1923

# On the correlation between the nitrate content of the soil and the chlorotic condition of maize

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Thesis Submitted for the Degree of M.Sc.

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Amherst.

May, 1923.

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## Introduction.

It is a commonly observed fact that there appears, at some seasons, a considerably marked difference in the growth of the same crops on different portions of the field. Particularly is this difference noticeable in rainy seasons on soils that are poorly drained. As we shall see later in the review of literature on the subject, the process of nitrification in soil is hindered by excessive moisture and the resulting lack of aeration.

The rainy season of 1922 afforded a good opportunity for studying these differences in the growth of maize on different portions of the same fields. The present investigation was planned with the view of bringing to light, if possible, any existing correlation between the nitrate content of the soil and the chlorotic condition of maize growing thereon, under field conditions.

## REVIEW OF LITERATURE.

### The importance of nitrogen in plant nutrition.

The importance of nitrogen as a nutritive element for plants is well-known since the early times of agricultural experimentation. Hence, it is only necessary to review briefly the literature dealing with the effect of the presence and absence of nitrogen in the soil, whether in nitrate or other form, on the growing plants, particularly on their colour.

Among the oldest experiments to determine the rôle of nitrogen in plant nutrition is the famous experiment of Boussingault(4)* with the Helianthus argophyllus, in which he found that the plant increased its dry weight 4.6 times without being supplied with nitrogen, but a similar plant supplied with potassium nitrate acquired a dry weight 198 times greater than that of the seed.

According to Hunt(61), the production of proteins is "about all that is positively known about the function of nitrogen in the growth of plants".

Voorhees(129) reported that his corn experiments showed in every case a decided gain in weight of dry matter due to application of nitrogenous fertilizers. His experiments(130) with sodium nitrate on forage crops showed that nitrates had a tendency to increase the yield as well as to hasten the period of development.

Wilfarth and Wimmer(137) observed that with a lack of nitrogen the leaves take on a light green to yellowish colour. With a lack of phosphoric acid, but with a plentiful nitrogen supply, the leaves become dark green; but when there is a great lack of phosphoric acid the edges of the leaves and later the entire leaf become brownish in colour. Lack of phosphoric acid and nitrogen together results in a reduction in the entire growth of the plant and the leaves remain small. They have also found(138) that lack of nitrogen does not seem to interfere with starch formation.

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* Reference is made by number to "Bibliography".

Jost(65) states that when nitric nitrogen is absent no noticeable increase in dry weight takes place "even though all other salts be present", hence, "nitric acid in a form capable of being absorbed from the soil is essential".

Snyder (113) says that yellowish leaves indicate a lack of nitrogen for chlorophyll formation. And Livingston (83) states that the "rate of supply of nitrates and similar bodies is surely often the limiting condition to the extent or rapidity of plant growth".

Roberts (106) attributes the darker green colour of wheat, barley, and maize plants in some parts of the field to the abundant supply of nitrogen and at least a moderate supply of phosphoric acid and potash.

Russell (103) remarks that "nitrogen starvation causes yellowing of the leaf, absence of growth, and a poor starved appearance generally; while the abundance of nitrogen leads to a bright green colour, to a copious growth of soft sappy tissue, and to retarded ripening". Thatcher (126) almost reiterates these words of Russell.

Duggar and Williamson (31) found that in nearly all cases nitrogen afforded a fair increase in the yield of corn. While Alderman(2), Gladwin (45), and Collison (17) all call attention to the fact that nitrogen causes a healthy and luxuriant growth and appearance of small fruits and apple orchards. The leaves become larger in size and more numerous; while the phosphorus and potash plots usually show a distinctly yellowish colour.

Hoagland (58) studied the relation of the nutrients to the growth of plants and expresses the opinion that "when the supply of nitrogen and other elements is constantly replenished, the plants may remain green almost indefinitely".

Emerson (51), in his investigation on the development of colour, (red, brown, purple, etc.), in maize found a striking contrast between the plants receiving complete nutrient solution and those supplied with only clear water or nitrogen-free solution. In these experiments plants were grown in glazed earthen jars in clean quartz sand practically free from nutrient elements except iron. Complete solutions of varying strengths and also one-element-free solutions were employed. One lot received distilled water only. At one month of age the plants receiving very strong solution began to wilt. Those supplied with distilled water and those receiving nitrogen-free solution both showed much colour at two weeks after germination, and soon afterward the seedlings were red to the tip of their leaves. Within six weeks the plants in these two lots were slenderer and shorter than those given complete solution. Their upper leaves were pale yellowish green, with much red, and the lower leaves were dead but still showing the red colour developed earlier.

It was also noted by Emerson that in the nitrogen-free lot, and to some extent in the phosphorus-free lot, the new growth seemed to take place at the expense of the older leaves. This rather confirms the view held by Hellriegel (57),-that plants can grow slowly for a time even in a deficiency of nitrogen, the younger organs having the ability to obtain their combined nitrogen from the older ones. The plants in the nitrogen-free lot first became light or yellowish green, then red, and finally died. These results obtained by Emerson correspond, in general, to those of Wilfarth and Wimmer previously reviewed (137).

Moreover, Emerson states that "the reddening of young plants in cold wet soils in spring, the greater development of colour in plants maturing in the cool weather of late autumn, and the excessive development of red in plants on very light sandy soils, are possibly all due to the plants' inability to get from such soils an adequate supply of nutrient salts, particularly of nitrates".

Czartkowski's (20) claim that a lack of nitrogen in available form may limit or check protein synthesis, thus leaving an excess of carbohydrates which may favour anthocyanin formation, is questioned by Emerson on the ground that, although phosphorus and sulphur are also necessary for protein synthesis, yet his own experiments afforded little or no evidence of such a relation between a lack of sulphur and colour development in maize, while lack of phosphorus does apparently bear some relation.

It will thus be seen that the effect of nitrogen on the development of green colour in plants, the intensity usually depending on the amount applied, is considered as an established fact. No one seems to entertain any doubt of the fact that the application of nitrogen to the soil will cause the vegetation to assume a green colour.

## Forms of nitrogen absorbed by plants.

Nitrogen occurs in several forms in the soil. In the uncombined form in soil air it constitutes the largest supply. The nitrogen of the organic compounds ranks next in quantity, ranging, according to Lyon and Bizzell (86), from 0.05 to 0.3 per cent on ordinary arable land and is "always slightly, but appreciably, soluble in soil water". In upland cultivated soils the nitrate nitrogen occurs in larger amounts than does the nitrogen of ammonium salts and nitrites; but in forest, swamp, and inundated soils these compounds form the greater proportion of the soil nitrogen than does the nitrate nitrogen.

Numerous experiments have been carried out by able investigators to demonstrate the utilization of various forms of nitrogen by agricultural plants, and certain facts have thus been definitely established. The utilization of the atmospheric nitrogen by leguminous plants, for instance, has been proved beyond question; but opinions still differ on the subject of the extent to which this form of nitrogen can be utilized by other plants. It is generally conceded that nitrate nitrogen is the form best suited to the use of most higher plants.

It is to Boussingault (4) and to Lawes and Gilbert (73) that we owe our knowledge of the importance of nitric acid as a nutrient to the green plants. Previous to this time, 1860, it was believed, mainly owing to the influence of Liebig, that ammonia was the chief source of nitrogen to the plants; and still earlier the humus had come to be considered as the source. It was not known, at that time, that ammonia in the soil is transformed into nitrates before it is absorbed by the plant. The comprehensive researches of Pitsch (102) and of Maze (88) have conclusively proved that the nutritive value of ammonia must not be entirely ignored; in the majority of green plants it is second only to nitrates in value, inducing a definite development and considerable increase in dry weight.

In the extensive experiments of Pitsch (102) it was shown that nitrate nitrogen was decidedly superior to ammoniacal nitrogen. The percentage of nitrogen in the plant was greater in case of ammonium salts as nutrients.

Pagnoul (99) experimented with mangolds, clover, and oats growing in sterilized sands, (a) without manure, (b) with sodium phosphate and potassium nitrate, and (c) with sodium phosphate, potassium chloride, and ammonium sulphate. The results showed that, under the conditions of the experiments, nitrogen was directly assimilated in the form of ammonia.

The experiments of Schloesing, Jr., (114) with buckwheat, and Tropaeolum minus, also indicate the ability of plants to utilize the ammoniacal nitrogen almost as readily as they do the nitric nitrogen, the development of buckwheat being essentially the same under both treatments.

There are, however, certain plants that prefer ammonium salts to nitrates, as Kellner (70 ) and also Kelley (69) proved to be the case with swamp rice. In the case of some plants, particularly maize and other Gramineae, ammonia is by no means of inferior value to nitrates, for Maze (88) was able to obtain as great an increase in dry weight in maize, using at most a one-half per cent solution of ammonium sulphate, as when he supplied it with a correspondingly strong solution of nitrate. It is believed that the injurious effect of ammonium sulphate is due to the use of too large amounts of the salt.

Hutchinson and Miller (63) found that peas obtained their nitrogen readily from either ammonium salts or nitrate of soda; but the wheat plants, although capable of assimilating the ammoniacal nitrogen, grow better in a solution containing nitrates.

Possibly the plants of different species vary in their ability to absorb ammonium compounds. Plants are found to contain nitrates and they are also found to contain ammonia. In his experiments with wheat and oats, Pitsch (102) found that with nitrates the yield of the crop was largely increased. Mitchell (93) found as much as 84 per cent increase in the yield of white potatoes, due to nitrates.

It may be, as Lyon and Bizzell (85) say, that "the formation of nitrates is merely a process that facilitates the absorption of nitrogen by reason of the ease with which nitrates pass through the semi-permeable membrane of the root-cells".

Whatever the explanation, the superiority of nitrates gives them a more important place in our study of soil fertility.

## The nitrifying power of soils.

Nitrifying power of soils is regarded generally as one of the important indices to their fertility. Thus in the work of Stevens, Withers, et al. (130) at North Carolina, it has been demonstrated that distinctly more good soils were possessed of a good nitrifying power than poor soils. Among the earlier investigators into the fertility of soils, Ehrenberg (32) and also Ashby (3) hinted at the close relationship existing between the nitrifying power of soils and their known fertility. Likewise, the results obtained by Lyon, Bizzell, and Conn (87) in their study of the different portions of an experimental field at Cornell Station show that the different yielding powers of these portions, although having apparently the same soil, are accompanied by the significant difference in their nitrifying power. They have also noted the minor differences in the chemical composition and the physical structure of those soils.

Vogel (128) found a direct correlation between productivity and the nitrifying powers of soils producing different crops. The yield on those soils ran parallel with the soils' power to make nitrogen (in the form of nitrates) available to plants. And he concluded that "in the large, the nitrifying energy of a soil affords us a useful explanation of its state of fertility".

In his investigations in San Joaquin Valley of California, Lipman (79) reports having observed that unusually fertile spots of the soil in grain fields (barley fields) possessed a higher nitrifying power, and also more citric acid soluble phosphoric acid and potash, than the surrounding area. The nitrifying power was as much as six to eight times greater than that of the poor soils. In conclusion he states: "I believe that a soil's nitrifying power, whether it be the cause or effect, is one of the prime factors in determining a soil's power to produce".

Kellerman and Allen ([illegible]), and Given (4[illegible]) have also called attention to the association of high nitrifying power and high productivity. Brown (8) has observed a close relationship between ammonifying and nitrifying powers of soils and their crop-producing capacity.

## The influence of crops on soil nitrates.

Besides the investigations made to find out the relationship between the nitrifying powers of soils and their crop-productivity, other numerous and extensive experiments have also been carried out to determine the influence - whether beneficial or otherwise,- of crops on the nitrate content of soils.

In their field experiments, King and Whitson (73) found that nitrates were highest during the growing season under corn, next under potatoes, and lowest under alfalfa and clover.

As the results of three-year experimentation on the irrigated soils of Utah, Stewart and Greaves (123 )found an exceptionally low concentration of nitrates in the alfalfa land. Cultivated fallow soil contained more nitrates at the end of the irrigation season than did the uncultivated fallow; but in the fall very little difference was noticed in the nitrate contents of these plats. At certain stages in the growth of the crop more nitrates were found in the soil under maize than in fallow soil. They also noted a steady decrease in the amounts of nitrates in soils under potato and maize from period to period, while that in alfalfa and fallow lands remained nearly constant.

Later, in another extensive set of experiments, Stewart and Greaves (124) found the nitrates to be most abundant under maize, next under potatoes, then under oats, and least under alfalfa.

Brown (7), in his analyses of the soil samples taken from neighbouring plats that for several years were continuously planted to maize and clover respectively, found that nitrification occurred more rapidly in soils under maize than under clover.

Brown and MacIntire (6) give the average nitrate content of the soils under different crops during the growing season as follows: under maize, 55.5 parts per million of dry soil; under oats, 18 parts per million; under wheat, 7.9 parts per million; and under grass, 1.4 parts per million.

Prescott, in Egypt, (104) found no accumulation of nitrates in soil under wheat and maize. Pot experiments with these crops showed that the root activities of the growing crop have some limiting effect on the production of nitrates in the soil. In both cases fallow plots accumulated considerably more nitrates than the cropped plots.

Lyon and Bizzell (86) found that the nitrate content of the soil under timothy, maize, potatoes, oats, millet, and soy-beans was different for each crop when grown on the same soil. They noticed a characteristic relationship between the crop and the nitrate content of the soil at different stages of growth. During the most active growing period the soil under maize frequently contained more nitrates than did the unplanted cultivated soil. They account for these phenomena on the assumption that nitrification is stimulated by "some process connected with the active growth and absorbing functions of some higher plants, particularly of maize", although there are indications that the maize plants take a large part of their nitrogen "in some form other than nitrates". The combination of these conditions may account for the higher nitrate content of the soil under maize.

Results obtained by Jensen (64) showed that during the first part of July the soil under maize contained more nitrates than did the fallow land.

A residual influence of crops on nitrification was noted by Wright (139) who found, in his pot experiments, that nitrification following maize, wheat, beets, soy-beans, and field peas showed evidence of inhibition, while that following barley and vetch showed evidence of stimulation.

The foregoing results are by no means all the work that has been done on the study of the relationship between the nitrate content of the soil and the crop growing on it; but they may be taken as a fair representation of the work embodied in the extensive literature on the subject. Suffice it to note here, however, that, although considerable attention has been directed towards the study of the effect of crops on the amount of nitrates in the soil, yet, so far as could be found out, very little, if any, attempt has been made to correlate the healthy or unhealthy growth of plants with the nitrate content of the soil sustaining them. Therefore the present investigation into that phase of the problem is thought justifiable.

The influence of moisture on nitrification.

Gainey (40) says: "Fertility in normal agricultural soils, in so far as the nitrate nitrogen is the limiting factor, is limited by those analytical (*) processes necessarily preceding nitrification rather than by nitrification itself". The processes of ammonification, nitrification, and nitrogen-fixation, being, as now generally accepted, due to the action of micro-organisms, are closely associated with the amount of moisture in the soil. Our text-books give considerable importance to the necessity of moisture and aeration for proper nitrification. Of course, moisture and aeration are not the only necessities of life for the bacteria in the soil; but in so many cases these two, which can be regarded as inseparables, constitute

(*) Probably refers to "biological".

the limiting factors in nitrate production. Still further reduction in the number of factors will give moisture the most dominating position, as its excess means the lack of aeration, and vice versa.

As early as 1887 Deherain (25) found the most active nitrification to occur when the soil was allowed to become partially dry between the applications of water. Later, in 1896, he (26) found a relationship between the rapidity of nitrification in fallow land and its moisture content, nitrification increasing with the water. Together with Demoussy (27) they found that the bacterial activity was at its maximum when a rich soil contained 17 per cent of water, but that it decreased when the proportion of water fell to 10 per cent or rose to 25 per cent. In the case of soils rich in humus a higher proportion of water was found to be necessary to retard the oxidation to any marked degree. In a later work, Deherain (28) concluded that 25 per cent of water was the optimum for nitrification. An insufficient supply of water checked both nitrification and nitrogen-fixation. In his experiments this inhibition took place when the moisture content was reduced to 16.5 per cent.

Naturally the optimum moisture for nitrification will be expected to vary with different kinds of soils. For Schloesing, Jr.,(114) found that nitrification and bacterial activity in general are less active in fine-grained, compact soils than in lighter, coarse-grained soils. Although this is generally attributed to the greater facility with which the air circulates in lighter soils and supplies the necessary oxygen, yet it is stated that in many cases it is not the air but available water that is deficient in heavy soils, the fine particles of which render it unavailable for the growth of the higher plants and also for nitrification.

Heavy soils must have a higher water content in order to make nitrification equally possible. Dafert and Bolliger(28) found that the difference in moisture content did not have to be great to produce a marked change in the process of oxidation in the soil, and that a distinctly measurable difference could be noticed with even one per cent variation in the moisture content.

Lawes (77) observed that in some fields of Rothamsted, nitrates increased in the fall after a heavy rain, evidently from better moisture conditions. Warington (131) said that the rate of nitrification increases somewhat with the proportion of water present, provided that "the moist soil still remains porous"; beyond this denitrification sets in. According to him Boussingault observed that soils with 60 per cent of water lost greater part of their nitrates within a few weeks.

Giustiniani (43) found that in sandy soils the rapidity of nitrification of ammonium sulphate was directly proportional to the amount of moisture present when this varied between 0 and 18 per cent. Lohnis (83) studied the transformation of nitrogen in soils, and found that the dry weather in July was especially injurious to nitrification, nitrogen assimilation, and decomposition of calcium cyanamid; but it had no effect on denitrification, and decomposition of bone meal and urea. He concluded that water to the extent of 50 - 80 per cent of the water-holding capacity of the soil is required for the uninterrupted progress of various changes in the soil nitrogen.

Warmbold (132) estimated the most desirable water content from the standpoint of preservation and accumulation of nitrogen in the soil to be 20 per cent. With 10 per cent and less there was either no increase or a marked decrease of soil nitrogen, the loss being particularly large in case of lighter soils.

Roche, in Egypt, (107) has shown that irrigation supplying from 15 to 25 per cent of water to a soil furnished the most favourable condition for nitrification. Teis (133), however, found no relationship, in humid areas, between the percentage of moisture and the nitrate production.

Fraps (36) experiments showed nitrification to be at its height in soils containing 55.6 per cent of their water-holding capacity. Excess of moisture almost stopped this process, and was more injurious than a deficiency of moisture.

Coleman )(16) found nitrification to be most active in loam soils with a moisture content of 15 - 20 per cent. It was greatly retarded when the water content was reduced to 10 per cent or increased to 26 per cent. He also noted that with a high moisture content soluble organic matter became injurious to nitrification.

Pouget and Guiraud (103) report that during the winter on the Algerian coast nitrification in place is not retarded except when the soil becomes water-logged by excessive rain. All forms of tillage which tend to increase aeration in soil promote nitrification and diminish denitrification.

The results obtained by Lipman and Brown (80) showed that ammonification in a loam soil increased with increased water content even up to 35 per cent of the weight of the soil; but nitrification was most active with only 15 per cent of water in the same soil; it was slightly less active with 10 per cent of moisture and was still quite marked with only 5 per cent of moisture. Sievers (117) found nitrification to proceed very slowly in Palouse silt loam with a moisture content below 15 per cent.

No definite correlation could be established by Jensen (64) between the amount of nitrates and the soil moisture, as the relation was different on different plats. Buckman (9), on the other hand, obtained a close relationship, and states that, under dry farming conditions, the formation of nitrates is greater with a good supply of water than where the soil is quite dry.

Such a distinct correlation was also observed by Gainey (39) who using soil made to its optimum moisture content, i.e. 2/3 saturated, found at times consistent relationship between nitrate accumulation and the moisture in soil. His figures indicate that an increase of one per cent moisture at or near the minimum for nitrification may cause an increase of 100 per cent in nitrate production. Under field conditions, 67 mgs. of nitrates per 100 gms. of soil have been formed with a moisture content of 32 per cent in soil, but nitrification fell rapidly with further increase in moisture.

Paterson and Scott(100), working with sandy and clay soils, found nitrification to be inactive in these soils while they still contained about three times more moisture than in their average air-dry condition. "At the lower limits of moisture less water starts nitrification in sand than in clay. At the higher limits of moisture less water stops nitrification in sand than in clay; while the optimum amount of water probably varies for each soil and is higher for clay, still for both soils it lies within the range of 14 - 18 per cent of dry soil". These figures are rather low for clay. They, too, noted that a rise above the optimum was more harmful than an equal fall below it.

According to Sharp (116) nitrification reached its maximum when the soil contained 19 per cent of moisture, the further increase to 25 per cent caused a reduction of 50 per cent in the rate of nitrification. (The exact character of the soil used in his experiments is not reported).

To Löhnis and Green (84) the "most significant cause of variation" in the rate of ammonification and nitrification " appears to be that of aeration". Both these processes took place more rapidly under aerobic than under anaerobic conditions. With inadequate aeration there was no formation of nitrates.

The investigations of Münter and Robson (96) showed that nitrification of organic nitrogen was more intense in sandy soils than in clay or loam when the degree of moisture is low, the difference decreasing with the increase of moisture. On the contrary, transformation of ammonium sulphate into nitrates occurred all the more readily the higher the water content in sandy soils as well as in loams and clays. A 6 per cent of moisture in sandy soil was more favourable to bacterial activity than the 8 per cent in clay.

Lyon and Bizzell (86) state that "changes in the moisture content or in the temperature of the soil after early summer had no important effect on the nitrate content of the soil under plants. On the uncropped soil an increase in moisture content was sometimes accompanied by an increase in nitrates and sometimes by a decrease".

In a recent publication of Lyon (85) data are presented which demonstrate the effect of mulching, scraping, and cultivation on the moisture and nitrate contents of the soil,(Dunkirk silty clay loam), and which indicate that increased moisture is not injurious to nitrate formation, if there is sufficient aeration of the soil — as, for example, caused by cultivation. Lyon found that the cylinders of soil taken from the field without disturbing the soil structure nitrified only slightly with a moisture content and temperature favourable to nitrate formation, while similar soil that had been aerated gave a large increase in nitrates. Under similar conditions more compact soil gave lower nitrates than a less compact soil. Bréal (5) had expressed the view that "the simple settling of soil should transform it from a nitrifying to a denitrifying medium".

The influence of moisture upon the nitrifying organisms is demonstrated in the work of Fraps (35) who found that the number of bacteria in soil varied inversely with moisture after the latter reached a certain limit. "Cold, dryness, and excessive moisture reduce the number of bacteria, while warmth, and favourable moisture content of the soil increase their number". Engberding (34) claimed that manure increased the number of bacteria in the soil, but he considered that the moisture had more influence on the bacteria in the soil than had the temperature.

Fraps (35) also showed that bacterial activity was periodic, rapid nitrification being preceded or followed by periods of less activity. This periodicity in the accumulation of nitrates in the soil may, according to Lipman and Brown (83), be due to temporary prominence of species especially capable of transforming large amounts of nitrates into protein nitrogen, as well as to a more rapid increase of various decay organisms and their intense utilization of nitrates for the building of their tissues.

King and Doryland (74) found that an excessive moisture greatly reduced the number of bacteria in the soil, and was detrimental to bacterial activity. They consider the periodic rise and fall in bacterial life and activity as being, to a certain extent, independent of moisture and temperature, but possibly due to the presence of bacterial by-products.

The work of Rahn at Michigan (105) with B. Mycoides, Bact. aceti, and Azotobacter shows that development increased with decreasing thickness of the layer of moisture, the maximum being reached at a thickness of 10 - 20 microns. If the moisture film becomes less than 10 microns in thickness the development of bacteria is retarded, owing to the fact that, although there exists an abundant supply of oxygen, yet the diffusion of food to the cells and the diffusion of metabolic products from the cells is not sufficient to allow the fastest metabolism. Further reduction in thickness of the moisture film may result in the cessation of diffusion and the death of the cells from starvation. Aeration and moisture are the two controlling factors in sand cultures; and aeration decreases with increasing moisture.

Koch and Pettit (75) found that denitrifying bacteria remain quiescent in soils with a water content below 25 per cent, but when the water reaches 25 - 30 per cent or more they become active and liberate considerable quantities of nitrogen from nitrates. Their results are confirmed by Von Caron (13) whose investigations in the presence of hydrogen tend to uphold the view that denitrifying bacteria are responsible for the loss of nitrates observed to occur in the presence of a source of energy,- such as dextrose, cellulose, straw, etc.-, and of nitrates with the exclusion of air. The hydrogen in this case is said to play the rôle of too high moisture content in the soil. And thus he infers that any means of excluding the air in soils may lead to the destruction of nitrates by these bacteria when present and other conditions are favourable.

The experimental data of Severin (115) also show that denitrifying process is more energetic in an atmosphere of hydrogen than under aerobic conditions.

Traaen (127), working with a loam soil, found that nitrification occurred best with a moisture content of 17.5 per cent, corresponding to 2/3 saturation. Considerable decrease in the rate of nitrification was observed when the moisture reached above 20 per cent or fell below 10 per cent.

McBeth and Smith (93) state that application of irrigation water reduced the nitrifying power of soils as determined by laboratory methods. No increase in nitric nitrogen took place when the soil contained as little as 5 per cent of moisture.

Greaves and Carter (48), on the other hand, found the nitrifying power of the soil to increase with the water applied up to 17.5 per cent. Above this it had a slight depressing effect upon nitrification, probably caused by the production of anaerobic conditions. The greatest increase of nitric nitrogen per unit of manure is produced when five tons of manure are applied; and likewise, the greatest increase per unit of water applied is found for the lowest application, - 7.5 inches. A close correlation was obtained between ammonification and nitrification. Water up to a certain limit increased ammonification, nitrification, and nitrogen-fixation, but depressed the number of colonies. This was rather interesting. The nitrifying power of the soil increased as the manure and water applied increased up to 25 tons of manure and 22.5 per cent of water. A close correlation was also noticed as existing between the bacterial activities of the soil receiving varying amounts of water and the crop produced upon the soil.

In a later publication Greaves (47) sums up the results of the experiments at Utah Station for the past ten years and states that in all the 25 soils studied the maximum nitrification and ammonification occurred when the soil contained 60 per cent of its water-holding capacity. Some of his results are presented in the following table, the soil which received 60 per cent of its water-holding capacity being taken as 100 :(*)

| Water in soil, per cent of water-holding capacity. | Ammonia produced. | Nitrates found. |
|---|---|---|
| 10 | 2 | 11 |
| 20 | 9 | 17 |
| 30 | 32 | 61 |
| 40 | 65 | 68 |
| 50 | 85 | 85 |
| 60 | 100 | 100 |
| 70 | 75 | 40 |
| 80 | 57 | 9 |
| 90 | 49 | 0 |
| 100 | 45 | |

From these results Greaves concludes that "an excessive quantity of water is more detrimental than is an insufficient water". He had previously (49) considered that "the optimum moisture for soil for the production of many of our staple crops is nearly 60 per cent of the water-holding capacity of the soil".

In their irrigation experiments, Harris and Butt (55) noted the tendency of the nitrates to vary inversely with the irrigation water

(*) Two separate tables of the original are here combined into one.

applied when no manure was applied to the soil. The nitrates of the manured plots showed a rather significant increase with a 6-inch application of water; but more than this amount caused a decrease.

Noyes and Conner (98) studied nitrification in acid soils and found that the degree of saturation of the soil affected the nitrates present. As a rule, more nitrates were found in soil kept at one half saturation than in soil kept one-fourth saturated. The soils that had been kept fully saturated with water for ten months contained no nitrates and formed no nitrates when incubated with ammonium sulphate. Under optimum moisture conditions both without and with lime and fertilizer treatments the nitrates after incubation varied with the aerobic counts.

Hutchinson and Milligan (63) found in their green manuring experiments that 3/8 saturation of the soil was the optimum moisture for nitrification of green manures under the conditions. The optimum water content, 16 per cent, for carbon dioxide formation was the same as that for nitrification of green manures.

A distinct correlation between the yield of wheat and nitrate content of the soil, (at seeding time), was observed by Call (11); also a correlation between nitrates and moisture, but very little, if any, correlation could be found between yield and water.

Russell's (109) observations indicate that the accumulation of nitrates occurred most rapidly in late spring or early summer. Losses of nitrates occurred in winter, especially were they marked during a wet winter. He considers the main loss as due to leaching rather than to denitrification. Whiting and Schoonover (125) also consider late spring and early summer as the period of the most active production and accumulation of nitrates. During this period optimum moisture and temperature conditions are supposed to be approached.

From the foregoing presentation of the results of numerous investigations it is quite manifest that the facility of nitrification varies with different kinds of soil, and that the optimum moisture for nitrification cannot be generalized for all soils. One thing, however, remains certain that, so long as it does not interfere with aeration, the increase in the degree of moisture is not detrimental to nitrification. It becomes so only when it reduces aeration to such a limit that the nitrifying organisms cannot obtain sufficient oxygen necessary for their proper development and activities. An excess of aeration, resulting in a lack of sufficient available moisture, may be equally injurious.

## The relation of nitrogen to chlorophyll formation. and the causes of chlorosis.

The effect of nitrogen in producing a green colour in plants will at once suggest a close relationship existing between nitrogen and the formation of chlorophyll, the green colouring matter of the plants. As has been proved by Molisch (94) and others, chlorophyll is a nitrogenous body, having the formula $C_{54}$ $H_{72}$ $O_5$ $N_4$ Mg.(41). It contains no iron(94), but yet iron is absolutely essential for its formation in plants(128). It is difficult to say whether the rôle of nitrogen, a constituent of chlorophyll, or that of iron, a non-constituent thereof, is the more important. In the absence of iron plants do not attain a green colour, the foliage becomes white or pale even in bright sunshine(65), the plants become etiolated and photosynthesis stops (126), and actual growth or increase in dry weight is impossible (65). A modicum of some salt of iron is sufficient(94) and indispensable for chlorophyll formation; but if larger quantities become distributed through the tissues the cells die.(111).

Sachs (111) states that organs not containing chlorophyll and plants entirely destitute of it - parasites and saprophytes - do not assimilate but absorb substances already assimilated; and growth is only possible as a result of assimilation. Like many others he considers chlorosis as due to a lack of iron. He found that maize seedlings, growing in iron-free solutions had their first three or four leaves green, several following were white at the base, but had green tips, and afterwards perfectly white leaves unfolded. On adding a few drops of sulphate or chloride of iron to the nutrient medium, the foliage recovered a deep green colour within twenty-four hours. When transferred to an iron-free solution, white leaves were again developed, which could again be brought to a normal healthy colour by the addition of iron.

E. Gris (50) was the first to trace the reason of these effects and he first found in 1843 that watering the roots of plants with a solution of iron, or even applying such a solution externally on the leaves, shortly developed a green colour where it was previously lacking. By microscopic examinations he found that in the absence of iron, the protoplasm of the leaf cells remains a colourless, yellow mass, destitute of chlorophyll. Under the influence of iron, grains of chlorophyll begin to appear and pass through various stages of their normal development. His results were later confirmed by those of Salm-Horstmar(113).

Pfeffer (101) declares that absence of iron probably inhibits the formation of chlorophyll only in an indirect manner, for iron does not enter into the composition of chlorophyll, For this reason "the non-formation of chlorophyll may be merely a pathological phenomenon, as, even in the presence of iron, the formation of chlorophyll may be partially or entirely suppressed when the plant is in unhealthy condition". To Jost (63) "it seems probable that like potassium and magnesium, iron is necessary to the formation of protoplasm, and that its absence is followed by chlorosis in the higher plants as a <u>secondary</u> effect". Riess, under Sachs' direction, demonstrated that manganese can not take the place of iron in the office of chlorophyll formation. (110)

Coulter, et al., (19) regard the iron salts and nitrates as favourable for chlorophyll development. Another statement in regard to the relation between nitrates and chlorophyll is made by Godlewski (46) who says: "since some time it was known that in green plants the nitrates persist in the organs kept in darkness, while they disappear in those that are exposed to light. As Th. Schloesing Jr., has shown, they are reduced to chlorophyll in the cells".

Lipman (79) claims that a low nitrifying power of soil may cause various physiological diseases in plants. Noll (97) remarks that besides the well-known action of the absence of light in producing etiolation of plants, the action of water under special circumstances may result in etiolation, and an inadequate supply of nitrogen and other important food constituents may likewise cause a similar effect.

Mazé (91) who claims that Boron, Al, Fl, I are indispensable to the development of maize in the same sense as are N, P, K, C, H, O, Ca, Mg, S, Cl, Si, and Zn, has found (89) that chlorosis could be artificially induced in this plant by a lack of iron, sulphur, manganese, etc. He also states (90) that chlorosis may result from a number of causes; besides an absence of iron or sulphur, an excess of lime may contribute to its development.

According to Duggar (30), lack of iron is only "one of the many conditions leading to pathological chlorosis". In the course of investigations on the influence of the phosphorus supply on several species of plants, Crone (21) has come to conclude that an excess of soluble phosphates, (in solutions more concentrated than N/100 or N/50), induces chlorosis, even in the presence of a liberal supply of iron as ferrous sulphate. He also found that ferrous sulphate was toxic while a corresponding phosphate was favourable to growth.

Juritz (67) analyzed the soils on which chlorosis occurred and found that it could not be due to a lack of plant food, it being in fact worse where the plant food was most abundant, nor could it be due to a lack of iron, excess of magnesia, or presence of parasitic fungi. There was however an excessive amount of calcium carbonate in those soils; but analyses did not indicate any direct relation between alkali and chlorosis. The conditions favouring chlorosis were apparently intensified by unsatisfactory moisture conditions in the soil and by the existence of a fairly impermeable substratum of marl.

Russell (103) makes the statement that where the amount of calcium carbonate becomes too high the plants tend to become chlorotic; and he cites Chauzit's (14) analyses showing that vines suffered badly when 35 per cent or more was present, but not when the amounts fell to 3 per cent. The evidence obtained by Molz (95) also tends to the same conclusion.

Clinton (15) reports that a lack of sufficient light or improper fertilization often appears to produce general chlorosis of plants. Likewise "an insufficient aeration of the roots in water-soaked soil may have a similar effect".

In his experiments with chlorotic corn Davis (24) found that about one-third of the plantlets produced from an ear of Reid's Yellow dent corn were chlorotic either entirely or some of the lower leaves. A second planting from the same ear produced thirty-eight plantlets, of which eleven were chlorotic: four had no chlorophyll, three had the first leaf chlorotic, the other four varied, having two or three chlorotic leaves. Further studies on the transferability of this disease led to the following conclusions:

1. Corn embryos may be chlorotic.
2. Chlorosis in corn may not be transferred to other corn plants by contact or by sap.
3. When corn plantlets are entirely chlorotic, they will not mature.

Hoffer and Carr (60) claim that maize plants with chlorotic leaves are characterized by low hydrogen-ion concentration and they contain immobile iron compounds within them. It is stated that sufficiently high hydrogen-ion concentration keeps the iron compounds moving and chlorosis does not occur.

Gile (48) found that chlorosis in pineapples is due to an excessive amount of carbonate of lime in the soil. He believes that chlorosis is not caused by an organic disease, but is the result of a disturbance in the mineral nutrition of the plant induced by the lack of iron in the ash or the small amount of iron in the presence of large amount of assimilable lime.

Demnt'ev (29) believes that chlorosis is due to injuries of the roots of plants, and that in most cases the parasites are responsible for this disease. It is believed that they lay bare the ends of the minute root-vessels which then allow the soil solution to enter directly and cause a high concentration in the leaves through evaporation. As a result, no chlorophyll formation can take place. No chlorosis is observed when the absorption of salts by plants takes place normally.

A study of the above-reviewed literature will manifest an inconsistency in many of the explanations of the causes of chlorosis in plants. This is due mostly to the unestablished definition of the term "chlorosis". Some botanists define it as "whitening due to lack of iron" (19); while there are others who use this term to cover partial or complete destruction of chlorophyll, or absence of it, in otherwise green plants. Therefore, it is apparent that nitrogen starvation is as much liable to cause a chlorotic condition of plants as the lack of magnesium, iron, or other elements necessary for proper chlorophyll formation.

## Digest of the literature reviewed.

The facts and suggestions brought out in the review of literature may be summarized as follows:

1. Nitrogen application generally imparts a green colour to the plants, and its absence causes the vegetation to assume a pale, yellowish appearance.

2. Some higher plants possess the ability to utilize the ammoniacal nitrogen almost as readily as they do the nitric nitrogen. Maize has been found to do almost equally well with ammoniacal nitrogen. But the nitric nitrogen is the form most readily available to most of the higher plants. The nitrogen of ammonia is second only to nitrates in value.

3. Nitrifying powers of soils correlate with their productivity, good soils having, in general, a higher nitrifying capacity than the poor soils.

4. The crops have some important influence on the soil nitrates. Most of the results obtained by numerous investigators tend to show that nitrates are higher under maize, during the growing season, than

under potatoes, oats, alfalfa or clover.

5. The moisture content of a soil plays an important role in nitrate production, and in ~~quite~~ many cases moisture is the limiting factor in bacterial activities in the soil. But the optimum water content for maximum nitrification depends on the kind of soil, the sandy soils requiring a low moisture content, while the fine-grained soils require considerable moisture. It may be safe, however, to conclude from the evidence presented that the optimum moisture for most of the agricultural soils ranges between 16 and 21 per cent. The excess of moisture is considered more detrimental to nitrification than an insufficient amount.

6. An increase in moisture content increases nitrification so long as the aeration of the soil is not interfered with. It becomes injurious only when it reaches the limit where the nitrifying bacteria in soil cannot obtain sufficient oxygen necessary for their proper development and activities.

7. Bacterial activity in the soil is periodic, rapid nitrification being preceded or followed by periods of less activity. This periodicity is supposed to be due to temporary and rapid increase of bacteria and various other decay organisms which utilize considerable amounts of nitrates for the building of their tissues.

8. The relation of nitrogen to chlorophyll is suggested by the fact that the latter contains nitrogen in its composition. But iron, though a non-constituent, is absolutely essential for chlorophyll formation.

9. Chlorosis of plants is attributed to many different causes among which may be enumerated the following;

1. Lack of iron, and in some cases of sulphur.
2. Excess of calcium carbonate.

3. Poor drainage.

4. Improper fertilization.

5. Excess of soluble phosphates.

6. Accumulation of immobile iron compounds in the leaves.

7. Parasites injuring the roots of plants.

10. This inconsistency in the explanations of the causes of chlorosis is mostly due to unestablished definition of the term, which some define as blanching due to lack of iron, and others as complete or partial destruction or absence of chlorophyll.

## PRESENT INVESTIGATION.

As has been stated before, the present investigation was planned with the purpose of studying the correlation, if such existed, between the nitrate content of the soil and the "chlorotic condition" of the maize plants growing thereon, under field conditions. By "chlorotic condition" is meant, not the absence of chlorophyll or blanching due to lack of iron, but a general yellowish, pale, sickly appearance, usually accompanied by a dwarfness of plants.

A similar investigation was accomplished by King (72) who found a consistently greater amount of nitrates in the soil under large plants than under comparatively small plants.

During the growing season of 1922, there was noticed considerable irregularity in the growth of plants in corn-fields in this locality. There could hardly be encountered a field sustaining a uniform or perfect growth. Figs. 1 and 2 represent the typical condition of the corn-fields during that season. At times rather extensive areas could be found where the plants suffered a chlorotic condition, while at a little distance around such spots the plants did at times wonderfully well. This poor growth usually occurred on poorly drained portion of the field.

Twelve such fields were studied, three samplings being made at intervals during the season. The differences in colour, size, and vigour of plants were carefully noted, together with drainage and other conditions of the fields.

No green-house experiments were attempted in this connection, the idea being to study the correlation as it exists in nature and under field conditions.

The economic importance of this problem lies particularly in the fact that serious losses in the yield of the crop occur as the result of such a chlorotic condition, the amount of loss depending on the extent of the affected area. The chlorotic plants do not mature generally; and those that may attain the stage of maturity produce ears such as those represented in figure 3.

In field No. 12, for instance, about three-fifth of the entire area of the field was affected by the chlorotic condition, and the yield was reduced to about one-half. One-half of the fields Nos. 8 and 10, produced nothing and the loss to the owners could hardly be over-estimated.

The existing correlation between the nitrates in soil and the chlorotic condition of the plants may also have a diagnostic value in that it may afford some information as to the need for improvement of the drainage condition of the field and the proper fertilization of the crop.

Fig. 1 Showing the typical condition of the corn-fields during the season of 1922. This particular field is No.12.

Fig. 2. Showing the good and poor spots of the field No 12, where the samples were taken for analysis.


Fig. 3 Typical condition of the ears of good and poor plants. Collected September 13,1922.

Character of the soils studied.

The soils of the fields under investigation have a glacial lake origin and belong to Hartford and Suffield series and their modifications. The apparent textures of the soils are given in Table I.

TABLE I. Texture of the soils of the fields under investigation.

| Field No. | Good surface (*) | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1 | F. S. L.** | F. S. | F. S. L. | F. S. |
| 2 | F. S. L. | F. S. | F. S. L. | F. S. |
| 3 | F. S. L. | F. S. L. | St. L. | Cl. L. |
| 4 | F. S. L. | V. F. S. | Peaty L. | Humus |
| 5 | F. S. L. | F. S. | St. L. | Cl. L. |
| 6 | F. S. L. | F. S. | St. L. | St. Cl. L. |
| 7 | F. S. L. | St. L. | F. S. L. | St. Cl. L. |
| 8 | S. L. | F. S. | F. Sl L. | Cl. L. |
| 9 | S. L. | St. L. | F. S. L. | Cl. L. |
| 10 | F. S. L. | F. S. | St. L. | Cl. L. |
| 11 | St. L. | Cl. L. | St. L. | Cl. L. |
| 12 | F. S. L. | St. L. | F. S. L. | St. Cl. L. |

(*) For desigations of samples, see "Sampling".

**F. S. - Fine sand or fine sandy.

V. - Very. L. - Loam St. - Silt or silty.

Cl. - Clay. S. - Sand or sandy.

Methods employed.

Sampling.

Soil samples were taken with an one and one-half inch augur, from portions of the field sustaining healthy and unhealthy growth, respectively. Borings were made at the foot of the plant. Six such borings from the upper seven inches were mixed and made into a composite sample of surface soil; likewise, borings from the lower seven inches constituted the composite subsoil sample. Samples from the portion of the field where the plants grew healthily were designated as "Good surface" and "Good subsoil", and those from the poor spots of the field, as "Poor surface" and "Poor subsoil".

Circumstances made it impossible to analyse these soils for nitrates in their fresh state. They were brought to the laboratory and dried in an electric oven at approximately 82° ~~degrees~~ C. for 48 hours, in order to stop, if possible, the activities of the nitrifying bacteria. King (71) studied the effect of drying on the amounts of nitrates and other soluble salts recoverable from the soils, and found that drying at 110° ~~degrees~~ C. caused a marked increase in the amount of these salts. Gustafson (51) also found a considerable increase of soluble salts in the soil as a result of drying at 105° ~~degrees~~ C. But in view of the fact that the present investigation is only a comparative one and that the samples are dried at a much lower temperature, at about 82° ~~degrees~~ C., any increase in the amount of nitrates would probably be too slight to cause serious discrepancies in the results.

Determination of nitrates.

The well-known phenol-di-sulphonic acid method, as described in the Bureau of Soils Bulletin 31, was used in nitrate determinations. The soil extract was obtained by filtering through a Pasteur-Chamberland filter.

The condition of the fields at the time of the first sampling.

The first sampling of the season was made on July 15, 1922, from ten of the fields, the remaining two being sampled three days later, on July 18. A brief description of the fields at this time is given below.

Field No. 1.

Samples were collected from this field on July 15, on which day the plants on "good" spots were about 2 - 2½ ft. high, while those growing on "poor" spots were 1 - 1½ ft. high and had a lighter colour, though not very much marked as contrasted with the colour of the plants on good spots. Drainage was fair in both places, and the topography, level.

Field No. 2.

A very marked contrast in colour, size, and vigour of plants growing on good and bad portions of this field was noted. The height of plants on good spots was 3½ - 4 ft., while the ones on poor spots were only about a foot or so in height. The condition of drainage in both spots was fair and practically the same, and the topography, nearly level.

Field No. 3.

The corn on good spots was 4 - 5 ft. high and very vigorous in growth. The plants on poor sections of the field were less than two feet in height and very sickly and pallid in appearance. Drainage of the good spots was very good and that of the poor spots, rather poor, although the topography was level.

Field No. 4.

The plants on good spots had attained a height of 3 - 4 ft., growing under excellent drainage conditions. The drainage of the poor spots, however, was very poor on account of a depressed or hollow topography of this field; consequently, the plants on this section suffered a chlorotic condition and were only 1 - 1½ ft. high.

Field No. 5.

A very marked difference in colour and growth of plants was found to exist between the plants of good spots that were about 4 ft. high and those of the poor spots which were hardly 1½ ft. in height. The surface drainage of the poor spots was fair, but the subsoil was very poorly drained. Topography was nearly level.

Field No. 6.

The corn was about 3-3½ ft. high on good spots and 1-1½ ft. high on poor spots. Drainage of the former was good, but that of the latter was rather poor, the topography being uneven.

Field No. 7.

Colour contrast was not marked on this field, although the size of the plants was not the same. Good plants were 3-4ft.high, while the poorer ones were only 2 ft. in height. Drainage was fair on poor surface soil, but rather poor in subsoil.

Field No. 8.

With a good drainage on good spots, the plants grew up healthily and were about 3 ft. high. The plants growing on poorly drained poor spots were comparatively dwarf, 1-1½ ft. high, and pale.

Field No. 9.

The drainage of the good spots was very good and the plants looked healthy and strong, and were about 4 ft. high. The surface drainage of the poor spots was also fair, but the subsoil was very poorly drained. The plants on this spot were only 1-1½ ft. high and very unhealthy in appearance. Topography was almost level.

Field No. 10.

Corn was about 5 ft. high and vigorously flourishing on good spots where drainage was excellent. The poor spots were very poorly drained, on account of an uneven topography, and the plants were less than 1½ ft. in height, and quite yellowish in colour.

Field No. 11.

There was a difference in size but not in colour of plants growing on this field. The good plants were 3½-4 ft. in height. Drainage conditions were not satisfactory under small plants, though the topography of the land was level.

Field No. 12.

Corn, on poorly drained portions of the field, was very dwarf and sickly in appearance.(See Figs. 1 and 2). It was noted that manure which had been in the soil for about five years was yet undecayed. The large plants were healthy and strong, and about 4 ft. high, drainage of the soil under them being fair. Topography was fairly level.

## Nitrate content of samples of Series A.

The samples first collected were designated as those of Series A; and their nitrate contents are given in Table II.

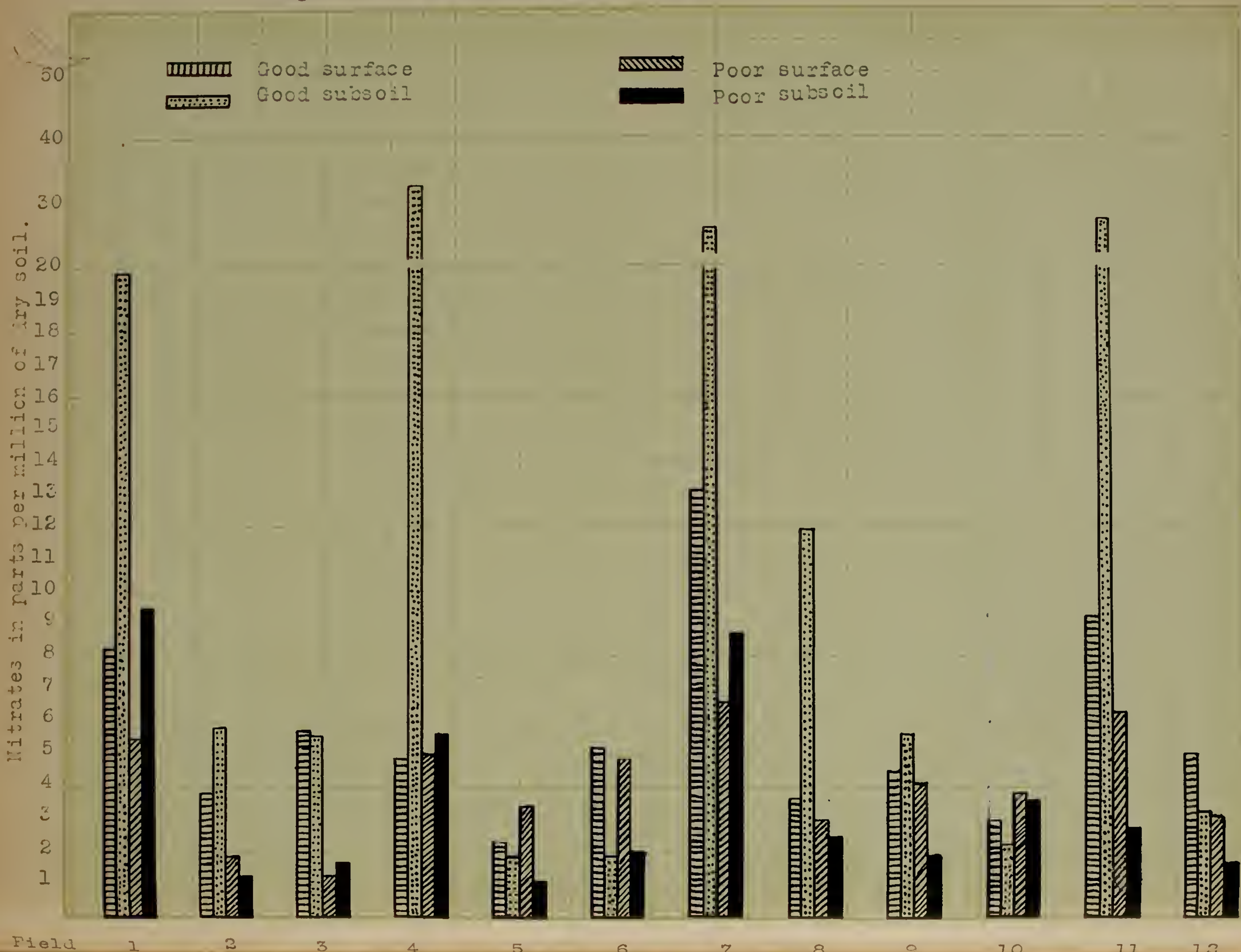
At the time of testing for nitrates a moisture determination was also made on the samples, already dried at about 82° ~~degrees~~ C., by drying the soil at 110 degrees C., and the results appear in Table III.

TABLE II. Nitrates in samples of Series A.
(Parts per million of dry soil)

| Field No. | Good Surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 8.32 | 19.87 | 5.45 | 9.54 |
| 2. | 3.83 | 5.81 | 1.94 | 1.37 |
| 3. | 5.76 | 5.60 | 1.33 | 1.72 |
| 4. | 4.92 | 32.59 | 5.08 | 5.62 |
| 5. | 2.33 | 1.90 | 3.42 | 1.08 |
| 6. | 5.10 | 1.92 | 4.94 | 2.00 |
| 7. | 13.06 | 25.75 | 6.61 | 8.67 |
| 8. | 3.71 | 11.95 | 3.01 | 2.50 |
| 9. | 4.43 | 5.74 | 4.09 | 1.98 |
| 10. | 3.01 | 2.34 | 3.85 | 3.63 |
| 11. | 9.34 | 27.16 | 6.39 | 2.78 |
| 12. | 5.06 | 3.38 | 3.11 | 1.77 |

As will be seen in Table II and the accompanying graph, fig. 4, nine out of twelve fields indicated a consistent correlation between the nitrate content of the soil and the healthy growth or chlorotic condition of corn. Eleven subsoils showed a higher nitrate content

Fig. 4 Showing nitrates in soils of Series A.



than their corresponding surface soils. The experiments of Fraps (37) at Texas Station had also shown that in some cases subsoils contained more nitrates than surface soils. This might be due either to leaching of nitrates from the surface into the lower depths of the soil or to better nitrifying capacity of the subsoils.

TABLE III. Moisture content of the samples at the time of nitrate determinations. (Series A.)

(per cent, on moist basis)

| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 3.23 | 1.57 | 3.90 | 1.67 |
| 2. | 2.97 | 1.67 | 1.99 | 1.47 |
| 3. | 1.72 | 1.35 | 1.93 | 1.18 |
| 4. | 3.80 | 2.31 | 10.70 | 10.21 |
| 5. | 3.02 | 2.38 | 3.11 | 2.78 |
| 6. | 1.80 | 1.02 | 2.52 | 2.19 |
| 7. | 2.32 | 2.46 | 2.75 | 3.14 |
| 8. | 1.50 | 1.44 | 1.80 | 1.34 |
| 9. | 1.62 | 1.43 | 1.86 | 1.38 |
| 10. | 1.83 | 1.26 | 2.20 | 2.03 |
| 11. | 1.33 | 0.96 | 1.20 | 0.81 |
| 12. | 1.10 | 0.84 | 1.63 | 1.03 |

There are, however, as will be noticed in the table, certain inconsistencies in the results, where poor soils showed a higher nitrate content than did good soils. Such discrepancies could best be explained in the light of knowledge of the character of the soil, topography of the land, and other external and internal factors.

There were found slightly more nitrates in poor surface soil from field No. 4 than in good surface soil of the same field, despite the fact that the actual drainage condition of the former was such as to be apparently inhibitive of nitrification. This was reflected in the chlorotic condition of the plants growing on poor spots. Oven-drying, however, probably altered the conditions in the sample and made them favourable for nitrification. A reference to the Table (III) of moisture determinations would manifest the fact that either this peaty poor surface soil was not dried sufficiently even after remaining in the oven for 48 hours at approximately 82° ~~degrees~~ C., or it absorbed moisture later on, although, as all other samples, it was kept in a nicely closed tin can. 10.70 per cent of moisture found in this soil at the time of testing for nitrates was possibly sufficient to favor nitrification during the interval between sampling and analysis.

Another striking exception occurred in case of soils from field No. 10. The excess of nitrates in poor soils over the amount found in good soils was possibly due to the fact that owing to the lighter texture of the soil of good spots and the uneven topography, nitrates leached down to the lower section of the field and there accumulated. But, apparently, poor drainage conditions made it impossible for plants to utilize the nitrates thus accumulated.

In general the results tend to establish a correlation between the nitrate content of the soil and the chlorotic condition of corn.

## General condition of the fields at the time of the second sampling.

The second sampling of the season was made on August 6, after an interval of 22 days, from the first ten of the fields, and on August 17, after an interval of 30 days, from the fields Nos. 11 and 12. These samples were designated as those of Series B. So far as was possible, samples were taken from the foot of the same plants, from where the Series A samples were previously collected, with the exception of field No.9 where the samples were taken from new places but from the same part of the field.

The weeks preceding the second sampling had been rather rainy, with a precipitation of about 3.39* inches during the interval between July 17 and August 6.

In general the plants on good spots made considerable growth, ranging from 7 to over 10 feet in height, and looked very strong and flourishing, and they were mostly in ear forming stage; while on poor spots the plants remained almost stationary. No growth or recovery of colour was noticeable, except in certain cases which are briefly considered here below.

The improved drainage conditions on some fields were accompanied by a disappearance of color contrast between other-wise good and poor plants. Thus, there hardly existed a difference in colour of plants on field No. 1. But while the good plants were about 5 ft. high and "in ear", the otherwise poor plants were comparatively small and not yet in ear, though they were quite healthy and had attained a height of nearly 4 ft.

On field No. 2, no growth took place in poor plants; and the good plants, though 5 ft. tall, had taken on a pale colour, due to the infestation of the field by strong and vigorous weeds, which robbed the plants of nitrates.

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* Taken from the meteorological bulletins of Mass. Expt. Sta.

The formerly poor plants on field No. 3 slightly recovered their green colour, and attained a height of about 4 ft., but their stalks were rather thin. The drainage of poor spots was still poor.

A considerable growth and recovery of colour were noticed in the "poor" plants on field No. 7. They were about 5 ft. high and looked almost as strong and healthy as the plants on good soil.

The drainage conditions were still rather poor on poor spots of the field No. 11. The plants, however, managed to grow considerably and attained a height of about 5 ft., but they were not yet in tassel while the good plants were 8-10 ft. high and their ears, forming.

The samples of the Series B were dried in the same way as those of Series A, and a moisture determination on them was made at the time of testing for nitrates. The results of moisture and nitrate determinations are given in Tables IV and V, respectively.

TABLE IV. Moisture content of Series B samples at the time of nitrate determinations.

(per cent, on moist basis)

| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 2.00 | 1.65 | 2.11 | 1.38 |
| 2. | 2.07 | 1.79 | 1.62 | 1.16 |
| 3. | 1.72 | 1.30 | 1.43 | 1.05 |
| 4. | 2.64 | 1.18 | 1.94 | 12.77 |
| 5. | 2.30 | 1.57 | 2.68 | 2.13 |
| 6. | 1.45 | 1.07 | 2.03 | 1.40 |
| 7. | 2.30 | 2.09 | 2.43 | 1.96 |
| 8. | 1.35 | 1.19 | 2.10 | 1.69 |
| 9. | 1.85 | 1.88 | 1.56 | 1.25 |
| 10. | 1.87 | 1.66 | 2.47 | 2.11 |
| 11. | 1.32 | 1.01 | 1.13 | 0.75 |
| 12. | 1.15 | 0.86 | 1.67 | 1.15 |

TABLE V. Nitrates in samples of Series B.

(parts per million of dry soil)

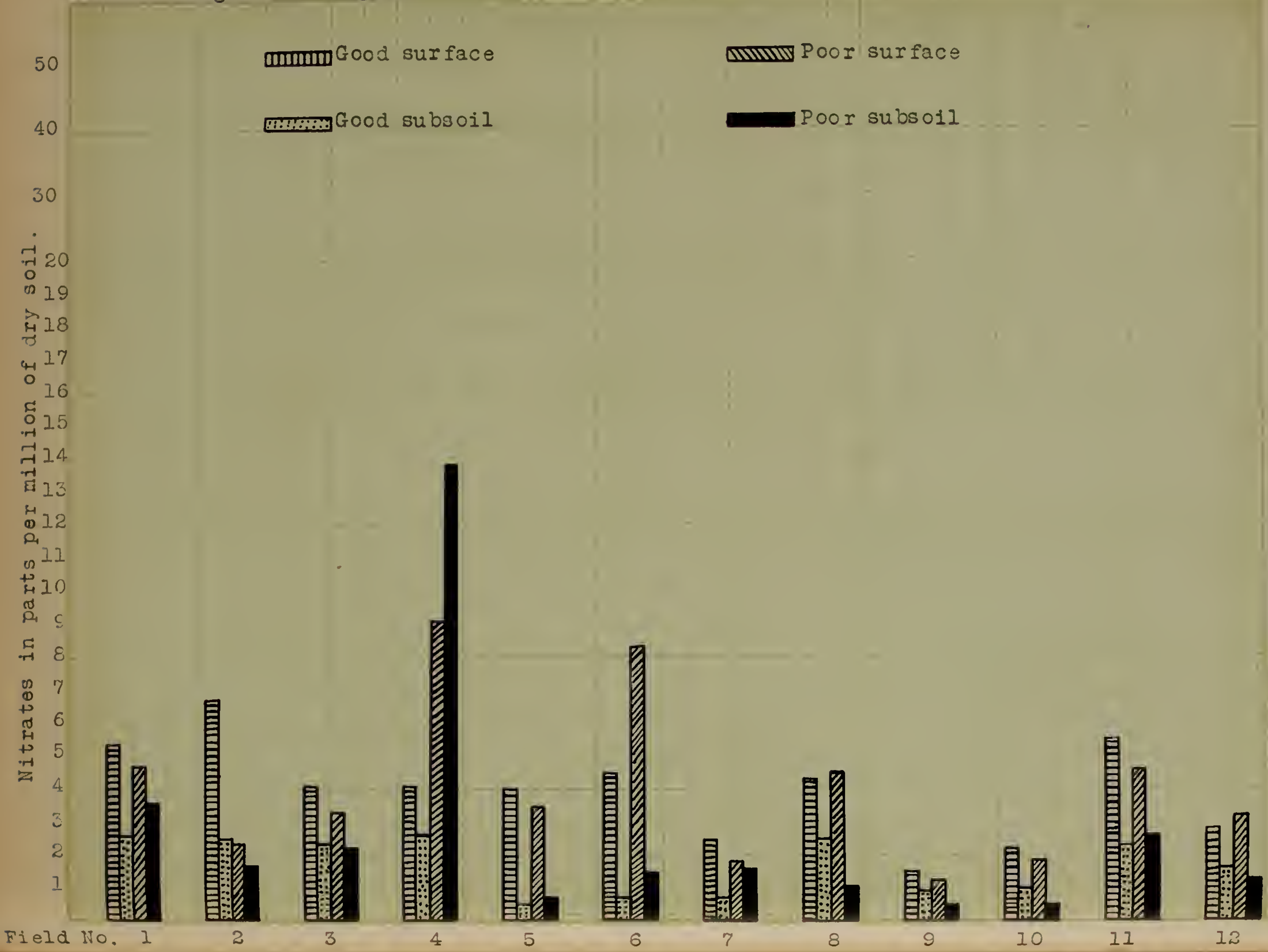
| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 5.27 | 2.60 | 4.76 | 3.60 |
| 2. | 6.67* | 2.55 | 2.29* | 1.16 |
| 3. | 4.08 | 2.38 | 3.30* | 2.32* |
| 4. | 4.13 | 2.63 | 9.06* | 13.81* |
| 5. | 4.01* | 0.50 | 3.46* | 0.76 |
| 6. | 4.57 | 0.70 | 8.35* | 1.47 |
| 7. | 2.57 | 0.92 | 1.80 | 1.63 |
| 8. | 4.31* | 2.48 | 4.56* | 1.12 |
| 9. | 1.58 | 0.97 | 1.27 | 0.50 |
| 10. | 2.19 | 1.02 | 1.90 | 0.51 |
| 11. | 5.48 | 2.27 | 4.61 | 2.62 |
| 12. | 2.83 | 1.61 | 3.21* | 1.31 |

*Indicates an increase as compared with corresponding ones in Series A. The rest is usually a decrease, or nearly so. See also Fig. 5.

The most striking fact brought out in these determinations is the considerable decrease of nitrates in "good subsoils". Possibly, this is due to absorption by plants and perhaps, in some measures, to leaching as well.

In nine cases out of twelve, there is a falling off in the amount of nitrates in "good surface" soils, accompanied generally by a marked growth of plants. Albrecht (1) also found that for maize nitrates accumulated until late in June but decreased very decidedly

Fig.5 Showing nitrates in soils of Series B.



thereafter. This period of decrease in soil nitrates corresponds with the period of the most active growth of maize.

On two of the three fields where an increase occurred in the nitrate content of good soils, this increase was accompanied by strong and healthy growth. On field No. 2, however, although the plants did attain a height of about 5 ft., yet they were pale in colour, owing to the condition of the field which was almost lost in weeds. The plants were evidently robbed of their nitrate supply by the infesting weeds. This robbing effect of weeds was also noticed by Call and Sewall (12).

On poor soils, on the other hand, an increase of nitrates was not accompanied by increased development of plants, except in the case of the field No. 3 where the formerly poor plants slightly recovered their colour and made an actual growth in size. The accumulation of nitrates in other poor spots might be the result of leaching from the lighter-textured good soils where the topography is not level, or it might be due to the fact that the unhealthy plants were not in a condition to utilize any nitrates either produced in place or otherwise accumulated.

The belief of Whitney and Cameron (136) in that the controlling factors in fertility were moisture and the physical condition of the soil and not the amount of plant nutrients would rightly apply to these poor spots which contained either slightly or considerably more nitrates than their corresponding good spots, but the drainage conditions of which were aggravated by excessive precipitation during the two weeks previous to sampling. Lyon (85) also made a statement to the same effect.

The concentration of the soil solution ~~would~~ seems also ~~seem~~ to have played an important rôle in the case of the poorly drained spots where an increase of nitrates but no growth was noticed. For Hall, Brenchley, and Underwood (52) showed that in nutritive solutions of varying dilutions, the growth of plants "varied directly, but not proportionally, with the concentration of the solution", irrespective of the total amount of available plant food in the soil.

The high nitrate content of the poor subsoil sample from field No. 4 is again probably due to a later nitrification, owing to its moisture content of 12.77 per cent at the time of nitrate determinations.

Taking the results of Series B as a whole, it will be noticed that they indicate a consistent correlation between the nitrate content of the soil and the chlorotic condition of maize plants. This consistency is more marked in case of the fields where topographical, drainage, and other conditions are nearly alike for both good and poor sections of the field.

General condition of the fields at the time of the third sampling.

The third samples of the season were collected on August 21 from first ten of the fields, and on September 1, from the remaining two. An interval of 15 and 14 days, respectively, elapsed since the dates of the second sampling of the fields.

The total precipitation during the period from August 6 until August 21 was 1.82 inches; but the last week of August had alone a precipitation of 1.91 inches.

The borings were made again as before at the foot of the same plants, whence the first and second samples were collected. On account of the recent cultivation the old places on field No.5 were lost from sight, and consequently, the present samples were taken from new places on the same part of the field.

At this time of the season the plants on good spots were generally tall, strong, vigorous, and in full ear, except there was noticed a pale colour and slackened condition in those of the fields Nos. 2 and 9, which were lost in weeds.

There was also observed a marked improvement in colour and size of the otherwise poor plants growing on poorly drained spots. Such, for instance, was the case with those on fields Nos. 1, 3, 7, 11, and 12. The poor plants also had started to form ears. In fact, no appreciable difference in colour, size, or vigour was noticeable in plants on field No. 7. The growth on this field was excellent and almost uniform. A little improvement in colour, but not in size, was noted on fields Nos. 4 and 6, in spite of poor drainage conditions.

No growth or improvement of any kind could be seen in the poor plants on fields Nos. 2, 5, 8, and 9. The owner of the field No. 10 apparently lost hope and patience with the poor plants; for he had cleared them off his land.

These samples also were dried at approximately 82 degrees C. for 48 hours. A moisture determination was made on them at the time of nitrate determinations. The results of these determinations are given in Tables VI and VII, respectively.

TABLE VI. Moisture content of Series C samples at the time of nitrate determinations.

(per cent, on moist basis)

| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 1.62 | 1.48 | 1.69 | 1.23 |
| 2. | 1.67 | 1.57 | 1.80 | 1.40 |
| 3. | 1.23 | 1.63 | 1.14, | 0.80 |
| 4. | 2.44 | 1.30 | 9.16 | 21.95 |
| 5. | 1.80 | 1.47 | 2.04 | 1.75 |
| 6. | 1.48 | 1.13 | 1.90 | 1.41 |
| 7. | 2.05 | 1.78 | 2.27 | 1.87 |
| 8. | 0.97 | 0.78 | 1.73 | 1.60 |
| 9. | 1.26 | 1.00 | 1.95 | 1.00 |
| 10. | 1.58 | 1.23 | 1.65 | 1.60 |
| 11. | 1.23 | 1.09 | 1.24 | 0.76 |
| 12. | 1.05 | 0.74 | 1.59 | 0.64 |

TABLE VII. Nitrate content of samples in Series C.

Parts per million of dry soil.

| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 0.10 | 1.73 | 3.85 | 1.11 |
| 2. | 4.70 | 2.03 | 5.52* | 0.61 |
| 3. | 0.65 | 1.07 | 1.67# | 1.41 |
| 4. | 3.45 | 1.32 | 3.08 | 6.80# |
| 5. | 4.19* | 0.81** | 3.35 | 0.66 |
| 6. | 5.65* | 1.06** | 9.46* | 1.42 |
| 7. | 2.76** | 1.02** | 51.39* | 5.67** |
| 8. | 4.04# | 1.26 | 1.58 | 0.40 |
| 9. | 3.14** | 2.07** | 2.45** | 0.40 |

Fig. 6 Showing nitrates in soils of Series C.

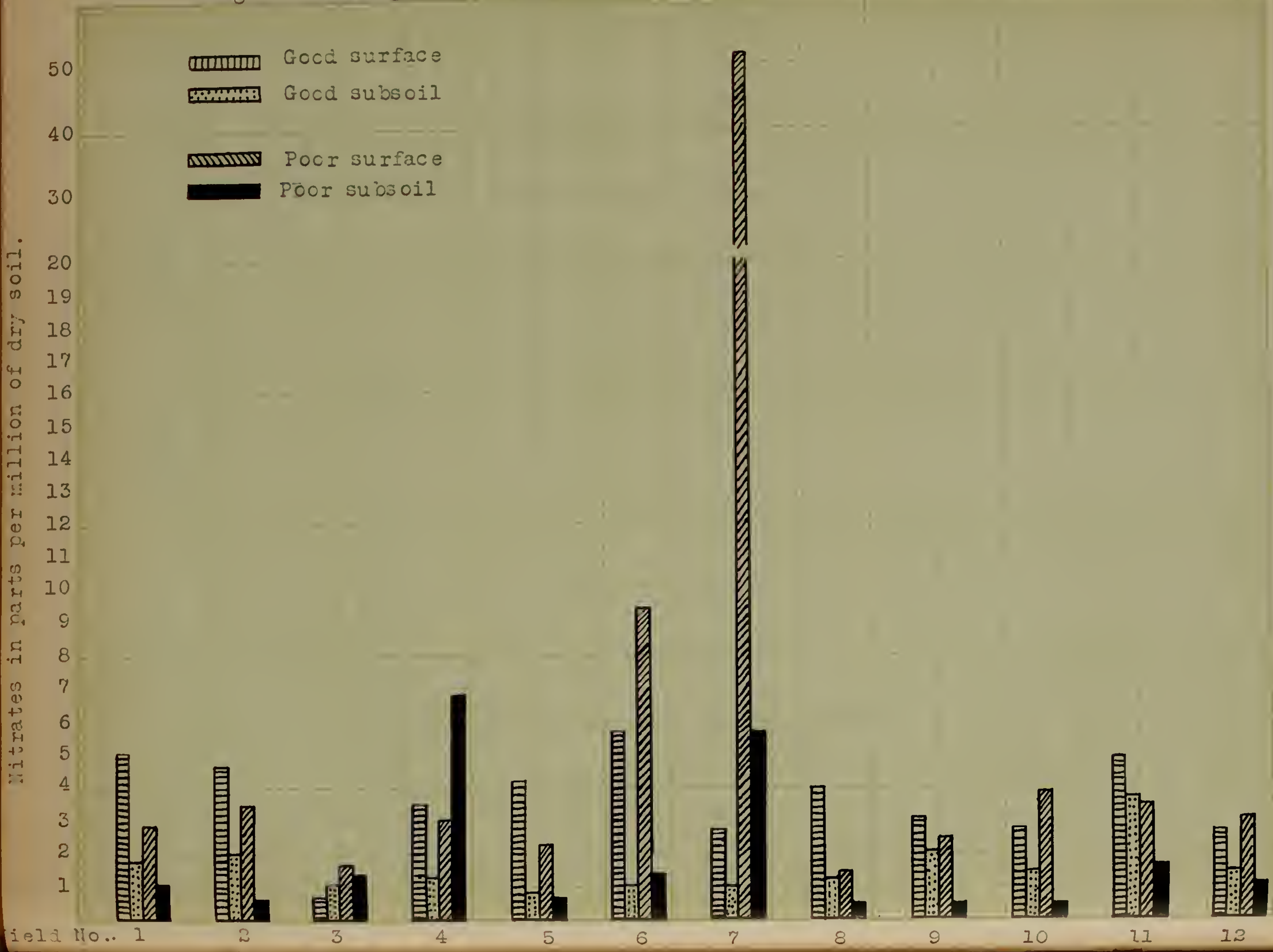


TABLE VII.-Continued.

| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 10. | 2.85** | 1.42** | 3.87* | 0.50 |
| 11. | 4.87 | 3.74** | 3.50 | 1.71 |
| 12. | 2.73 | 1.41 | 3.10 | 1.11 |

* Denotes increase over Series A and B.
\# Denotes increase over Series A only.
** Denotes increase over Series B only.
**

As will be seen in Table VII and Fig. 6, the results indicate the existence of a definite and rather consistent correlation, the good soils having, in general, more nitrates than the corresponding poor soils.

A slight increase of nitrates in poor surface soil of the field No. 2 was not accompanied by growth or healthiness of plants, owing most probably to the fact that the infesting weeds might have robbed them of nitrates. Even the good plants did not possess a very healthy colour.

The utilization of nitrates by the good, vigorous plants of field No. 3 had been extremely active, causing a decided decrease in the amount of nitrates in good soil as compared with the results of the preceding series; there was also a marked decrease of nitrates in poor soils, due to their absorption by the recovering "poor" plants. As mentioned before, there was observed a marked improvement in colour and size of the "poor" plants on this field, which fact is clearly reflected in the results.

The excessive amount of nitrates in poor subsoil from the field No. 4 might again be due to leaching from the lighter-textured good soil, on account of an uneven topography and the previous rainy weeks, or to a later nitrification,-which fact may be suspected from the amount of moisture, 21.95 per cent, which the sample was found to contain at the time of nitrate determination. In fact, a little improvement in colour but not in size of the poor plants on this field was noticed.

The higher nitrate content of poor soils from field No. 6 could be attributed to the accumulation of unutilized and leached nitrates. While the unusual increase of nitrates in the poor soils of field No. 7 could not be easily explained, yet it is sufficientto observe that no contrast in colour or growth of plants existed between those growing on good soil and those growing on poor soil.

No importance could be attached to the slightly higher nitrate content of the poor surface soil of field No. 10, as the plants on poor spots were all cut and cleared off the land. This accumulation may also be the result of leaching.

The colour of the poor plants on field No. 12 was distinctly improved; and the slight decrease of nitrates as compared with the results in Series B indicated the utilization of these nitrates by the plants.

The existence of a correlation between the deficiency of nitrates in the soil and the chlorotic condition of maize plants is thus demonstrated by the results of Series C, as well as those of the preceding series.

Naturally, there are ~~quite~~ many discrepancies in the results, but the importantefactor of absorption must always be kept in mind in order to appreciate the occasional increase of nitrates in poor spots. Burd (10), and Stewart (121) have shown that, even though good uncropped soils may contain considerably more nitrates and other salts than the poor uncropped soils, both good and poor soils are reduced to the same general level at the time the crop is growing. It is considered that the productivity of the good soils lies in their ability to elaborate additional solutes as rapidly as the plants require them, and to sustain normal losses due to absorption.

The results of all three series are combined and presented in Table VIII and Fig. 7.

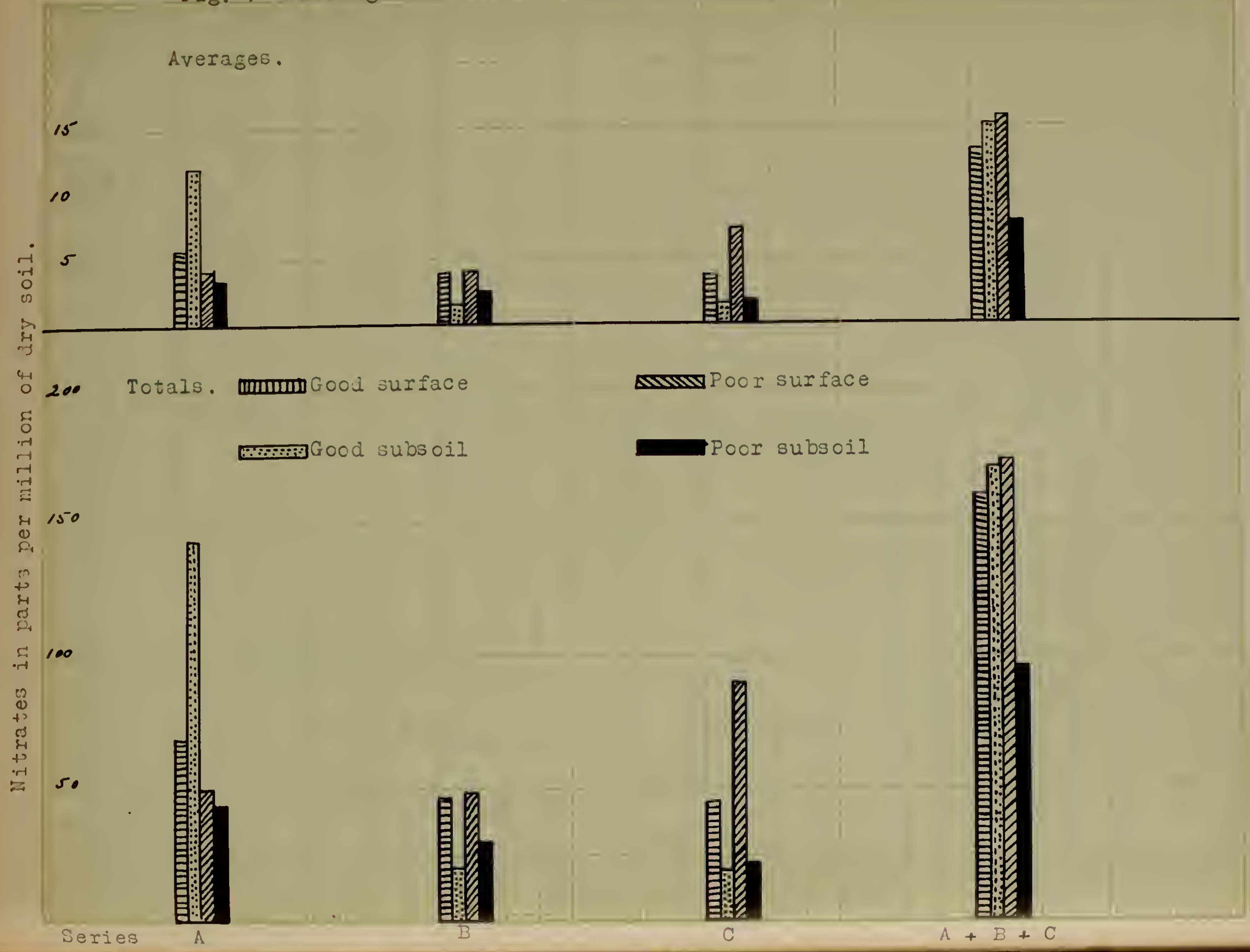
TABLE VIII. Total nitrate content of different series.

(Parts per million, dry soil)

| Series | Good surface Total | Good surface Average | Good subsoil Total | Good subsoil Average | Poor surface Total | Poor surface Average | Poor subsoil Total | Poor subsoil Average |
|---|---|---|---|---|---|---|---|---|
| A. | 68.87 | 6.74 | 144.01 | 12.00 | 49.22 | 4.10 | 42.66 | 3.55 |
| B. | 47.69 | 3.97 | 19.63 | 1.63 | 48.57 | 4.04 | 30.81 | 2.56 |
| C. | 44.13 | 3.68 | 18.99 | 1.58 | 88.82 | 7.40 | 21.80 | 1.81 |
| A+B+C. | 160.69 | 13.39 | 182.63 | 15.22 | 186.61 | 15.55 | 95.27 | 7.94 |

It will be noted that the combined results of Series A show a decidedly high nitrate content in good soils, but in the other series the poor soils contain, on the whole, more nitrates than the good soils. This is due to the fact that the poor spots of field No. 4 were found to contain larger amount of nitrates than the good spots of the same field. This was explained on the ground that nitrates might have leached down to this portion of the field and there accumulated, or that nitrification might have occurred later in the samples.

Fig. 7 Showing the total nitrate content of different series.



The excess of the combined amounts of nitrates in poor soils of Series C. over the amount in good soils is apparently due to the unexplained excessive increase of nitrates in the poor spots of the field No. 7, accompanied by the recovery of the previously chlorotic plants and the total disappearance of differences in colour and vigour of the plants growing on this field. Also, it is due to the higher nitrate content of the poor soils from field No. 4. The average of the combined results of the three series seems to be in favour of the good soils, with the exception of the results of poor surface soils. There has been a gradual, and in some cases rapid, decrease of nitrates in the soils during the season.

The last observation of the fields.

On September 16, a last observation was made of the condition of the fields, which is briefly considered here.

On field No. 1 both good and poor corn were in the "dough" stage and the colour of plants was quite good; but the ears of the poor plants had imperfect rows and kernels.

The plants were all cut and removed from the fields Nos. 2, 3, 4, 5, and 10.

The ears of the good plants on field No. 6 were in milky stage and their rows and kernels were perfect. The plants were about 2-3 feet high on poor spots, and their leaves were quite yellowish.

The plants on field No. 8 were 8-10 feet tall, strong, and green. Their ears were perfect and in milky stage. There was no apparent difference between the good and "poor" plants.

The good corn of the fields Nos. 8 and 9 was in the dough stage and perfect. Poor plants were cut and removed.

On field No. 11 the poor plants were 8-10 feet high, and had a nice green colour, in spite of poor drainage. The ears were in "watery" stage. The good plants were taller and stronger than the poor plants and their ears were in milky stage.

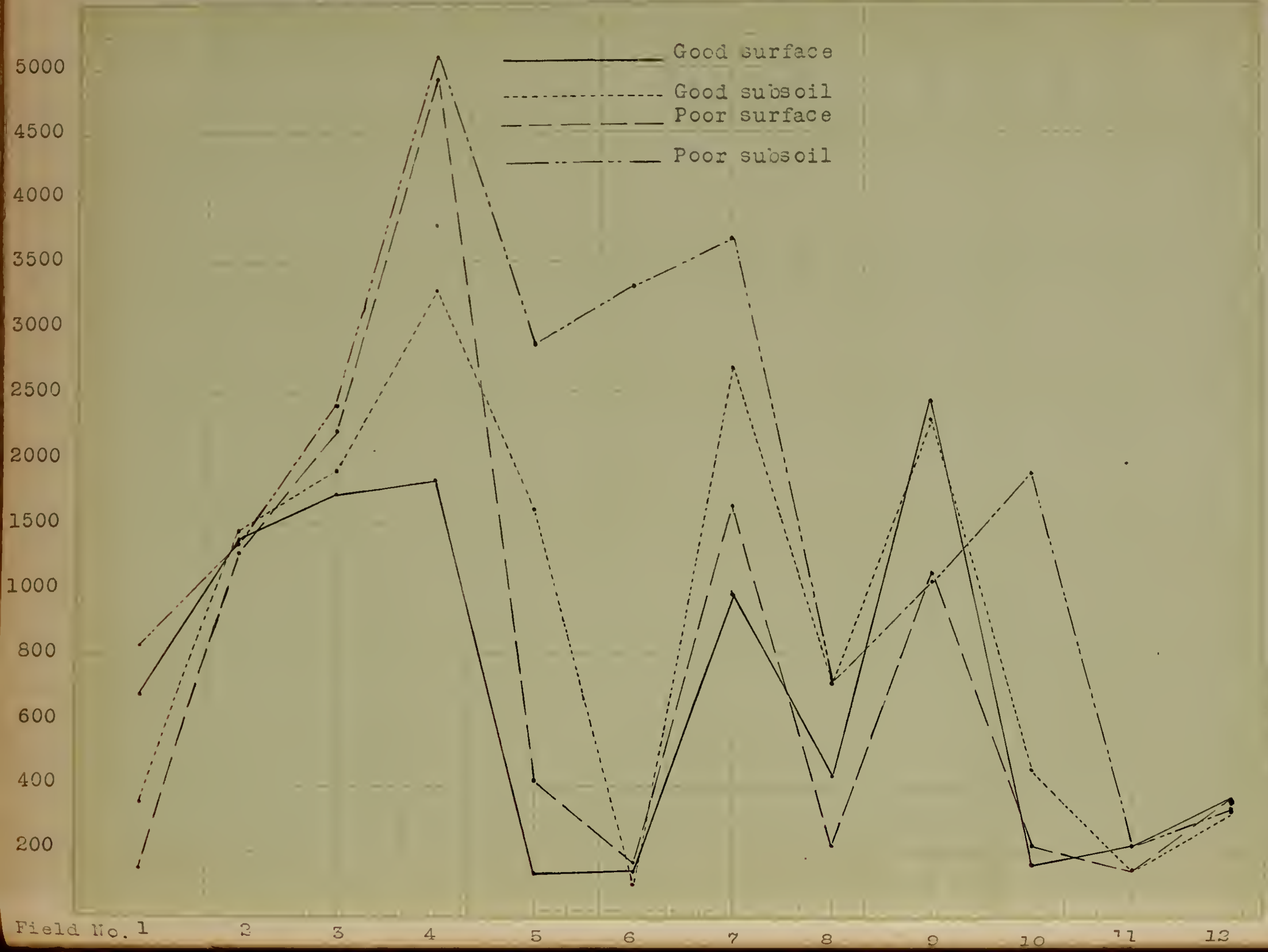
The plants on the poor spots of field No. 12 were weak and very dwarf and sickly in appearance. Drainage was very poor on this part of the field. The good plants were strong and 8-10 feet tall; their ears were in dough stage. Drainage of the good spots was better. (See Fig. 3 for the condition of the ears).

## Acidity Determinations.

In view of the recent tendency to consider some of the physiological diseases of the higher plants-such as root-rot of corn- as due to soil acidity or toxicity, the injurious effect of which is attributed to the excessive concentration in soil of the soluble aluminum (56)(18), a set of determinations was undertaken in this connection to study this phase of the problem.

Kratzmann (76) found that aluminum salts of 0.005 per cent concentration hindered the growth of maize and some other higher plants, but very dilute solutions, 0.0001 per cent, on the other hand seemed to serve as stimulants to growth. He noticed also that even nitrate of aluminum, when highly concentrated, was badly toxic to growth. Hoffer (59) especially pointed out the probable relationship between the accumulation of iron and aluminum compounds in corn plants and the disease. He found considerable accumulation of these compounds in the nodal tissues of the plants that suffered from

Fig. 8 Showing lime requirements of soils of Series B. Pounds CaO per acre.



root-rot. He seemed to consider the excessive amount of aluminum in the soil as one of the chief causes of this disease.

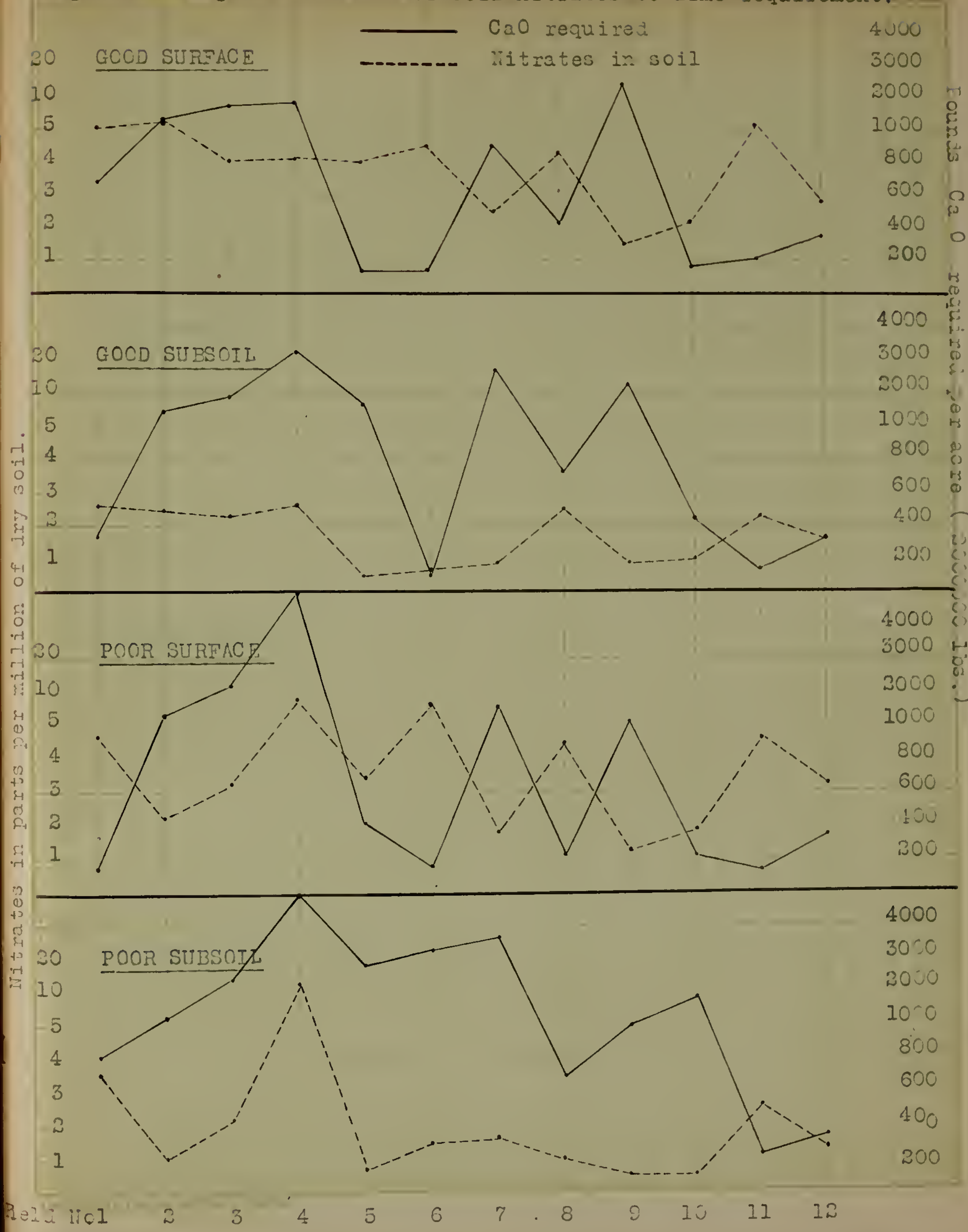
Hopkins' method was employed in these toxicity determinations, because of the fact that it is said to give a fairly good indication of the amount of soluble aluminum in the soil. The soil samples of Series B were used for this purpose and the results are presented in Table IX and Fig. 8

TABLE IX. Lime requirements of soils of Series B.
(pounds CaO per acre)

| Field No. | Good surface | Good subsoil | Poor surface | Poor subsoil |
|---|---|---|---|---|
| 1. | 632 | 348 | 154 | 928 |
| 2. | 1383 | 1458 | 1290 | 1362 |
| 3. | 1727 | 1900 | 2222 | 2419 |
| 4. | 1829 | 3279 | 4910 | 5089 |
| 5. | 143 | 1613 | 419 | 2885 |
| 6. | 143 | 107 | 161 | 3304 |
| 7. | 985 | 2688 | 1630 | 3691 |
| 8. | 439 | 717 | 217 | 717 |
| 9. | 2419 | 2276 | 1111 | 1040 |
| 10. | 161 | 448 | 217 | 1382 |
| 11. | 217 | 143 | 143 | 222 |
| 12. | 358 | 330 | 358 | 340 |

It will be seen that the so-called "acidity" is not confined to poor portions of the fields and that these poor spots do not necessarily require more lime than do the corresponding good spots.

Fig.9 Showing the relation of soil nitrates to lime requirement.



In these determinations it was observed that the turbidity in the solution after neutralization, probably due to the precipitation of aluminum compounds, was almost unnoticeable in case of the soils with a lime requirement of less than 200 pounds of CaO per acre, but it was increasingly marked with increasing lime requirements.

This acidity or toxicity, however, does not seem to have checked nitrification to any great extent. Although Hall et al.(53) and Coville (20) attribute the injurious effect of soil acidity chiefly to the inhibition of nitrification and other bacteriological processes, yet, that such is not the case and that nitrate production occurs in so-called acid soils has been demonstrated in the work of Temple (125), White (134), Fred and Graul (37), Noyes and Conner (93), Stephenson (119), and others. In Fig. 9 is given a graphical presentation of the relation between the lime requirement of the soil and its nitrate content. It would seem as if there exists a correlation between these two properties of the soil. In certain cases high acidity is accompanied by small amount of nitrates; but this is in no way proportional. It cannot be said definitely, however, that toxicity has played an important role in decreasing the nitrate production, and thereby bringing about the chlorotic condition of plants.

Statistical Study of the results of nitrate determinations.*

TABLE X. .Showing the odds in favour of the good spots

| Series | | Odds |
|---|---|---|
| A | Good surface vs. Poor surface | 41 to 1 |
| | " subsoil vs. " subsoil | 76 to 1 |
| | " total vs. " total | 83 to 1 |
| B | Good surface vs. Poor surface | 8 to 1 |
| | " subsoil vs. " subsoil | 10 to 1 |
| | " total vs. " total | 3 to 1 |
| C | Good surface vs. Poor surface | 2 to 1 |
| | " subsoil vs. " subsoil | 22 to 1 |
| | " total vs. " total | 4 to 1 |

Explanation of table X.

The results of the field No. 4 are altogether excluded from this statistical study, on account of the fact that the excessive amounts of nitrates found in the poor soils of this field could not be adequately explained. Also the fields in which the colour contrasts disappeared within the latter part of the season are ommitted. Therefore, the above presented results are only from the fields explained below:

Series A includes all except the field No. 4.
Series B includes the fields Nos.2, 3, 5, 6, 8, 9, 10, and 12.
Series C includes only six fields; they are fields Nos.2, 5, 6, 8, 9, and 12.

* Student's formula is used. See Biometrika, Vol. 6, Pp. 1 - 25.

## Summary and conclusions.

During the season of 1922, twelve fields on which there was a considerable irregularity in the growth of corn plants on different portions of the same fields were studied with the purpose of determining the correlation, if any, between the nitrate content of the soil and the "chlorotic condition" of the plants on this soil.

"Chlorotic condition" is defined in this work, not as the absence of chlorophyll or blanching due to lack of iron, but as the yellowish, pale, and sickly appearance of plants, usually accompanied by retarded or inhibited growth.

Nitrate determinations generally showed an excess in favour of the "good" portion of the field, where the plants were healthy and strong. There were, however, some exceptions to this rule and in certain cases there were found more nitrates in "poor" soils than in "good" soils, possibly due to leaching from the lighter-textured and well-drained good soils into the poorly drained spots of the field on account of an uneven topography, and the resulting accumulation of nitrates in the latter spots. It might also be due to the inability of the "chlorotic" plants to utilize the nitrates thus accumulated. There was, undoubtedly, a heavy and rapid absorption of nitrates by the healthy plants, thus adding to the complications of the problem. The difference in the amounts of nitrates produced in "good" and "poor" soils would be very much accentuated if the difference in nitrogen absorption of the healthy and unhealthy plants were considered, due to a heavier absorption of nitrates by healthy plants.

The increase of nitrates in some poor soils observed from time to time often resulted in either slight or very marked recovery of the otherwise poor plants, thus diminishing and sometimes entirely removing the contrats of colour, size, and vigour between the good and poor plants.

The "chlorotic" plants did not mature generally, and the development of their ears, if any were present, was far from normal.

Acidity or toxicity determinations did not indicate a consistent relationship between the lime requirement of the soil and the chlorotic condition of plants. Although there seemed to exist, in certain cases, a correlation between the acidity and nitrate content of the soil, yet this was in no way proportional; and it could not be stated with any certainty that toxicity checked nitrification, thereby causing the "chlorotic condition" of plants.

The limited number of fields studied and the confinement of the investigations to only one season, which has been unusually rainy, in addition to numerous other important factors which could not be taken up within the scope of this work, make it difficult to draw definite conclusions. It is believed, however, that the evidence tends to the existence of a rather consistent correlation between the deficiency of nitrates in the soil and the chlorotic condition of maize growing upon it. This correlation, as brought out by the statistical study of results, is more distinctly manifested during the earlier stages of growth than in the latter part of the season.

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## Acknowledgments.

The author wishes to express his gratitude to Dr. A. B. Beaumont, under whose supervision this work is performed, for the keen interest he has taken in this work and for his generous assistance throughout the investigations. It is also a pleasure to acknowledge indebtedness to Director S. B. Haskell, Professors Orton L. Clark and John B. Abbott for reading the manuscript and making valuable criticisms and suggestions; also to Professor Charles A. Michels and other members of the Agronomy Department for the interest they have shown and the friendly suggestions they have made from time to time.

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On the Correlation Between the Nitrate Content of the Soil and the Chlorotic Condition of Maize

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