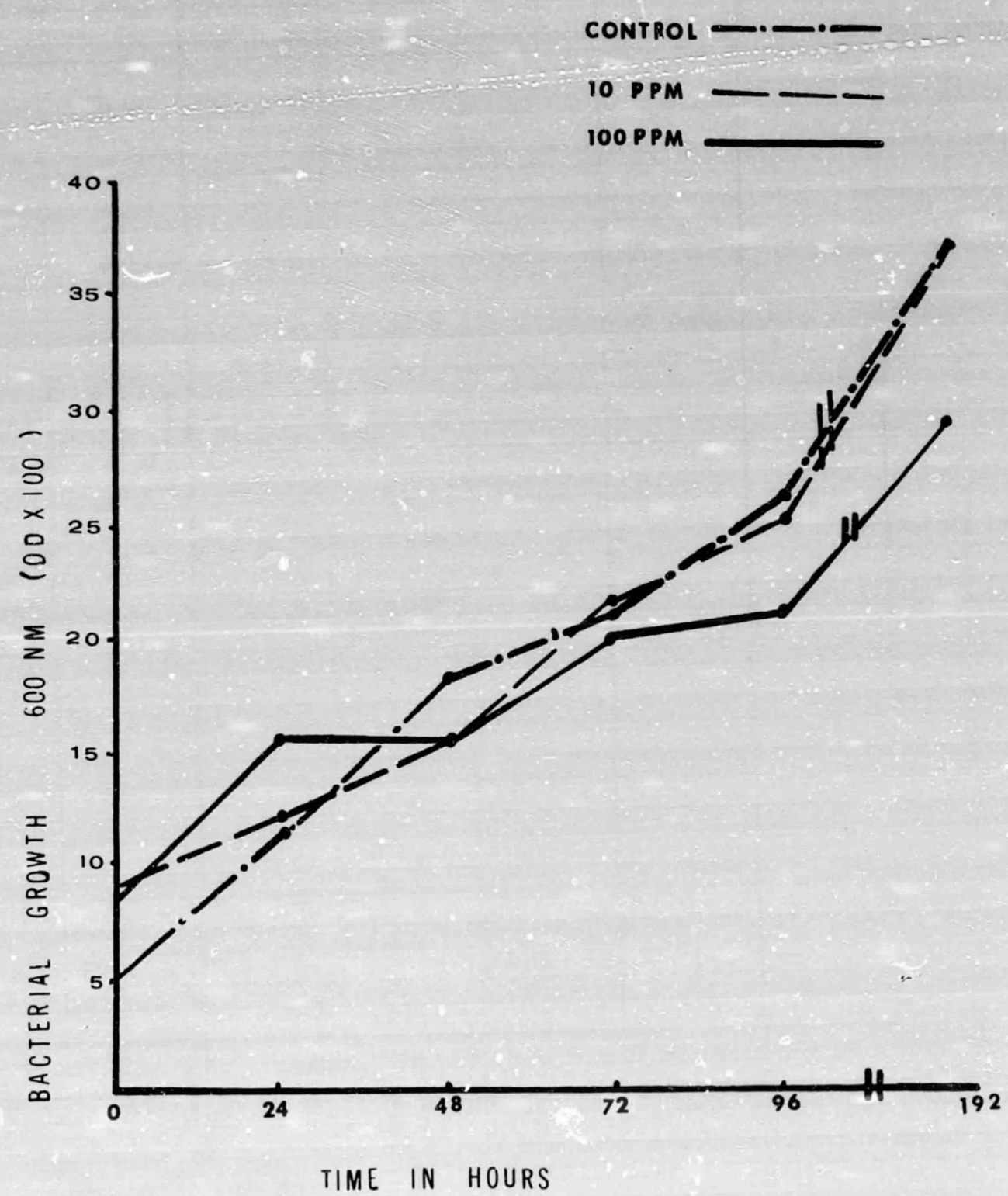


Fig. 13. Growth of Azotobacter chroococcum in various concentration of ammonium perchlorate.

Conclusions: (Table 9, Figures 12, 13)

1. In all the concentrations of ammonium perchlorate (1 ppb to 100 ppm range) growth of Azotobacter increased in direct proportion to time of incubation.
2. Growth of these bacteria reached the highest in 10 ppb, and was also better than control in 1 ppb.
3. In 10 ppm concentration, bacterial growth was very similar to control during the entire period of growth (192 hours).
4. Growth inhibition decidedly took place in 100 ppm, since there was a marked decline in the curve.

LONG-TERM EFFECTS OF AMMONIUM PERCHLORATE ON SOIL CHEMISTRY

A field experiment was set-up at N.ST.L., Bay St. Louis, Mississippi, where a 50^2 meter plot of land was cleared with the help of a tractor. Sixtyfour 1^2 meter plots were marked with wooden pegs, and a buffer zone between each plot (1/2 meter X 1 meter) was maintained. The 64 plots were further divided into 4 groups, each one consisting of 16 one-meter^2 plots. Fortyeight of these plots were treated with 0.5, 5.5 and 55.0 g ammonium perchlorate homogeneously mixed with surface soil. The rest 16 plots were kept as control. The plots were based on Completely Randomized Block Design.

Soil samples were removed with an auger and sent for analyses to Mississippi State Chemical Laboratory, Mississippi State, Mississippi. Total nitrogen and chloride contents

of soil were determined. Soluble chlorides were determined by Bolhard Method of Titration and total nitrogen as described in: "The Method of Analysis for the Association of Analytical Chemistry, AOAC Procedure 2.052".

In the final report of 1975, we have given results of pH analyses of soil taken after 2 months, which did not differ from each other statistically. Similarly, there was no significant difference in nitrogen and chloride contents of soil after 4 months. However, only chloride contents of soil were significantly higher in the first and second month samples. In Table 10 results of soil analyses performed after 4 months are given and Table 10 is a summary of results obtained by Analysis of Variance for the comparison of means.

Table 10-Total nitrogen (%) and Chloride contents of soil (ppm) in samples taken after 4 months of initial treatment.

NH_4ClO_4 g/m^2	12 MONTHS		16 MONTHS		22 MONTHS	
	TKN (ppm)*	Cl^-(ppm)**	TKN (ppm)*	Cl^-(ppm)**	TKN (ppm)*	Cl^-(pp
0.00	630	12.0	400	40.0	300	43.0
0.00	630	12.0	300	55.0	250	29.8
0.55	630	20.0	400	55.0	400	46.2
0.55	630	24.0	300	95.0	300	66.0

5.5	700	18.0	300	80.0	400	43.0
5.5	700	20.0	300	50.0	400	50.0
55.0	560	16.0	400	35.0	250	59.4
55.0	665	16.0	300	40.0	400	33.0

*Calculated from chloride determination
**TKN=Total kjeldahl nitrogen

Table 11---Results of Analyses of variance obtained for chloride contnents of soil measured at different intervals.

No. of months	Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
12	Treatments	3	9.5	3.17	0.12
	Error	4	110.0	27.50	
	Total	7	119.5		
	Table value F 0.95 (3,4) = 6.59				
16	Treatments	3	1712.5	570.83	1.66
	Error	4	1375.0	345.75	
	Total	7	3087.5		
	Table value F 0.95 (3,4) = 6.59				
22	Treatments	3	380.5	126.83	0.78
	Error	4	647.0	161.75	
	Total	7	1027.5		
	Table value F 0.95 (3,4) = 6.59				

Conclusions: (tables 10 and 11)

1. No statistically significant difference was obtained in chloride contents of soil, which was analyzed after 12, 16 and 22 months of the initial treatment.
2. Statistical analysis for nitrogen data was not done, however, the results are explicitly clear. No change in nitrogen contents of soil occurred any time after the initial treatment with ammonium perchlorate.
3. Soil pH was determined for all samples including the last taken (after 22 months), which revealed no significant difference.
4. Supporting data confirm our statement that the soil chemistry is not affected by the treatment of ammonium perchlorate.
5. Plant germination and growth experiments have shown that the toxicity of ammonium perchlorate is still persistent only in the highest treatment level (55 g active ingredient per square meter of soil). However, there is insignificant effect of this compound in lower treatment levels, i.e., 5.5 and 0.55 g/m^2.
6. Contrary to plant growth, microorganisms were unaffected even in the highest concentration of ammonium perchlorate. In most cases, their growth increased in treated cultures, presumably because ammonium perchlorate provided additional growth factors.

BIOGAS PRODUCTION

Alligator weeds, Alternanthera philoxirides were collected from a cooling pond located at Crosby Chemical Company, Picayune, Mississippi. They were transported to the laboratory in large plastic bags with nominal amount of water. The plants were chopped in ½ inch lengths, and 1000 g of this material was placed in several narrow-mouth bottles (10 liter capacity). The bottles were sealed with two-hole stoppers; one outlet was fitted with a rubber septum for easy accessibility in taking gas sample for chromatographic analyses. The other outlet was connected to another sealed bottle with a tflon tubing. This bottle contained water and 5 ml of saturated Phenol Red indicator. The displacement of water in the second bottle provided convenient method of monitoring the volume of gas produced.

To assess the effect of reducing agents as well as ammonium perchlorate, several treatments were made. Two weeks after the active formation of gas, samples were analyzed by Fisher Hamilton Gas Partitioner, at N.S.T.L. Environment Lab., Bay St. Louis, Mississippi.

The following particulars apply to the gas-analyzer at the time of sample analyses: Pyrex column 6' X 4', packing material: 30/60 Type 13 X molecular sieves; column temperature: 30°C; injector and detector temperature: 100°C; range-10^{-11}; attenuation: 512; carrier gas: nitrogen; flow rate: 40 ml/min.

recorder scale: 1 ma; sample size: 6 µl.

Table 12--Methane-gas production by alligator weeds, treated with various ingredients.

Treatment No. & Description	Initial Date	Volume displacement (ml)	%Methane	Date sample analyzed
1. Control	7/10/75	1,950	75.0	9/2/75
2. Control	"	2,100	62.3	8/18/75
3. Control	"	no change	86.0	8/27/75
4.0.15% NH_4ClO_4	"	"	2.1	-
5. 0.05% "	"	very little	10.5	8/18/75
6. 0.005% "	"	"	0.0	"
7. 40% chick manure	"	1,000	40.4	"
8. 20% chick manure	"	19,00	69.3	"
9. 20% chick manure	"	little change	52.0	"
10. 30% rumen content	"	no change	15.0	"

Conclusions:

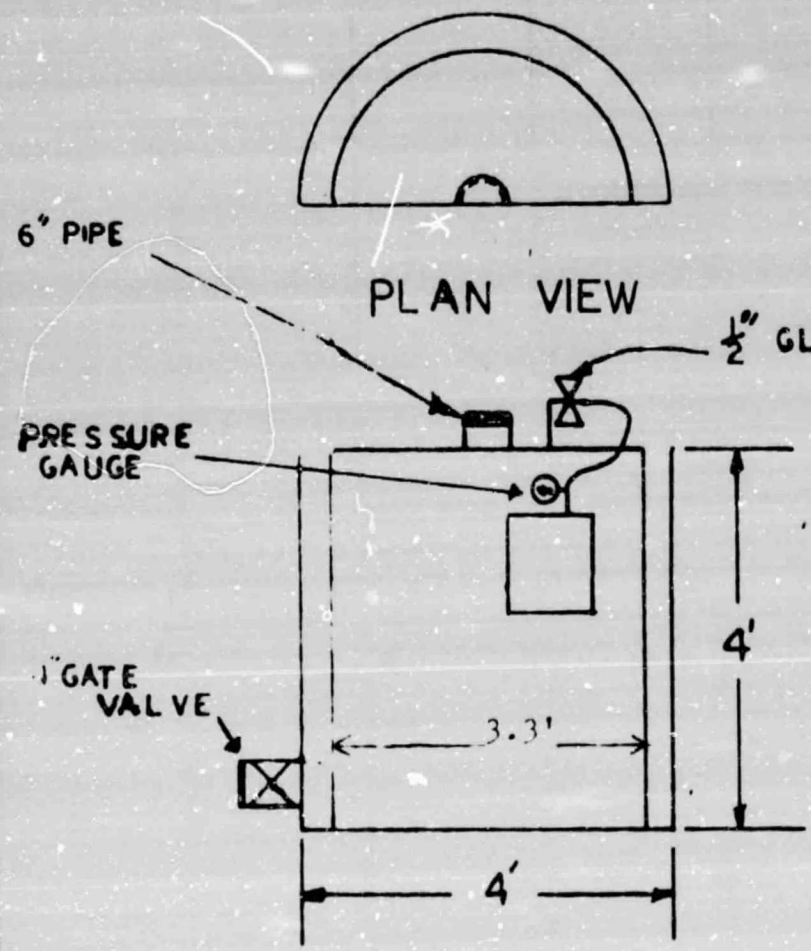
1. No conclusive results were obtained from the above mentioned data which may be due to the following:

   A. Facilities for analyses of gas were not available at the University campus. Bottles containing the fermenting material were transported more than 200 miles for analyses, which may have resulted in gas leakage or intro-duction of air, in presumably air-tight bottles whose stoppers were thoroughly sealed with a rubber glue.

   B. It is inexplicable why one experimental set-up yields a large amount of gas while the other, although identically similar fails to do so (Pers. comm. with Dr. Paul Smith, Chairman, Department of Microbiology, University of Florida, Gainesville, Florida). This may be due to the fact that

NOTE

TANK CONSTRUCTED FROM 20 GA. BLACK IRON STEEL RESTING ON A 4" CONCRETE SLAB.



UNIT WT.
APPOX. 315 LBS

ELEV VIEW SIDE ELEV

PREPARED FOR ALCORN STATE UNIVERSITY	2 TANKS	DRAWN BY J C WASHINGTON
DESIGNED BY R L BUTLER	SCALE ½"=1'-0"	DATE 1/10/75

methanogenic bacteria are highly sensitive to presence of oxygen.

2. Replications on a large scale are needed to obtain meaningful data from these experiments.

Addendum to Methodology

A bio-gas digester was constructed as described in Figure 14. It also did not produce any methane, probably because of air-leakage in the system.

_A_C_K_N_O_W_L_E_D_G_M_E_N_T_S_

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