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"Biodegradation of Rocket Propellent Waste, Ammonium Perchlorate"

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During the past year efforts were made to study the biodegradation rate of ammonium perchlorate (active ingredient of rockets). The impact of this compound on the environment was also studied in the form of growth, metabolic rate and total biomass of selected animal and plant species.

Preliminary results of this project were sent for publication in the January issue of STAR Journal (Space Technology & Aerospace Reports). Two scientific reports were presented during the thirtysixth annual meeting of the Association of Southeastern Biologists (ASB) held at Virginia Polytechnic Institute & State University, Blacksburg, Virginia (April 16 to 19, 1975). Published abstracts of these papers appeared in the ASB Bulletin (Vol. 22, No. 2, pp 62 and 70).

Brief methodolgy and detailed results are presented as follows:

I Seed germination
- A. Corn
- B. Soybean
- C. Wheat

II Plant growth rate
- A. Corn

III Photosynthesis
- A. Elodea
- B. Natural phytoplankton

IV Microbial respiration
- A. Aquatic
- B. Soil inhabiting

V Chemical properties of soil

A. Total Nitrogen
B. Chlorides (NaCl equivalent)

VI Microbial growth
A. Nitrogen fixing bacteria, Azotobacter chroococcum
B. Chlamydomonas sp.

VII Total biomass
A. Treated plots
B. Control plots

SEED GERMINATION

Soybean, Wheat and Corn:

Seeds of soybean and wheat were soaked in 2% sodium hypochlorite solution for 10 minutes for surface sterilization. Germination was done in petri dishes at 24°C ± 3 °C. Each dish contained 10 seeds per concentration; and 10 replications in each concentration were made. Serial dilutions of 1% ammonium perchlorate stock solutions were made to obtain the desired concentrations. Percent germination and seedling heights of soybean and wheat were recorded after 168 hours.

Corn seeds were grown in styrofoam cups containing 320 g soil. Each cup contained 4 seeds, and replicated ten times. Ammonium perchlorate was mixed with soil homogeneously to one group of seeds, and to other group treatment was made in aqueous solution form. Seedling height of each germinated seed was measured for 4 weeks and also the pH of soil. These seeds were similarly treated for surface sterilization.

Results: The germination success and seedling height were inversely proportional to increase in ammonium perchlorate concentration (Table 1, Figure 1).

Table 1---Percent germination and seedling height of soybean and wheat.

NH_4ClO_4 Conc. in water(ppm)	Ave. seedling height (mm)	% Germination
	SOYBEAN	
0.0	37.0	81.2
0.1	29.0	70.0
1.0	28.0	63.0
10.0	21.0	60.0
100.0	13.0	48.0
1000.0	8.0	30.0
	WHEAT	
0.0	30.8	77.8
0.1	37.4	70.9
1.0	36.7	61.8
10.0	34.7	56.3
100.0	22.9	55.8
1000.0	17.1	54.9

In corn, significant reduction in seedling height was noticed. Ammonium perchlorate in aqueous form was less in toxicity than salt form, when applied in the same conc.(Table 2 No conclusive reason can be given for this difference. Possibly, this compound dissociates in water and does not interfere as much in plant's physiology. The pH of soil did not vary greatly in 4 weeks, which is evident from Table 3.

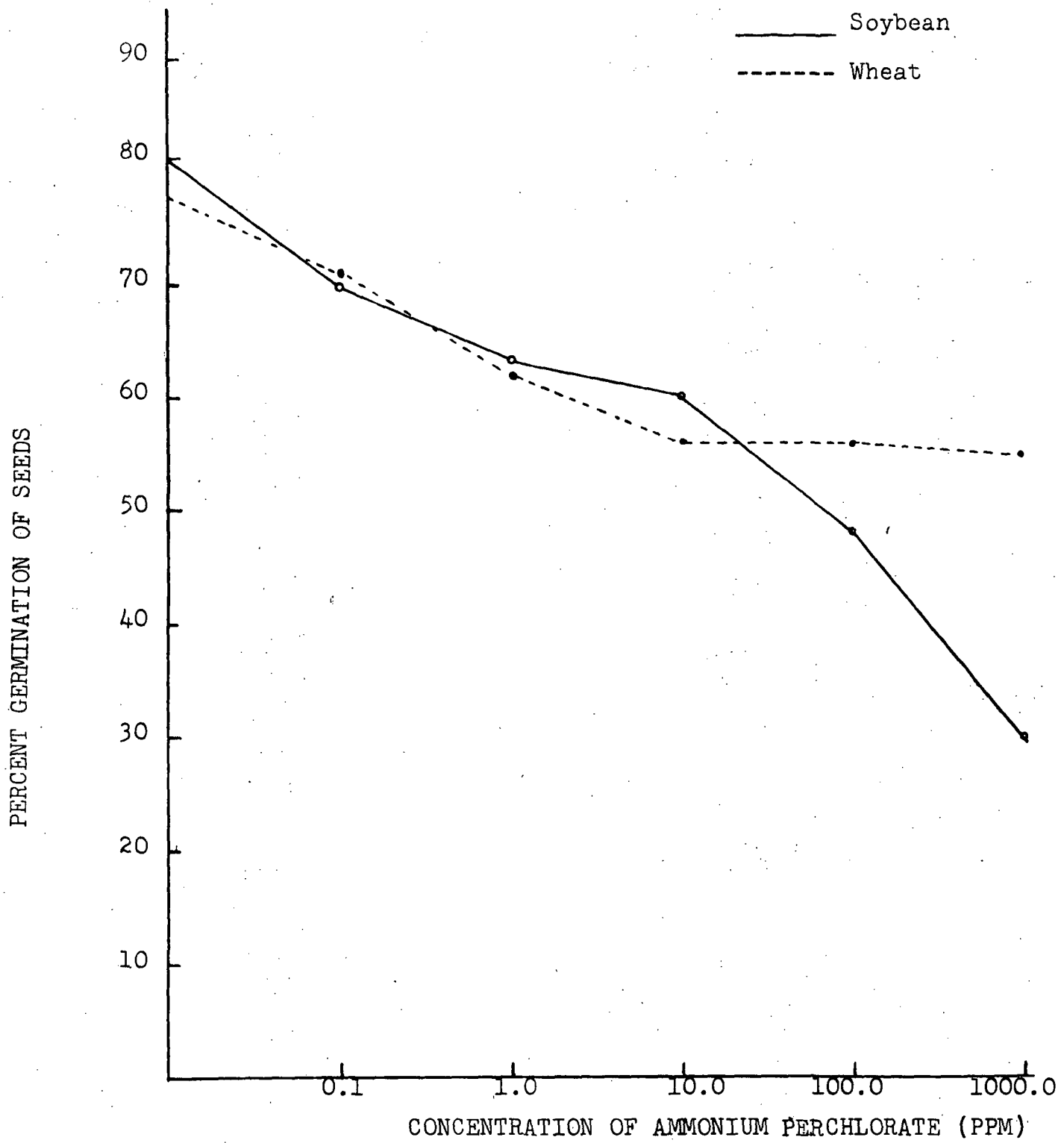

Figure 1---Percentage germination of soybean and wheat seeds treated with ammonium perchlorate.

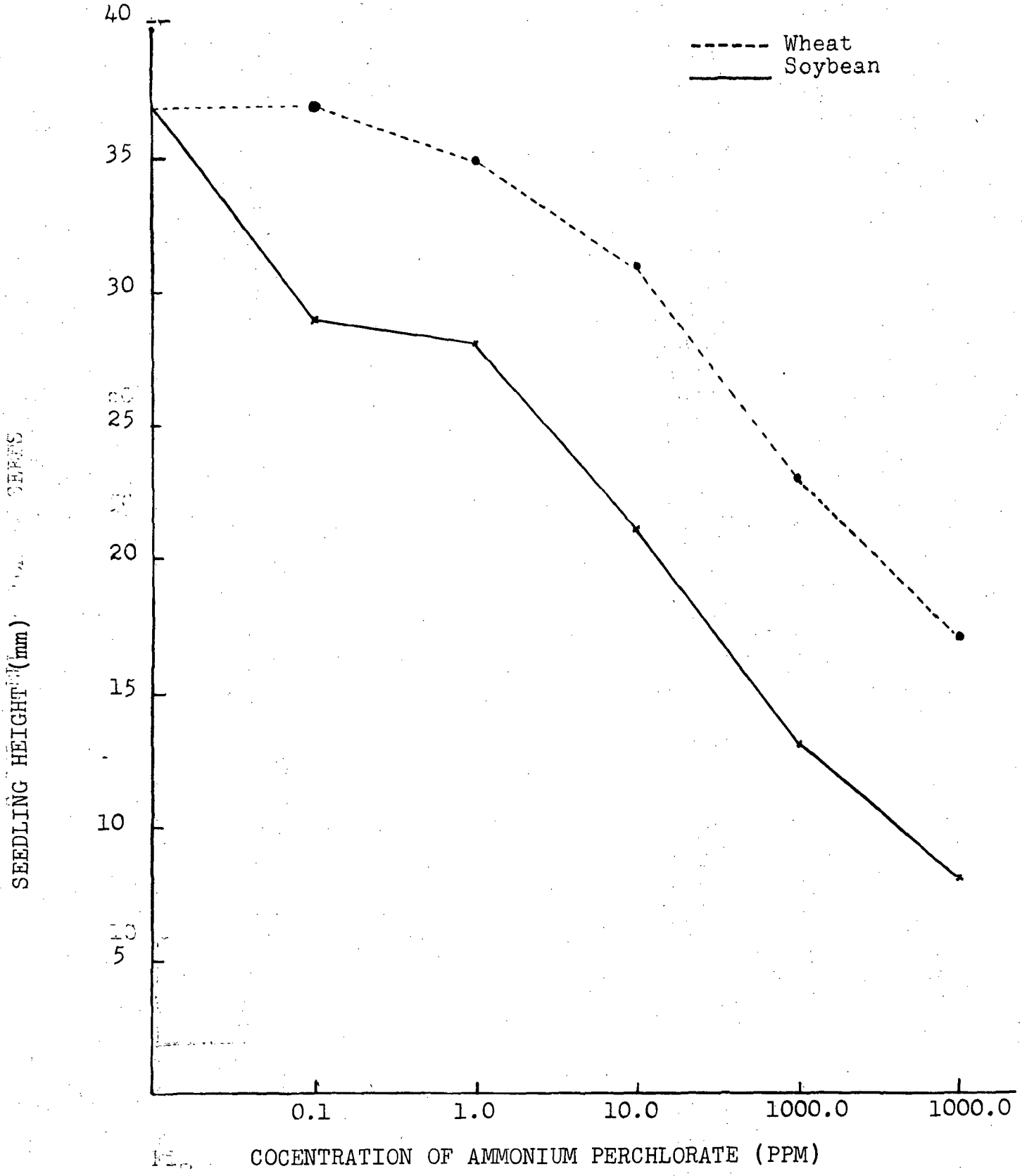

Figure 2---Seedling height of wheat and soybean treated with various concentrations of ammonium perchlorate.

Table 2--- Average plant height (mm) of corn seedlings treated with aqueous and salt form of ammonium perchlorate.

Concentration of NH_4ClO_4		Weekly growth height (mm) measured for 4 weeks							
Aqueous (ppm)	Salt form per 1000g soil	First (Aq)	(Sa)	Second (Aq)	(Sa)	Third (Aq)	(Sa)	Fourth (Aq)	(Sa)
Control	Control	15	15	49	64	85	107	110	119
1.0		12		23		37		46	
10.0	0.001g	9	3	16	6	24	10	19	15
100.0	0.01g	11	3	15	4	17	6	17	6
1000.0	0.1g	4	0	6	3	6	3	6	3

Table 3---Soil pH recorded at monthly intervals.

NH_4ClO_4 Conc. (Aqueous form) ppm.	Week I	Week II	Week III	Week IV
0.0	6.70	6.70	6.70	6.75
1.0	6.70	6.65	6.60	6.60
10.0	6.70	6.65	6.65	6.65
100.0	6.65	6.60	6.60	6.60
1000.0	6.00	6.00	6.00	6.00

PHOTOSYNTHESIS

Elodea twigs were acclimatized in the laboratory for 1 week before testing their photosynthetic activity. The plants were treated by placing them in wide-mouth gallon jars for 72 hours to insure proper exposure of ammonium perchlorate. Initial oxygen concentration of test solutions was recorded, and twigs were placed in the B.O.D. bottles for 6 hours. The bottles were kept in a water bath to maintain similar temperature and light conditions. Six B.O.D. bottles for each test solution were used, and each such test was repeated 5 times. Oxygen production by Elodea was computed on the dry weight basis, expressed as O_2 ppm/hr/gm. Elodea twigs at the completion of the test were dried in a thermostatic electric oven at 60°C for 48 hours and weighed on a Mettler balance. The temperature around the B.O.D. bottles did not change more than 3°C during the experiments.

Photosynthetic activity of Spirogyra was measured, identically in a similar way.

Natural phytoplankton was collected at the time of 'bloom' from a pond located within the university campus of Alcorn State. The water was stored in 20-gallon aquaria, acclimatized for 72 hours and kept in a well-lighted room. Test solutions were made directly by the pond water. Oxygen production was measured similarly, by B.O.D. bottle technique. Oxygen was measured by an electrode type YSI Model 51A oxygen meter; and experessed as O_2 ppm/hr.

Results Ammonium perchlorate reduced photosynthesis of Elodea in 0.1 ppm and higher concentrations, however, slight increase was noticed in 0.01 ppm (Figure 3). Similar phenomenon was recorded for Spirogyra. Oxygen production was higher in 0.1 and 1.0 ppm test solutions, then declined markedly in 100.00 ppm and almost disappeared in 1000.00 ppm (Figure 4). It is not known, if possibly this compound in very low concentrations triggers the photosynthetic machinery of the plant.

Photosynthesis was also increased in treated natural phytoplankton, similarly. In control, oxygen production was 1.95 ppm/hr; while higher in treated plankton, i.e., 2.16 and 2.14 ppm/hr in 0.1 and 1.0 ppm ammonium perchlorate.

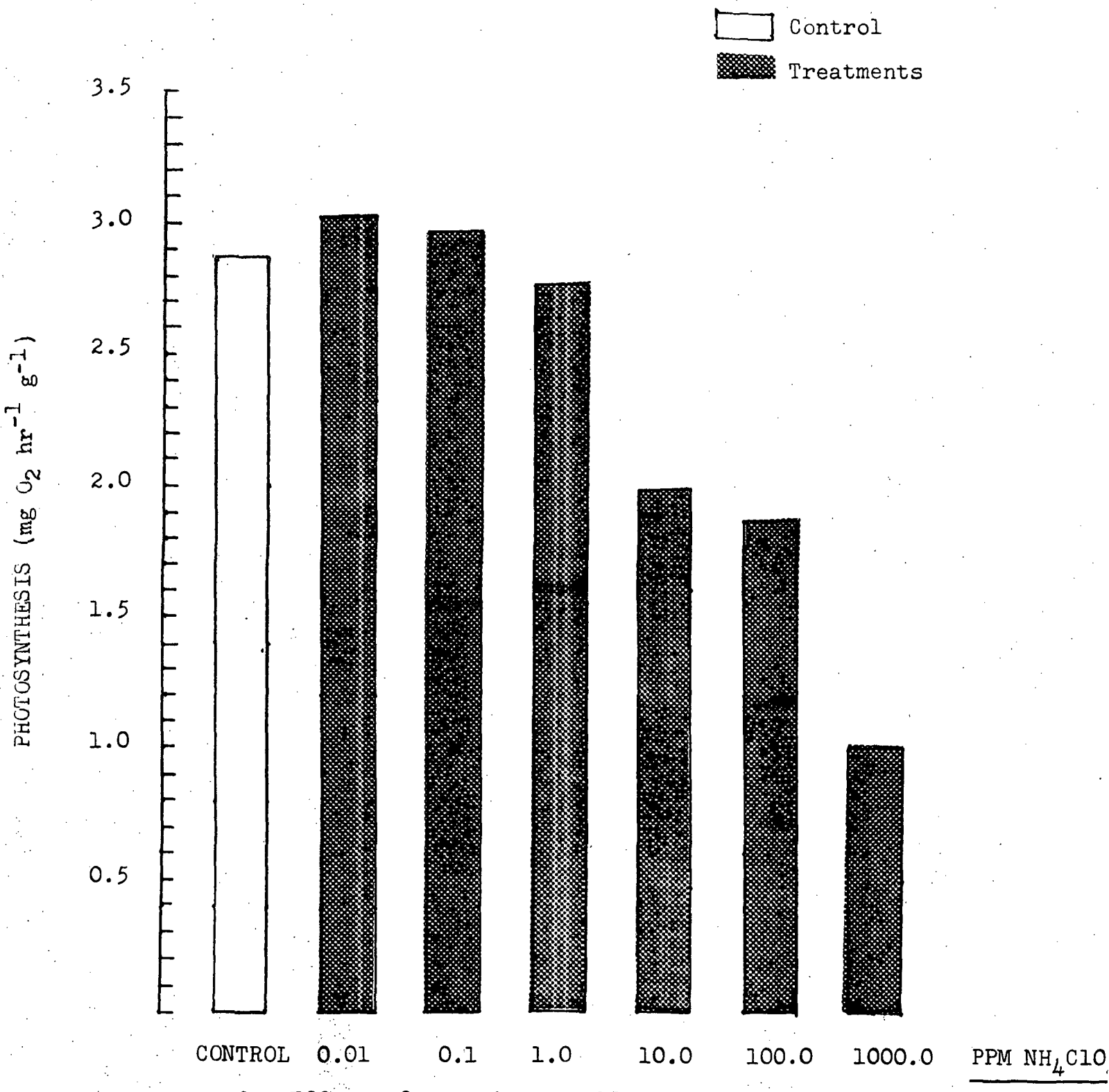

Figure 3---Effect of ammonium perchlorate on photosynthesis of Elodea.

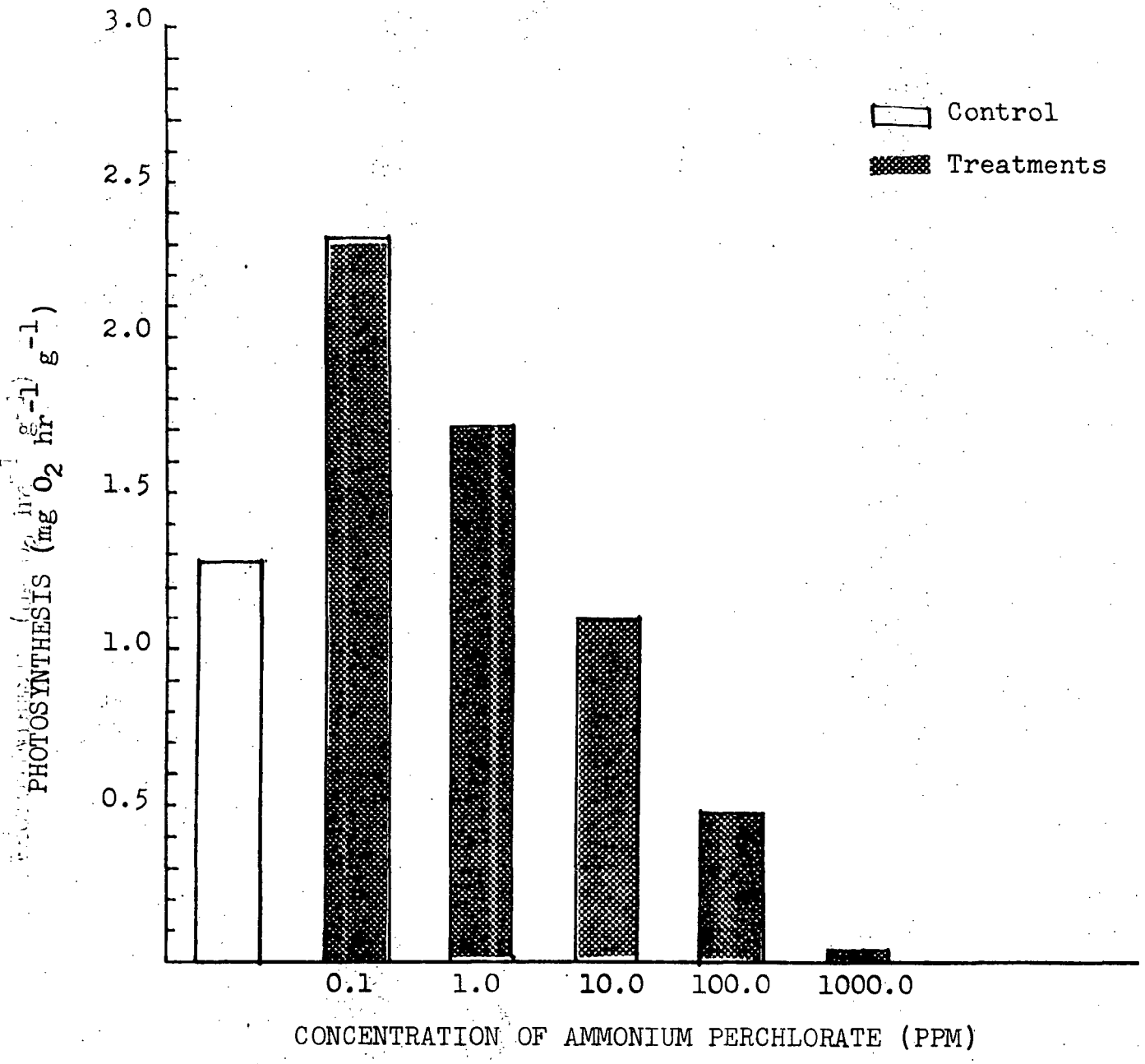

Figure 4--- Effect of ammonium perchlorate on photosynthesis of Spirogyra.

However, oxygen production decreased in 10 ppm ammonium perchlorate solution (1.93 ppm/hr). In all the 3 kinds of plants tested, increase in photosynthetic activity was noticed, if the amount of ammonium perchlorate did not increase above 1.0 ppm. Unless further studies on biochemical effects of this compound on plant's photosynthetic mechanism are carried out, no satisfactory reason for this increase can be given.

RESPIRATION

Respiration of aquatic microorganisms was measured, basically by using the same technique. Natural pond water was collected during the month of December, when the least phytoplankton is expected. The water was filtered through cotton to remove floating objects, and acclimatized in the laboratory for 72 hours. Ammonium perchlorate was mixed directly with the pond water to make test solutions of 0.01, 0.1, 1.0, 10.0, and 100.0 ppm concentrations. The B.O.D. bottles were placed in completely dark place to prevent photosynthesis by phytoplankton presence. The average oxygen consumption per hour by the control microorganisms was 0.0055 ppm. No significant change (statistically) was present in treated microorganism's respiration. Oxygen consumption was measured after 108 hours. Five B.O.D. bottles for each concentration were used, and 3 replications were made.

Respiration of soil microorganisms

Ammonium perchlorate was homogeneously mixed with moist soil, at the rate of 0.5, 1.0, 1.5, 2.0 and 3.0%. Respirometers were made by connecting two conical flasks (filtering type, 250 ml). In each respirometer unit, one flask contained 100 gm treated soil and the other 100 ml sodium hydroxide solution. One of the respirometer unit served as a control, since no ammonium perchlorate was added to the soil. Another such set served as a 'blank', since one flask had sodium hydroxide and the other had only air. It was done to compensate for the absorption of carbon-dioxide of the atmosphere. All the respirometers were kept at room temperature for 72 hours, before measuring the CO_2 production.

At the conclusion of 72 hour incubation period, 25 ml aliquots of $NaOH$-Na_2CO_3 solution was obtained from each flask and mixed with generous amount of barium chloride solution, to precipitate out barium carbonate. To determine the amount of unused sodium hydroxide (which did not react with carbon-dixide); unreacted sodium hydroxide was back titrated with standard HCl. Phenolphthalein was used as an indicator. Similarly, CO_2 production was measured for the blank and control respirometers. The following relation was used to calculate the amount of CO_2 evolved due to microbial respiration, expressed as milligrams of carbon:

Milligram carbon= (B-V)NE, where:

V = Volume (ml) of acid to titrate alkali in solution to the end point.

B = Volume (ml) of acid to titrate alkali in carbon-dioxide collector of control, to the end point.

N = Normality of acid.

E = Equivalent weight of carbon in the reaction.

Results

The control soil microorganisms produced 0.84 mg C, compared to the treated soil: 0.84, 0.87, 0.96, 1.08, and 1.14 mg C, in the ascending order of ammonium perchlorate concentration (Fig. 5). It is evident that the respiration increased consistently with ammonium perchlorate concentration. This does not necessarily imply that the presence of this chemical had an adverse affect on microbial growth. Similar increase in respiration has previously been recorded for certain invertebrates (De la cruz and Naqvi, 1973) exposed to Mirex insecticide. This was interpreted by the authors as due to natural response of an animal to the toxic stress. Naqvi and de la Cruz (1972) report similar stimulation of respiration rate of certain freshwater invertebrates exposed for 24 hours to 1 ppm Mirex. To ascertian the effect of ammonium perchlorate of bacterial growth, separate tests were conducted, which are reported in the following section of this report.

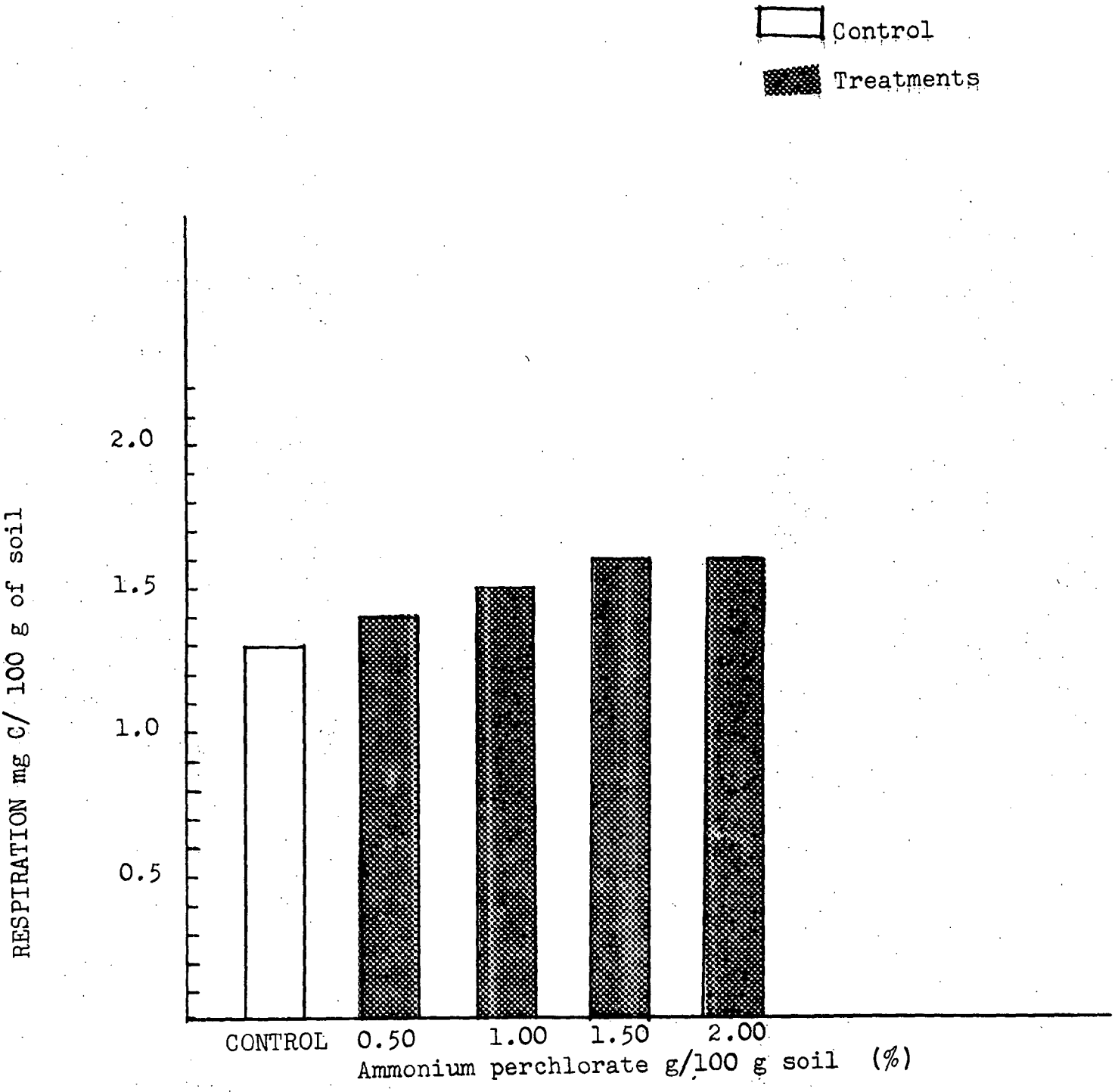

Figure 5---Respiration of soil microorganisms.

MICROBIAL GROWTH

In order to assess the effect of ammonium perchlorate on microbial growth, aquatic microorganisms were grown on nutrient medium. Inoculation was made by 1 ml of pond water, pre-exposed to a certain concentration of this compound. Individual colonies of microorganisms were counted for each treatment level (Conc. of NH_4ClO_4). There was a great variation in the number of colonies, which did not follow any specific trend. Therefore, these tests were discarded.

Pure culture of Azotobacter chroococcum was inoculated to liquid nutrient medium. This medium was autoclaved before inoculation and was mixed with known amount of 0.1% stock solution following the serial dilution technique, so as to obtain the desired concentrations of ammonium perchlorate. The inoculated medium was kept at 24°C, and bacterial growth was measured every 24 hrs on Spectrophotometer 21 (Log absorbance 600 nm) upto 144 hrs. During the first 72 hrs the growth was measured for ammonium perchlorate concentrations ranging from 1.0 ppb to 500.0 ppm, however, in the next 72 hours the growth was recorded for 1.0 ppb to 500.0 ppb (Fig. 6).

Results

In general, bacterial growth increased upto 120 hrs(Table 4) in cultures containing 1 and 50 ppb ammonium perchlorate, in comparison to the control. However, the growth was

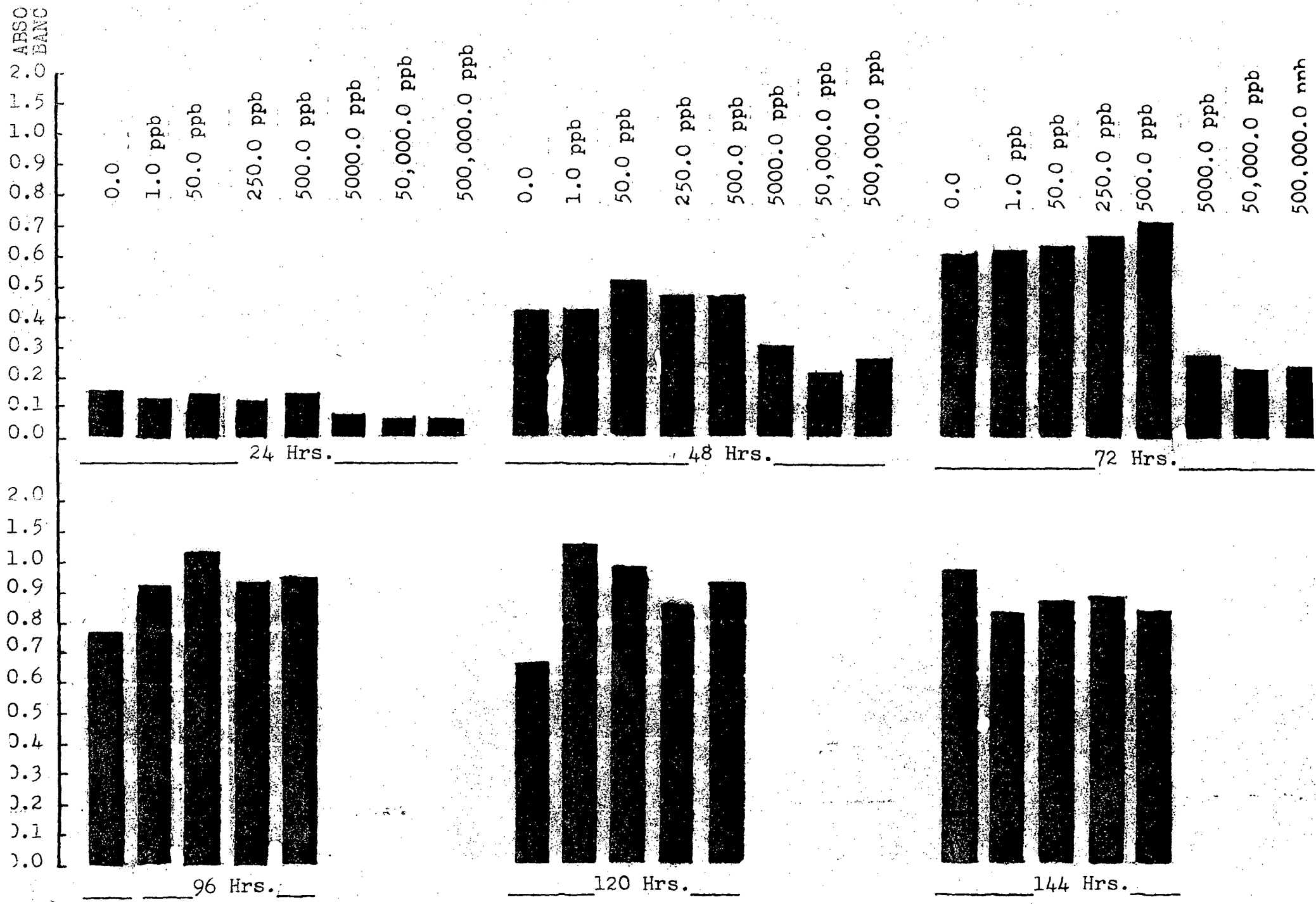

Fig.6 Growth of N-fixing bacteria (Azotobacter chroococcum) in defined media (Log Absorbance 600 nm) containing varying concentrations (ppb) of ammonium perchlorate.

Table 4---Growth of Azotobacter chroococcum measured by Spectronic 20, for a period of 144 hrs.

Conc. NH_4ClO_4 (ppb)	Hrs. Incubation	% Transmittance (600 nm)	Optical Density
0.0 (Control)	24	72.5	0.1397
"	48	40.0	0.3980
"	72	26.0	0.5850
"	96	18.0	0.7450
"	120	23.0	0.6380
"	144	11.5	0.9390
1.0 ppb	24	78.0	0.1078
"	48	38.0	0.4200
"	72	26.0	0.5850
"	96	13.0	0.8860
"	120	09.5	1.0220
"	144	16.0	0.7960
50.0 ppb	24	76.0	0.1192
"	48	31.5	0.5020
"	72	24.5	0.6110
"	96	10.0	1.0000
"	120	11.0	0.9590
"	144	14.5	0.8380
250.0 ppb	24	80.5	0.0942
"	48	36.5	0.4380
"	72	22.5	0.6480
"	96	12.5	0.9030
"	120	14.5	0.8380
"	144	14.0	0.8540

Table 4--- Contd.

500.0 ppb	24	74.5	0.1278
"	48	35.0	0.4500
"	72	20.0	0.6800
"	96	12.0	0.9210
"	120	12.5	0.9030
"	144	15.0	0.8100
5,000.0 ppb (5.0 ppm)	24	89.0	0.0505
"	48	52.5	0.2798
"	72	55.7	0.2537
50,000.0 ppb (50.0 ppm)	24	90.6	0.0434
"	48	64.0	0.1939
"	72	60.0	0.2218
500,000.0 ppb (500.0 ppm)	24	91.8	0.0374
"	48	57.8	0.2384
"	72	57.2	0.2422

suppressed after 120 hrs. This may be due to delayed mortality effect of this compound. This cannot be ascertained unless further data are obtained. Growth was curtailed in 50,000 ppb (=50 ppm) and greater concentrations, even at 24 hrs incubation. Increase in growth upto 120 hrs (1 ppb to 500.0 ppb) is yet to be explained, and no conclusion can be made at present.

It is suggested that this work shall be continued with certain modifications: (i) Ammonium perchlorate will be mixed with the autoclaved culture, instead of prior to incubation (ii) Growth will be measured for a period of 3 weeks instead of 1 week (iii) Pure cultures of other bacteria will be tested (iv) Concentration of ammonium perchlorate higher than 50 ppm shall not be included.

Chlamydomonas spp. Pure culture of this organism was obtained from a biological supply house. Laboratory stock of Chlamydomonas was maintained in Knop's solution. All precautions were taken to avoid contamination in the stock solution. Separately, ammonium perchlorate was added to freshly prepared Knop's solution, and desired concentrations were thus prepared. Pure culture of Chlamydomonas was then inoculated to test solutions which were contained in 250 ml conical flasks. The organisms were allowed to grow 72 hrs before the first growth was measured by Spectronic 20. The inoculated media were kept in subdued light, near a window.

Results

Chalmydomonas generally followed the same trend as Azotobacter chroococcum. In all the concentrations of ammonium perchlorate, growth increased substantially after 96 hrs of incubation. However, the rate of growth differed, slightly (Table 5). The highest amount of growth took place in culture which was exposed to 1ppb NH_4ClO_4. Change in O.D. from zero to 96 hours of incubation is given in Table 5, which can be considered as 'growth range'. It is evident that the growth range was greater in treated Chlamydomonas of 1.0 and 10.0 ppb. Interestingly enough, the growth of bacteria (Azotobacter) had also increased when the treatment level was small, but inhibition of growth took place in higher concentrations.

Even at this stage, no conclusive remarks concerning the effect of this compound can be made. Subsequent work will show if this difference in growth rates is a regular feature or just accidental. We are presently in the process of measuring growth of Chlamydomonas, however, additional experimentation is needed.

Growth of unicellular algae is sometimes measured by gross photosynthesis. We also intend to use Chlamydomonas cultures for this purpose in our future studies, which will complement this part of our work.

Table 5--- 96 hr growth of Chlamydomonas spp in various concentrations of ammonium perchlorate.

Conc. of NH_4ClO_4 (ppb)	Treatment Time (Hrs)	Ave. O.D.* at 600 nm	Ave. growth Range
0.0	0.0	0.07	
0.0	24.0	0.11	
0.0	48.0	0.18	0.18
0.0	72.0	0.21	
0.0	96.0	0.25	
1.0 ppb	0.0	0.05	
" "	24.0	0.10	
" "	48.0	0.15	0.21
" "	72.0	0.20	
" "	96.0	0.26	
10.0 "	0.0	0.08	
" "	24.0	0.13	
" "	48.0	0.21	0.20
" "	72.0	0.25	
" "	96.0	0.28	
100.0	0.0	0.11	
" "	24.0	0.15	0.11
" "	48.0	0.22	
" "	72.0	0.24	
" "	96.0	0.22	
1.0 ppm	0.0	0.11	
" "	24.0	0.15	
" "	48.0	0.20	0.14
" "	72.0	0.25	
" "	96.0	0.25	
10.0 "	0.0	0.09	
" "	24.0	0.12	
" "	48.0	0.16	0.15
" "	72.0	0.21	
" "	96.0	0.25	
100.0	0.0	0.09	
" "	24.0	0.16	
" "	48.0	0.16	0.12
" "	72.0	0.20	
" "	96.0	0.21	

* Average reading taken from 6 replicates.

CHEMICAL PROPERTIES OF SOIL

A field experiment is in progress at N.S.T.L NASA Test Site, Bay St. Louis, Mississippi. A 50 meter X 50 meter plot of land was clearded on June 17, 1974; its soil was broken mechanically and grass etc. was allowed to dry for 1 week. Sixty-four plots (1^2 meter) were demarcated by wooden pegs, and a buffer zone of $\frac{1}{2}$ meter between the adjacent plots was left. These plots were further divided into 4 larger blocks each one consisting of 16 one square meter plots. Forty-eight of these plots were treated randomly with 0.5, 5.5, and 55.0 gm ammonium-perchlorate (16 plots per treatment). The last 16 plots were control, which did not receive any treatment (Fig. 7).

Two pounds surface soil from each plot was mixed manually by shaking in a glass wide mouth 1-gallon jar, and spread evenly. Soil samples for the analyses of total Nitrogen and Chlorides were taken after 1, 2 and 4 months intervals. The next sampling will be resumed after 12 months for those plots which were intially sampled after 1 month, followed by 16 and 20 months for the other two.

Soil samples were dried in an oven at 110°C for 24 hours, ground thoroughly and sieved through a 40-mesh/inch sieve. All samples were taken by an auger (1" diameter) from the upper 6-inch surface soil. Analyses of soil samples were

1 B	5 C	9 A	13 D	17 D	21 B	25 A	29 C
2 D	6 A	10 A	14 C	18 A	22 C	26 B	30 D
3 C	7 B	11 D	15 B	19 B	23 A	27 B	31 C
4 D	8 C	12 B	16 A	20 A	24 D	28 D	32 C
33 D	37 C	41 D	45 D	49 D	53 D	57 B	61 C
34 C	38 B	42 A	46 A	50 C	54 C	58 C	62 B
35 B	39 D	43 B	47 D	51 A	55 A	59 A	63 D
36 B	40 C	44 C	48 A	52 A	56 B	60 B	64 B

Fig. 7 LAY-OUT OF EXPERIMENTAL PLOTS AT NASA TEST SITE

A: 55 g ammonium perchlorate/ square meter
B: 5.5 g " " " " "
C: 0.0 g Control plots
D: 0.55 g ammonium perchlorate/ square meter

performed by the Mississippi State Chemical Laboratory personnel at Mississippi State University. These samples were extracted (100.0 gm dry weight) with water, and soluble chlorides were determined by Bolhard Method of Titration. The total itrogen was determined according to: "The Method of Analysis for the Association of Analytical Chemistry, AOAC Procedure 2.052" (pers. communication with Dr. E. Bailey, Chief Chemist, I.A.S. Div. Mississippi State Chemical Laboratory).The pH of each sample was also determined.

Results

Decidely, there was no statistically significant difference between the control and treated plot pH readings, measured for 2 month samples. The control plots had an average pH of 5.33, while the 3 different treated plots had 5.27, 5.26 and 5.25 for 0.55 g, 5.5 g, and 55.0 g treatments, respectively.

The nitrogen of soil samples did not differ significantly in samples taken after 4 months, but the chlorides increased significantly after 1 month (significant at 0.01% level), less significantly after 2 months (0.05% level) and was insignificant after 4 months. (Tables 5 and 6). This suggests that if the compouund is released in a terrestrial environment, it would not change the chloride levels for a longer period of time.

Table 6---Total Nitrogen (%) and Chloride (NaCl equivalent) levels of soil samples taken at 3 intervals.

Amt. NH_4ClO_4	ONE MONTH		TWO MONTHS		FOUR MONTHS	
(Gm/meter^2)	Nitrogen (%)	Chlorides (ppm)	Nitogen (%)	Chlorides (ppm)	Nitrogen (%)	Chlorides (ppm)
0.00	0.049	0.25	0.060	0.58	0.060	0.20
0.00	0.049	0.21	0.046	0.29	0.073	0.33
0.00	0.049	0.17	0.049	0.45	0.073	0.10
0.55	0.063	0.41	0.067	0.33	0.083	0.43
0.55	0.056	0.33	0.070	0.29	0.068	0.36
0.55	0.056	0.17	0.049	0.83	0.070	0.16
5.50	0.063	0.33	0.053	0.66	0.030	0.10
5.50	0.049	0.17	0.056	0.58	0.060	0.16
5.50	0.053	0.12	0.042	0.41	0.075	0.30
55.0	0.046	0.25	0.063	1.10	0.100	0.16
55.0	0.049	0.66	0.070	2.10	0.065	0.33
55.0	0.063	1.60	----- *	1.90	0.068	0.36

*None detected (This method)

It is clearly evident from the data available on nitrogen that it did not change due to treatment with ammonium perchlorate. There was a slight increase in nitrogen percentage over a period of 4 months, in general. As stated earlier, there was no significant change in the pH of soil, but the chloride contents did increase mostly in the first month and subsided thereafter.

This part of our work is being continued for another year to record changes in soil chemistry after 12 months of initial exposure. Visual observation of the treated plots indicates clearly the toxicity of this compound at its highest level of concentration, where the natural vegetation has not returned back as yet since the initial treatment date (June 17, 1974).

Increase in the amount of chlorides is explicable on the basis of the chemical composition of this compound which has the highest amount of chlorine. However, it is not known how exactly the chlorides combine with other chemical constituents and affect the biochemical machinery of living cells.

Table 7---Analyses of variance for chloride contents of soil taken at different intervals.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
		ONE MONTH SAMPLES		
Treatments	3	3.2748	1.0916	10.8833**
Error	8	0.8027	0.1003	
Total	11	4.0775		
Table value	F 0.95(3,8) - 4.07	F 0.99(3,8) - 7.59		
		TWO MONTHS SAMPLES		
Treatments	3	1.1204	0.373	6.2063*
Error	8	0.4812	0.0601	
Total	11	1.6016		
Table value	F 0.95 (3,8) - 4.07	F 0.99 (3,8) - 7.59		
		FOUR MONTHS SAMPLES		
Treatments	3	0.0216	0.0072	0.09
Error	8	0.6086	0.0761	
Total	11	0.6302		
Table value	F 0.95 (3,8) - 4.07	F 0.99 (3,8) - 7.59		

Table 8---Average total nitrogen (%) of samples taken at different intervals of time.

Conc. NH_4ClO_4 gm/meter square	ONE MONTH	TWO MONTHS	FOUR MONTHS
Control	0.049	0.052	0.069
0.55	0.058	0.062	0.074
5.50	0.055	0.050	0.055
55.0	0.052	0.066	0.078

TOTAL BIOMASS

Quantitative analysis of natural vegetation was done on May 21, 1975. A total of 16 plots (4 from each treatment and control) were harvested. All vegetation was removed above the soil surface, cleared of attached debris and dried at 102°C for 24 hours.

Results

The weight of dried plants is given in Table 8, and shown graphically in Figure 7. The plant growth was retarded in each treatment, however, there was no difference in 0.55 and 5,50 g ammonium perchlorate treatments. The biomass was reduced more than 5 times in the treatment where ammonium perchlorate was mixed with soil @ 55.0 g per square meter. In the other two treatments the re uction in biomass weight was more than 1½ fold. More data shall be acquired for biomass to substantiate our findings more conclusively.

Table 9---Total biomass (gms) of control and treated plots (average of 4 plots), harvested on May 21, 1975.

NH_4ClO_4 gm/sq.m	Weight (gm)	Fold diff. with control
Control	268.0	
0.55	172.0	1.56
5.50	172.0	1.56
55.0	51.0	5.25

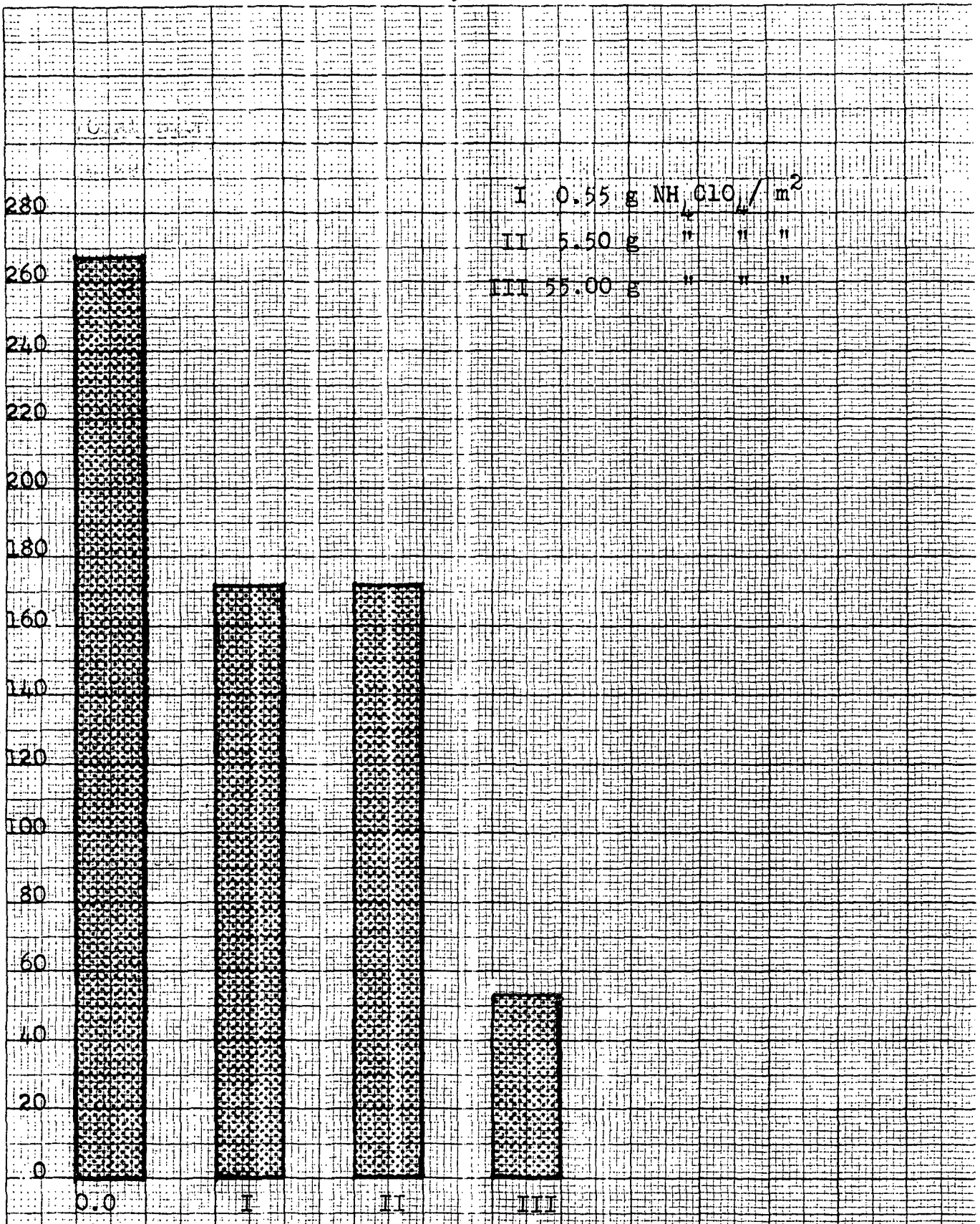

Figure 7. Average biomass of 4 blocks each, located at NASA test site, Bay St. Louis, Mississippi.

RESEARCH IN PROGRESS AND FUTURE PROJECTIONS

1. We continuing to see the effects of ammonium perchlorate on plant growth and germination. Commercially important plant species shall be included in later work.
2. Pure cultures of Chlamydomonas and Euglena shall be used to measure gross plant metabolism by computing oxygen gain due to synthesis and loss due to respiration. Growth rate of these organisms in various concentrations of ammonium perchlorate is being measured by Spectronic 21.
3. Bioassay work is in progress; mosquitofish, Gambusia affinis are being used to determine the toxicity of this compound. LC_{50} values will be calculated by a computer program of probit analysis (Daum and Kilcreas, 1966).
4. Long-term studies on biodegradability of this compound are in progress. In the next 12 months, soil samples from treated plots will be analyzed for determining the change in chlorine and nitrogen of soil.
5. Work on biodegradation of ammonium perchlorate is being enforced by studies on compost derived from plant material, which will be laid underground at NASA test Facility, Bay St. Louis, Mississippi. Periodically, samples will be analyzed to note changes in nitrogen and chloride contents, as well as feasibility of using this cmpost in lieu of artificial fertilizer. Data are expected to exhibit effect of ammonium perchlorate on biodegradability of soil micro-organisms.

6. Studies on bio-gas production by methanogenic bacteria are in progress. Efforts are being made to see if ammonium-perchlorate affects anaerobic digestion of sludge or the growth of methanogenic bacteria.

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N.B.

Half-yearly report of Grant NSG-8005 was sent to the following persons:

1. Mr. William Wolverton, N.S.T.L. NASA Test Facility, Bay St. Louis, Mississippi.

2. Mr. Marion Kent, Assistant Director, University Affairs, George C. Marshall Space Flight Center, Huntsville, Alabama.

3. Mr. Thomas A. Bryant, ONR Representive, Office of Naval Research, University of Huntsville Alabama Research Institute, Huntsville, Alabama.

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