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To the Graduate Council:

I am submitting herewith a thesis written by William Eagleton Black entitled "The Evaluation of Urea as a Nitrogen Fertilizer on Wheat." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

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Major Professor

We have read this thesis
and recommend its acceptance:

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Accepted for the Council:

E. Winters
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THE EVALUATION OF UREA AS A NITROGEN FERTILIZER ON WHEAT

A THESIS

**Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science**

by

William Eagleton Black

August 1955

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CHAPTER I

INTRODUCTION

Nitrogen fertilization has become an important farming practice in the past seventy-five years. During the latter part of the Nineteenth Century the use of sodium nitrate increased rapidly in Europe and America (3). Shortly after 1893 ammonium sulfate, a by-product of the coke industry, appeared on the American market.

Urea, imported from Germany, was used to a limited extent from 1924 to 1936, primarily in the preparation of commercial, mixed fertilizers. A product having the name Uramon was placed on the market in 1938. It consisted of urea treated with cocoa shell meal and rock phosphate dust which coated the individual particles. Since World War II the production of nitrogen fertilizers has increased rapidly, and today there are many products available including urea fertilizers which have appeared under trade names such as Floranid, Urear, Calsi-Urear, and Agramon (15).

While some studies have been made of urea as a nitrogen fertilizer, there is much to be learned of its behavior in soils and its relative efficiency under different soil and crop conditions. The purposes of this investigation have been:

- (1) To evaluate urea as a nitrogen fertilizer on wheat under several soil conditions in Tennessee, and

- (2) To determine rates of urea conversion to ammonia and nitrate and to determine the influence of certain factors which affect this conversion.

CHAPTER II

LITERATURE REVIEW

Urea was discovered in 1773 by Roulle as a compound occurring in urine of mammals. It has been classed chemically as an organic compound because before 1828, when Wohler synthesized urea, it had been found in nature only as a product of life processes. "Vital" energy had been considered necessary for production of organic compounds; urea is of interest historically as the first organic compound to be synthesized (15).

Pasteur was the first to recognize, in 1860, that ammonia formation from urea is brought about by living organisms. It was later established that many groups of bacteria, actinomycetes, and fungi are capable of decomposing urea (19). While it was generally assumed that these agents in soil were directly responsible for this change, Conrad and Adams (5) obtained data which suggest that the reaction may be in part catalytic rather than completely biological. Conrad (4) found evidence in later studies that urea hydrolysis in soil was similar to the reaction brought about by the enzyme urease. Urea splitting activity in incubated soil was reduced to less than half its original value. On the other hand the growing of crops resulted in about a three-fold increase over the original activity.

"Urea" bacteria (bacteria whose metabolism is closely connected with the transformation of this substance) are divided into cocci and bacilli. The former are usually destroyed at 60 - 70°C. whereas the

latter, because of their ability to form endospores, can withstand heating at 90 - 95°C. for several hours. The upper pH limit of urea hydrolysis is given as 6.6 for Urobacillus duclauxii, 7.0 for Urobacillus maddoxii, and 8.1 for Urobacillus pasteurii (19).

Ladler and Hoare (10) measured the rate of urea hydrolysis catalyzed by urease, in buffer solutions of various pH values and at different temperatures. They found the maximum hydrolysis rate at 30°C. and pH 6.2.

Sizer (15) used carbon dioxide evolution to estimate the rate of urea hydrolysis over a wide range of temperatures, using soybean urease. At all temperatures carbon dioxide liberation was a linear function of time.

A number of studies of crop response to different nitrogen fertilizers have been conducted. Andrews (1) in Mississippi compared five nitrogen fertilizers in two tests on cotton and found that urea ranked first in the one test and third in the other. In corn experiments conducted at the same time urea ranked first.

In a Maine experiment with potatoes, Brown et al. (2) found urea to be the most effective of six nitrogen fertilizers in one six-year test and one three-year test. In a second three-year test, it was fourth in a group of seven fertilizers. Differences in yield, however, were small.

In a study conducted by Tidmore and Williamson (17) in Alabama, the response of cotton to six forms of nitrogen was measured in 222 experiments. Urea ranked fourth in effectiveness in Cecil, Hartsell,

and Clarksville soils, and third in Holston. In Decatur, urea and nitrate of soda shared first place in producing highest yields.

Hoffman (9) in experiments with strawberries during 1951 and 1952 at the Wheeler Nursery found larger, stronger root systems and more plants per acre in plots treated with urea than in those treated with ammonium nitrate.

Foliar application of urea to plants is of wide interest, and considerable research has been done in this field. Foote and Batchelder (7) in experiments on Hannchen barley found no significant difference in yield when urea was applied as a foliar spray and ammonia sulfate was applied to the soil. However, when a comparison was made using ammonium nitrate as a soil fertilizer and urea as a foliar spray, the yield of the barley which had received the soil fertilizer was significantly greater than that which had received the foliar application.

Volk and McAuliffe (18), working with tobacco at the North Carolina Agricultural Experiment Station, studied the rate of absorption of urea from foliar application. The rate was three to ten times greater during the night than during the day. As sucrose was increased in the urea spray, absorption of urea by the plant tended to decrease.

Foy et al. (8) in experiments using urea as a foliar spray on corn undertook to determine the cause of injury to the leaves. They suggested that the injury may be due to some product of ammonia metabolism, such as amino acids and polypeptides, and that sucrose may decrease the injury.

Jones (10) suggests that leaf injury in citrus trees sprayed with

urea is due to the presence of biuret.

Fisher (6), in work with foliar applications on apple trees, found that the healthy leaves showed more resistance to burning than other leaves.

CHAPTER III

FIELD EXPERIMENTS AND RESULTS

Field experiments to evaluate urea as a nitrogen fertilizer on wheat were located on the Blount County Farm near Knoxville, the Plateau Experiment Station at Crossville, and the Middle Tennessee Experiment Station at Spring Hill. The soils at each location were Decatur silt loam, Tilsit silt loam, and Maury silt loam respectively. The experimental design was randomized block with four replications.

Composite soil samples were collected from each experimental site for determination of available phosphate and potash in order that these nutrients could be applied in sufficient quantity for optimum crop growth.

The variety of wheat seeded at all locations was Seneca. The plots at the Middle Tennessee Experiment Station and at Blount County Farm were seeded on October 15, 1953, and those at Crossville on September 30, 1953. Korean Lespedeza was seeded in the plots at the Middle Tennessee Experiment Station and at Blount County Farm but failed. Red Clover was seeded in the plots at the Plateau Experiment Station, but was not harvested.

Urea and ammonium nitrate were applied at the time of seeding at rates ranging from 0 to 75 pounds nitrogen per acre. Part of the nitrogen was applied in the fall, part in the spring.

The experimental plots at the three localities were examined periodically during the spring and summer season. Throughout the

growth period, the wheat at the Middle Tennessee Experiment Station showed little difference among the plots. When mature the wheat had lodged on all plots, but remained upright for about six inches around the edge of each plot, producing a border effect. While the fifteen pound rate of ammonium nitrate gave the highest yield, there was no significant difference between treatments (Table I, Figure 1).

At the Plateau Station, the plots showed wide contrasts in growth rate during the season, especially those which had applications of nitrogen at the lower rates. While there was little visible difference between the wheat receiving urea or ammonium nitrate, the wheat receiving the ammonium nitrate outyielded that which had the urea at the sixty and seventy-five pound rate (Figure 2). The analysis of variance (Table II) of this experiment indicated a significant difference between treatments and between replications. Treatment sums of squares were further broken down and indicated the difference to be between rates rather than sources.

The Knoxville Station presented easy access, and frequent observations were made of the experimental plots. There was little difference during the growing season in the appearance of wheat receiving either of the fertilizers (Table III, Figure 3). Slight lodging occurred at the sixty and seventy-five pound rates of both carriers.

TABLE I

YIELDS OF WHEAT FERTILIZED WITH UREA AND AMMONIUM NITRATE AT SEVERAL RATES
 MIDDLE TENNESSEE EXPERIMENT STATION, NEAR
 SPRINGHILL, TENNESSEE, 1954
 MAURY SILT LOAM

Treatment No.	Treatment	Grain Yield Bu./Acre				
		Replication				Average
		1	2	3	4	
1	0-0-50 Check	41.8	47.8	49.1	46.1	46.2
2	15-0-50 Urea	30.4	49.3	46.1	51.2	44.3
3	30-0-50 Urea	41.0	49.3	44.4	49.6	46.1
4	45-0-50 Urea	41.8	45.4	46.2	48.3	45.4
5	60-0-50 Urea	40.6	41.7	35.0	44.4	40.4
6	75-0-50 Urea	37.2	45.1	33.2	47.8	40.8
7	15-0-50 Ammonium Nitrate	45.6	53.4	49.3	51.0	49.8
8	30-0-50 Ammonium Nitrate	46.8	44.2	50.7	49.6	47.8
9	45-0-50 Ammonium Nitrate	44.7	46.6	40.0	47.4	44.7
10	60-0-50 Ammonium Nitrate	39.1	44.2	45.9	47.1	44.1
11	75-0-50 Ammonium Nitrate	29.2	42.2	33.7	42.4	37.9

ANALYSIS OF VARIANCE

	D/F	Var.	F-Value
Treatments	10	47.82	3.29
Replications	3	142.98	9.84*
Error	30	14.53	

*Highly significant.

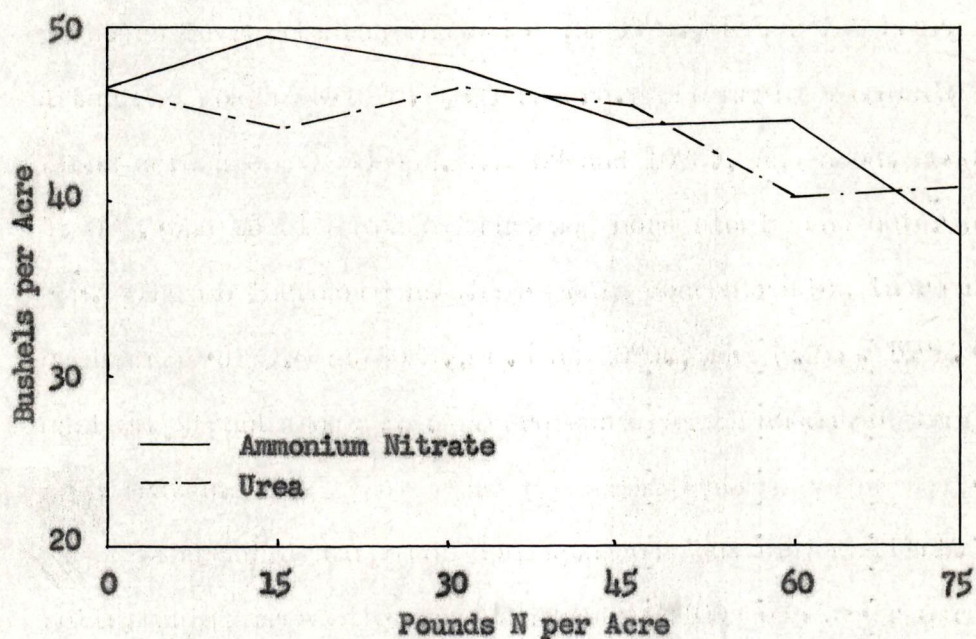


Figure 1. Yields of wheat fertilized with urea and ammonium nitrate at several rates, Middle Tennessee Experiment Station, near Springhill, Tennessee, 1954, Maury silt loam.

TABLE II

YIELDS OF WHEAT FERTILIZED WITH UREA AND AMMONIUM NITRATE AT SEVERAL
RATES, PLATEAU EXPERIMENT STATION, NEAR
CROSSVILLE, TENNESSEE, 1954
TILSIT SILT LOAM

Treatment No.	Treatment	Grain Yield Bu./Acre				
		1	2	3	4	Average
1	0-80-50 Check	26.3	26.7	27.7	28.4	27.3
2	15-80-50 Urea	22.4	31.3	33.1	41.4	32.1
3	30-80-50 Urea	34.2	33.3	32.7	38.7	34.7
4	45-80-50 Urea	38.3	36.8	40.8	43.1	39.8
5	60-80-50 Urea	39.3	38.3	33.7	43.9	38.8
6	75-80-50 Urea	36.4	46.8	40.2	44.7	42.0
7	15-80-50 Ammonium Nitrate	27.3	28.6	35.2	35.8	31.7
8	30-80-50 Ammonium Nitrate	35.6	33.5	39.3	34.2	35.7
9	45-80-50 Ammonium Nitrate	34.6	35.8	43.9	43.3	39.4
10	60-80-50 Ammonium Nitrate	39.3	41.8	42.4	46.6	42.5
11	75-80-50 Ammonium Nitrate	43.9	41.8	59.6	44.9	47.6

L.S.D. 5% = 5.7 Bu.

C.V. = 10.4%

ANALYSIS OF VARIANCE

	D/F	Var.	F-Value
Treatments	10	134.29	8.67*
N x No. N	1	451.67	29.16*
Sources N	1	36.10	2.33
Rates	4	200.06	12.92*
Sources x Rates	4	54.87	.89
Replications	3	86.24	5.57*
Error	30	15.49	

*Highly significant.

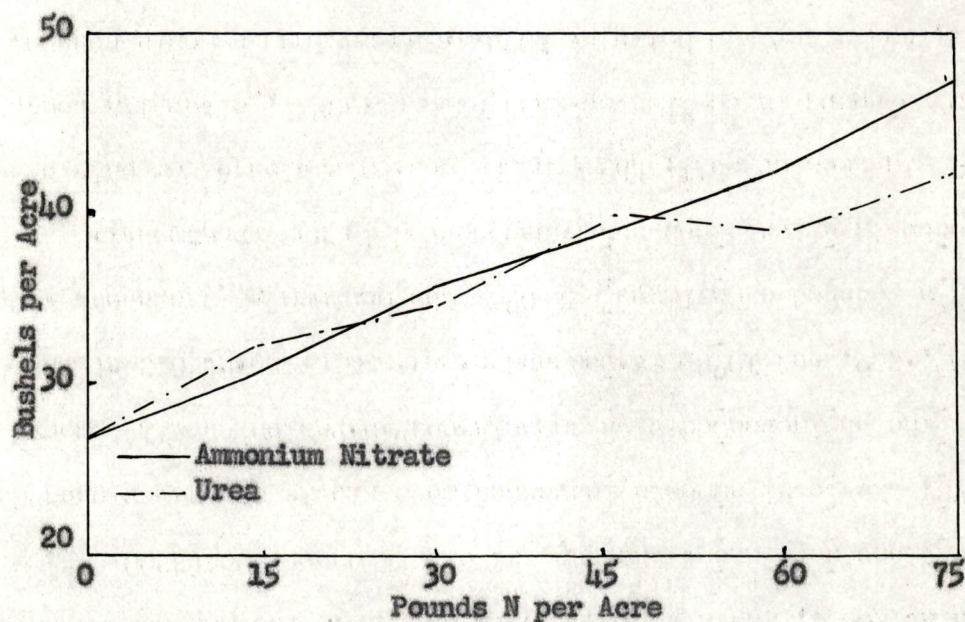


Figure 2. Yields of wheat fertilized with urea and ammonium nitrate at several rates, Plateau Experiment Station, near Crossville, 1954, Tilsit silt loam. (L.S.D. 5% = 5.7 bu.)

TABLE III

YIELDS OF WHEAT FERTILIZED WITH UREA AND AMMONIUM NITRATE
AT SEVERAL RATES, BLOUNT COUNTY FARM, NEAR
KNOXVILLE, TENNESSEE, 1954
DECATUR SILT LOAM

Treatment No.	Treatment	Grain Yield Bu./Acre				
		1	2	3	4	Average
1	0-80-50 Check	42.0	49.5	48.7	48.7	47.2
2	15-80-50 Urea	48.6	48.8	42.0	62.9	50.6
3	30-80-50 Urea	40.6	39.4	41.0	40.2	40.3
4	45-80-50 Urea	44.2	48.2	46.3	46.2	46.2
5	60-80-50 Urea	37.8	37.2	50.9	45.1	42.8
6	75-80-50 Urea	39.1	41.6	39.8	46.8	41.8
7	15-80-50 Ammonium Nitrate	47.0	48.2	39.9	45.8	45.2
8	30-80-50 Ammonium Nitrate	51.6	40.3	48.2	41.9	45.5
9	45-80-50 Ammonium Nitrate	38.4	37.8	51.8	42.8	42.7
10	60-80-50 Ammonium Nitrate	46.4	37.5	55.1	40.2	44.8
11	75-80-50 Ammonium Nitrate	37.8	48.2	42.8	45.5	43.6

ANALYSIS OF VARIANCE

	D/F	Var.	F-Value
Treatments	10	32.30	1.15
Replications	3	29.65	1.05
Error	30	28.20	

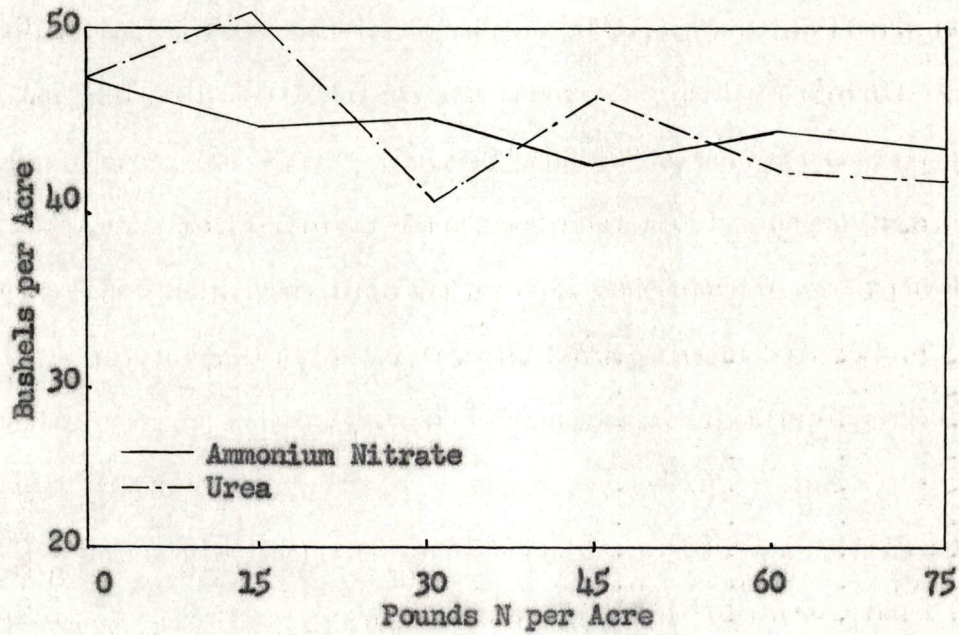


Figure 3. Yields of wheat fertilized with urea and ammonium nitrate at several rates, Blount County Farm, near Knoxville, 1954, Decatur silt loam.

CHAPTER IV

LABORATORY EXPERIMENTS AND RESULTS

Three experiments were conducted on the conversion of urea to ammonia and nitrate nitrogen in soils. The soils came from the experimental plots where the urea-ammonium nitrate experiments on wheat were conducted.

The first study was conducted with surface samples from Decatur and Tilsit soils which were treated with nitrogen from urea at the rate of 200 pounds per acre (100 ppm.). The samples were incubated at 26°C. and replicates analyzed for pH, ammonia, and nitrate nitrogen every seven days. Results are recorded in Figures 4 and 5. Detailed procedures are outlined in Appendices 1, 2, and 4.

In Decatur soil there was a rapid increase in ammonia for the first seven days with little increase noted in the nitrate content. After this initial increase in ammonia concentration, there was a decline over the remaining twenty-one days with a sharp decrease the last fourteen days. The concentration of nitrates over the last twenty-one days steadily increased. The soil pH dropped from 6.4 to 5.5 over the twenty-eight day period.

The rate of conversion of urea to ammonia was much slower in the Tilsit than in the Decatur soil. A period of fourteen days elapsed before there was maximum concentration of ammonia. The nitrate concentration was greater at seven days than in Decatur, about the same at fourteen days, a little lower at twenty-one and twenty-eight days. The

soil pH dropped from 4.8 to 4.2 during the time of the experiment.

The second study on the conversion of urea was conducted with surface samples from each of three soils, Decatur, Tilsit, and Maury. Nitrogen from urea was applied at the rate of 4500 pounds per acre (2250 ppm.) to all samples. Samples were incubated at 0°, 10°, 20°, 30°C. for intervals of 168 hours to 216 hours. Replicates were withdrawn every twenty-four hours and analyzed for ammonia and nitrate nitrogen and pH. The results of this study are shown in Figures 6, 7, and 8. Detailed procedures are outlined in Appendices 1, 2, and 3.

In Tilsit soil, the maximum concentration of ammonia occurred after seventy-two hours in replicates incubated at 30°C., after 144 hours at 20°C., and after 192 hours at 10°C. The maximum ammonia concentrations were about the same at each temperature (3200 - 3400 ppm.). At 0°C. the total ammonia increased more slowly and never exceeded 1000 ppm. The pH increased with ammonia concentration at all temperatures. At 30°, 20°, and 10°C. it reached a maximum of about 8.2 at the time ammonia reached a maximum. At 0°C. the maximum pH was about 7.0. No increase in nitrate content was observed in any treatment.

In Decatur soil, the maximum concentration of ammonia occurred after 96 hours in replicates incubated at 30°C., after 144 hours at 20°C., and after 240 hours at 10°C. The maximum ammonia concentrations were about the same at 20° and 10°C. temperatures (about 3400 ppm.). At 30°C. the maximum ammonia concentration was about 2500 ppm. At 0°C. the total ammonia increased more slowly and never exceeded 1300 ppm. The pH increased with ammonia concentration, reaching a maximum of 9.0

at 30°C. and about 8.1 at 20°C. and 10°C. At 0°C. the maximum pH was about 7.5. There was no appreciable increase in nitrate content at any temperature.

In Maury soil, the maximum concentration of ammonia occurred after 96 hours in replicates incubated at 30°C., after 168 hours at 20°C., and after 240 hours at 10°C. temperature. The maximum ammonia concentrations were about 2500 ppm. at 30° and 10°C., and about 2000 ppm. at 20°C. At 0°C. the total ammonia increased more slowly and never exceeded 800 ppm. The pH increased as the ammonia concentration increased with a maximum pH of 9.0 at 30°C., 8.5 at 20°C., and 8.2 at 10°C. At 0°C. the maximum pH was about 7.8. There was a small amount of ammonia converted to nitrates.

The third study was conducted on Decatur and Tilsit surface soils, using urea nitrogen at a rate of 100 pounds per acre (50 ppm.) at 20° and 30°C. The peak production of ammonia nitrogen occurred at seventy-two hours in Decatur soil at both temperatures, followed by a decline over the remainder of the culture. The ammonia nitrogen was converted to nitrates slowly for the first twenty-four hours, after which conversion was very rapid for the duration of the experiment. The conversion of urea to ammonia in Tilsit soil was much more rapid at 30° than at 20°C. The peak ammonia production was reached at seventy-two hours at 30°C. as compared to 120 hours at 20°C. The conversion of the ammonia to nitrates was slower in the Tilsit soil. After ninety-six to 120 hours there was a rapid increase in nitrification. The nitrate nitrogen reached its peak at 168 hours for the 30°C. and at 192 hours

for the 20°C. temperature. In both soils there was a slight drop in the pH. Results of this study are shown in Figures 9 and 10. Detailed procedures are outlined in Appendices 1, 2, and 3.

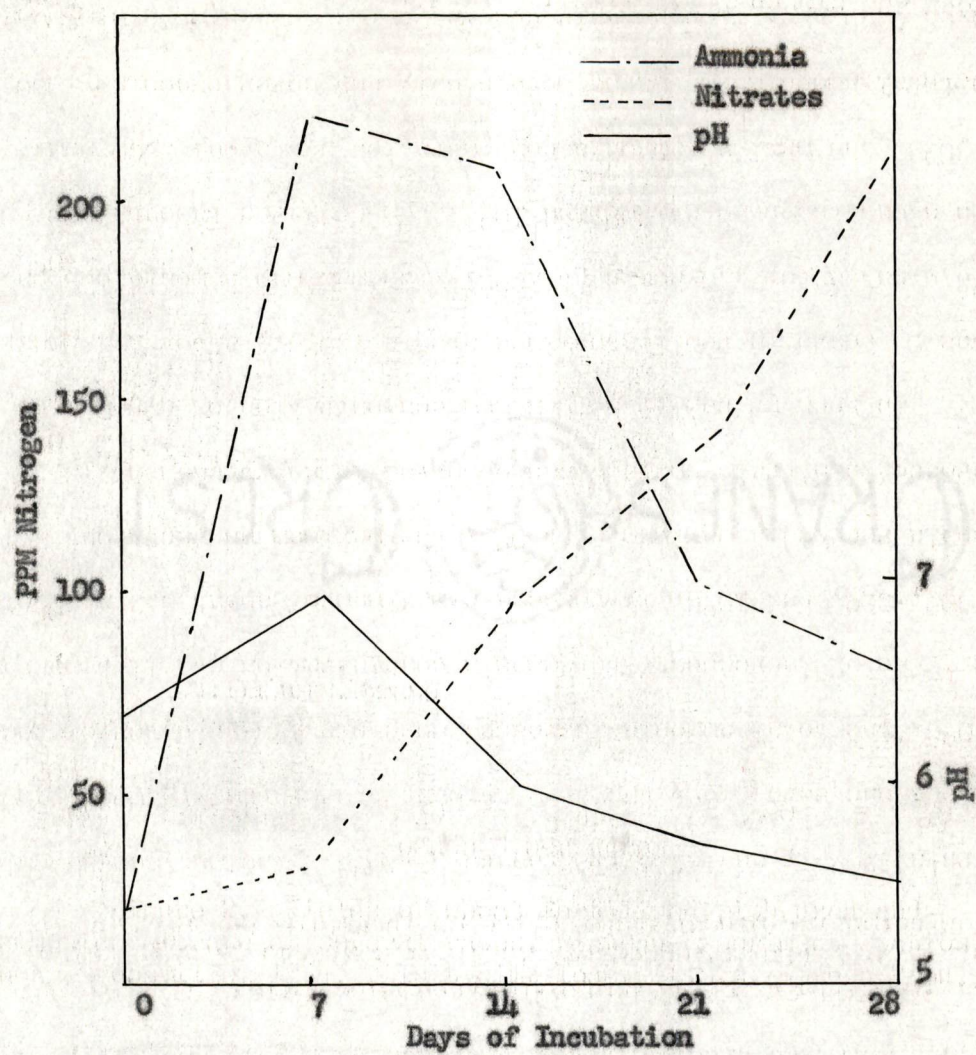


Figure 4. Levels of ammonia and nitrate nitrogen and pH in Decatur silt loam incubated at 26°C.

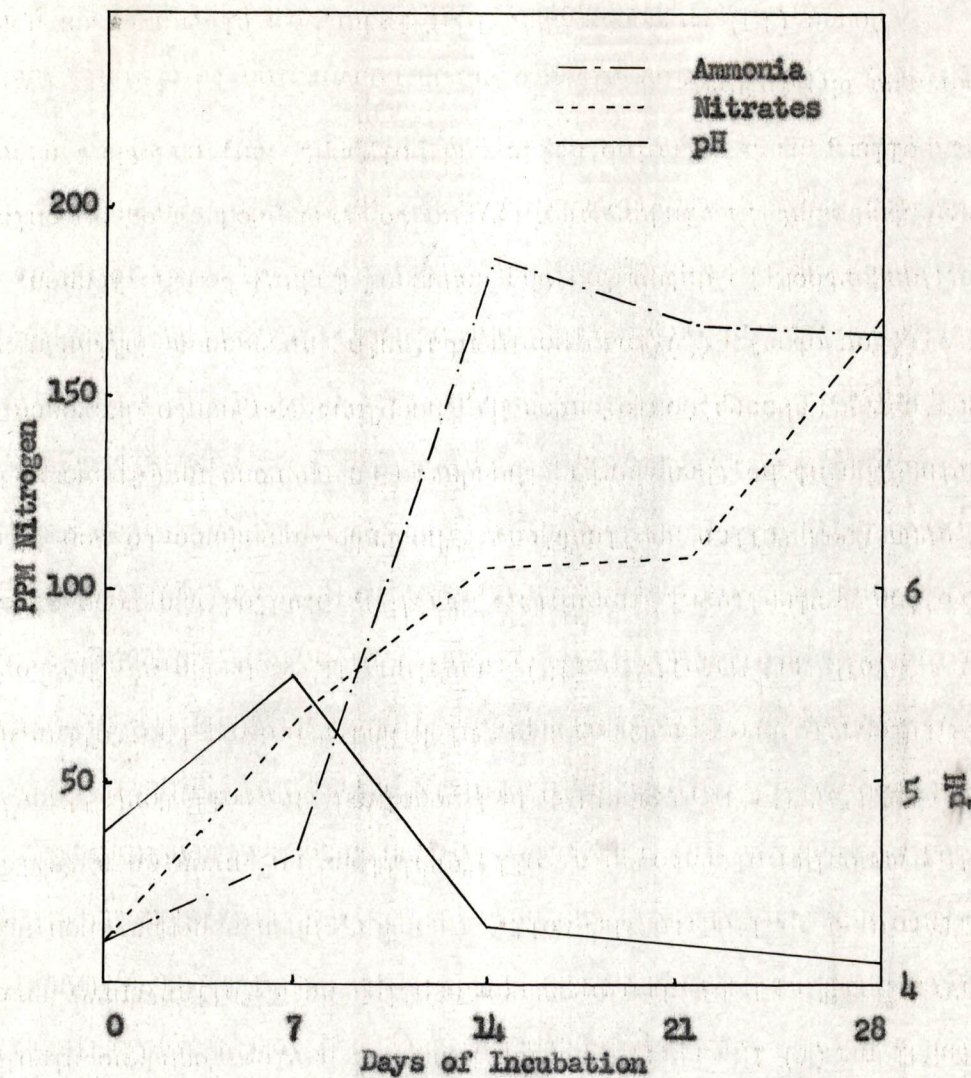


Figure 5. Levels of ammonia and nitrate nitrogen and pH in Tilsit silt loam incubated at 26°C.

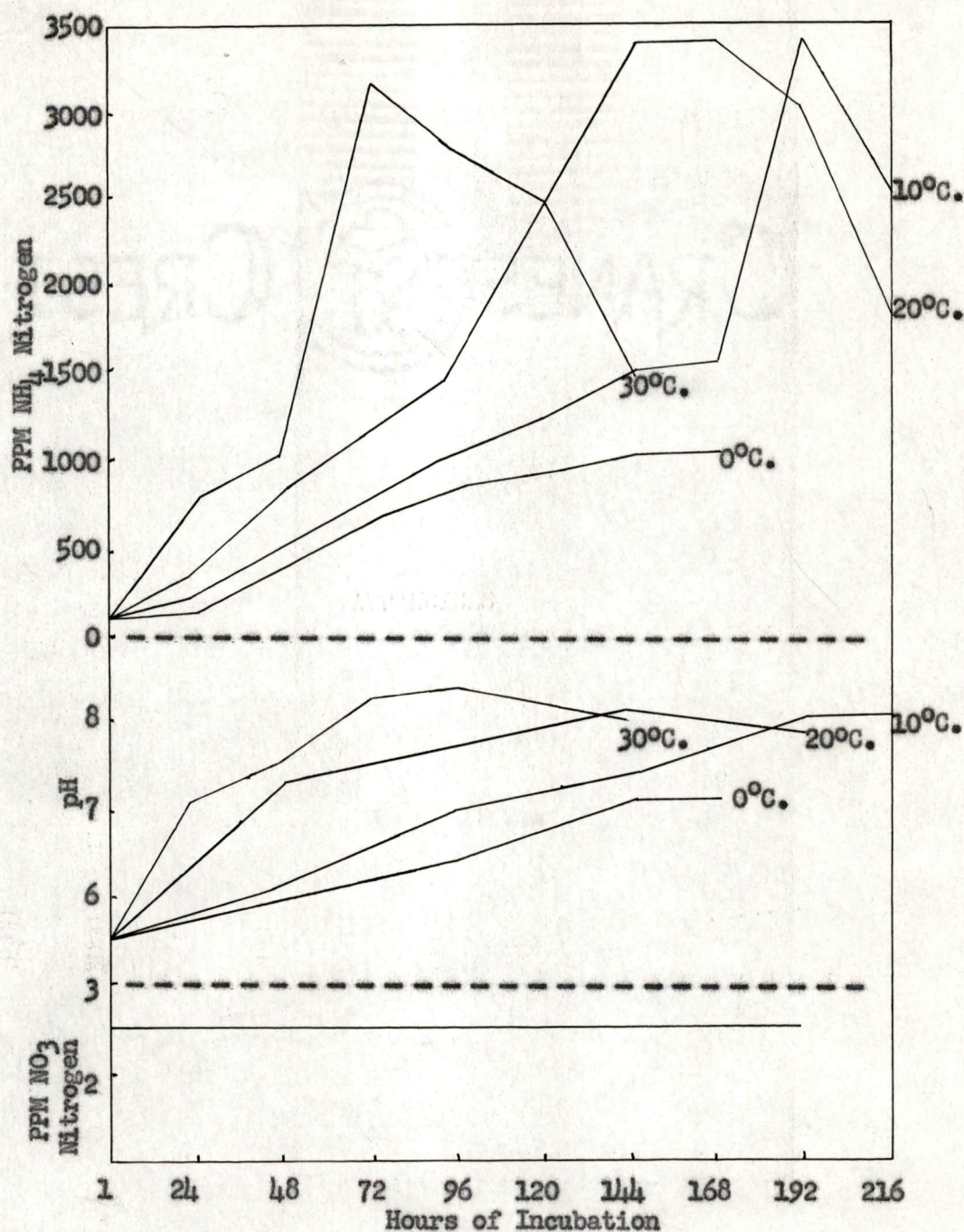


Figure 6. Levels of ammonium and nitrate nitrogen and pH in samples of Tilsit silt loam which were treated with 4500 pounds per acre of urea nitrogen and incubated at different temperatures over varying periods of time.

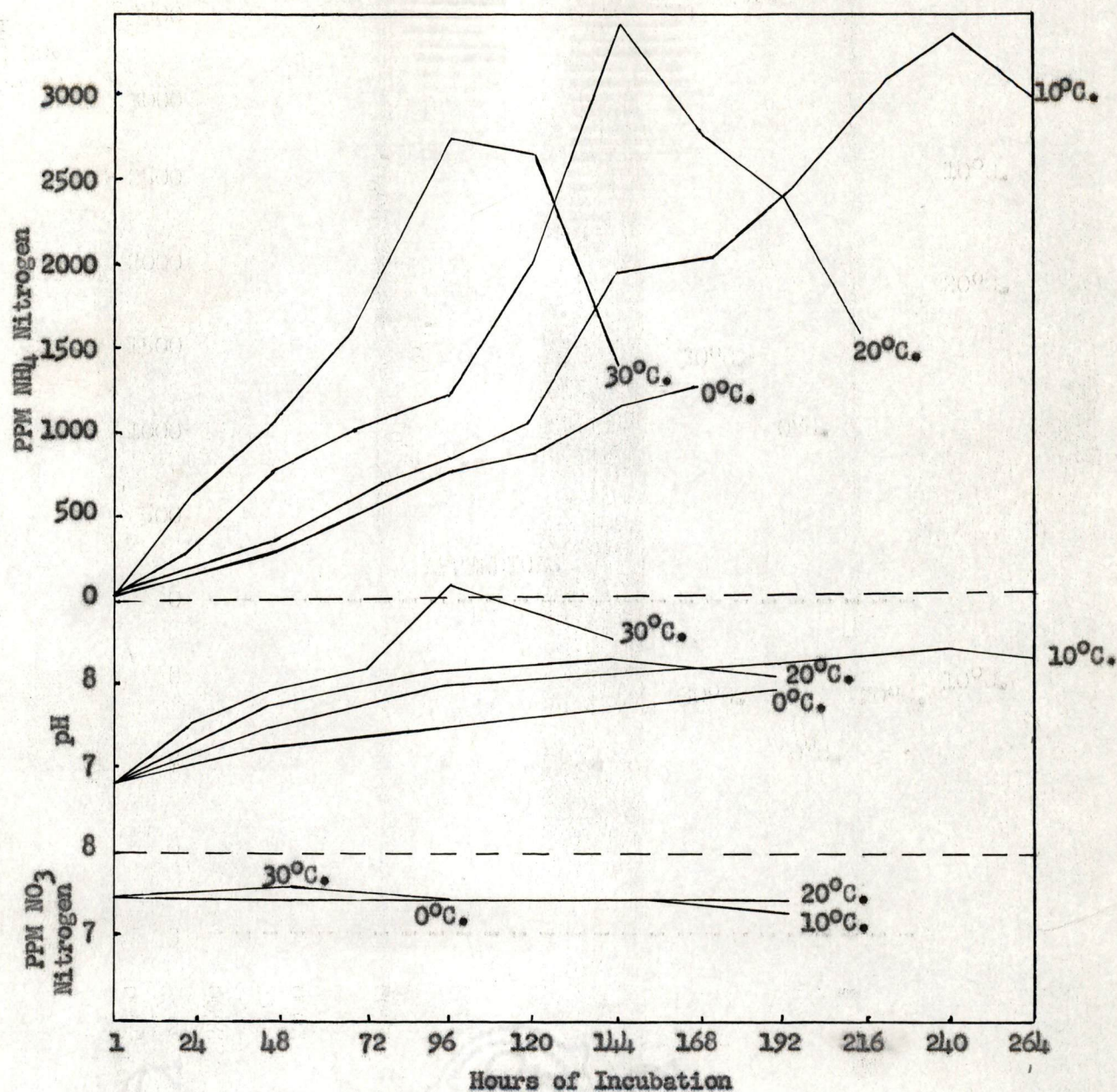


Figure 7. Levels of ammonium and nitrate nitrogen and pH in samples of Decatur silt loam which were treated with 4500 pounds per acre of urea nitrogen and incubated at different temperatures over varying periods of time.

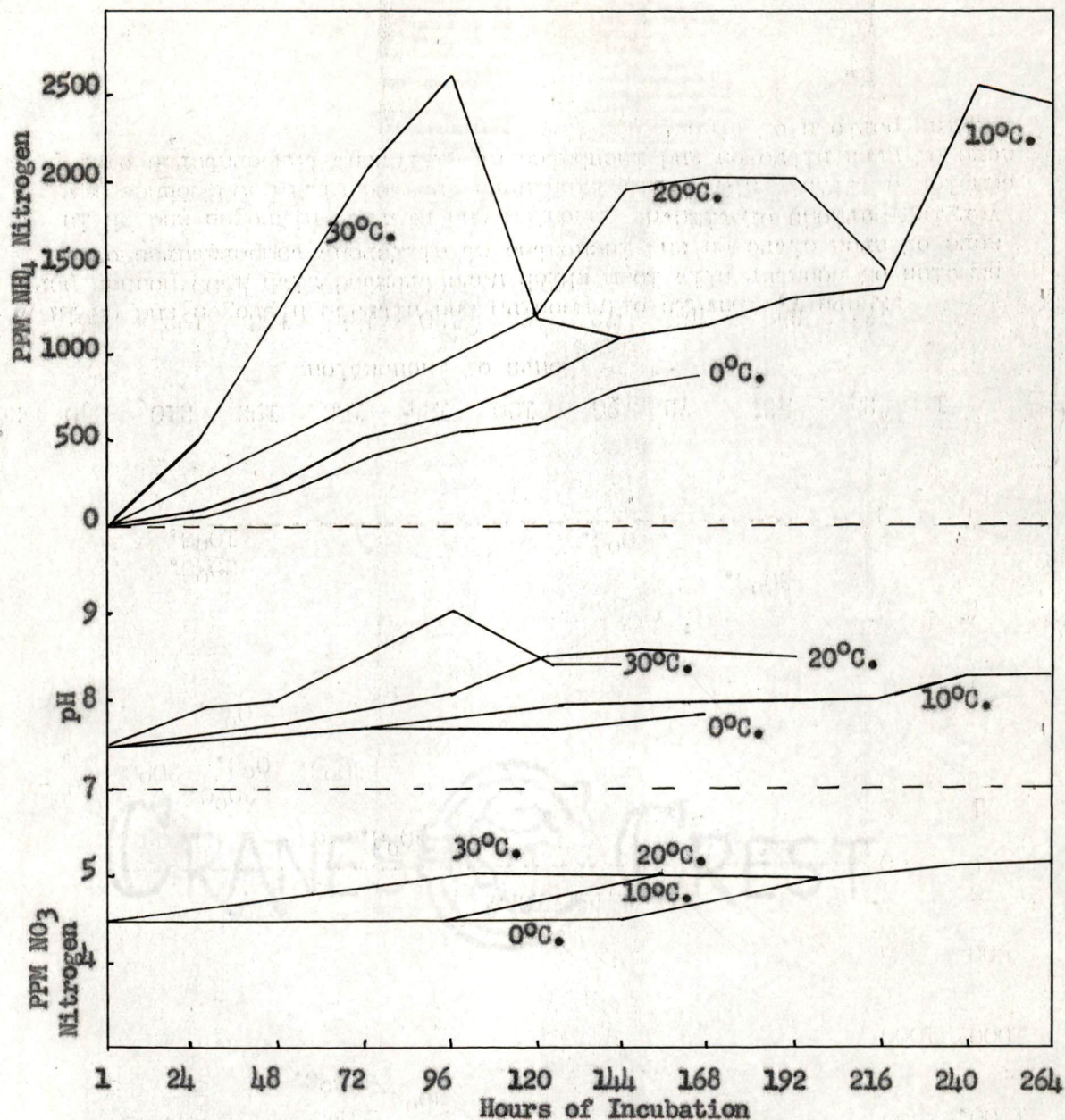


Figure 8. Levels of ammonium and nitrate nitrogen and pH in samples of Maury silt loam which were treated with 4500 pounds per acre of urea nitrogen and incubated at different temperatures over varying periods of time.

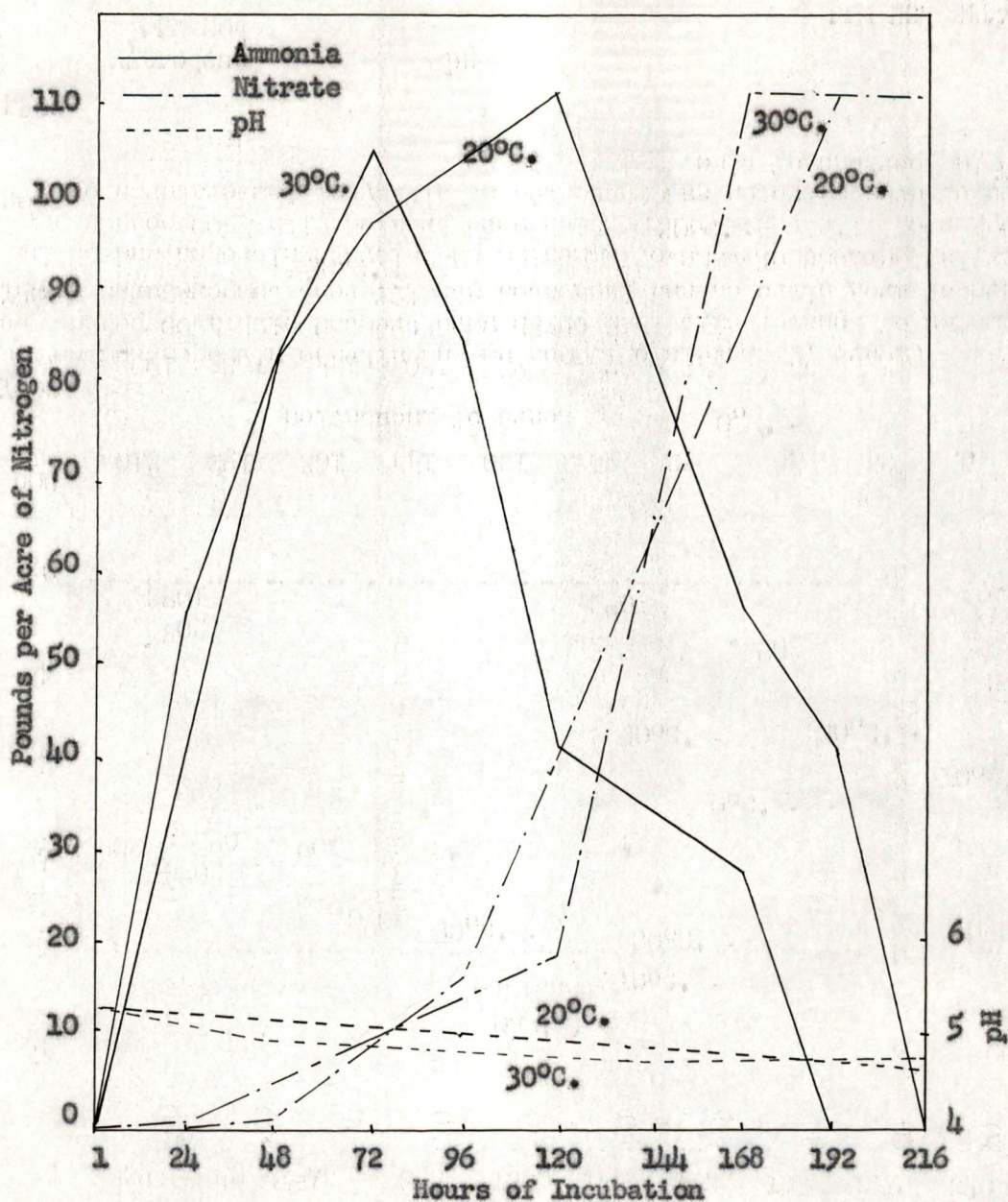


Figure 9. Levels of ammonia and nitrate nitrogen and pH in Tilsit silt loam which was treated with urea at the rate of 100 pounds of nitrogen per acre and incubated at 20°C. and 30°C. for 216 hours.

CRANES & CREST

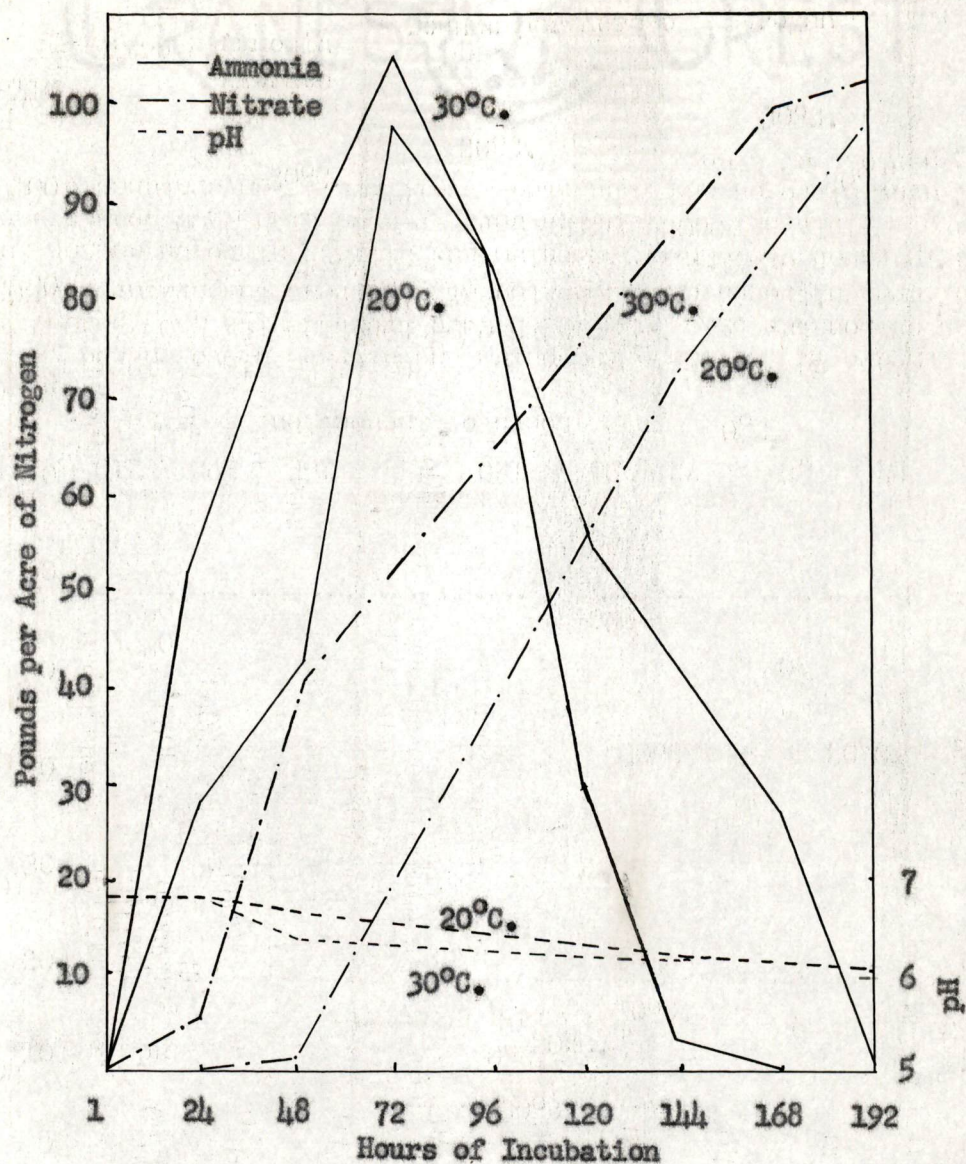


Figure 10. Levels of ammonia and nitrate nitrogen and pH in Decatur silt loam which was treated with urea at the rate of 100 pounds of nitrogen per acre and incubated at 20°C. and 30°C. for 192 hours.

CHAPTER V

DISCUSSION OF DATA

There was no significant response of wheat to nitrogen in either the Decatur or Maury soils (Figures 1 and 3). The experimental plots of the former were located on an old alfalfa sod and those of the latter were a part of a barn lot which had been reclaimed for use in crop production. The previous use explains in part the lack of response. On the other hand the wheat grown on the Tilsit silt loam responded to applications of nitrogen in both carriers. There was a significance between treatments; however, when the treatments sums of squares were further broken down, it was found that rates of nitrogen accounted for this significant difference and not the different nitrogen carriers.

It was found that, when urea nitrogen was applied to the soil at the rate of 200 pounds per acre in the prilled form, ammonification took place much more rapidly in the Decatur soil than in Tilsit. This may have been caused by the presence of more active basic materials in this soil as indicated by the pH. Plants grown in Decatur soil are able to obtain more ammonia earlier than those grown in Tilsit.

From the data obtained it is evident that the urea was converted to the nitrate form within a period of four weeks in Decatur soil, while at this time there still remained in the Tilsit soil quite a large amount of exchangeable ammonia on the colloid particle. This may be of a great advantage in the growing of many crops where the nitrogen stress is

not as great at the times when greatest leaching takes place. Although there is danger that urea may be leached in Tilsit soil before it is ever converted to ammonia, once the urea is hydrolyzed it may be retained as ammonia much longer in this soil than in Decatur soil due to the different rates of nitrification in the two soils.

In the study conducted using urea nitrogen at a 4500 pound per acre rate, it was found that, as the temperature was raised, urea was converted to ammonia at an increasingly rapid rate. Peak conversion was reached as the pH reached 8.2. There are definite pH limits for the bacteria which convert urea to ammonia (19), and there was definite indication that these microorganisms were either killed or inactivated as the pH reached 8.2. More ammonia was produced in the soil with a lower pH. Urea at about this same rate is being used now in some areas for the sterilization of tobacco beds (3).

It is of importance to notice the sharp drop in ammonia after the peak had been reached. This may be caused by the use of considerable amounts of ammonia by other soil organisms, and a small amount of ammonia may be lost by volatilization. There is also the possibility that nitrogen may be evolved in the elemental state when the urea is used in a slightly acid soil and there is a presence of nitrites. This is not necessarily caused by adverse soil conditions, but is strictly a chemical reaction (12).



When this high concentration of urea is used, there is a possible displacement of calcium, potash, and other elements from the surface soil to a location lower in the profile. This could be advantageous in cases where deep rooted crops are grown. This displacement may be brought about by the mass action of the ammonia produced by the hydrolysis of the urea. It is reasonable to assume that phosphate may become more available through the formation of ammonium phosphate.

When urea nitrogen was applied at the 100 pound per acre rate, there seemed to be much faster conversion to ammonia than at the other rates studied. One reason may be that the urea was applied in solution to this culture as compared to being applied in the prilled form in the 200 pound per acre culture. There was also a very rapid conversion of ammonia to nitrate nitrogen, which may be of great benefit when nitrogen is needed immediately by the crop being grown. While the rapid conversion suggests little residual effect from this fertilizer material on crop growth after the season of its application to the soil, there is, however, probably an indirect influence to consider, for under some conditions during its first season of application the urea nitrogen may so stimulate plant growth, even weed growth, after crops are harvested or laid by that the increased plant residue and root growth given to the soil will carry their influence through the succeeding crops.

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APPENDICES

APPENDIX 1

PROCEDURE FOR CONVERSION STUDY

Equipment and materials

1. Constant temperature incubator or water bath.
2. 125 ml. conical flask.
3. Ammonia free water.
4. Standard solution of urea of a known concentration.
5. Spectrophotometer.
6. Beckman pH meter.

Procedure

1. Place 10 grams of soil in 125 ml. conical flask.
2. Moisten to near saturation.
3. Add urea of known concentration.
4. Place in a constant temperature incubator or water bath at the temperature desired for study.
5. Place enough cultures in the incubator or water bath to allow withdrawal at various times during the period of study.
6. Determine ammonia by colormetric or volumetric method.
7. Determine pH by the glass electrode method.
8. Determine nitrate by the phenol disulphonic acid method.

APPENDIX 2

DETERMINATION OF AMMONIA BY VOLUMETRIC METHOD

Equipment and materials

1. Suction apparatus.
2. Buchner funnel (9 cm.).
3. Suction flask.
4. No. 42 filter paper (9 cm.).
5. 10% acidified potassium chloride.
6. 500 conical flasks.
7. Kjeldahl distillation apparatus.
8. Kjeldahl flasks.
9. Distilled water.
10. 10 N. sodium hydroxide.
11. 4% boric acid.
12. Standard sulfuric acid.
13. Methyl red indicator.

Procedure

1. Extraction procedure is the same as that used for the determination of ammonia by colorimetric method. One hundred grams of soil are extracted with 500 ml. of acidified potassium chloride (pH 2).

2. The leachate from the extraction is placed in an 800 ml. Kjeldahl flask.

3. Add 80 ml. of 10 N sodium hydroxide.

4. Connect 500 ml. conical flask containing 25 ml. of 4% boric

acid to receive the distillate.

5. Connect Kheldahl flask to distillation unit, swirl to mix.
6. Distill approximately 400 ml. of the leachate over.
7. Back titrate with standard sulfuric acid, using methyl red indicator.
8. Calculate percentage of nitrogen from the amount of acid used.

APPENDIX 3

DETERMINATION OF AMMONIA COLORMETRICALLY

Equipment and materials

1. 10% potassium chloride acidified to pH 2.5.
2. Nessler's Reagent.
3. 10% sodium tartrate.
4. 125 ml. conical flask.
5. Suction apparatus.
6. Distilled water.
7. Spectrophotometer.
8. Shaking apparatus.
9. Buchner funnels (9 cm.)

Procedure

1. Place 10 grams soil in a 125 ml. conical flask.
2. Add 50 ml. acidified 10% potassium chloride and shake for 15 minutes.
3. Filter through Buchner funnel under slight suction.
4. Rinse out flask with 50 ml. acidified potassium chloride and pour through Buchner funnel.
5. Place .50 ml. of this leachate in a 125 ml. conical flask.
6. Add 0.45 ml. of distilled water.
7. Add 1.00 ml. of 10% sodium tartrate and swirl to mix.
8. Add 2.5 ml. of Nessler's Reagent and swirl again.
9. Allow to stand for 25 minutes.
10. Read on spectrophotometer using a 410 wave length and calculate.

APPENDIX 4

DETERMINATION OF NITRATES COLORMETRICALLY

Equipment and materials

1. Spectrophotometer.
2. Phenol 2, 4-disulphonic acid.
3. 4 N. ammonia hydroxide.
4. Potassium nitrate (for standards).
5. Nitrate extracting solution.
6. Hot plate.
7. Evaporating dishes (3-inch).
8. Conical flasks, 500 ml.
9. Calcium hydroxide.
10. Magnesium carbonate.
11. Funnels.
12. Filter paper.
13. Fuming sulfuric acid.
14. Concentrated sulfuric acid.

Procedure

1. Place 50 grams of soil in 500 ml. conical flask.
2. Add 250 ml. extracting solution.
3. Place on shaker and shake for 10 minutes.
4. Add .4 gram calcium hydroxide and 1 gram magnesium carbonate.
5. Shake for 5 more minutes to precipitate the copper and silver.
6. Filter, discarding the first 20 ml. of the filtrate.

7. Set up a series of aliquots (2, 5, 10, and 15 ml.) of the standard nitrate (10 ppm.) in 3-inch porcelain evaporating dishes.

8. Measure a 10 ml. portion of the filtrate into a 3-inch porcelain evaporating dish.

9. Place all evaporating dishes on hot plate and evaporate to dryness in an atmosphere free of nitric acid fumes.

10. Allow to cool.

11. Add 3 ml. of the Phenol 2, 4-disulphonic acid to the dishes, rotating the dishes to insure contact with all salts.

12. Allow to stand 10 minutes.

13. Add 15 ml. distilled water.

14. Make alkaline with ammonium hydroxide as indicated by appearance of yellow color.

15. Transfer to 100 ml. volumetric flask and add water to mark.

16. Read on spectrophotometer using 410 wave length and calculate.