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FOREWORD

The work herein described was undertaken at the Oklahoma Agricultural College, Stillwater, Oklahoma, State Agricultural and Mechanical College.

The work was outlined at the suggestion of Dr. L. L. Lewis, head of the Department of Bacteriology, and was done under his direction. Acknowledgment and thanks are accorded him for kindly help and counsel. To Dr. W. P. Schuler, Assistant Bacteriologist, for timely help and valuable suggestions the author acknowledges his gratefulness.

Object Of The Investigation.

The object was to find out the effect of applications of different amounts of lime and of manure on the numbers of bacteria and their activities in soil which had been continually cropped and unfertilized for twenty years, as shown by pot experiments with the use of cotton-seed meal and dried blood as ammonifying material and dried blood and ammonium sulphate as nitrifying material. Effort was also made to determine by the results of the experiments, the relative availability of the dried blood, cotton-seed meal and the ammonium sulphate.

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LIMING

Like many of the so-called "lost arts", known to the ancients years prior to the Christian era, the beneficial effects of liming were recognized though not understood. Brown (1) tells us that almost a century ago Johnston spoke of lime as the "basis of all good husbandry".

With the increasing knowledge of man due to thorough and painstaking search for truths, we have come to understand more thoroughly and appreciate more fully the role played by lime in the soil and are constantly seeking to explain the old and seek new truths regarding this phenomena over which so many facts and theories have been evolved.

The science of Soil Bacteriology particularly has developed a new field to related sciences, such as chemistry and physics, until today we know that the results due to liming are dependent upon four great determining forces; viz. physical, chemical, physiological, and bacteriological. It is from a standpoint of the latter factor on which this work is based, although the others are not to be ignored.

Historical.

So much valuable data has already been accumulated and received in this connection that to relate all would merely be a repetition of what has been said. However a short review is not considered out of place.

The production of carbon dioxid, in soils, has long been known to represent decomposition of arganic matter. The effect of lime on soils was shown by Peterson (1871) (2), later in 1886 by Wollny (3), who proved that the addition of lime increased the production of carbon dioxid. Later Ebermayer (4), Hilgard (5), Hartwell and Kellogg (6) confirmed the results obtained by Peterson and by Wollny. Chester (7) found that lime applied at the rate of 1,000, 2,000, 3,000 lbs. per acre increased the number of bacteria on nutrient agar. He further (8) showed that application of 4800 pounds gave the highest increase and he concluded that the action of the lime was not direct but that it served to neutralize the soil acidity, making the conditions more favorable to bacterial growth.

The effect of lime on ammonia production was shown by Remy (9 & 10), later by Ehrenberg (11) and Wohltmann, Fischer and Schneider (12) and by Lipman (13) to give an increase in the ammonifying power of the soils treated.

That liming of soils materially effects an increase in the nitrifying bacteria was pointed out as early as 1871 by Peterson, already referred to in connection with his work on carbon dioxid production. Confirmation of such results has been ably shown by other investigators, among whom may be mentioned Pritchard (14), Ewell & Woley (15), Wohltmann, Fischer & Schneider (16), Remy (17), Lipman & Brown (18), Brown (19) and by Lipman and Blair (20).

MANURING

Barnyard manure added to the soil brings about numerous changes. It adds to the nitrogen content; 1 ton on an average containing 10 to 12 pounds of nitrogen and potassium, and 2 or 3 pounds of phosphorus, it changes the physical condition of the soil such as increasing the granulation of a clay loam while it tends to make a sandy soil less porous by binding the particles together. These changes affect the water holding capacity of the soil, the capillarity, aeration and temperature, all of which have a direct bearing on the bacterial flora and their activities. Furthermore manure is rich in bacteria. It therefore adds millions of them to the soil. Many of these may die off or be inhibited in their activities because of a new environment, while others multiplying will decompose the constituents of the manure and by so doing greatly alter the organic and inorganic substances in the soil. Therefore, such changes will be both qualitative and quantitative. The chemical reactions which bring about such changes will also liberate heat, thereby changing the soil temperature, which in turn will have direct bearing on the numbers and physiological activities of the bacteria.

Whatever the changes, the addition of manure will greatly change soil conditions through the control of the factors mentioned and these will all be reflected in the crop produced.

Historical.

Remy (21) (1902) and Fischer (22) (1909) showed that the number of bacteria was materially increased by the addition of manure. Caron (1895) (23) found that the crop grown and the cultural methods, as well as manure added, regulated the numbers of bacteria in the soil. King (24) found that an excessive moisture content decreased the bacterial activities while an increased temperature increased their activities.

Hiltner & Störmer (25) found a marked increase in numbers with the addition of manure. Likewise Brown (26) found an increase in numbers, with applications of manure up to 16 tons, and that the ammonifying and nitrifying powers of the soil were also increased. The greatest increase was noted when applications of from 8 to 12 tons per acre were applied.

That manure was especially beneficial to nitrifying organisms was brought out by Heinz (27) and Hiltner (28). Withers (29) reported a reduction of nitrates in the soil when the quantity of manure applied was excessive on the soil, very coarse grained and poorly aerated. Barthel (30) found that nitrification did not take place in the presence of large amounts of soluble organic matter. Millard (31) was unable to find any nitrifying bacteria in manure while Niklewski (32) claimed that nitrification occurred in solid stable manure when the amount of liquid present was not too great. Gerlack & Vogel (33) have shown that there are varieties of bacteria in the soil having the ability to convert ammonia, nitrates, and nitrites into insoluble proteins, such synthetic reactions accounting in part for the disappearance of nitrates; aside from other losses, such as denitrification, loss through percolation, utilization by the crop. Greaves and Carter (34) found that in calcareous soils the greatest number of organisms were noted where 25 tons of manure per acre were applied that the ammonifying powers of the soil increased with manure applied up to 25 tons per acre, but that the greatest increase per ton of manure was obtained in soil receiving 5 tons. The nitrifying powers increased as the manure applied was increased up to 25 tons and 22.5 percent water.

AMMONIFICATION

Ammonification is defined as the production of ammonia by bacteria, either from protein substances or their cleavage products. It is a very important phase in the cycle of transformation to which nitrogen is subject. The process is by no means simple, but involves many complicated reactions. The combined nitrogen in protein material passes through an unknown series of intermediate changes and ultimately the nitrogen of a decaying mass, in a large measure, assumes the condition of ammonia.

Müntz and Condon (34) (1893) were first to demonstrate that ammonification in soils is a biological process. They showed that no ammonia was formed in sterile soils, also that moulds as well as bacteria were capable of producing ammonia from nitrogenous organic matter. Later Marchal (35) not only confirmed the work of Müntz and Coudon, but showed that various organisms exhibit marked differences in their ability to split up proteins into ammonia. Of these B. mycoides stands foremost, about half the organic matter placed at its disposal being transformed into ammonia. It is interesting to note in this connection that while the demomposition of proteins is accomplished by hydrolysis due to bacterial enzymes, (proteolytic enzymes) yet in the above connection Marshal working with egg albumen showed that an apparent oxidation of the protein molecule took place according to Voorhees and Lipman (36). He assumes the following equation:

$C_{72}H_{112}N_{18}SO_{22} + 77\ O_2 = 29\ H_2O + 72\ CO_2 + SO_3 + 18\ NH_3$ as representing what takes place in the production of the ammonia.

From a standpoint of actual conditions in the field ammonification serves as an index to the relative amount of plant food for it tends to regulate the production of soil nitrates. It is indicative of the physiological activities of the bacterial flora of any given soil and as conditions such as mechanical and chemical composition, moisture, temperature, air supply, fertilizer treatment and methods of tillage, cropping, etc. regulate this factor, the amount of ammonia produced under a given set of conditions is of great value in determining controlling methods of treatment in endeavoring to regulate soil fertility.

NITRIFICATION

Nitrification refers to the oxidation of ammonia or nitrites to nitrates.

The incessant drain on the natural nitrate supply for the manufacturing of gun powder caused by the wars of the seventeenth and eighteenth centuries by countries involved (England and France) gave rise to an increasing demand for nitrates.

That England had shut off the supply that France depended upon from the saltpeter beds of India compelled her to tax her internal production to an alarming degree. We are told that the peasants annually paid a part of their taxes in saltpeter. Prizes were offered by the Government for methods of producing saltpeter, all of which stimulated investigation and brought forth valuable facts by which agriculture has since profited. So it is the arts of war instead of peace that are responsible for much of our early knowledge of this subject.

Early theories regarding the natural formation of nitrates were purely from a chemical point of view and of which many were advanced. These have been ably reviewed by many authors and would be out of place here. Suffice to say, however, that no great light was shed upon the solution of the problem until chemical and bacteriological methods were combined in the search for the truth.

Pasteur, in 1862, said that ferment action was responsible for nitrification processes and Müller observed the rapid formation of nitrates from ammonia in sewage while no such change took place from pure solutions of this gas.

It is to Schlösing and Müntz (37) that we owe our first knowledge of experimental evidence on the subject. They filled a glass tube with quartz sand mixed with a small amount of lime and then passed sewage through it. The sewage passed through unchanged for twenty days, at the end of which time nitrates appeared and increased in amount. When the tube and contents were treated with chloroform the formation of nitrates stopped. Upon adding a little fresh garden soil the process of nitrification was renewed. This showed that the chloroform had stopped the activities of something in the sewage and that the fresh soil contained the something essential to a renewal of the process. That it was due to micro-organisms was evident. This successful demonstration led others to attempt the isolation of the organisms in pure culture. This led to much painstaking work on the part of others, such as Warington (38), Celli, Marino Zuco (39) and the Franklands (40), but it finally remained for Winogradski (1890) (41) to solve the problem and to demonstrate wherein others had failed. He proved that the organisms do not develop on ordinary gelatine, but only upon inorganic media. For this purpose he employed silicate jelly properly supplied with nutrient inorganic salts. Later other investigators proved that agar deprived of its soluble organic matter, gypsum, sandstone disks, filter paper pads, etc., could be effectively used as solid media. Today the isolation of nitrous and nitric ferments stands out preeminently as one of the greatest achievements contributed to the science of agricultural bacteriology and has served greatly towards the solution of problems in the interest of increasing and maintaining the fertility of the soil.

Since the ammonifying and nitrifying power of a soil is a measure of the activities of certain groups of beneficial micro-organisms acting on the insoluble and unavailable plant food, and through these activities setting up, such anabolic changes as to render soluble and available this food material it was the purpose to measure by this means, other things being equal, the material effect of varied liming and manuring upon the physiological activities of the micro-organisms influenced by such.

PLAN OF EXPERIMENT

The soil chosen for the investigation was a clay loam from a one-half acre plot which for 20 years previous had received no fertilizer in the form of manure, etc., the only source of enrichment being from the decomposition of plowed under stubble from wheat which had been successively grown each year for 20 years.

Sampling.

The samples were gathered in June after the crop was cut. To obtain a representative of the entire plot seven samples from equally distributed points were taken as follows: With a sterile spade (flamed in an alcohol lamp) the surface debris was first gently scraped away over approximately a four foot radius. Approximately 100 pounds, to a depth of 8 inches were removed and placed in sheet iron tubs which had previously been thoroughly scalded and allowed to dry inverted. Each sample was sieved separately through a large sand sieve, the first few sievings in each case being thrown away. When finished samples were successively returned to their original containers.

Treatment.

The containers were taken to the greenhouse where a composit of the entire set was made as follows:

First the contents of each container was spread upon a separate sterilized newspaper whereupon a thorough mixing with the hands was made, the hands being washed and dried with a clean towel before eabh mixing of the successive samples. Each sample was quartered down opposite quarters being taken after mixing until the mass was reduced to approximately 25 pounds. The seven samples were then mixed by taking a handful of each at the time with thorough mixing of the successive added portions. When all had been added the compost was thoroughly mixed after the manner of the originals.

The composit was divided into 10 portions of 11 kilograms each and placed in thoroughly clean, scalded and dried five gallon stone jars. A small hole had been made in the bottom of each and a piece of broken sterile flower pot placed over same before soil was added. These "pots" were numbered as incidated below. Sterile newspapers were spread on a table in the greenhouse and the pots placed thereon when each received the following treatment:

1 & 2 - nothing

3 & 4 - lime 5.4 grams.

5 & 6 - " 10.8 grams

13 & 14- manure 64.8 grams

15 & 16- " 129.6 grams.

The added materials were thoroughly mixed with the soil. The moisture was determined on a remaining portion and that of all pots was adjusted to approximately 2/3 saturation, using sterile cistern water. This was adjusted every two weeks by weighing the pots and soil and adding sterile cistern water to make up the loss, 1 c.c. being added for each gram lost. After each addition of water the soil in each pot was stirred and worked with a clean sterile spatula. The pots were kept at an average temperature of 60-65°C. At the end of 30, 60 and 90 days respectively representative samples of approximately 1,100 grams from each pot were taken after dumping contents on a sterile newspaper, thoroughly mixing and quartering down by same procedure as aforementioned. These were placed in sterile fruit jars and removed to the laboratory. Moisture determinations were immediately made and dilution plates poured from each. The remaining portions save 50 grams placed in the ice box for infusions to be made later, were spread out separately on newspaper, covered with the same and allowed to air dry for three days. They were then ground in a mortar and sieved through an 80-mesh sieve, all under sterile precautions when possible. Ammonification and nitrification determinations were made from 100 gram portions of such soil infusions of those portions of the original which were placed in the ice box being used as inoculum.

EXPERIMENTAL METHODS
BACTERIAL COUNTS

As stated counts were made immediately after samples were brought to the laboratory.

Under sterle precaution 10 gram infusions in 100 cc of sterile distilled water were made and revolved by hand for 5 minutes and sucessive dilutions made from this viz:

10 grms soil in	100 cc	sterile water			=	dil.	1 - 10	(a)
1 cc of (a)	" 99	"	"	"	=	"	1 - 1000	(b)
1 cc of (b)	" 99	"	"	"	=	"	1 - 100,000	(c)
10 " " (c)	" 90	"	"	"	=	"	1 - 1,000,000	(d)

Duplicate plates were poured from the (c) and (d) dilutions using 2/10 and 5/10 cc portions. Results from dilution (d) were the only ones used as in all cases dilution (c) gave growths that were too thick to be representative. All plates were incubated at 28° C and counts were made 5 days after pouring, using a hand lenze and a Jeffers counting plate. The medium for growth was as follows:

Dextrose - 10.0 gms.

K 2H Po 4 - 0.5 "

Mg So4 - 0.2 "

Peptone (Whitte's) - 0.1 gms.

Agar -20.0 "

Water - 1000. cc

The reaction of the medium was adjusted to +0.5 % Sterilization was accomplished by heating three sucessive days in the Arnold Sterilizer for periods of 15 minutes each.

Titration of media was done at room temperature according to the standard methods as set fourth by the American Public Health Association. (43)

Sterilization of Glassware

Sterilization of all glassware, petri dishes, test tubes, pipetts, etc., except tumblers used in the Ammonification and Nitrification experiments, was accomplished by means of the hot-air sterilizer, at 150-160° centegrade for two hours. Tumblers were subjected to the play of the free flame of a Bunsen burner on the inside and on the outside rim. After being filled with soil etc. Sterile porous covers were placed thereon.

Chemicals Used

The chemicals used throughout the investigation were Merck's C.P. Salts of Standard Purity.

Ammonification Experiments

100 gram portions of the respective air dried sieved soil were placed in tumblers and arranged thus :

1 & 2 nothing

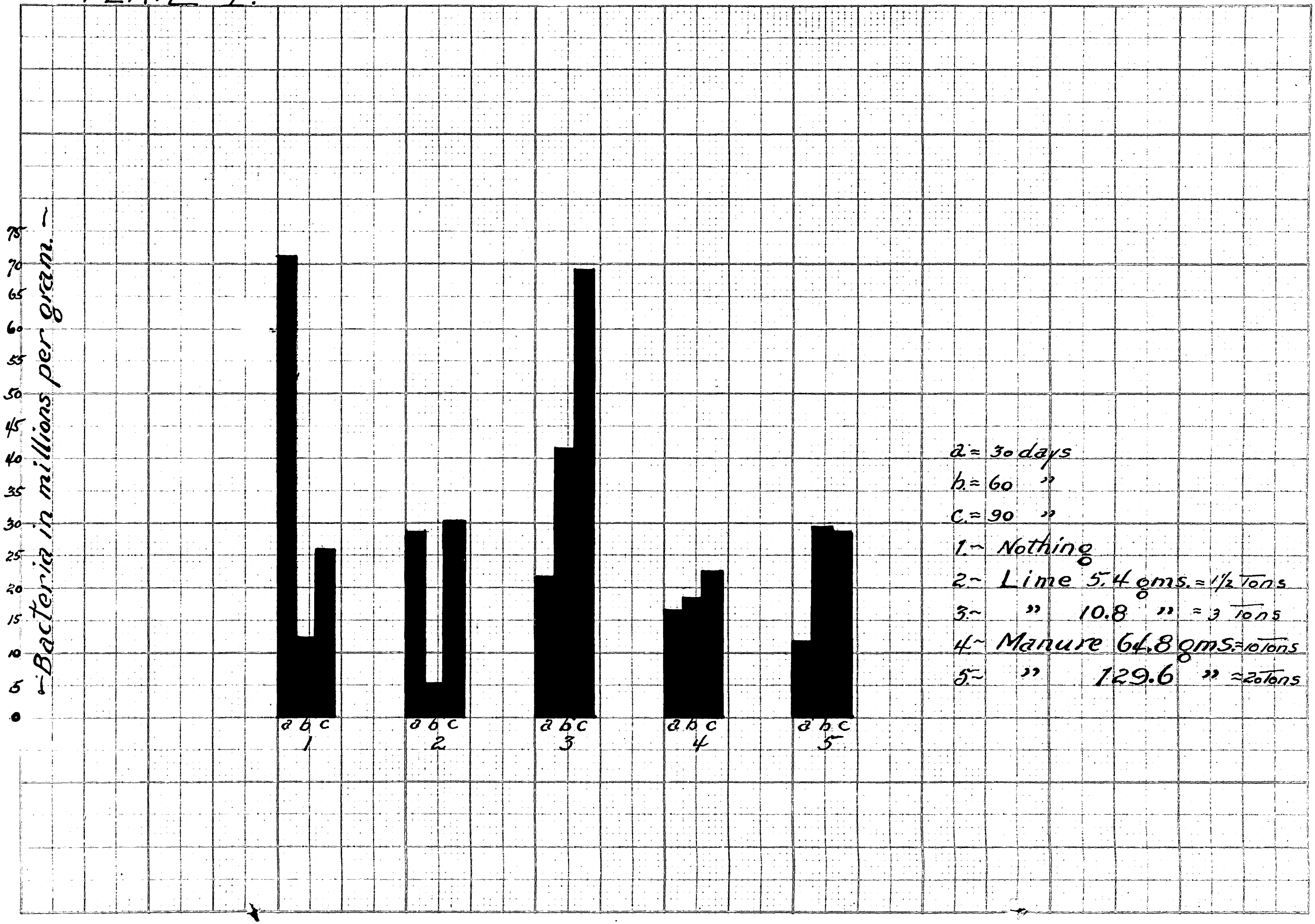
3 & 4 Dried blood 5 grams

5 & 6 Cotton seed meal 5 grams.

The materials were stirred thoroughly by means of a sterile glass rod which was cut short enough to remain in the tumbler after the porous cover was put on. The mixture was then treated with an infusion, 10cc of the original fresh soil, (kept in the ice box) by revolving for 5 minutes, 50 grams of the soil in 100 cc of sterile water. Enough sterile water was then added to bring the moisture content up to the optimum for that soil, 12 cc additional being added for those portions contaning dried blood and cotton seed meal. They were covered with porous earthen-ware covers and incubated at 28° C for 8 days, at the end of which time they were transferred to distillation flasks and the ammonia determined by the magnesium oxide method. (44)

(Note) Water used in both Ammonification and Nitrification determinations was distilled and tested free from ammonia and nitrates.

~PLATE~I.



~Bacteria in millions per gram.~
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
a b c
1
a b c
2
a b c
3
a b c
4
a b c
5
a = 30 days
b = 60 "
c = 90 "
1~ Nothing
2~ Lime 5.4 gms. = 1/2 Tons
3~ " 10.8 " = 3 Tons
4~ Manure 64.8 gms. = 10 Tons
5~ " 129.6 " = 20 Tons

Nitrification Experiments.

The same proceedure was observed here as in the Ammonification experiments, and arranged thus:

1 & 2 - Nothing

3 & 4 - $(NH_4)_2 SO_4$ - 100 mgs.

5 & 6 - Dried blood - 200 "

These received similar treatment to the ammonification tumblers except that only enough water was added to bring the soil up to the optimum for that soil. The tumblers and soil were weighed and the weights recorded every 10 days, they were re-weighed and the loss in moisture replaced with sterile water. They were incubated four weeks, at the end of which time warm water and about 2 grams of lime were added, stirred well, allowed to settle, filtered by decantation five times through double filter papers into 500 cc graduated flasks. Finally the residue was thrown on the paper, washed and after cooling to room temperature the filtrate was made up to 500 cc and the nitrates determined in 25 cc portions according to the Phenol Sulphonic acid method for nitrates.

(Note) Cold water was first used on several washings but it was found on testing the residue (44) that all nitrates were not removed. Using the same amount of hot water removed all the nitrates.

Effect of Treatment and Time On the Number of Bacteria In the Soil

Effect of Lime

Following the diagram on plate I we find that different treatment and time play an important role in the numbers of bacteria in the soil under consideration.

At the first sampling 30 days, one and one half tons of lime* to the acre caused a marked decrease over the check from 71 million to 28,900,000 while an increase in lime content to 3 tons per acre gave a further decrease to 21,400,00. The moisture content it will be noted in table I is nearly constant from 13.6 to 13.8. This marked and gradual decrease in the numbers confirms to the work of

* (Note) The lime added is calculated as $CaCO_3$, the wt. of soil at 3,500,000 lbs per acre (45)

TABLE I MOISTURE AND BACTERIAL COUNTS FROM POTS AT END OF 30, 60 AND 90 DAYS.

Pot Composit Number	Lab. No.	30 DAYS			60 DAYS			90 DAYS		
		Moisture percent	Bacteria in millions per gram of dry soil	Average	Moisture percent	Bacteria in millions per gram of dry soil	Average	Moisture percent	Bacteria in millions per gram of dry soil	Average
1 & 2	a	15.00	64,705			20,747			34,325	
			77,647	71,176	13,60	4,149	12,448	12.60	17,602	25,596
3 & 4	b	17.20	*			4,640			45,104	
			28,985	28,985	13.80	5,801	5,220	22.40	15,464	30,284
5 & 6	c	16,20	23,866			52,204			*	
			19,093	21,480	13.80	30,162	41,183	13.20	69,124	69,124
13 & 14	d	10.00	16,666			29,205			*	
			contaminated	16,666	14.40	7,009	18,102	11.80	22,676	22,676
15 & 16	e	11.80	11,338			47.169			34,722	
			11,338	11,338	15.20	11,792	29,480	13.60	23,148	28,935

*Lost

Fisher, (46), who showed that lime at first caused a depression in 7 days. Beckwith and Vass (47) that lime at the rate of 2 tons per acre may cause no marked gain or may cause a depression in the numbers in certain types of soils in thirty days.

The Second Sampling

At the end of 60 days a decrease in numbers is noted in the check pots and where one and one half tons of lime has been added while a marked increase is seen where 3 tons of lime have been added the numbers increasing 28,700,000 over the check and 35,900,000 over the one and one half ton application of lime.

The Third Sampling

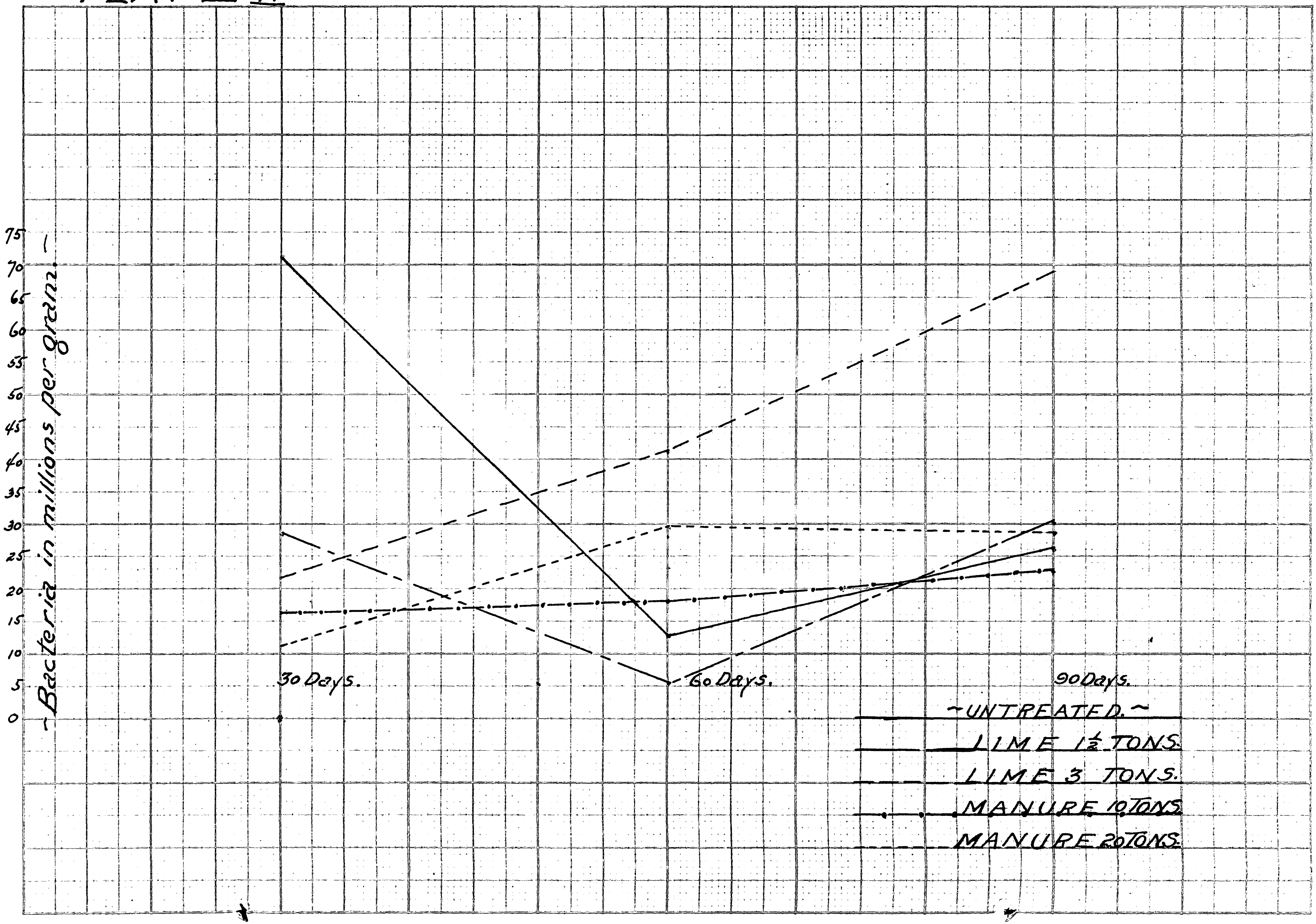
At the third sampling 90 days, a gradual raise in numbers is noted in the case of where one and one half tons of limestone were applied there being an increase of 4,600,000 and a marked increase of 33,900,000 where 3 tons of limestone per acre was applied. It is striking to note also that in the application of 3 tons of lime per acre there is a marked rise in numbers at the end of 60 and 90 days these being greater than in either the check pots or where one and one half tons of limestone were added. The curve is almost a straight line. The greatest gain was found at the end of 90 days where 3 tons of lime per acre was applied. This conforms somewhat to the work of Chester (7) who as already mentioned found the greatest increase with 4000 pounds of lime.

The Effect of Manuring

At the time of first sampling at the end of 30 days application of 10 tons per acre showed a marked decrease from 71 million in the untreated pots to 16,600,000 or a reduction in number of 54,400,000 while the application of 20 tons per acre gave a still further decrease in the same length of time. The next sampling at the end of 60 days showed an increase of 6 million over the check where 10 tons of manure were applied and of 17 million where 20 tons were applied. At the third sampling there was a slight decrease of 2 million over the check where 10 tons per acre were applied and a slight increase of 3,400,000 where 20 tons were applied.

The greatest number of bacteria seem to be in favor of the 60 and 90 day

-PLATE-II.



75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-Bacteria in millions per gram.-
30 Days.
60 Days.
90 Days.
~UNTREATED.~
LIME 1½ TONS.
LIME 3 TONS.
MANURE 10 TONS.
MANURE 20 TONS.

periods where 20 tons of manure were applied and the greatest gain at the end of 60 days with 20 tons of manure.

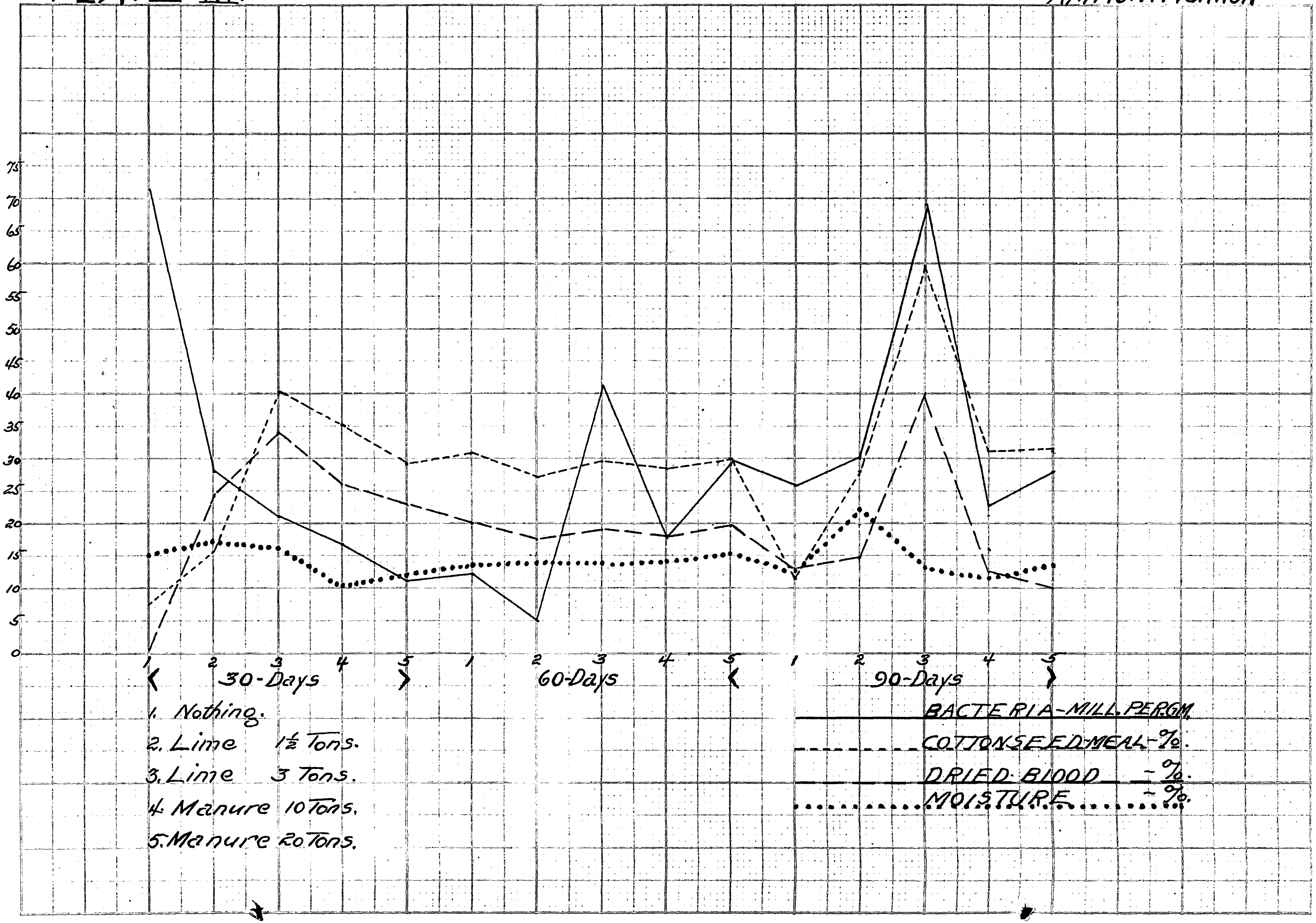
Comparison of Treatment

A striking comparison between the lime and manure for the same and different periods of time is shown in the curves on Plate II.

It is noticeable that at the end of 30 days all treatments gave lower numbers than the untreated portions. The lowest number was attained with the application of 20 tons of manure while the highest was attained with $1\frac{1}{2}$ tons of lime. At the end of 90 days there is a very marked decrease in the untreated portions and a still further decrease where $1\frac{1}{2}$ tons of lime was applied. The highest count was attained where 3 tons of lime were applied while 20 tons of manure gave an increase over either the untreated, the $1\frac{1}{2}$ tons of limed portion or that receiving 10 tons of manure. There is also a striking parallelism between the curve for the pots receiving 3 tons of lime and those receiving 20 tons of manure. An increase in numbers at the end of 60 days being noted over the untreated pots and over their former bacterial content. At the end of 90 days there is a slight raise in numbers in the untreated, portions while those portions receiving applications of $1\frac{1}{2}$ tons of lime, 10 and 20 tons of manure respectively show a slight but unappreciable gain over the untreated, although there is a fair increase in all treatments except the 20 tons of manure application which shows a slight decrease over the 30 day content. The $1\frac{1}{2}$ tons lime application show a greater though slight gain than manured pots while the three ton applications give a marked increase in numbers.

There is a gradual and marked increase for the 30, 60 and 90 day periods, where 3 tons of lime were applied and a fluctuation giving very much lower numbers where all other treatments were considered for the entire period.

~PLATE~III. ~AMMONIFICATION~



The Relationship of Treatment To Bacterial Numbers and Their Ammonifying Activities As Shown by Dried Blood and Cottonseed Meal.

Tables 2, 3, 4, 5, and 6 show in detail, the results obtained at the three samplings, also the various treatments applied.

Examining plate III, the curves indicate that while the bacterial count of the check pots were relatively higher at 30 days than in any other instance, yet the percentage of ammonification, as indicated both by the dried blood and cottonseed meal was less. For the same period where three tons of lime were applied the ammonifying power was greatly increased, even though the number of bacteria decreased.

Where manure was applied we note a gradual decline in numbers in both instances and a decrease in ammonifying power. And when the number of bacteria are lowest, at the end of 60 days where 1½ tons of lime were applied, the degree of ammonification also decreased. The same at 90 days shows a slight decrease for dried blood and a like increase for cottonseed meal. It is interesting to note that the degree of ammonification for dried blood and cottonseed meal are nearly the same in the 90 day check pots and that this is the lowest reached except in the 30 day checks. The highest degree of ammonification was reached after 90 days with the application of 3 tons of lime. The bacterial content was 69 million per gram; 39.7% of dried blood was ammonified and 59.6% cottonseed meal . We also note that for the same period with applications of 10 tons of manure there is a decline in bacterial numbers and their activities. It is noticeable also that in 11½ instances out of 15-(77%) the ammonification and bacterial content were somewhat parallel, i. e. a rise or fall in numbers gave a corresponding increase or decrease in the percentage of ammonification. In 3½ instances (23%) there was no correlation between the numbers and degree of ammonification. This is particularly noticeable in the first 30 days the pots were run; in the checks and where 1½ and 3 tons of lime were applied. It is also to be noticed that the cottonseed meal was more readily available than

than the dried blood since in 13 instances out of 15 or 87% it gave higher yields of ammonia nitrogen. This fact is probably due to the high carbon content of the dried blood which makes it more resistant to decomposition.

TABLE 2. AMMONIA NITROGEN RECOVERED FROM UNTREATED PORTIONS AT END OF 30, 60 AND 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment Pots Received	Lab.No.of tumblers	Added grams	Nitrogen in Material added Mgs.	Ammonia nitrogen recovered from 100 gms.of air dry soil: 8 days Mgs.	Average Mgs.	Average increase over check Mfg.	% Ammonia nitrogen recovered
1 & 2	30	Nothing	1-a	Nothing		9.52 9.52	9.52		
"	30	"	2-a	D.B. 5	685.35	15.98 14.00	14.99	5.47	0.80
"	30	"	3-a	C.S.meal 5	375.40	24.18 40.20	32.19	26.72	7.12
"	60	Nothing	1-b	Nothing		2.24 2.24	2.24		
"	60	"	2-b	D.B. 5	685.35	132.96 128.46	130.71	128.47	18.75
"	60	"	3-b	C.S.meal 5	375.40	117.26 117.26	117.26	115.02	30.64
1 & 2	90	Nothing	1-c	Nothing		2.80 2.80	2.80		
"	90	"	2-c	D.B. 5	685.35	102.26 87.84	95.05	92.25	13.40
"	90	"	3.c	C.S.Meal 5	375.40	49.86 49.86	49.86	47.06	12.54

TABLE 3. AMMONIA NITROGEN RECOVERED

FROM PORTIONS RECEIVING 1½ TONS OF LIME PER ACRE -- AT END OF 30, 60 and 90 DAYS.

Composit: Pot No.	Time Pots were run days	Treatment Pots Received	Lab.No. of tumblers	Added grams	Nitrogen in Material added Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil		Average increase over check Mgs.	% Ammonia nitrogen recovered
						8 days Mgs.	Average Mgs.		
3 & 4	30	1½ tons lime	4-a	Nothing		6.20 10.80	8.50		
"	30	" " "	5-a	D.B. 5	685.35	171.62 171.62	171.62	163.12	23.80
"	30	" " "	6-a	C.S.meal 5	375.40	70.88 70.88	70.88	62.38	16.62
3 & 4	60	1½ tons lime	4-b	Nothing		13.44 13.44	13.44		
"	60	" " "	5-b	D.B. 5	685.35	146.68 122.32	134.50	121.06	17.66
"	60	" " "	6-b	C.S.meal 5	375.40	114.74 114.74	114.74	101.30	26.98
3 & 4	90	1½ tons lime	4-c	Nothing		2.10 2.10	2.10		
"	90	" " "	5-c	D.B. 5	685.35	104.22 104.22	104.22	102.12	14.90
"	90	" " "	6-c	C.S.meal	375.40	90.36 89.66	90.01	87.91	23.42

TABLE 4. AMMONIA NITROGEN RECOVERED FROM PORTIONS RECEIVING 3 TONS OF LIME PER ACRE -- AT END OF 30, 60 AND 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment Pots Received	Lab.No. of tumblers	Added grams	Nitrogen in Material added Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil: 8 days Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil: Average Mgs.	Average increase over check	%Ammonia nitrogen recovere
5 & 6	30	3 tons lime	7-a	Nothing		5.46 5.46	5.46		
"	30	" " "	8-a	D.Blood 5	685.35	237.56 234.38	235.97	230.55	33.64
"	30	" " "	9-a	C.S.Meal 5	375.40	174.28 155.94	165.11	159.65	40.40
5 & 6	60	" " "	7-b	Nothing		1.54 1.54	1.54		
"	60	" " "	8-b	D.B. 5	685.35	133.10 132.40	132.80	131.26	19.17
"	60	" " "	9-b	C.S.Meal 5	375.40	134.72 93.20	113.96	112.42	29.94
5 & 6	90	" " "	7-c	Nothing		132.40 136.18	134.29		
"	90	" " "	8-c	D. B. 5	685.35	370.42 443.40	406.91	272.62	39.79
"	90	" " "	9-c	C.S.Meal 5	375.40	378.06 338.06	358.11	223.82	59.62

AMMONIA NITROGEN REVOCERED

TABLE 5. FROM PORTIONS RECEIVING 10 TONS OF MANURE PER ACRE -- AT THE END OF 30, 60 and 90 DAYS.

Composit Pot No	Time Pots were run days	Treatment Pots Received	Lab.No. of tumblers	Added grams	Nitrogen in Material added Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil: 8 days Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil: Average Mgs.	Average increase over check	% Ammonia nitrogen recovered
13 & 14	30	10 tons manure	10-a	Nothing		91.78 91.78	91.78		
" "	30	" " "	11-a	Dried blood 5	685.35	236.24 269.70	266.47	174.69	25.48
" " " "	30	" " "	12-a	C.S.Meal 5	375.40	234.38 215.48	224.93	133.15	35.46
13 & 14	60	" " "	10-b	Nothing		9.38 6.86	8.14		
" " " "	60	" " "	11-b	Dried blood 5	685.35	130.44 133.80	132.12	123.98	18.09
" "	60	" " "	12-b	C.S.Meal 5	375.40	117.40 116.20	116.80	108.66	28.94
13 & 14	90	" " "	10-c	Nothing		125.80 125.80	125.80		
" "	90	" " "	11-c	Dried blood 5	685.35	232.98 238.16	235.57	109.77	16.02
" "	90	" " "	12-c	C.S.Meal 5	375.40	242.22 242.22	242.22	116.42	31.01

TABLE 6. AMMONIA NITROGEN RECOVERED FROM PORTIONS RECEIVING 20 TONS OF MANURE PER ACRE AT THE END OF 30, 60 AND 90 DAYS.

Composit Pot No	Time Pots were run	Treatment Pots Received	Lab.No. of tumblers	Added grams	Nitrogen in Material added Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil: 8 days Mgs.	Ammonia nitrogen recovered from 100 gms. of air dry soil: Average Mgs.	Average increase over check	% Ammonia nitrogen recovered
15 & 16	30	20 tons manure	13-a	Nothing		19.20 19.20	19.20		
" "	30	" " "	14-a	Dried blood 5	685.35	207.62 219.96	213.79	194.59	28.35
" "	30	" " "	15-a	C.S.Meal 5	375.40	132.40 127.08	129.74	110.54	29.45
15 & 16	60	20 tons manure	13-b	Nothing		5.46 6.86	6.16		
" "	60	" " "	14-b	Dried blood 5	685.35	142.90 143.02	142.96	136.80	19.96
" "	60	" " "	15-b	C.S.Meal 5	375.40	116.70 117.40	117.05	110.89	29.53
15 & 16	90	20 tons manure	13-c	Nothing		121.88 131.68	126.78		
" "	90	" " "	14-c	Dried blood 5	685.35	215.76 215.76	215.76	88.98	10.06
" "	90	" " "	15-c	C.S.Meal 5	375.40	246.66 265.76	256.21	129.43	31.81

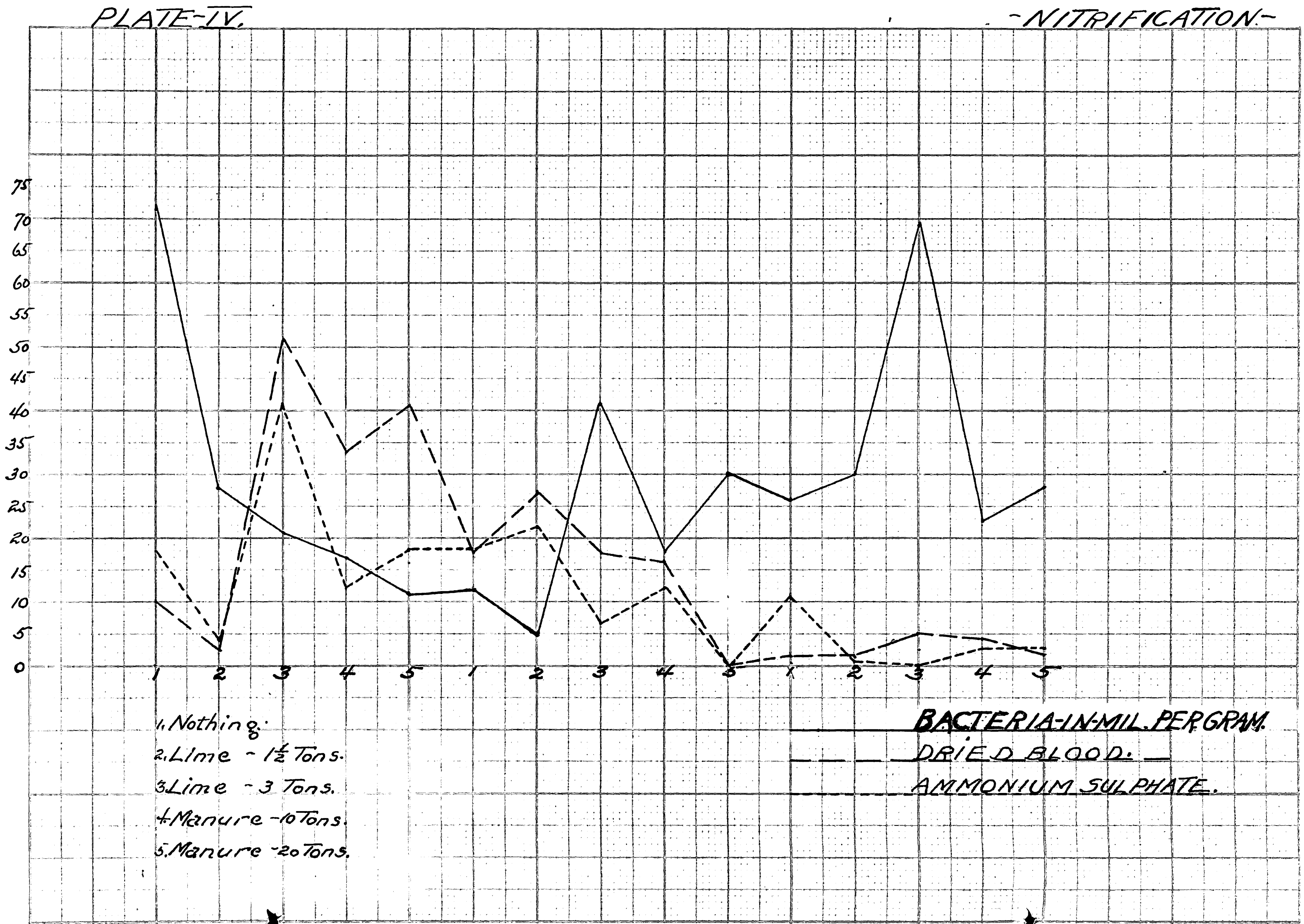


PLATE-IV.
-NITRIFICATION-
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
1. Nothing.
2. Lime - 1½ Tons.
3. Lime - 3 Tons.
4. Manure - 10 Tons.
5. Manure - 20 Tons.
BACTERIA-IN-MIL. PER GRAM.
DRIED BLOOD.
AMMONIUM SULPHATE.

The Relationship of Treatment To Bacterial Numbers and Their Nitrifying Activities As Shown by Ammonium Sulphate and Cottonseed Meal.

Tables 7, 8, 9, 10 and 11 show in detail the resubts obtained at the three samplings, also the various treatments applied.

Examining Plate IV we are at once struck by the gradual and increasing decline in the nitrifying properties of the soil for each and for the entire period; with the exception of the 30 day period, where 3 tons of lime were applied. In all other cases, i. e. the 60 and 90 day periods both the applications of $1\frac{1}{2}$ and 3 tons of lime gave lower results than in the previous instance. This decrease is probably due to the nature of the soil organisms present which in the time consumed over the 30 day period caused denitrification and a loss of nitrate either by volatilization of elemental nitrogen or through the formation of ammonium carbonate.

There may have been certain groups of acid formers which in time were able to overcome the action of the lime in which case the nitrifying organisms were inhibited in their activities or killed off. Beckwith and Vass (47, p.p.27-28) found in nitrification experiments with certain Oregon soils showed that applications of lime had a retarding effect on the nitrifying power of the micro-organisms present. They also found that in this instance there was no correlation between the ammonifying and nitrifying power. This is noticeable in the above instance.

Studying the curves from the same viewpoint as the ammonification ones we note the following: In the check pots at the end of 30 days the nitrification was relatively lower than the bacterial content. For the same period where 3 tons of lime were applied there is an increase in the nitrifying power along with a decrease in bacterial content. This runs parallel with the results obtained in the ammonification experiments. The action of 3 tons of lime to the acre decreasing the number of bacteria but increasing both the amount of ammonia and of nitrate nitrogen produced. This may be explained on the assumption that while the numbers originally present were greatly reduced by the treatment, yet their numbers are relatively large at this period and it seems feasible to believe that under the condition imposed by the time and treatment involved, considering the ammonification and nitrigication results as a whole, the relative numbers of both ammonifyers and nitrifyers had reached their optimum physiological efficiency. In no other instance is this comparison to be noted. When manure was applied, at the rate of 10 tons per acre, we note a decline in numbers and in the nitrifying power, while a slight increase is seen over this where 20 tons of manure were applied. Both the ammonifying and the nitrifying powers decrease where 10 tons of manure were applied but the same relationship does not exist where 20 tons were applied. For the 60 day period where 3 tons of lime were applied there is a close approach to the ammonifying and nitrifying powers as indicated by dried blood; the ammonia nitrogen being 19.17% the nitrate nitrogen being 17.91%. There is a marked decrease in the nitrifying power over the check as indicated by ammonium sulphate, while the check and the dried blood are the same.

In all other instances the nitrifying powers were considerably lower than the ammonifying powers and rapidly declined with time. In the 90 day treatments there is no comparison between the ammonifying and the nitrifying properties since the ammonia produced is far in excess of the nitrates formed. It is interesting to note that in 60% of the instances dried blood gave higher results than did ammonium sulphate.

Tables 12 and 13 are arranged for the convenience of showing the percentages of nitrogen ammonifyed and the nitrogen nitrated under all conditions of treatment for eawh 30 day period.

TABLE 7

NITRATE NITROGEN RECOVERED
FROM UNTREATED PORTIONS AT END OF 30, 60 AND 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment Pots received	Lab.No. of tumblers	Added mgs.	Nitrate N recovered for every 100 gms. of air dry soil 28 days -Mgs.	Increase over check mgs.	Nitrate N in materials added mgs.	Nitrate N Recovered mgs.	Total Nitrogen in materials added exclusive of nitrates Mgs.	% of nitrogen nitrated
1 & 2	30	Nothing	1-d	Nothing	1.25					
1 & 2	30	"	2-d	$(NH_4)_2SO_4$ 100	5.00	3.75	None	3.750	20.77	18.05
1 & 2	30	"	3-d	D.Blood 200	4.16	2.91	0.092	2.818	27.41	10.28
1 & 2	60	"	1-e	Nothing	5.00					
1 & 2	60	"	2-e	$(NH_4)_2SO_4$ 100	*					
1 & 2	60	"	3-e	D.Blood 200	10.00	5.00	0.092	4.908	27.41	17.91
1 & 2	90	"	1-f	Nothing	1.32					
1 & 2	90	"	2-f	$(NH_4)_2SO_4$ 100	3.57	2.25	None	2.250	20.77	10.83
1 & 2	90	"	3-f	D Blood 200	1.92	0.60	0.092	0.508	27.41	1.85

*Lost

NITRATE NITROGEN RECOVERED

TABLE 8. FROM PORTIONS RECEIVING $1\frac{1}{2}$ TONS OF LIME PER ACRE -- AT END OF 30, 60 AND 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment Pots received	Lab. No. of tumblers	Added mgs.	Nitrate N recovered for every 100 gms. of air dry soil 28 days Mgs.	Increase over check mgs.	Nitrate N in materials added mgs.	Nitrate N Recovered mgs.	Total Nitrogen in materials added exclusive of nitrates Mgs.	% of nitrogen nitrated
3 & 4	30	$1\frac{1}{2}$ tons lime	4-d	Nothing	4.16					
3 & 4	30	" " "	5-d	$(NH_4)_2SO_4$ 100	5.00	0.84	None	0.840	20.77	4.04
3 & 4	30	" " "	6-d	D. Blood 200	5.00	0.84	0.092	0.748	27.41	2.73
3 & 4	60	$1\frac{1}{2}$ tons lime	4-e	Nothing	2.50					
3 & 4	60	" " "	5-e	$(NH_4)_2SO_4$ 100	7.00	4.50	None	4.50	20.77	21.67
3 & 4	60	" " "	6-e	D. Blood 200	7.00	4.50	0.092	4.408	27.41	27.54
3 & 4	90	$1\frac{1}{2}$ tons lime	4-f	Nothing	1.92					
3 & 4	90	" " "	5-f	$(NH_4)_2SO_4$ 100	2.08	0.16	None	0.160	20.77	0.77
3 & 4	90	" " "	6-f	D. Blood 200	*					

* Lost

TABLE 9. NITRATE NITROGEN RECOVERED FROM PORTIONS RECEIVING 3 TONS OF LIME PER ACRE AT END OF 30, 60 and 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment: Pots received grams	Lab.No. of tumblers	Added mgs.	Nitrate N recovered for every 100 gms. of air dry soil 28 days Mgs.	Increase over check mgs.	Nitrate N in materials added mgs.	Nitrate N Recovered mgs.	Total Nitrogen in materials added exclusive of nitrates Mgs.	% of nitrogen nitrated
5 & 6	30	3 tons lime	7-d	Nothing	2.50					
5 & 6	30	" " "	8-d	$(NH_4)_2SO_4$ 100 gm.	10.00	8.5	None	8.500	20.77	40.92
5 & 6	30	" " "	9-d	D.Blood 200	16.66	14.16	0.092	14.068	27.41	51.29
5 & 6	60	3 tons lime	7-e	Nothing	4.00					
5 & 6	60	" " "	8-e	$(NH_4)_2SO_4$ 100	5.00	1.00	None	1.000	20.77	4.81
5 & 6	60	" " "	9-e	D.Blood 200	9.00	5.00	0.092	4.908	27.41	17.91
5 & 6	90	3 tons lime	7-f	Nothing	2.08					
5 & 6	90	" " "	8-f	$(NH_4)_2SO_4$	2.08	0.00	None	0.00	20.77	0.00
5 & 6	90	" " "	9-f	D.Blood 200	3.57	1.49	0.092	1.398	27.41	5.10

TABLE 10

NITRATE NITROGEN RECOVERED FROM PORTIONS RECEIVING 10 TONS OF MANURE PER ACRE AT END OF 30, 60 and 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment Pots received grams	Lab. No. of tumblers	Added mgs.	Nitrate N recovered for every 100 gms of air dry soil 28 days Mgs.	Increase over check mgs.	Nitrate N in materials added mgs.	Nitrate N: Recovered mgs.	Total Nitrogen in materials added exclusive of nitrates Mgs.	% of nitrogen nitrated
13 & 14	30	10 tons manure	10-d	Nothing	2.50					
" "	30	" " "	11-d	$(NH_4)_2SO_4$ 100	5.00	2.50	None	2.500	20.77	12.03
" "	30	" " "	12-d	D. Blood 200	12.50	10.00	0.092	9.080	27.41	33.53
13 & 14	60	" " "	10-e	Nothing	2.50					
" "	60	" " "	11-e	$(NH_4)_2SO_4$ 100	5.00	2.50	None	2.500	20.77	12.04
" "	60	" " "	12-e	D. Blood 200	7.00	4.50	0.092	4.408	27.41	16.08
13 & 14	90	" " "	10-f	Nothing	3.12					
" "	90	" " "	11-f	$(NH_4)_2SO_4$ 100	2.50	0.62	None	0.620	20.77	2.99
" "	90	" " "	12-f	D. Blood 200	1.92	1.20	0.092	1.108	27.41	4.04

TABLE 11

NITRATE NITROGEN RECOVERED
FROM PORTIONS RECEIVING 20 TONS OF MANURE PER ACRE -- AT END OF 30, 60 AND 90 DAYS.

Composit Pot No.	Time Pots were run days	Treatment Pots received grams	Lab.No. of tumblers	Added mgs.	Nitrate N recovered for every 100 gms of air dry soil 28 days Mgs.	Increase over check mgs.	Nitrate N in materials added Mgs.	Nitrate N Recovered mgs.	Total Nitrogen in materials added exclusive of nitrates Mgs.	% of nitrogen nitrated
15 & 16	30	20 tons manure	13-d	Nothing	2.50					
15 & 16 15	30	" " "	14-d	$(NH_4)_2SO_4$ 100	6.25	3.75	None	3.750	20.77	18.05
15 & 16	30	" " "	15-d	D.Blood 200	13.80	11.30	0.092	11.208	27.41	40.89
15 & 16	60	" " "	13-e	Nothing	4.00					
15 & 16	60	" " "	14-e	$(NH_4)_2SO_4$ 100	4.00	0.00	None	0.000	20.77	00.00
15 & 16	60	" " "	15-e	D.Blood 200	9.00	5.00	0.092	0.092	27.41	00.34
15 & 16	90	" " "	13-f	Nothing	2.50					
15 & 16	90	" " "	13-f	$(NH_4)_2SO_4$	*					
15 & 16	90	" " "	13-f	D.Blood 200	3.12	0.62	0.092	0.528	27.41	1.93

* Lost

TABLE 12. SHOWING THE PERCENTAGE OF NITROGEN

Ammonified for Each 30 day Period

Time-Days	CHECK		LIME - 1½ TONS		LIME - 3 TONS		MANURE - 10 TONS		MANURE - 20 TONS	
	D.B.	C. M.	D. B.	C. M.	D. B.	C. M.	D. B.	C. M.	D. B.	C. M.
30	00.80	7.12	23.80	16.62	33.64	40.40	25.48	35.46	28.35	29.45
60	18.75	30.64	17.66	26.98	19.17	29.94	18.09	28.94	19.96	29.53
90	13.40	12.54	14.90	23.42	39.79	59.62	16.02	31.01	10.06	31.81

TABLE 13. SHOWING THE PERCENTAGE OF NITROGEN NITRATED FOR EACH 30 DAY PERIOD

Time: Days:	CHECK $(NH_4)_2SO_4$	D.B.	LIME - 1½ TONS $(NH_4)_2SO_4$	D.B.	LIME - 3 TONS $(NH_4)_2SO_4$	D.B.	MANURE - 10 TONS $(NH_4)_2SO_4$	D.B.	MANURE - 20 TONS $(NH_4)_2SO_4$	D.B.
30	18.05	10.28	4.04	2.73	40.92	51.29	12.03	33.53	18.05	40.89
60	*	17.91	21.67	27.54	4.81	17.91	12.04	16.08	00.00	00.34
90	10.83	1.85	00.77	*	00.00	5.10	2.99	4.04	*	1.93

* Lost

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SUMMARY OF RESULTS

1. Applications of lime at the rate of one and one-half and three tons per acre to soils which have undergone cropping without rotation or the application of artificial fertilizer, cause at first a decrease in the number of bacteria, later an increase.

2. Applications of three tons of lime per acre to such soils produce a greater increase in the number of bacteria for the same length of time than applications of lime at the rate of one and one-half tons per acre.

3. Barnyard manure added at the rate of ten and twenty tons per acre bring about conditions similar to those as stated in "1".

4. Where lime at the rate of three tons per acre was added, there was a greater increase in the numbers of bacteria than where manure was added at the rate of ten and twenty tons per acre.

5. Applications of manure at the rate of 20 tons per acre are more favorable to the increase in the number of bacteria in such soils than additions of lime at the rate of one and one-half tons per acre.

6. As a whole three tons of lime give a greater increase in the number of bacteria for the same length of time than any other applications of either lime or manure, while the addition of 20 tons of manure per acre ranks next to the three tons of lime in bringing about an inerease in bacterial numbers.

7. The addition of one and one-half tons of lime per acre brings about a decrease in the ammonifying power of the soil, while additions of lime at the rate of three tons per acre increase the degree of ammonification, although the number of bacteria may be decreased.

8. Applications of manure in amounts up to 20 tons per acre cause a decrease in the numbers of bacteria and in the ammonifying activity of the soil.

9. There is no direct correlation of bacterial numbers and the ammonifying power of the soil for the first thirty days. After this time a rise or fall in

the numbers of bacteria gives a correspodding rise or fall in ammonifying power.

10. Cotton seed meal and dried blood as a source of nitrogen show a marked parallelism in relation to the nitrogen ammonified.

11. Cotton seed meal is more readily and completely ammonified than dried blood.

12. Separate applications of one and one-half and three tons of lime, ten and twenty tons of manure per acre to the soil, cause a marked decrease in the nitrifying powers of soil, or a loss of nitrates after thirty days.

13. Where a decrease in the nitrifying power or a marked loss of nitrates is occasioned, there is no correlation between the ammonifying and nitrifying power of the soil.

14. An increase of decrease in bacterial numbers does not necessarily indicate the same for the ammonifying and nitrifying powers of the soil.

15. There is a fair parallelism in the amount of nitrogen nitrated by dried blood and by ammonium sulfate.

16. Dried blood is more readily and more completely nitrated than is ammonium sulfate.

17. Soil such as the above is more benefited by applications of three tons of lime per acre, which give the greatest maximum degree of ammonia and nitrate nitrogen within thirty days from application.

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