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All manuscripts submitted should be addressed to J. C. Gilman, Botany Hall, Iowa State College, Ames, Iowa.

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SOME CONTRIBUTIONS TO SOIL MICROBIOLOGY AND THEIR INFLUENCE ON THE DEVELOPMENT OF THIS SCIENCE

F. B. SMITH

From the Soils Department, University of Florida

Received May 26, 1941

"Soil Microbiology or Soil Bacteriology as it is called in some quarters, is a science which by its nature and status is certainly a distinct branch of science. It has many complex relationships but is most closely allied to Soil Science or Pedology as a part of the large group of agricultural sciences. It is developed from Bacteriology and Botany, from Chemistry and Geology, from Zoology and Pedology. It utilizes all these sources. But it has developed a technique all its own, it has accumulated facts, correlated and classified them, drawn deductions from them, and definitely demonstrated its place among present day sciences."—*P. E. Brown.*

It has been said that history is the biography of the world's great men. The history of the development of a science consists of the contributions to our knowledge in that field. If Soil Microbiology is considered as that branch of science which treats of the micro-organisms in soils, their character, life history, and functions and their relationship to the fertility or crop-producing power of the soil, then a list of the contributions which have served to form our modern concept of the science might include, among others, titles such as "Bacterial Activities in Frozen Soils," "Micro-organisms in Some Soil Profiles in Iowa," "Effects of Manganese Salts on Ammonification and Nitrification," "Soil Inoculation With Azotobacter," "Phosphorus Assimilation by Certain Soil Molds," "The Importance of Mold Action in Soils," "Bacteriological Effects of Liming," "The Effects of Different Cropping Systems on Bacterial Action in Soils," "Bacteria at Different Depths in Some Typical Iowa Soils," and "Legume Inoculation." One need search no further to find proof in the belief that soils are dynamic, alive, and that changes are constantly taking place which may have far-reaching significance. It would be far more difficult to appraise the influence of these contributions than to list some of their implications.

A knowledge of the soil population, biological interactions, and the effects of such factors as moisture, temperature, reaction, and food supply on numbers and kinds of organisms, are topics still very much alive today. It may be inferred from these contributions that the population of the soil was expected to vary and that the findings for one soil type did not necessarily apply to all other soil types. The soil type was defined as a soil which, wherever it occurs, is relatively uniform in all its profile characteristics, *including its biological properties.* It was the placing of the investigations in soil microbiology upon such a scientific basis that led to its early recognition as a science. On the other hand, the practical side of soil microbiology was emphasized in recognizing the importance of the relation to soil fertility or the crop-producing power of the soil.

In these and other investigations reported by P. E. Brown and his associates, the whole field of the science was covered. In addition to the work on the numbers and kinds of micro-organisms in different soil types, the different horizons, and under different seasonal conditions, the effects of soil treatments, such as farm manures, fertilizers, lime, crop residues, and cropping systems, on numbers, species, activity, and the physiological efficiency of different groups were studied. Important findings on the inoculation of soil with Azotobacter and the occurrence and distribution of Azotobacter in Iowa soils were reported. The relation of nitrogen, phosphorus, and calcium in soils and in pure cultures on growth and nitrogen fixation by Azotobacter was the subject of numerous studies.

The efficiency of cultures for the inoculation of legumes was carried as a project over a number of years and unpublished reports before the name of the legume bacteria was changed to Rhizobium indicate a belief in the existence of several species of this genus. Definite experimental evidence was obtained showing physiological differences in the alfalfa, red clover, and soybean organisms. Studies on the utilization of carbon compounds by Rhizobia and the effects of fixed nitrogen, reaction of medium and the minor elements on the growth and efficiency of these organisms were made. The associative growth of legumes and nonlegumes, the nature of the symbiotic relationship between the bacteria and legumes, the mechanism of nitrogen fixation, and the form of nitrogen fixed were subjects under investigation from time to time.

The importance of mold action in soils was recognized, and as a result of the emphasis on this subject a number of contributions to our knowledge of the occurrence and action of fungi in soils were made. "A Summary of the Soil Fungi," by Gilman and Abbott, the most complete work of this nature, is one of the many contributions along this line.

In these contributions one encounters in numerous places the implication that micro-organisms, being plants, are better suited to test the fertilizer needs of soils than chemical methods. The relationship of phosphorus to the fixation of nitrogen by Azotobacter, the assimilation of phosphorus by various soil fungi and bacteria, and comparisons of chemical and biological methods were subjects investigated.

The decomposition of organic matter and the formation of humus in soils was carried as a project over a number of years, and the problem was investigated from many different angles. The chemical nature of organic matter, its reactions and relation to the mineral fraction of soil, the organisms involved, their end products, methods of measurement and rate of disappearance, the relation of organic matter to microbiological activity, soil fertility, water movement in soils, and soil conservation were studied. The influence of the carbon-nitrogen ratio of organic matter on the rate of decomposition and the fixation of carbon in soils; the influence of decomposing organic matter on the pH and the exchange capacity of the soil; the decomposition of leguminous green manures in limed and acid soils and the influence of lime on the respiratory capacity of the soil were

investigated, and reports appeared from time to time setting forth the results of these investigations. These reports have figured largely in our present concept of the importance of organic matter as a soil constituent, its transitory nature and its practical importance in soil management.

Many other phases of the science, such as nitrification, sulfofication, ferrification, denitrification, nitrate assimilation, and the isolation and identification of soil micro-organisms, were investigated, but sufficient has been said undoubtedly to show the extent of the work done, the scope of the investigations, and to indicate the practical importance and scientific significance of the results which have been obtained.

SOIL RESPIRATION STUDIES ON THE DECOMPOSITION OF NATIVE ORGANIC MATTER[1]

WALTER B. BOLLEN[2]

From the Department of Bacteriology, Oregon Agricultural Experiment Station

Received May 31, 1941

"The life of microorganisms in the soil is the result of the phenomenon of assimilation in its entirety."—Stoklasa.

A physiological consequence of the assimilative activities of microorganisms in the soil is the excretion of CO_2 as a metabolic waste product. Since the food element consumed in largest proportion by the majority of soil microbes is carbon and since most of this is oxidized to yield energy, CO_2 is the outstanding product of their activity. Various intermediate products may be formed, but these are later attacked by other microorganisms and sooner or later are completely oxidized.

The various plant and animal residues which constitute soil organic matter are subject to more or less rapid decomposition according to their energy content and availability as determined by structure and composition. Wollny (43) was the first to show that practically all the CO_2 in the soil, aside from that produced by roots of higher plants, was directly due to the activity of micro-organisms in effecting this decomposition. Stoklasa (28), who did some of the first fundamental work in soil respiration, calculated that certain bacteria produce in 1 hour 2 to 2½ grams of CO_2 per 100 grams of cell substance on the dry basis; more recent data (Rahn (20)) indicates some bacteria and yeasts acting on sugars are much more active, fermenting two to three times their own weight of substrate and producing up to one-half their own weight of CO_2 per hour. Van Suchtelen (36) measured the amount of CO_2 given off by various soils under different conditions and found production of the gas to be closely proportional to numbers of bacteria present. Later investigations both here and abroad have been concerned with methods of studying CO_2 production in soils and with the amount produced from different materials and under varying conditions; these are summarized by Waksman and Starkey (41). Soil respiration studies have thus attained importance as a measure of decomposition processes and as an index of soil fertility.

A supply of rapidly decaying organic matter in the soil insures desirable microbial activity, greater availability of nitrogen and other plant food elements, and the maintenance of good tilth. Since these consequences mean better crop yields and since decomposition results in loss of organic matter, a permanent system of soil fertility must include practices by which crop residues or manures are regularly added to the soil.

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[2] Associate Bacteriologist.

Numerous investigations (16) have shown that crop yields closely correlate with the organic matter content of the soil. It is also well established that the rate of loss of organic matter is influenced by inherent soil properties, environmental factors, and any treatment which affects microbial action in the soil. Hence, a study of soil organic matter and its rate of decomposition is of great practical importance.

OBJECT OF INVESTIGATION

A series of studies on CO_2 evolution from various soils, with and without the addition of organic materials and supplemental treatments, have been in progress at this station for a number of years. These have been complemented by chemical and microbial analyses made on the original soil samples and on the soils from the respiration apparatus during and at the close of the incubation period. In this way it was hoped to gain information on the effect of factors, both artificially impressed and inherently fixed by soil type, on the rate of humus formation, accumulation and decomposition. Data from these investigations have been segregated and some are here presented as a preliminary report showing the rate of decomposition of the native organic matter of certain similar and dissimilar soil types occurring in Oregon and elsewhere along the Pacific coast, and indicating the influence of moisture and available nitrogen, factors more or less subject to practical control.

SOILS STUDIED

Soils from distinct taxonomic groups as well as similar soils within a group were included in these studies. The ones selected include seven acid soils from the Willamette Valley, two alkaline soils from California, and one "alkali" soil from eastern Oregon. Samples, except where otherwise noted, were taken along fences or from other areas approaching virgin soil conditions as nearly as possible and were taken to a depth of 6 inches after removing surface litter or sod. Each sample was passed through a 2-mm. screen, mixed well, and stored in a large can for one to two weeks before setting up the respiration experiment.

ACID SOILS

All the acid soils were obtained in the Willamette Valley and are pedalfers representative of several groups developed under a mild subhumid climate from alluvial valley-filling materials derived mainly from basaltic rocks. They are noncalcareous, faintly to definitely acid, and range in texture from light to heavy. Their important pedologic characters are presented in the following outline:

RECENT SOILS: friable soils on friable subsoils, with little profile modification.

1. *Newberg loam:* This is a first bottom soil, formed in swift water, with coarse subsoil. It is a brown mellow loam averaging 12 inches in depth on a brownish sandy loam subsoil. Under virgin con-

ditions the soil contains a moderate supply of organic matter, but under cultivation this is soon lost. The soil is subject to overflow and reworking by high water and has a ridged or billowy relief. Drainage is good to excessive, and the soil dries out quickly. It is a productive soil but applications of manure are especially valuable because of the effect in increasing moisture-holding capacity.

2. *Chehalis loam, and*

3. *Chehalis sandy loam:* These are second bottom soils formed in backwater, with heavier subsoil. They are brown mellow soils 9 to 12 inches deep on stratified subsoil which, to a depth of about 4 feet, is the same or slightly heavier in texture and somewhat lighter in color than the surface soil. The soil is subject to overflow almost every year, but drainage takes place rapidly after the backwater subsides. A relatively low supply of organic matter is present.

OLD VALLEY FILLING SOILS: Mature soils with compact subsoils.

4. *Willamette silty clay loam:* This soil consists of maturely weathered, old valley-filling deposits which have developed under good drainage. The surface soil has an upper layer, 1 or 2 inches thick, of brown, loose granular loam containing organic matter in varying stages of decomposition. The subsurface layer, extending to a depth of about 10 inches, consists of a dark brown, firm, silty clay loam which breaks into fine clods when dry. The surface soil is leached of readily soluble minerals and colloids, which have infiltrated into the subsoil, and it is moderately acid. To a depth of from 30 to 35 inches, the upper subsoils consist of a vesicular, heavy silty clay loam; this grades into the lower, denser subsoil which, at approximately 4 feet, contacts dense, slightly compact, yellowish clay loam or silty clay loam parent material of varying thickness overlying gravel deposits.

5. *Sifton gravelly fine sandy loam:* This is a black-brown prairie island soil with a strongly developed horizon of humus accumulation; it differs from the true chernozems in lacking a horizon of carbonate accumulation. Soils of the Sifton series are high in finely divided, partly decomposed organic matter and are popularly termed "loose land." They are very dark brown or black when moist. When the dry soil is cultivated a cloud of dust which settles as a sooty deposit is formed. The surface soil to a depth of approximately 15 inches is a dark brown, loose fine sandy loam containing gravel ranging to 2 inches in diameter. Nearly 30 per cent of the sample collected for this work would not pass a 2-mm. screen. The subsoil, which extends to about 3 feet, is similar to the surface soil but contains less organic matter and becomes grayish brown in color. Underlying this is a highly porous and gravelly fluvio-glacial deposit of parent material.

6. *Dayton silty clay loam:* To a depth of 8 or 10 inches this soil is a dull brownish-gray, slightly compact silty clay loam. The area sampled was covered by sod, and the first 2 inches were dark gray

and somewhat granulated; below this the remainder of the A horizon progressively lightened in color due to increasing penetration of podzolic flour. Plowed areas of this soil when dry have a dingy white appearance and it is locally called "white land." Minute ortstein concretions are also distributed in the A subhorizon. The subsoil, extending to about 3 feet, consists of bluish-gray plastic impervious clay irregularly mottled with yellowish-brown clay loam. Soils of the Dayton series are derived from heavy compact alluvial clay and silt, have weathered under poor drainage, and are strongly acid. Nikiforoff (14) describes them as glei-meadow podzols. They contain a moderate supply of organic matter, decomposition of which is retarded by a water-logged condition during the winter and spring rainy seasons and by an extremely dry compact condition during the summer.

HILL SOILS: residual.

7. *Aiken silty clay loam:* This is a red podzolic soil of lateritic derivation. To a depth of 10 inches the surface soil consists of a red silty clay loam; it is granular in structure, and contains numerous iron-cemented pellets. The surface, 1 to 1½ inches, is tinged with brown due to an appreciable content of organic matter. The subsoil is a less silty clay, slightly redder in color; it is compact and dense when moist but breaks down to a coarse cloddy structure when dry. Extending upward into the lower subsoil from the parent basaltic bedrock, which occurs at about 5 feet, are many partly weathered, angular fragments which produce rust-brown mottles. Although the soil is sticky when wet, it is friable and easily cultivated when the moisture content is favorable.

ALKALINE SOILS

Two slightly alkaline soils were obtained from a citrus grove in the San Joaquin Valley. These had been under cultivation for a period of years without addition of organic manures.

8. *San Joaquin sandy loam:* This is a secondary pedalferic soil formed from transported material of granitic origin under semiarid conditions. It has a mature profile with well-developed hardpan at 28 to 30 inches. The surface soil, about 6 inches deep, is a dark reddish-brown sandy loam high in colloidal material and poor in organic matter. It is decidedly sticky when wet, but the presence of coarse gritty fragments and mica particles mask plasticity. The subsoil is a red heavy clay; it is gritty with coarse angular fragments and sand, but a high colloidal content exerts predominating influence on structure and consistence. The soil is definitely basic in reaction but noncalcareous.

9. *Porterville clay adobe:* Soils of the Porterville series are secondary pedocals formed under semiarid climate from transported material derived primarily from basic igneous rocks. The parent material was largely soil eroded from hillsides and washed to its present position. The midmature profiles developed under semiarid

climate and are characterized by calcareous concentrations. In the area sampled, the surface soil, 6 inches deep, is chocolate-brown, heavy in texture, and has a typical adobe structure. On drying, the adobe blocks break by secondary cracking into small angular granules to a depth of several inches; this results in an unstable structure, forming what is locally termed a "dry-bog." It is readily puddled by working when wet. The reaction is slightly basic and the organic matter content is relatively low. The subsoil is similar to the surface soil in color and texture but contains light streaks and stains due to lime accumulation which increases with the depth to form segregated plates and lens. Drainage is poor. The soil is so impervious that once saturated it dries very slowly.

"ALKALI" SOIL

10. *Vale heavy loam*[3]*:* The "alkali" soil, obtained from the Vale Branch Experiment Station in eastern Oregon, is a strongly salinized heavy loam, dispersed and moderately compact. The native vegetation is chiefly greasewood *(Sarcobatus vermiculatus)*, sage brush *(Artemisia tridentata)*, and salt grass *(Distichlis spicata)*. From 0 to 5 inches the profile consists of grayish-brown dispersed loam that is sticky when wet but friable under proper moisture conditions. The 5 to 20-inch horizon is similar in texture but lighter in color; when dry the upper 5 inches exhibits columnar structure which grades to a nut structure below. From 20 to 40 inches is indurated calcareous alkaline heavy loam to clay loam pan. Below this is light yellow-brown, more friable material streaked with sand; this rests on parent sand and gravel alluvium derived from the Payette formation. The soil developed under a hot arid climate and is heavily salinized with native alkali salts. An analysis of the sample gave the following results:

WATER SOLUBLE SALTS	PPM
Total	3,150
CO_3	1,019
HCO_3	0
S as SO_4	79
Cl	108
Na	1,200
Ca	76

REPLACEARLE BASES	EQUIV. PPM
Exchange capacity	242
Ca and Mg	25
Na	203
K	15

The reaction is strongly alkaline (pH = 9.8), and the organic content is low.

[3] This soil has not been mapped by the Soil Survey Division, and the name here used is not official.

TABLE 1

CHEMICAL ANALYSES OF SOILS STUDIED*

Soil	Organic Matter	Kjeldahl Nitrogen	C:N†	pH		S as SO_4		N as NO_3§		P as PO_4	
				Original	Incubated‡	Original	Incubated	Original	Incubated	Original	Incubated
	Percentage	*Percentage*				*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
1. Newberg loam	2.35	0.15	9.0	5.7	5.8	2	2	8	28	0.0	1.0
2. Chehalis loam	1.65	0.10	9.6	6.8	6.7	0	0	11	55	0.2	2.1
3. Chehalis sandy loam	0.99	0.06	9.6	6.6	6.4	1	25	37	32	0.7	0.1
4. Willamette silty clay loam	3.71	0.23	9.3	5.9	5.9	2	0	19	45	0.3	2.1
5. Sifton gravelly fine sandy loam	9.55	0.63	8.8	5.3	5.0	1	0	28	110	0.0	0.0
6. Dayton silty clay loam	3.24	0.18	10.4	5.6	5.5	7	30	55	38	3.5	1.3
7. Aiken silty clay loam	4.51	0.19	13.7	6.0	5.7	1	27	70	63	0.5	0.1
8. San Joaquin sandy loam	0.51	0.04	7.4	8.4	8.0	0	16	143	36	1.5	1.3
9. Porterville clay adobe	1.58	0.15	6.1	8.1	8.2	31	29	81	72	0.3	1.4
10. Vale heavy loam	1.31	0.05	15.2	9.8	9.6	79	83	2	5	0.1	0.1

* Data expressed on basis of water-free soil.

† Carbon calculated from organic matter, using C = organic matter ÷ 1.724.

‡ "Incubated" refers to samples removed from respiration apparatus at close of the respiration period.

§ N as NO_2 was also determined; in all cases the concentration was less than 1 ppm.

It is of interest to compare this soil with the two alkaline soils from California. The data for organic matter and C:N ratio in Table 1 show that while the Vale soil is low in total nitrogen and in organic matter, it has the widest C:N ratio; the California soils, also low in organic matter and total nitrogen, have the narrowest C:N ratios. All three are semi-arid soils developed under similar moisture conditions, with an average annual precipitation of 8 to 9 inches, much of which occurs during the winter months. At Vale the average temperature during November to March is 30° F.; at Porterville, in the San Joaquin Valley, the average during the same period is 50° F. In the latter locality climatic conditions thus favor a rapid and extensive decomposition; in the Vale region low temperature inhibits decomposition when moisture is favorable, while during warmer periods moisture is limiting. Thus is expressed an outstanding influence of climate on soil organic matter through its influence on "living nature." (12) This influence is further reflected on the rate of CO_2 evolution when optimum conditions are established, as is shown later.

METHODS

Duplicate 1-kg. portions of each soil, on the water-free basis, were placed in jars of a respiration apparatus similar to that of Potter and Snyder (17). The moisture content of the soils was adjusted to 50 per cent of the saturation capacity: of the required amount of water a portion, insufficient to cause danger of puddling, was mixed with the soil before transferring it to a jar; the soil was then placed in the jar and caused to settle as uniformly as possible by tapping the jar on the table, after which the remainder of the water was poured on around the edge. Slight pressure rather than suction was used to pass CO_2-free air over the soil; the pressure did not exceed that necessary to force the air through a 4-inch column of approximately N/1 NaOH used as absorbent in a test tube. This procedure has the advantage of maintaining the pressure over the soil as well as over the absorbing solution at approximately normal atmospheric pressure. The apparatus was incubated at room temperature, which, during the course of the various experiments, showed an extreme variation of 20° to 32° C. but generally ranged with $\pm$ 2° of 23° C. as indicated by a recording thermograph. The greater variations in almost every case were not more than a few hours in duration, and their temporary influences can be disregarded in long-continued experiments. Absorbent was changed at daily intervals during the first 5 days, then at gradually increasing periods until the final determination, which was made 30 days after the one preceding. The absorbed CO_2 was determined by double titration, using thymol-blue and brom-phenol-blue as recommended by Little and Durand (10). Results are expressed in milligrams of carbon as CO_2 evolved per kilogram of soil. The incubation period of the experiments from which data are given varied from 91 to 115 days; the curves of Figures 1 and 4 have been arbitrarily extrapolated or shortened as required to 100 days.

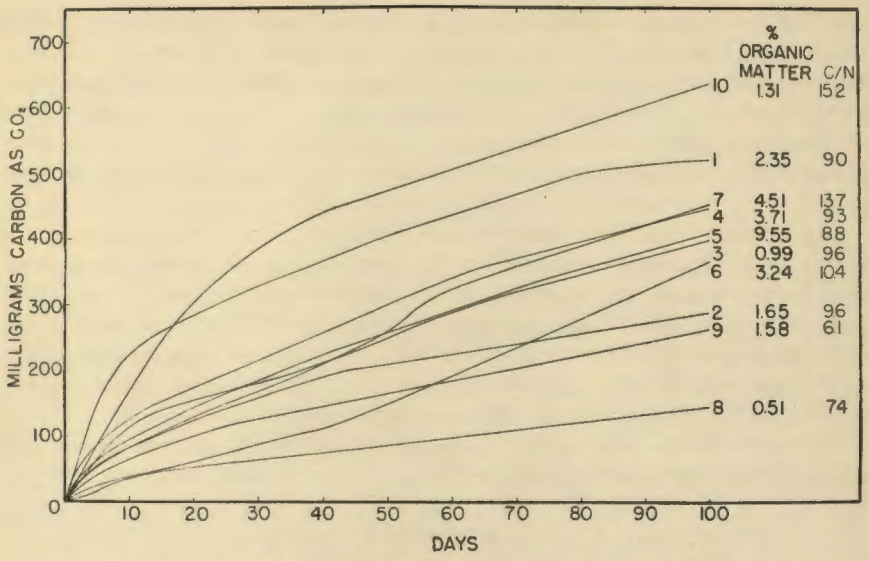

FIG. 1. Decomposition of Native Organic Matter in Different Soils. 1—Newberg loam, 2—Chehalis loam, 3—Chehalis sandy loam, 4—Willamette silty clay loam, 5—Sifton gravelly fine sandy loam, 6—Dayton silty clay loam, 7—Aiken silty clay loam, 8—San Joaquin sandy loam, 9—Porterville clay adobe, 10—Vale heavy loam.

Moisture was determined by heating samples to constant weight at 105° C. Saturation capacity was determined by placing the soil in gooches, flooding from below by immersing almost to the rim in water, and allowing free drainage to occur in a saturated atmosphere; as soon as free water failed to appear within 2 minutes on the bottom of a draining gooch after wiping dry, it was heated to constant weight at 105° C.; from the weight of the dried soil and the weight of water lost, the percentage of water held by the soil was calculated on the water-free basis.

Chemical and microbial analyses were made as follows:

A 1:5 suspension of the soil was prepared with sterile distilled water and shaken for 30 minutes; the coarser particles were then allowed to settle, and a 1-cc. sample was withdrawn for the preparation of dilutions for plate counts. Determination of pH, by means of the circulating hydrogen electrode (2), was made on another portion. The remaining suspension was then filtered, and the filtrate was analyzed. Association of Official Agricultural Chemists methods of analysis for waters were used for the determination of nitrate and nitrite. Sulfate was determined by the turbidity method of Schreiner and Failyer (24). Truog and Meyer's modification (31) of the Deniges method for phosphate was used.

For the microbial counts, peptone-glucose-acid agar was used for molds, and sodium albuminate agar, as described by Fred and Waksman (5), was used for bacteria and actinomyces. Duplicate plates from appropriate dilutions were poured in 150-mm. petri dishes; the number of colonies counted after incubation at 28° C. ranged from approximately

50 to 250. Numerous control plates, amounting to at least 10 per cent of the total poured, were used. Azotobacter were determined by the soil-paste plaque method, using 5 per cent starch and 1 per cent powdered $CaCO_3$.

The original soil samples were analyzed for organic matter by Rather's (21) method, and for total nitrogen by the Kjeldahl procedure (1).

Analytical results are given in Tables 1 and 2. At the close of the experiment samples of each soil were removed from the respiration apparatus and analyzed for micro-organisms, water-soluble anions, and pH; these results provide more direct comparison of the various soils since each had been maintained at an equivalent moisture percentage on the basis of water capacity. Only soil No. 6 lost an appreciable amount of moisture, 2.9 per cent, during the incubation period.

All data presented are averages of duplicate determinations which checked reasonably in each instance.

DECOMPOSITION OF NATIVE ORGANIC MATTER

Few previous studies on respiration of the soil's own organic matter have been reported. Isaac (7), using respiration periods of not over sixteen days, found that geological character of parent material was a definite though minor factor in determining differences in respiratory power of different soils; different types of vegetative covering appeared to exert a greater modifying effect. Vandecaveye (34), in a more extended experiment, observed greater CO_2 production in Palouse silt loam than in Helmer silt loam, two soils developed from identical parent materials under similar climate but under different vegetation. Data on untreated soils included as controls in experiments set up to study decomposition of added organic matter are available from numerous reports, but since the soils in most cases are incompletely described, particularly as concerns moisture capacity, the results are not directly comparable on the desired basis.

The results presented in Figure 1 show that under like temperature and equivalent moisture conditions the amount of CO_2 evolved from decomposition of native organic matter is determined largely by three factors: (1) the amount of organic matter present, (2) the soil texture, (3) the C:N ratio. The influence of the first factor is obvious. Production of CO_2 is not, however, proportional to carbon content (41). The amount of organic matter, as well as its nitrogen content, depend largely upon climate, while vegetation, topography, parent material, and age are influential in the order given (8). Texture is important because aeration increases as the square of particle size. The importance of the C:N ratio follows from nutritional requirements of organisms active in the decomposition; next to carbon, and aside from oxygen and hydrogen which are always abundantly supplied by organic matter and water, the food element required in largest amount is nitrogen. Hence, an insufficiency of avail-

able nitrogen limits microbial development, and nitrogen is most commonly the limiting food element.

The C:N ratio of humus as well as of fresh organic matter controls to a great degree the rate of mineralization and liberation of plant nutrients (26, 37, 38). In fresh vegetable material the ratio ranges from about 80:1 for nonleguminous to 40:1 for leguminous plants; the former will not be rapidly decomposed unless additional nitrogen is available in the soil, whereas leguminous material contains more nitrogen than required by the micro-organisms active in the decomposition, and the excess will be liberated as ammonia. Humus in surface soils has a C:N ratio approximating 10:1 on the average, but ratios of 3.5:1 to 35:1 have been reported (9). A wide ratio, such as 20:1, indicates the organic matter has not

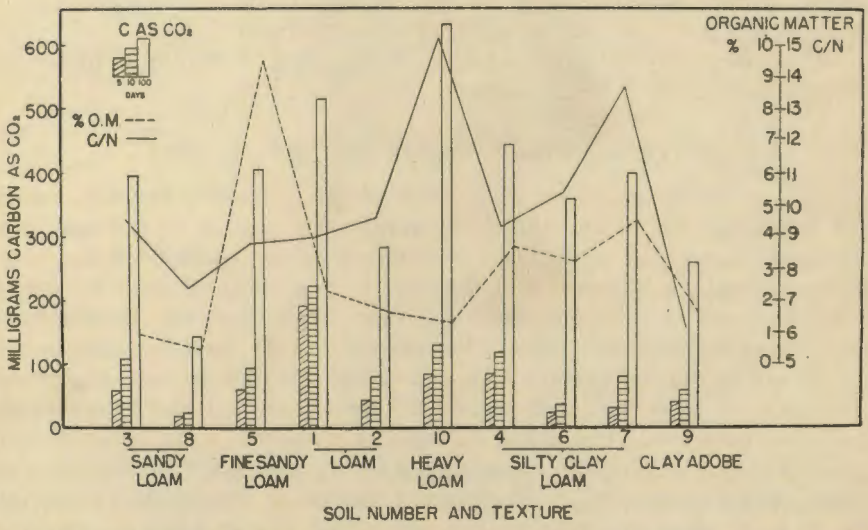

FIG. 2. Relation of Organic Matter, C:N Ratio, and Texture to Soil Respiration. 1—Newberg loam, 2—Chehalis loam, 3—Chehalis sandy loam, 4—Willamette silty clay loam, 5—Sifton gravelly fine sandy loam, 6—Dayton silty clay loam, 7—Aiken silty clay loam, 8—San Joaquin sandy loam, 9—Porterville clay adobe, 10— Vale heavy loam.

undergone extensive decomposition and that it can still support rapid development of micro-organisms under favorable soil conditions. A ratio of 10:1 or less indicates an advanced stage of decomposition; such residual material is resistant and is subject to attack only by the autochthonous microflora (4, 42), which is physiologically adapted to its utilization, and further decomposition is slow.

In Figure 2 is shown graphically the relation of organic matter, C:N ratio, and texture to CO_2 evolution in the soils studied. The San Joaquin sandy loam contained the least amount of organic matter as well as a very narrow C:N ratio and gave the lowest CO_2 evolution. The lowest C:N ratio occurred in the Porterville clay adobe soil. This soil contained three times as much organic matter as the San Joaquin sandy loam and, despite

a narrower C:N ratio and its heavy texture, gave off about twice as much CO_2; all the other soils, having wider C:N ratios, ranked above it. Sifton soil, with by far the highest organic matter content, also had a relatively low C:N ratio and was only intermediate in respiratory power. In contrast to these soils, the Vale heavy loam, next to the lowest in organic matter but with the widest C:N ratio, gave the greatest CO_2 production; as was shown in a previous paper (3). CO_2 from mineral sources contributed only 10 per cent of the total evolved, an amount insufficient to change the relative position of this soil in respiratory intensity. In these four soils, which present an extreme range in texture, in supply of organic matter, and in C:N ratio, the dominant factor in controlling respiration appears to be the C:N ratio.

Respiration in Aiken silty clay loam soil showed a response correlating with a comparatively high organic content and a wide C:N ratio. Texture is the dominant factor in Newberg loam. For the remaining soils, predominating influence can hardly be ascribed to any of the three factors.

The order of the various soils according to respiratory power varied little during the course of incubation. Figure 1 shows the soils which were high or low during the first few days were also high or low at the close. There is some crossing of the curves; in general, the shifts involved only two closely ranking soils. At the beginning the rate of CO_2 production was relatively greater in the coarser soils. Later, it dropped in these soils and increased slightly in the heavier soils. This is emphasized in Figure 2.

The microbial counts on the whole show relatively small variations between the different soils before, as well as after, the prolonged incubation. In the original samples the number of molds correlated to some extent with the organic matter. With few exceptions the bacteria and actinomyces were relatively constant throughout. In field soils bacterial numbers and nitrate production correlate closely with CO_2 evolution, as has been shown by the pioneer work of Russell and Appleyard (22, 23) and by numerous more recent experiments. Under uniform conditions in the laboratory the most extensive changes take place during the first few weeks; after prolonged incubation the soil tends to return to an equilibrium similar to the original condition (17, 41). Data in Tables 1 and 2 show this tendency. Unfortunately, no analyses were made during the incubation period.

Nitrates showed appreciable increase or slight decrease in most cases. In Sifton soil a large increase correlating with organic content was obtained. In San Joaquin sandy loam, which was lowest in total nitrogen and organic matter, the exceptionally high nitrate content became markedly lowered during incubation; there is no apparent correlation of this change with the other data. Nitrate accumulation in Vale heavy loam was negligible, possibly due to the wide C:N ratio or to high alkalinity, which inhibits nitrifying bacteria (27).

Changes in pH were comparatively small. In most cases it decreased. In the "alkali" soil the decrease of 0.2 pH was accompanied by a 40 per

TABLE 2

MOISTURE RELATIONS AND MICROBIAL ANALYSES OF SOILS STUDIED*

Soil	Water Capacity	Moisture		Molds		Bacteria		Actinomycetes		Azotobacter‡	
		Original	Incubated†	Original	Incubated	Original	Incubated	Original	Incubated	Original	Incubated
	Percentage	*Percentage*	*Percentage*	*Thous.*	*Thous.*	*Millions*	*Millions*	*Millions*	*Millions*		
1. Newberg loam	60.0	20.1	30.2	38	39	5.1	4.8	0.6	0.6	+	+
2. Chehalis loam	40.0	19.0	20.2	55	70	2.1	4.9	0.5	0.6	+	+
3. Chehalis sandy loam	36.2	4.6	18.0	59	20	1.7	2.3	0.3	0.6	+	+
4. Willamette silty clay loam	54.2	27.2	25.5	56	24	3.4	1.4	2.6	2.2	++	++
5. Sifton gravelly fine sandy loam	98.0	31.1	48.8	365	43	4.7	2.5	2.9	0.7	0	0
6. Dayton silty clay loam	50.8	8.5	22.5	57	73	2.2	3.8	0.6	2.7	+	+
7. Aiken silty clay loam	54.2	17.1	26.8	52	37	0.9	0.9	0.2	6.0	+	+
8. San Joaquin sandy loam	15.0	4.6	7.3	13	20	9.3	3.3	1.3	1.8	+++	+
9. Porterville clay adobe	45.2	10.3	27.4	13	70	3.7	6.3	0.8	1.7	+++	+
10. Vale heavy loam	57.0	17.6	28.1	10	20	1.4	2.0	0.2	0.6	0	+

* Data expressed on basis of moisture-free soil.

† "Incubated" refers to samples removed from respiration apparatus at close of the respiration period.

‡ Development on soil-starch plaques; 0 = no colonies; + few colonies; +++ = many colonies.

cent decrease in concentration of normal carbonate. Since hydrogen-ion concentration is an important factor controlling activities, it follows that CO_2 production should reflect this influence. Various reports have shown this to be so (28, 30, 35), but other factors may have predominating and masking influence, as in the present study.

Sulfates and water-soluble phosphate showed increases as well as decreases, but there is no consistent correlation with organic matter or CO_2 production.

EFFECT OF MOISTURE

Moisture is one of the environmental factors controlling development of micro-organisms. As available water in the medium falls below a certain minimum, diffusion is retarded, growth is slackened, and finally, the organism assumes a dormant or spore stage which retains life for a longer or shorter period characteristic of the species under the environment as fixed by temperature, pH, and other factors. Above some maximum percentage of water the physiology of the organism will be affected chiefly by the resultant effects on free oxygen supply; in soil layers of appreciable thickness, saturation with water retards or eliminates aerobic action and results in a preponderance of anaerobic and facultative species. The controlling factors, as pointed out by Rahn (19), are aeration and thickness of moisture film. Optimum aeration and optimum thickness of moisture film cannot co-exist in arable soils, since the particle size is so small that an optimum film, from the standpoint of diffusion rate, is attained only in the water-logged state. As particle size decreases, thickness of capillary films decrease, although moisture capacity increases. The moisture capacity of soils varies not only with texture, but also with structure and organic matter content. For this reason the optimum soil moisture for a particular species or association of species varies with different soils, even those of similar texture. For general soil microbial activity, the optimum is commonly considered to be that amount of moisture present when the soil is approximately 50 per cent saturated.

Since the CO_2 evolved in soil respiration results from the combined activities of all microbes present, anaerobes as well as aerobes, and autotrophes as well as heterotrophes, and since each kind possesses characteristic respiratory powers, it is evident that the optimum moisture for this general microbial function is determined by a complexity of interrelationships. While such definite functions as ammonification, nitrification, and nitrogen fixation have been found to have definite moisture optima (60, 60, 70 per cent, respectively (6)), an optimum range rather than an optimum point would seem more likely to apply to CO_2 evolution. Within this range a change in moisture content might increase respiratory action of some species while decreasing that of others.

Early studies showing the influence of moisture on CO_2 evolution are summarized by Löhnis (11). Stoklasa (29) found CO_2 production of a soil to be increased by increasing the moisture to 50 per cent of the satura-

tion capacity. Waksman and Starkey (41), for the optimum in carbon dioxide evolution studies, added water to bring the soil moisture to 50 per cent of the moisture-holding capacity. Vandecaveye (32) adjusted moisture to the normal field capacity as expressed by Shaw (25). With an arid soil Oberholzer (15) found that the rate at which added organic matter decomposed increased with increasing moisture up to almost complete saturation.

To study the effect of moisture on decomposition of native soil organic matter, Chehalis loam and Sifton gravelly fine sandy loam were selected

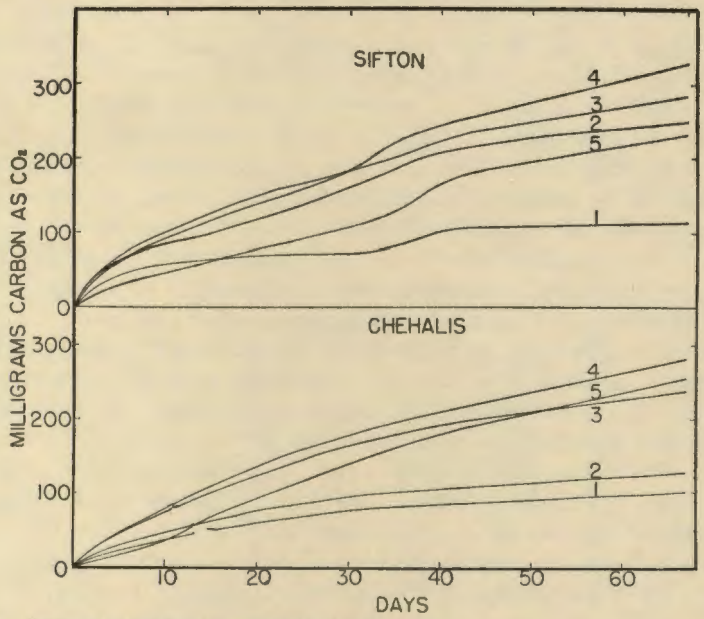

FIG. 3. Effect of Moisture on Soil Respiration.
Sifton gravelly fine sandy loam: 1—Wilting point, 2—25% saturation, 3—50% saturation, 4—75% saturation, 5—100% saturation.
Chehalis loam: 1—Wilting point, 2—25% saturation, 3—50% saturation, 4—75% saturation, 5—100% saturation.

because, while not greatly different in texture, they differ widely in moisture capacity and wilting point.[4] Moisture capacity was determined by the gooch procedure previously described, and wilting point was determined by the sunflower seedling method (44). The respective values for Chehalis loam were found to be 8 per cent and 40 per cent; for Sifton gravelly fine sandy loam, the values were 14 per cent and 98 per cent. The moisture in 1-kilogram portions of the soils was adjusted to the wilting point, 25 per cent, 75 per cent, and 100 per cent saturation . The respiration study, using the methods previously described, was carried on

[4] William H. Tomsheck, graduate student in soils, assisted in obtaining the data presented in this section.

for 68 days. After 30 days, 200 grams of soil were removed from each bottle for chemical and microbial analyses; subsequent CO_2 production in the remaining 800 grams was calculated to the original basis of 1 kilogram of water-free soil. Disturbance of the soil in the apparatus due to removal of the sample had a marked effect in temporarily increasing the rate of CO_2 evolution in the Sifton gravelly fine sandy loam, but not in Chehalis loam. This effect was probably due to both the higher organic matter content and the higher moisture capacity, which bears an inverse relation to aeration, of the Sifton soil. The effect of increased aeration during preliminary preparation of the soil is similarly expressed by the rate of respiration during the first 10 days, as may be seen from Figure 3; here, also, Chehalis loam showed least response.

In both soils CO_2 evolution increased as moisture was increased up to and including 75 per cent of the saturation capacity. Slowest respiration occurred with moisture at the wilting point except during the first 10 days, when respiration was lowest in the saturated soils. The subsequent increase in CO_2 from the saturated Chehalis loam was due to an increase in rate, indicating an adjustment or change in the microflora; the curve for saturated Sifton soil, on the other hand, gained ascendancy because the driest soil soon fell in rate of respiration. Saturated Chehalis loam soon approached and then paralleled the same soil with moisture at three-fourths saturation, which was optimum throughout. For the Sifton soil one-half saturation was optimum until the disturbance produced by sampling at 30 days; after this the 75 per cent saturated soil was superior. Differences between the soils at 50 per cent and 75 per cent saturation were not appreciable till after the first 10 days. While differences in moisture produced similar response in both soils, the effects were quantitatively greater in the soil with greater water capacity.

On the basis of these results optimum moisture for CO_2 evolution in prolonged respiration experiments is near 75 per cent of the saturation capacity.

From the results of analyses made at 30 and 60 days, only data for nitrates, total C as CO_2 evolved, and pH are given (Table 3).

Nitrate production did not correlate with CO_2 evolution at 30 days but was in fair agreement at 68 days. Since nitrification is an autotrophic transformation, consuming rather than liberating CO_2 in primary metabolism, and since it represents only a small portion of the total soil microbial activity, it can exert *per se* only a negligible influence on soil respiration. The effect of moisture on the process, which is aerobic, is likely to be indirect insofar as it controls aeration. The data show this to be true in the extreme case of complete saturation; denitrification was complete in 30 days, but CO_2 evolution was vigorous. At the same time there was little difference in the amounts of nitrate produced in either soil at 25, 50, and 75 per cent saturation. Nitrates increase with moisture up to 75 per cent saturation at the 68-day analysis. Anomalous results were obtained with both soils at the wilting point.

TABLE 3

INFLUENCE OF MOISTURE ON NITRATES AND CO_2 EVOLUTION*

SOIL AND MOISTURE CONTENT	N AS NO_3		TOTAL C AS CO_2 EVOLVED		pH	
	30 days	68 days	30 days	68 days	30 days	68 days
	ppm	*ppm*	*mg.*	*mg.*		
Chehalis loam	11†				6.8†	
Moisture at 8% = wilting point	54	30	75	101	6.6	6.6
Moisture at 10% = 25% saturation	34	31	89	127	6.7	6.7
Moisture at 20% = 50% saturation	44	53	156	236	6.6	6.7
Moisture at 30% = 75% saturation	47	55	176	276	6.7	6.6
Moisture at 40% = 100% saturation	0	0	127	250	7.3	7.3
Sifton gravelly fine sandy loam	28†				5.3†	
Moisture at 14.0% = wilting point	27	125	71	128	5.3	5.2
Moisture at 24.5% = 25% saturation	69	94	165	273	5.2	5.1
Moisture at 49.0% = 50% saturation	62	107	183	321	5.1	5.0
Moisture at 73.5% = 75% saturation	58	139	187	380	5.2	5.0
Moisture at 98.0% = 100% saturation	0	0	107	316	5.9	5.8

* Data expressed on basis of moisture-free soil.

† In original sample.

The plate counts for micro-organisms in Chehalis loam showed considerable irregularity. The maximum number of molds at both 30 and 68 days occurred in the half-saturated soil. Bacteria and actinomyces were highest in 25 per cent saturated soil at both times. Numbers of molds, bacteria, and actinomyces were considerably less for all other treatments and did not fluctuate greatly; at 68 days all counts were generally higher. There was little difference between numbers in saturated soil and soil at the wilting point.

Sifton soil gave higher mold counts than Chehalis loam, but the bacteria and actinomyces in most cases were lower. The moisture content giving maximum numbers in this soil was 75 per cent of the saturation capacity; this was true at both 30 and 68 days. In neither soil was the influence of moisture on microbial numbers, as revealed by plate counts, outstanding.

Of the other analytical data obtained in connection with the moisture experiment, only the pH values merit mention. Only in the saturated soils did changes of more than 0.2 pH occur. Saturation of Chehalis loam changed the original pH 6.8 to pH 7.3 in 30 days; the same value obtained at 68 days. Saturated Sifton soil changed from pH 5.3 to 5.9 in 30 days to pH 5.8 at the close of the experiment. These decreases in acidity may have been due to ammonia accumulation, inasmuch as nitrification was inhibited by complete saturation.

EFFECT OF AVAILABLE NITROGEN

The effect of various added nitrogen compounds on decomposition of native organic matter in four soils was studied by the respiration procedure previously outlined. Curves showing the effects on CO_2 evolution are given in Figure 4. In plotting results, data for the control were subtracted from the corresponding data for the treated soil in all cases. The control is thus plotted as a straight line at zero; curves above the line indicate stimulation, while those below show a depression.

A slight stimulation from nitrogen added as ammonium sulfate was obtained in Chehalis sandy loam and Vale heavy loam. This effect persisted for 10 days in the former and about 25 days in the latter, after which the respiration intensity dropped in comparison with the controls. The response of the Vale soil correlates with its wide C:N ratio (Table 1); addition of available nitrogen permitted greater microbial development at the expense of excess available carbon, for reasons discussed in the previous section. In Chehalis sandy loam the C:N ratio is normal, but the supply of organic matter is low; apparently the nitrogen of the humus compounds in this soil were less available than the carbon, so that added nitrogen enabled more micro-organisms to grow.

With Newberg loam, striking results were obtained with urea and with sulfamic acid (HSO_3NH_2). The curve for the urea-treated soil, however, includes the additional carbon as CO_2 evolved from its own decomposition, so that any stimulation due to the nitrogen is indicated only by

an increase over this amount. Urea is rapidly hydrolyzed by a variety of soil bacteria (39) to ammonium carbonate, which decomposes to ammonia, carbon dioxide, and water. This ammonification is much more rapid than the ensuing nitrification. Analyses made on the urea-treated soil during respiration showed that while the urea was 93 per cent nitrified in 90 days it was completely ammonified in 10 days. By calculation, the amount of carbon as CO_2 liberated by ammonification of 1 gram of urea is 200 mg. The highest point on the curve (No. 8, Figure 4) occurred at 10 days; the

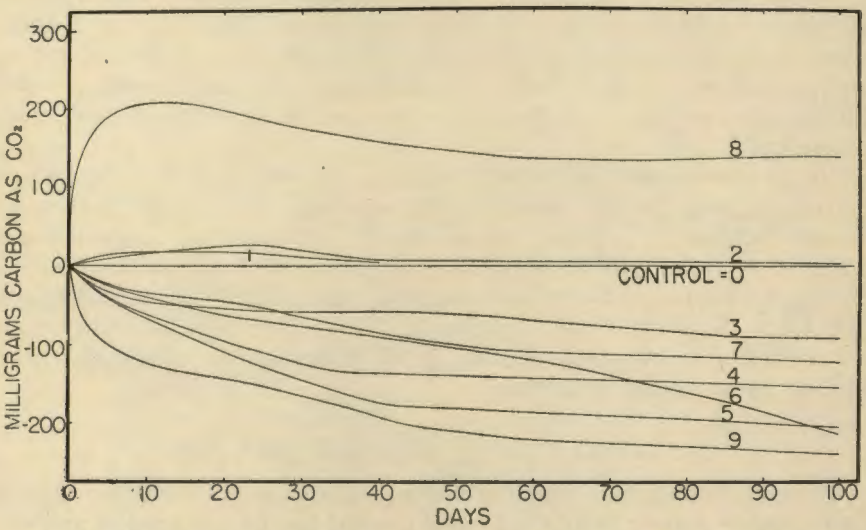

FIG. 4. Effect of Added Available Nitrogen on Decomposition of Native Organic Matter. 1—Chehalis loam + $(NH_4)_2SO_4$ equivalent to 21 ppm N. (Discontinued at 40 days), 2—Vale heavy loam + $(NH_4)_2SO_4$ equivalent to 64 ppm N., 3—Willamette silty clay loam + $(NH_4)_2SO_4$ equivalent to 106 ppm N., 4—Willamette silty clay loam + $(NH_4)_2SO_4$ equivalent to 250 ppm N., 5—Willamette silty clay loam + KNO_3 equivalent to 250 ppm N., 6—Newberg loam + $Ca(NO_3)_2$ equivalent to 150 ppm N., 7—Newberg loam + $(NH_4)_2SO_4$ equivalent to 300 ppm N., 8—Newberg loam + urea equivalent to 467 ppm N., 9—Newberg loam + HSO_3NH_2 equivalent to 433 ppm N.

reading of 208, minus 200 from decomposition of the urea, leaves a net increase over the control of only 8 mg. of carbon as CO_2 due to the added nitrogen. While this is within the experimental error it is nevertheless significant that urea caused no depression in respiration in this soil as did ammonium sulfate, calcium nitrate, and sulfamic acid.

During the first 50 days, ammonium sulfate equivalent to 300 ppm N. and calcium nitrate equivalent to 150 ppm N. produced similar effect, but from 50 to 100 days the ammonium sulfate remained parallel to the control while the calcium nitrate continued depressive. Sulfamic acid produced a marked depression which was immediately evident and persisted during the first half of the incubation period. This cannot be interpreted solely on the basis of added nitrogen: subsequent studies[5] have

[5] To be published later.

shown that sulfamic acid, when applied to alkaline as well as to acid soils, is toxic to germination and growth of sunflowers, that it temporarily reduces bacterial numbers, and that it strongly depresses CO_2 evolution. The toxic effect is apparently specific since sulfite liquor, which is highly sulfonated, is nontoxic even in much greater quantities.

The depressive effect of ammonium sulfate on respiration in Willamette silty clay loam was closely proportional to the amounts applied, the equivalent of 250 ppm N. reducing CO_2 evolution about as much more as the 106 ppm N. reduced it below the control. As with Newberg loam, the nitrate was considerably more depressive than the ammonium salt.

According to Waksman (40) the decomposition of humus of only wide C:N ratio is favored by the addition of inorganic fertilizers. In line with this the wide C:N ratio of the Vale soil affords a reasonable explanation for the stimulation observed. The depressing action noted in the Newberg and Willamette soils, however, is accounted for less readily. Potter and Snyder (17) also observed that both ammonium sulfate and sodium nitrate, used in amounts equivalent in nitrogen to 106 ppm and 100 ppm respectively, decreased the quantity of CO_2 evolved from Miami silt loam, and that the nitrate was more depressive. Possible explanations of lowered respiration intensity in the presence of these nitrogen compounds are: (1) they are absorbed by the humus complex in such a way as to temporarily immobilize previously decomposable carbon compounds; (2) the added nitrogen, being directly assimilable, relieves the microbes from the necessity of decomposing organic nitrogeneous compounds for their nitrogen requirements. The latter seems more probable. Reasons why nitrates are more depressive than ammonium salts are not clear; possibly the explanation involves oxidation-reduction potentials and availability as influenced by relative diffusion rates of anions and cations. From the standpoint of availability within the cell it would seem that ammonium nitrogen would be preferable to nitrate, since the latter must be reduced before it can be synthesized into tissue.

SUMMARY AND CONCLUSIONS

Decomposition of native organic matter in ten soils, including similar and dissimilar types, was studied by the respiration method. The influence of moisture and added available nitrogen compounds on respiration in some of these soils was also investigated.

Texture, organic matter content, and C:N ratio were found to be controlling factors in CO_2 evolution.

In four soils which differed widely in texture, organic matter, and C:N ratio, the last-named exerted predominating influence on both the rate and amount of CO_2 production.

The optimum moisture content for soil respiration was found to be approximately 75 per cent of the saturation capacity. This was true in two soils of similar texture but greatly different saturation capacity. Differences in moisture produced similar respiratory response in both

soils, but the effects were quantitatively greater in the soil with greater water capacity. It is suggested that for CO_2 production as a general microbial function, the optimum moisture may range between rather wide limits, whereas for strictly aerobic processes it is more sharply defined since aeration is inversely proportional to soil moisture.

Addition of available nitrogen increased respiration in a soil containing organic matter of wide C:N ratio. Depressive effects were obtained in other soils; nitrates were more depressive than ammonium sulfate. Possible reasons for these results are discussed.

The microbial population of the various soils, which present a wide range in origin, profile development, texture, and organic matter, was not greatly dissimilar either at the beginning or at the close of prolonged incubation. Outstanding respiratory differences developed during the first few days of incubation. Had chemical and microbial analyses been made during this time, changes correlating with CO_2 evolution would probably have been obtained. As carried out later, however, the analyses revealed only that different soils, as well as variously treated soils, attain a similar and relatively constant microbial and chemical equilibrium. This is likewise indicated by the similarity in slope attained by all respiration curves as incubation proceeded.

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A PLACE FOR THE PLATE COUNT METHOD AS APPLIED TO SOIL[1]

NORMAN JAMES AND MARJORIE L. SUTHERLAND

From the Department of Bacteriology and Animal Pathology, University of Manitoba

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The plate method of estimating numbers of bacteria is almost as old as the science of bacteriology. It had its beginning in Koch's liquefiable solid medium developed in 1881. This new medium revolutionized the procedure for studying bacteria. A small amount of material under investigation was mixed with the melted gelatine medium. On cooling, the medium solidified and trapped the organisms. There, they developed to form well-isolated colonies that usually contained only one species. These could be studied as pure cultures or counted. When the procedure was carried out quantitatively the counts could be used to give an estimate of the number of bacteria per gram of the original substance. The procedure was simple and soon was applied to soil, whose population responded in large numbers to the new technique. It was accepted without seriously questioning the validity of the resulting estimates. There was not even a standardized procedure, although most investigators followed the principles laid down for counting bacteria in milk. Each laboratory had its own system of preparing dilutions and favored certain media as most suitable for the purpose intended. The method was of value only if put to practical use. Accordingly, attempts were made to apply plate counts of bacteria to problems of soil fertility and crop production. One medium and set of cultural conditions produced one estimate. Another medium or set of conditions gave one quite unlike the former. By varying these factors it was possible to obtain estimates of populations with vastly different metabolic properties. The method was used extensively. Apparently, the potential information to be derived from plate counts of bacteria from soil was almost limitless.

Herein is to be found the reason for the failure of the method to produce the results expected. It was accepted by the soil scientist interested in the solution of practical problems before it had been proven capable of producing an estimate that was reproducible within definite limits. In the early twenties of this century the accuracy of estimates produced by the plate count method was questioned, and procedures were outlined for evaluating such estimates. It became apparent that the simple procedure followed in most laboratories produced a result of doubtful value. The findings of one investigator with respect to the

[1] Contribution from the Department of Bacteriology and Animal Pathology, The University of Manitoba, Winnipeg, Canada. A series of papers on this study is to be found in the Canadian Journal of Research, C.

relation of numbers of bacteria to a specific effect frequently were at variance with those of another. Too, there was evidence of apparent wide fluctuations in numbers of bacteria from samples taken at intervals from a given plot. This failure to confirm results, coupled with the gradual acceptance of the concept of a rapidly changing population in soil, led to an attitude of hopelessness in the minds of many workers. Consequently, counts were made and reported in a perfunctory manner along with data pertaining to biological activity and chemical and physical tests. There was little attempt to interpret counts. In short, the method was relegated to a place of minor importance in most laboratories, and other lines of approach were developed.

Large scale platings carried out at The University of Manitoba have furnished data that illustrate many reasons for the skepticism about the procedure. Composite samples from each of 36 plots were plated weekly for fourteen weeks during 1936. There was little evidence of consistency in the relation of the estimates to different fertilizer treatments or crops. To add to the confusion, estimates from 36 subsamples from one well-mixed composite of the residue from all samples were found to vary as widely as the estimates from the 36 samples from different plots. This led to a detailed consideration of the method.

THE ERRORS OF THE METHOD

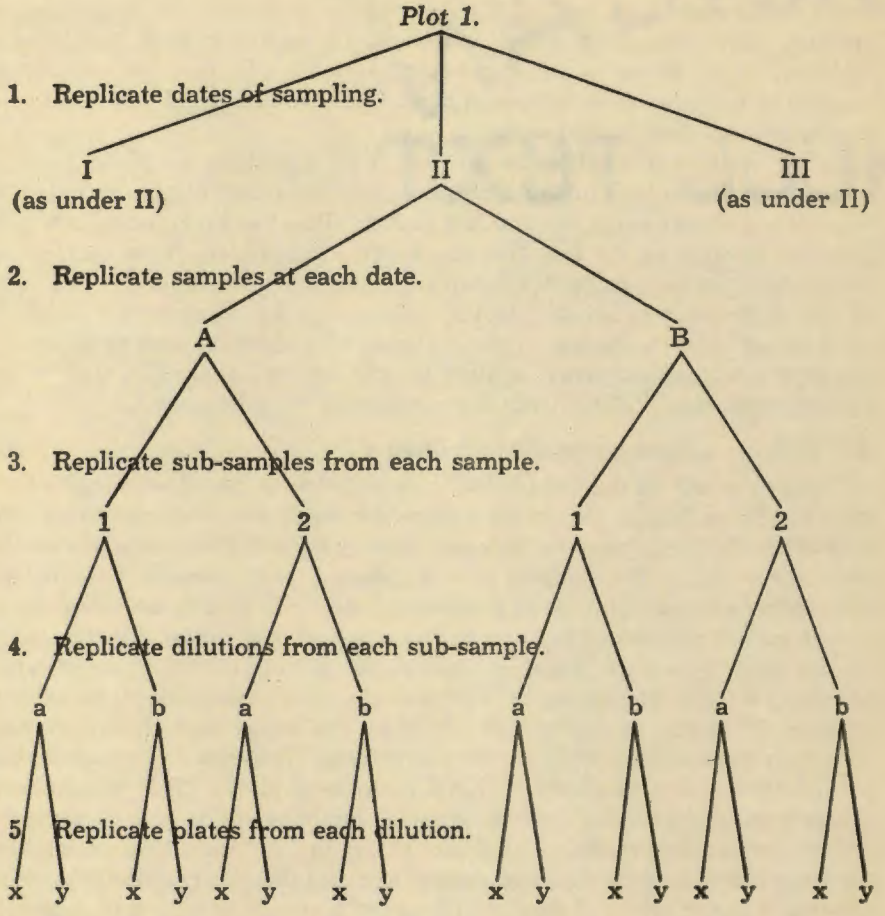
The plate count procedure, as applied to plots or fields, consists of several sampling steps, each of which is subject to experimental errors. The following diagram shows the sources of variation in the method.

Since every estimate of the population in a plot is made from counts on plates and consequently is subject to errors at every step shown above, it is obvious that real differences in the bacterial population may be obscured completely by variations arising from these sources of error. Hence, it is essential that the amount of variation at each sampling step be determined, and that the sampling procedure at each step be such that differences among estimates are as small as practicable. This implies the use of a design for the experiment that provides data for measuring variation of this nature, which means at least duplicate determinations at each step.

The information available on each source of variation shown in the diagram may be considered briefly.

1. The error of replicate plates.

Every estimate of the bacterial population is made from counts on plates. Hence, the error of replicate plates is a part of every estimate, whether of a dilution, a subsample, a sample, or a plot. When duplicate or replicate determinations have been made, the mean square for replicate plates may be calculated by analysis of variance on all the data for the plot. This value may be large or small. The same data may be used to provide a X^2 (chi square) value for each set of replicate plates.



If the distribution of these X^2 values conforms to expectancy for the Poisson series, the variation among counts from replicate plates is due to random sampling and there is little hope of reducing the mean square for replicate plates by changing the procedure. A poor distribution of X^2 values, on the other hand, usually indicates the presence of a factor that causes wide variation among counts in too many sets of replicate plates. This latter condition does not appear to be associated with the technique of plating, since in this laboratory the procedure used regularly for plating produced counts that gave a X^2 distribution conforming to expectancy in an experiment in which pure cultures of bacteria were added to sterile soil just before plating. Further, the same result was obtained with the normal soil flora in samples plated within 6 hours after the samples were obtained from the experimental plots. On the contrary, the variation among counts from replicate plates from one dilution was found to be wide in too many sets of counts when samples

were broken up and held in the laboratory overnight or longer before plating. The disturbing effect increased in intensity with the time of holding. The presence of pin-point colonies of bacteria or certain species of fungi on some plates in a set likewise increased the number of sets of counts that varied widely.

The failure to randomize the order of pipetting to plates, adding medium, piling plates in the incubator, and counting might be expected to produce a large error of replicate plates. This has been found not to be the case in spite of the fact that these procedures have been carried out deliberately in one order in all our experiments. Accordingly, the effect of the systematic order of the procedure may be disregarded; and the differences among replicate plates from one dilution accepted as the minimum laboratory error, against which the significance of differences among replicate dilutions from one subsample may be tested.

2. Differences among replicate dilutions.

An estimate of the population in a dilution is based on counts from plates. Accordingly, the mean square for replicate dilutions in general will not be less, and may be several times as large as the mean square for replicate plates. The success of the diluting procedure in keeping the differences among dilutions at a minimum depends largely on maintaining a uniform suspension of the soil in the water of the initial dilution blank at the time repeated transfers are made to the next higher dilution blanks. At the beginning of our studies the usual procedure was to suspend 25 grams of soil in 250 ml. of sterile water and shake mechanically on a to-and-fro shaker for 5 minutes. This gave a mean square for dilutions about three times that for replicate plates. This weakness in the procedure has been overcome under our conditions of experimentation by using a suspension of 50 grams of soil in 1,250 ml. of water, shaking for 10 minutes on a mechanical shaker and agitating as repeated transfers are made to the series of final dilutions for plating. Under this condition the mean square for dilutions is only slightly larger than that for replicate plates in two experiments completed recently.

The range in the number of colonies allowable on plates is worthy of consideration, since it is conceivable that an estimate based on a dilution yielding a count of 20 colonies per plate may not agree with another from the same sample based on a dilution giving 200 colonies per plate. This discrepancy may be associated with the wide differences in the number of colonies in plates with the same area and amount of food material. The effect may be attributable to: (a) failure of some colonies to grow on the crowded plate because of lack of available nutrients, (b) failure to grow because of the presence of toxic products of metabolism produced by certain members of the population, or (c) stimulation of some colonies by products and possibly growth stimulants produced by other kinds of bacteria.

Obviously, the only method of regulating the number of colonies on plates is by changing the strength of the dilution plated. This effect has

been tested in our laboratory. Each subsample was diluted in three different strengths calculated to give theoretical counts in the ratio of 3:5:8. Counts from pairs of dilutions were correlated. It was readily apparent that the dilutions did not give proportional counts. The high count gave an estimate relatively lower than the low count from a dilution of the same sample; and this discrepancy increased with the size of the counts. At the same time the mean square for dilutions on the basis of the number per gram of dry soil for each dilution was about twice that for replicate plates. However, the correlation between pairs of dilutions was so strong that counts from one dilution could be adjusted to make the estimate interchangeable with that based on counts from another dilution of different strength. The mean square for dilutions on the basis of adjusted data was only slightly larger than that for replicate plates. These findings make it obvious that the effect associated with different numbers of colonies on plates varies in a regular manner; and that the design of an experiment should provide a means for minimizing this effect. Accordingly, the use of three dilutions of different strengths gives three chances of having the most suitable dilution at each date of sampling from one source in an experiment extending over a crop season. Too, it furnishes a means of compensating for differences in the number of colonies per plate.

3. Differences among replicate subsamples.

Similarly, an estimate of the population in a subsample is based on counts from plates made from dilutions. It is subject to the effects of differences among plates and among dilutions, and in addition to the variation arising from the process of subsampling. The success of the subsampling process in furnishing a representative portion of the sample depends upon the ease of reducing the sample to uniformly small particles and the efficiency of mixing. These are functions of the texture of the soil and its moisture content. In the first studies on this project in our laboratory, when the samples of soil were broken and mixed by hand, the mean square for subsamples was many times that for replicate plates. This variation among subsamples has been reduced appreciably under our conditions by grating the soil mechanically through a four-mesh-per-inch wire screen under slight pressure and mixing in a revolving drum for 10 minutes. While the mean square for subsamples was larger than that for replicate plates in samples handled in this way, it is possible that this variation has been reduced as much as is practicable.

4. Differences among replicate samples.

An estimate of the population in a sample is subject to the effects of differences introduced by all the preceding sampling steps; as well as to the difficulty of obtaining samples with the same population levels from the plot. The variation among replicate samples is a function of soil heterogeneity in the area sampled. There is little hope of changing this variability. One may assume that under certain conditions this discrepancy may be overcome by using composite samples consisting of larger

numbers of cores taken from points determined at random from the plot. This assumption has not been substantiated under our conditions. On the contrary, the variation in estimates among samples consisting of 24 cores was about the same as that among samples composed of 6 cores. The same was true for composite samples of 18 and 12 cores. This might be interpreted as indicating that the variability within a core 6 inches long and 1 inch in diameter was as great as that among cores. Under this circumstance there could be little chance of reducing differences among samples by using a larger number of cores per sample. Further, the problem of soil heterogeneity in a plot or field may be linked with that of variability in moisture content. A strong correlation between moisture expressed in percentage and bacteria expressed in millions per gram is known to exist. Since in our experiments the variation in moisture among samples taken from a plot at one time has been shown to be wide enough to cause marked differences in estimates, it is obvious that a portion of the differences among samples may be accounted for by differences in their moisture content. This points to the need for a moisture determination on every sample plated; and also for considering the effect of moisture in the interpretation of an estimate of the bacterial population.

5. Differences among replicate dates of sampling.

Obviously, differences among estimates of the population in a plot made on different dates result from all the sources of variation already considered; and also from the effects of day-to-day or seasonal variation. These changes in numbers of bacteria may be associated with differences in one or many environmental factors. In our laboratory, changes in the moisture content of the plot have been found to account for a large portion of the mean square for differences among dates of sampling. In some plots an effect associated with the kind of crop and the stage of its development has been shown to be responsible for another equally large portion. Still, other effects may be found to account for other portions of the mean squares for differences among dates. A few of these follow: differences in the temperature of the soil on the dates of sampling, differences in length of time since the temperature was at its maximum, differences in the length of time after a rain, and differences in the amount of rainfall. It is apparent that the fluctuations in estimates of the population of a plot result from the combined response to changes in a number of factors. The portion of the mean square for differences among dates attributable to each factor must be known; and the possible effect of other disturbances must be realized. Then only, the residual or remaining portion of the mean square for differences among dates may be interpreted in relation to the specific effect under consideration.

PRINCIPLES AND NOT PROCEDURES

The principles governing the revision of the method for estimating numbers of bacteria are more important than the details of the laboratory procedure. The method as applied to soil deals with biological populations

varying at random and living in a medium that lacks homogeneity. The procedure consists of a series of steps involving random sampling and, as well, additional variation at each step. The practice of replication makes it possible to consider the amount of disturbance at each source of error as it affects the experiment as a whole. Revisions of the method must be considered with respect to the precision of the final estimate. Certain refinements regarding the tolerance of dilution blanks and pipettes may produce theoretical improvements that may be within the limits of experimental error for the method. Another refinement in the procedure, such as using a larger proportion of water to soil in the original dilution, may yield a significantly better estimate. This means that revisions must be rated in terms of relative importance and with due consideration to practicability. Further, the use of replicates gives a final estimate based on an average of two or more random samples at each step in the procedure. The number of replications will be determined by the precision desired in relation to the amount of labor and materials involved in obtaining the estimate. The last general principle to be considered involves the interpretation of the result. Estimates have been found to be subject to sampling variation at many places in the procedure. Too, the population has been shown to be responsive to various environmental factors. The problem of whether the difference in estimates represents response to a specific effect under investigation, or merely is the result of sampling variation or a combination of other effects or both, must be settled by a method of handling data that provides a mathematical value for each effect. Statistical methods have been developed for treating such data and for determining whether the variation among estimates represents only sampling variation or, in addition, response to some other factor. This method of analysis depends upon replication at each sampling step. It provides a constant check on known effects and a means for testing new ones. Only after such consideration can an estimate of the bacterial population in soil have real value.

A PLACE FOR THE METHOD

In spite of the recognized limitations of the plate count method, a series of estimates from a plot obtained by a procedure based on the principles outlined will have value in establishing the relationship of bacteria in soil to problems of importance to agriculture. This can be stated with confidence because of three facts.

1. Replication of samples at each step involving random sampling from a biological population results in a better estimate than one based on counts from one dilution only.

2. This replication provides a means of measuring the variation at each step in the procedure. Each source of discrepancy may be considered in true perspective with regard to the final estimate and the technique improved where serious disturbances are found.

3. The method is extremely sensitive in demonstrating responses

to changes in moisture and cropping. It is probable that it is equally responsive to other environmental factors.

These facts may be interpreted in relation to the inadequacy of the method in the past. They may be used to explain many of the inconsistencies that have been charged against the method. In the light of present knowledge, it is conceivable that an estimate could be so affected by failure to provide for the difficulties of sampling, or to consider response to environmental factors, that a real response to an effect under investigation could not be detected.

The same facts may be considered with respect to the use of the method in the future. In an experiment carried on recently under the conditions at The University of Manitoba, 60 to 90 per cent of the mean squares for the so-called seasonal fluctuations in the bacterial population were found to be associated with response to changes in moisture and in the development of crops. This extreme sensitivity to environmental conditions should make the method valuable for investigating the relationship of the microflora of the soil to plant growth in experimental plots. This type of study has many applications with respect to such problems as crop rotations, the use of fertilizers, and the control of soil-borne plant disease organisms. Too, it may be used to demonstrate responses by widely differing populations in the soil. Now that the soundness of the procedure is established, more emphasis may be placed on the use of specific media for various populations. In this respect it is highly probable that a carefully chosen selective medium may show that the population developing on it responds more markedly to a specific effect than does the general population to be found on a nonselective medium. This opens the road to a vast field, as yet unexplored. It involves the use of media containing products of the decomposition of plant materials and of root secretions.

When estimates based on single samples are considered, the marked response to moisture and cropping becomes a large and serious error. Since there is no means of estimating and removing the effects of these population responses in single estimates from plots, the sensitivity of the experiment is reduced sharply. This is because the response to these factors increases the only error available for testing the significance of the effect considered. A biological response to differences in chemical composition of the soil could not be detected unless it was several times as large as the combined variation resulting from random sampling at each step and the responses to moisture and cropping. Any smaller, even though real, population response would be masked completely by the large error of the experiment. The same reasoning applies to single samples from widely separated areas representing different soil types. The error in an experiment of this kind is likely to be so large that only an extreme response to an effect could be detected.

The sensitivity of the method in demonstrating response of the population to certain environmental factors, even with a nonselective medium,

gives reason for hope that it may be adapted to detect deficiency in mineral elements in soil with a precision not equalled by any chemical or biological method used for that purpose today. It is conceivable that a medium may be developed for growing a population so highly specialized in growth requirements as to respond to differences in amounts of certain of these elements that are within the limits of experimental error of recognized procedures.

Whether the method based on the principles outlined is used in intensive experimental plot studies or whether it is adapted along some of the lines indicated, undoubtedly it will find application in the problems so aptly referred to by the late Dr. Brown as he neared the end of a life devoted to this "fascinating and important science," when he expressed his views as follows[2]: ". . . it is particularly important that the true character of the soil and the occurrence and activities of micro-organisms in it should be recognized and appreciated. . . . Much remains to be learned of the processes in the soil, of the relationships between various micro-organisms, of the dependence of the organisms upon soil conditions and of the relations of the different processes to crop growth, to soil conservation, and to permanent agriculture."

[2] Soil Science 40:49-58, 1935.

INFLUENCE OF THE DECOMPOSITION OF ORGANIC MATERIALS ON SOME PROPERTIES OF ALKALINE-CALCAREOUS SOILS

W. P. Martin and W. A. Kleinkauf[1]

From the Arizona Agricultural Experiment Station

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The influence of decomposing organic materials on the physical, chemical, and microbiological properties of soils has received the attention of many investigators in recent years (1, 3, 4, 6, 10, 12, 13, 17, 18, 19, 20, 23, 26, 27, 28, 29, 30, 31, 38). Among the benefits of organic matter additions to soil are: improved structure, increased water-holding and base-exchange capacities, decreased susceptibility to erosion, increased availability of nutrients such as phosphorus, stimulated production of "auximones," decreased pH values and more rapid reclamation of alkali soils, and increased activity of the micropopulation.

Dr. P. E. Brown and his colleagues, in a series of papers dating from 1915, contributed much of the pioneer work in this field of study (7, 9, 24, 25, 33, 34). Doctor Brown emphasized particularly the importance in the growth of crops of available nitrogen (7), and revealed the relationship between decomposing organic matter and groups of soil micro-organisms physiologically active in nitrogen transformations (9). He and his colleagues established a relationship between the nitrogen present in organic materials and their rate of decomposition (25), and the influence of such material on the nitrogen content of the soil (24). These workers showed further that the base-exchange capacity of the soil can be increased by organic matter additions (24), and that the decomposition of these materials in the soil markedly increases the infiltration rates (34).

In the alkaline (pH 7.3-9.4), calcareous ($CaCO_3$, 2-20 per cent) soils of the Southwest, the decomposition of organic matter creates problems peculiar to the region. Since the annual precipitation is scarcely more than 10 inches, irrigation is necessary for crop production. Temperatures average approximately 80° F. from May to September, and 55° F. the balance of the year. These environmental conditions cause a rapid, comparatively complete decomposition of any added organic materials with the result that little accumulation occurs, and the organic matter content of the soil seldom exceeds 1 per cent.

Because of the rapid decomposition, and consequent slight accumulation of organic residues in the Southwest, many farmers question the economy of organic matter additions. It was deemed advisable, therefore, to initiate a study of the rates of decomposition of typical plant materials in this region to correct this misconception, and in addition, to study the influence of the decomposition process on the soils' pH value, nitrate-

[1] Assistant Soil Microbiologist and Graduate Assistant, respectively.

nitrogen content, and structure under laboratory conditions. The information thus obtained may serve as a foundation upon which to establish a program for improving the crop-producing power of the soils of this area. Such preliminary studies are reported in the present investigation.

EXPERIMENTAL

METHODS AND PROCEDURE

Three soils typical of scattered agricultural regions of Arizona were selected: (a) *Pima clay loam* from Safford (taken from a field fertilized annually for the past ten years with manure at the rate of 12 tons per acre); (b) *Laveen sandy loam* from the State Agricultural Experiment Farm at Mesa; and (c) *Superstition sand* from the Yuma mesa. *Clarion silt loam*,[2] representative of soils of more humid regions, was used for comparison. Descriptions of each soil type may be obtained from the appropriate Soil Survey reports (8, 35). The soils, freshly collected from the field, were screened through a 10-mesh sieve and thoroughly mixed to insure uniform sampling.

Two leguminous and two nonleguminous plant materials, sesbania and sour clover, and hegari and Markton oats, respectively, were chosen for the experiment. These plant materials are typical of those commonly added in large amounts to Arizona soils. The plants were gathered just prior to maturity, dried in the shade, and ground to pass a 20-mesh sieve. Each of the plant materials was incorporated with 150 grams of soil in glass tumblers at the rate of 2 per cent and was uniformly mixed. Enough distilled water was added to each sample to bring moisture up to optimum (approximately 65 per cent of the water-holding capacity). Untreated samples of each soil were incubated also. The tumblers, fitted with loose covers, were placed in a thermostatically-controlled incubator at 30° C. From time to time, enough water was added to maintain the samples at optimum moisture content.

Duplicate samples of each soil treatment were removed periodically from the incubator, the soil in each thoroughly mixed, and aliquots taken for determination of the pH value, nitrate-nitrogen and total carbon contents, and degree of particle aggregation.

Fifty-gram samples (on a dry-weight basis) of the mixed soil were transferred to wide-mouth bottles; 250 ml. of distilled water were added, and the bottles were shaken for 20 minutes on a mechanical shaker. Determinations of pH were made on this 1:5 soil-water suspension with a Beckman pH meter. The suspension was then filtered. Nitrates were determined in the filtrate by the phenoldisulfonic acid method (2). When necessary, the solutions were clarified and decolorized by Harper's cupric sulfate-calcium hydroxide method (16). The color comparisons were made with a Cenco photelometer.

[2] Obtained through the courtesy of Prof. B. J. Firkins, Department of Agronomy, Iowa State College, Ames, Iowa.

One-to-three grams of each soil were used for the total carbon determinations, depending on the carbon content; analyses were made by the Walkley-Black modification (40) of Schollenberger's dichromate titration method (32).

The effect of the organic materials on soil structure was deduced from the degree of aggregation of the soil particles. An adaptation, proposed by Gerdel (15), of the Bouyoucos hydrometer method of mechani-

TABLE 1

CHANGES IN CERTAIN PROPERTIES OF PIMA CLAY LOAM WITH TIME DURING THE DECOMPOSITION OF ORGANIC MATERIALS

Treatment	Incubation Time in Days	Total Carbon Content (%)	pH	Nitrate-N Content (ppm)	Particles Less Than 0.05 mm. (%)
No treatment	0	1.24	8.46	52.0	42
	3	1.25	8.45	50.4	42
	7	1.25	8.45	58.4	39
	15	1.26	8.47	55.8	38
	28	1.24	8.42	90.5	38
	49	1.22	8.38	94.8	38
	70	1.23	8.40	116.0	39
	133	1.24	8.35	148.5	37
Sesbania (2%)	0	2.12	7.88	52.0	42
	3	1.91	8.05	3.2	41
	7	1.82	8.18	26.6	36
	15	1.72	8.11	214.1	33
	28	1.67	8.01	339.5	32
	49	1.64	7.92	378.0	31
	70	1.57	7.90	412.0	30
	133	1.57	7.92	565.0	29
Sour clover (2%)	0	2.02	7.85	52.0	40
	3	1.82	8.08	20.0	38
	7	1.68	8.31	58.7	34
	15	1.60	8.14	274.6	34
	28	1.58	8.02	393.0	33
	49	1.56	7.95	442.0	31
	70	1.48	7.97	460.0	32
	133	1.45	7.91	594.0	30
Hegari (2%)	0	2.14	7.95	52.0	40
	3	1.95	8.00	1.5	36
	7	1.87	8.16	0.6	34
	15	1.76	8.52	0.7	32
	28	1.64	8.49	1.8	30
	49	1.62	8.36	27.7	30
	70	1.54	8.43	54.7	30
	133	1.51	8.22	151.5	26
Markton oats (2%)	0	2.18	8.06	52.0	40
	3	2.05	8.09	1.4	34
	7	1.96	8.33	0.6	33
	15	1.87	8.60	0.5	27
	28	1.70	8.58	0.9	28
	49	1.63	8.48	7.4	30
	70	1.49	8.43	73.9	29
	133	1.48	8.31	122.0	24

TABLE 2

CHANGES IN CERTAIN PROPERTIES OF CLARION SILT LOAM WITH TIME DURING THE DECOMPOSITION OF ORGANIC MATERIALS

Treatment	Incubation Time in Days	Total Carbon Content (%)	pH	Nitrate-N Content (ppm)	Particles Less Than 0.05 mm. (%)
No treatment	0	2.02	5.40	60.0	38
	3	1.98	5.29	58.2	36
	7	1.86	5.30	58.9	34
	15	1.84	5.25	61.5	34
	29	1.89	5.22	91.5	35
	50	1.87	5.19	107.5	32
	71	1.87	5.04	105.5	32
	133	1.79	4.92	161.0	28
Sesbania (2%)	0	2.88	5.34	60.0	39
	3	2.60	6.34	4.1	37
	7	2.52	7.00	0.9	34
	15	2.41	7.23	9.1	32
	29	2.36	7.07	13.7	27
	50	2.33	5.96	164.5	24
	71	2.21	5.21	300.0	21
	133	2.18	4.58	509.0	19
Sour clover (2%)	0	2.79	5.39	60.0	40
	3	2.65	6.18	46.9	39
	7	2.47	6.85	16.8	33
	15	2.32	7.48	6.8	32
	29	2.26	7.49	8.0	30
	50	2.18	6.75	54.5	26
	71	2.05	6.05	218.0	26
	133	2.06	5.19	440.0	26
Hegari (2%)	0	2.92	5.35	60.0	38
	3	2.66	5.75	6.2	36
	7	2.54	6.71	0.7	32
	15	2.42	6.14	0.5	26
	29	2.30	5.99	0.9	23
	50	2.26	5.87	9.1	21
	71	2.15	5.34	71.4	19
	133	2.05	5.02	163.0	14
Markton oats (2%)	0	2.93	5.40	60.0	38
	3	2.62	5.86	3.7	35
	7	2.46	6.63	0.2	30
	15	2.39	6.12	0.6	22
	29	2.34	6.01	4.3	20
	50	2.21	5.53	60.5	18
	71	2.21	5.21	300.0	21
	133	2.04	4.94	187.5	15

cal analysis (5) was used to determine the degree of aggregation. The soil was put into Bouyoucos cylinders with 1,000 ml. of distilled water and let stand overnight. The following morning the cylinders were turned end-over-end 20 times by hand, and promptly replaced on the laboratory desk. The percentage of particles less than 0.05 mm. in diameter was determined by taking a hydrometer reading after the suspension had been allowed to stand exactly 40 seconds. Proper temperature corrections were applied to all hydrometer readings.

A comparison of the percentages of particles less than 0.05 mm. in diameter in the untreated and in the treated samples reveals the influence of organic matter decomposition on the water-stable aggregates of the soil. It was necessary to bring the soil samples to optimum moisture 24 hours prior to analysis to obtain consistent results by this method.

RESULTS

Notwithstanding a wide range of values for the physical and chemical properties of the three alkaline, calcareous soils used (texture, sand to clay loam; total carbon, 0.04-1.24 per cent; total soluble salts, 300-2,000 ppm; nitrate-nitrogen, 2.5-52 ppm; pH value, 8.46-8.79), nearly identical changes, differing only in magnitude, were observed in the three soils during decomposition of the organic materials.

For the sake of brevity, the results for Pima clay loam only are presented here, and these data together with those obtained from Clarion silt loam are given in Tables 1 and 2.

RATE OF DECOMPOSITION

The decrease in carbon content in the variously treated Pima clay loam samples is presented in Figure 1. These curves, typical of those obtained with the other soil samples, show that the rate of decomposition of the plant substances was most rapid during the first two weeks of decomposition. Complete decomposition had not yet occurred at the end of 133 days of incubation, the duration of the experiment.

The data in Tables 1 and 2 and Figure 1 indicate that the various materials decompose at different rates in any given soil, and that the rates vary from soil to soil. The extent of decomposition, in percentage of the carbon originally added, is shown for various incubation periods in Table 3. The 15-day period was chosen for comparison because the rates of decomposition prior to and following the fifteenth day of incubation are quite different; thus the data indicate the influence of the initial rate on the extent of decomposition observed at the end of 133 days.

Initially the decomposition rate was more rapid in the Pima clay loam for the leguminous than for the nonleguminous plant materials, whereas the rates were about equal in the Clarion silt loam. After 133 days of incubation, however, a greater percentage of the nonleguminous carbon had been oxidized than of the leguminous. Sour clover decomposed more rapidly than sesbania and almost as quickly as the oats and hegari; the latter two substances decomposed at approximately the same speed.

Chemical analyses of the plant materials showed 45 per cent of the sour clover to be water soluble, whereas only 25 per cent of the sesbania was soluble. That the sour clover should decompose more rapidly than the sesbania is evident, therefore, since Waksman and Tenney (39) and others have shown that the water-soluble fraction of organic materials decomposes most rapidly.

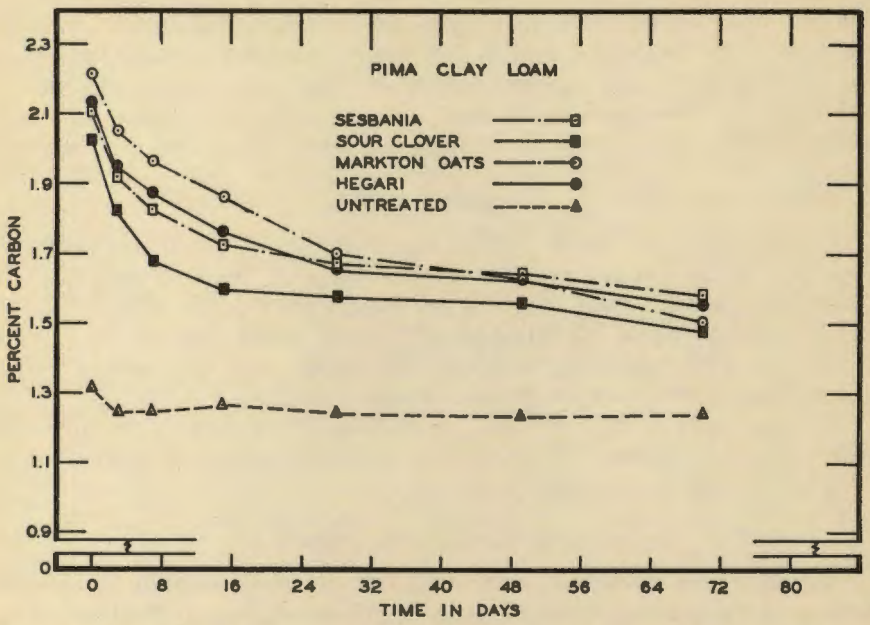

FIG. 1. Changes in carbon contents of samples of Pima clay loam during the decomposition of organic materials.

The rapid rate of decomposition of the nonleguminous materials may be explained as follows: Since a smaller percentage of nitrogen is present in the oats and hegari (1.35 per cent) than in the clover and sesbania (3.5 per cent), less protoplasm can be synthesized at any given time by the heterotrophes attacking the organic materials; consequently,

TABLE 3

TOTAL CARBON LOSS WITH TIME IN PIMA CLAY LOAM AND CLARION SILT LOAM SOILS VARIOUSLY TREATED WITH ORGANIC MATERIALS

Treatment	Incubation Time in Days	Pima Clay Loam		Clarion Silt Loam	
		Gms. C per 100 Gms. Soil	Loss in Weight (%)	Gms. C per 100 Gms. Soil	Loss in Weight (%)
Sesbania	0	0.85	0	0.86	0
	15	0.48	45.5	0.39	54.6
	133	0.33	62.5	0.16	81.4
Sour clover	0	0.78	0	0.77	0
	15	0.36	53.9	0.30	61.0
	133	0.21	73.1	0.04	94.9
Hegari	0	0.90	0	0.90	0
	15	0.52	42.2	0.40	55.6
	133	0.27	70.0	0.03	96.5
Markton oats	0	0.94	0	0.91	0
	15	0.63	33.0	0.37	59.4
	133	0.24	74.5	0.02	98.0

over a long period of time a larger carbon loss would result from the nonleguminous than from the leguminous substances.

The fact that the organic additions decomposed more rapidly in the *acid* Clarion silt loam than in the *alkaline* Pima clay loam is of particular interest. In general the assumption has been made that organic matter decomposes more quickly in alkaline than in acid soils by virtue of factors other than the higher prevailing temperatures in arid regions (28). The data presented herein, however, indicate that such is not the case since identical experimental conditions were used in the comparison of the two types of soils. Stephenson (36), too, found the decomposition of organic materials to be more rapid in acid than in alkaline soils.

If the molds are more numerous in acid than in alkaline soils, as is commonly believed, and are more active organic-matter decomposers than the other soil micro-organisms, an explanation for the above findings is apparent.

pH EFFECTS

Changes in the pH values of the two soils concurrent with decomposition of the organic materials may be noted in Tables 1 and 2, and in Figures 2 and 3.

The organic matter additions had a marked effect on the pH values not only initially but during the entire course of decomposition. In the Pima clay loam, an average initial lowering of 0.5 of a pH unit occurred; the sesbania and sour clover caused an average lowering of 0.6 of a pH unit compared with 0.4 of a pH unit for the oats and hegari.

Following the initial depression in the alkaline soil, the pH values of all treated samples rose quickly, and in those treated with oats and hegari assumed a position slightly above that of the untreated samples, remaining constant thereafter or decreasing slightly to that of the untreated samples. In the case of the clover- and sesbania-treated samples, however, the rise in pH value following the initial decrease was interrupted at the end of seven days and again started to decrease. Following the twenty-eighth day of incubation, the pH values of these samples remained nearly constant at a value approximately 0.4 of a pH unit below that of the untreated samples.

In contrast to the effects noted for the Pima clay loam, the pH value of the Clarion silt loam was not appreciably affected initially by any of the organic substances. Their decomposition during incubation, however, very markedly affected the pH value. Within three days, the pH had risen about 0.5 of a pH unit in the case of the hegari- and oat-treated samples, and nearly 1 pH unit in the leguminous-treated samples. The maximum pH rise for the former was from pH 5.35 to pH 6.70, and was observed after seven days of incubation. After this maximum had been reached, the pH values decreased gradually throughout the incubation period to a value nearly the same as that of the untreated samples,

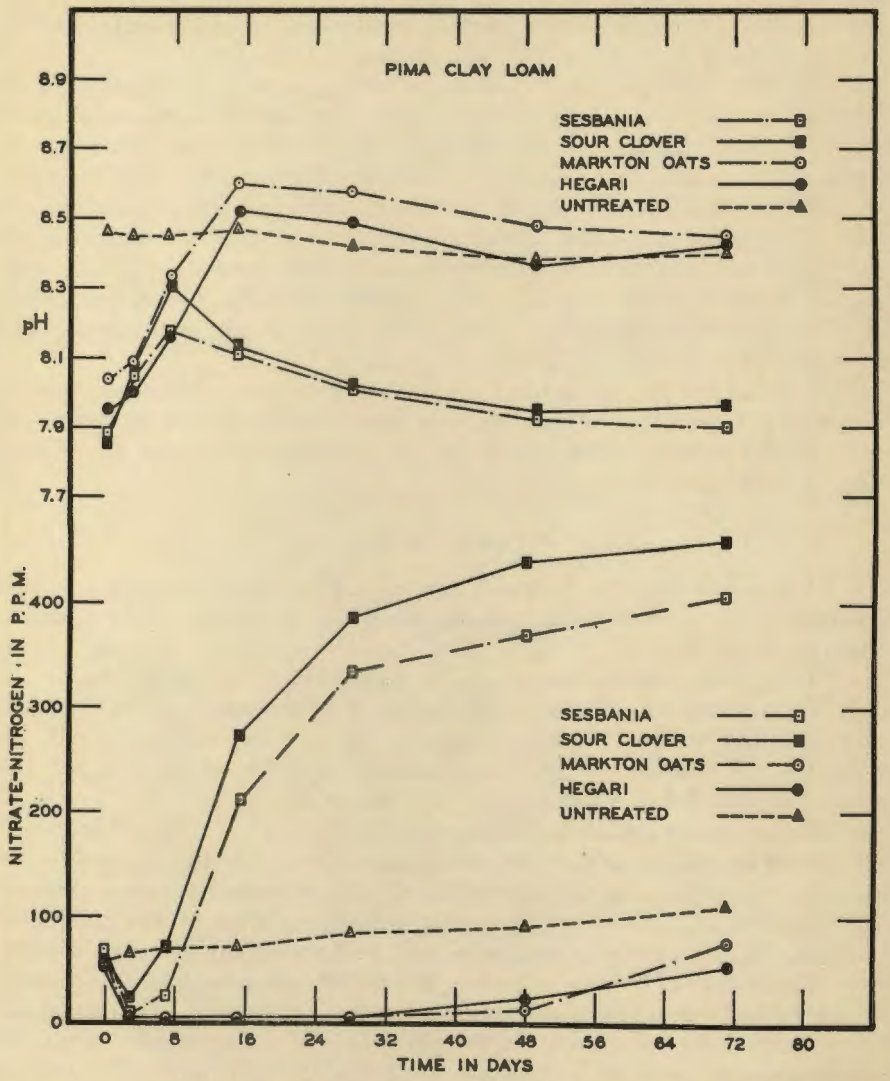

FIG. 2. Changes in pH values and in nitrate-nitrogen contents of samples of Pima clay loam during the decomposition of organic materials.

namely, pH 4.92. The maximum pH rise for the sesbania- and sour clover-treated samples, on the other hand, did not occur until after 15 days of incubation; the highest value reached by the sesbania-treated samples was pH 7.23, that by the sour clover-treated samples, pH 7.49. These values represent an average increase of over 2 pH units for the legume-treated samples. After reaching the maxima, these curves also show decreases in pH values, with the sesbania-treated samples decreasing more quickly than those treated with sour clover. At the end of the

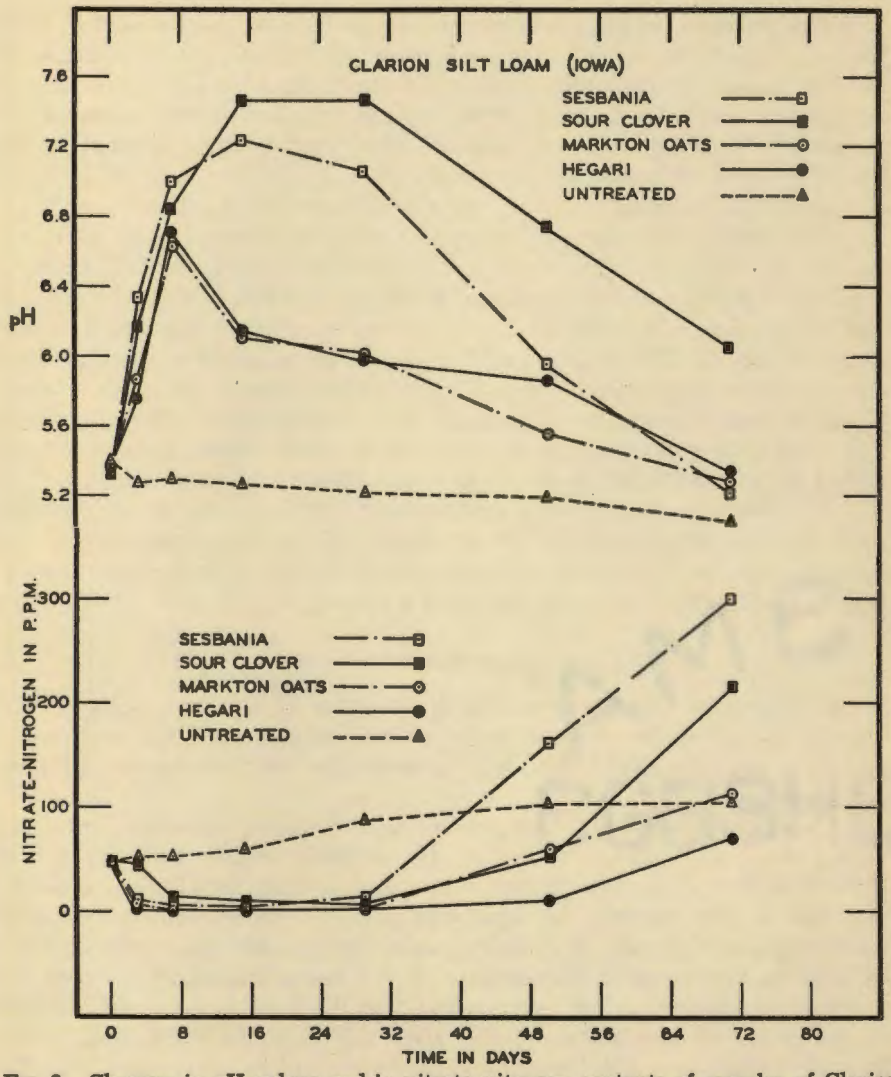

FIG. 3. Changes in pH values and in nitrate-nitrogen contents of samples of Clarion silt loam during the decomposition of organic materials.

incubation period, the sesbania-treated samples had a pH value 0.34 of a pH unit *lower*, and the sour clover-treated samples a value 0.27 of a unit *higher* than the control samples.

The foregoing pH effects are very striking, particularly in view of the observations of Oberholzer (28) that organic matter decomposition does not produce appreciable changes in the pH value of alkaline-calcareous soils. They are in agreement, however, with the findings of Conrad (13), and those of Dyal *et al.* (14).

The changes in pH reported here may be related to the following fundamental factors: (a) the production of considerable quantities of carbon dioxide during incubation, (b) the release of ammonia and its subsequent oxidation to nitric acid, (c) the inherent acidity of the water extracts of organic material, and (d) the high calcium content of the leguminous plant materials (approximately 1 per cent) which tends to increase the alkalinity of the soil as it is released during decomposition.

It is true, of course, that before any marked change in pH value can occur, the buffer capacity of the soil must be overcome. A suspension of 2 grams of the nonleguminous plant materials in 500 ml. of distilled water had an average pH value of 6.5; the corresponding value for oats and hegari was 6.25. The addition of 2 grams of these materials to the Pima clay loam samples probably explains the initial drop in pH with this soil when the values were determined on the 1:5 suspension. The absence of a corresponding increase in the pH value when these materials were added to the acid Clarion silt loam may indicate buffer action.

The changes in pH values subsequent to those occurring initially undoubtedly resulted from the products of the decomposition of the organic materials. Of these products, ammonia and its subsequent oxidation to nitric acid is probably the most effective.

NITRATE-NITROGEN CHANGES

Changes in the nitrate-nitrogen contents of the two soils during decomposition of the organic materials is depicted in Figures 2 and 3 as well as changes in pH. This dual presentation on each figure facilitates comparisons.

The rapid microbial decomposition of the added carbonaceous materials caused an initial decrease in the nitrate-nitrogen content of both soils. The low C:N ratio in the leguminous materials (15.8 in sesbania and 14.0 in sour clover), the rapid dissolution of the carbonaceous materials, and the high rate of nitrification of the released ammonia resulted in nitrates beginning to accumulate in the incredibly short time of one week of incubation. After two weeks of incubation nitrates were present to the extent of over 200 ppm, and after 133 days, over 550 ppm, in the legume-treated samples. Sour clover decomposed more rapidly than the sesbania, had the narrower C:N ratio, and showed the most rapid accumulation of nitrates.

The leguminous-treated samples of Clarion silt loam differed markedly in nitrification behavior from the correspondingly treated samples of Pima clay loam. Nitrate accumulations did not begin until the twenty-eighth day of incubation, and then at such a slow rate that an additional 12 days elapsed before the nitrate content of the sesbania-treated samples equalled that of the untreated samples and an additional 28 days in the case of the sour clover samples. When nitrates first appeared in the Clarion silt loam on the twenty-eighth day of incubation, over 60 per cent of the nitrogen in the corresponding samples of Pima

clay loam had already been oxidized to nitrates. Since dissipation of carbon was most rapid in the Clarion silt loam (Table 3), these results seem to indicate that the nitrifying bacteria are much more active in the alkaline Arizona soil.

In spite of the fact that sour clover contained the higher nitrogen content and decomposed more rapidly than sesbania in the Clarion silt loam, nitrates accumulated less rapidly in this soil treated with sour clover than with sesbania. No immediate explanation is offered for this unexpected observation.

The nonleguminous materials affected the nitrates in a similar manner in both soils. As anticipated, nitrates disappeared immediately and did not reappear for nearly seven weeks. The nitrate-nitrogen contents of the oat- and hegari-treated samples did not equal or approach that of the untreated samples until after ten weeks of incubation. In all instances, nitrates accumulated slightly more rapidly in the samples treated with Markton oats than in those treated with hegari.

CORRELATION BETWEEN NITRATE FORMATION AND CHANGES IN pH

A correlation exists between the rate of accumulation of nitrates in the soil samples treated with the leguminous plant materials, and changes in the pH values of the soils, as shown in Figures 2 and 3. In the Pima clay loam, for example, the sesbania- and sour clover-treated samples showed a distinct reversal in the pH trend when nitrates first began to accumulate after 7 days of incubation; thereafter the pH values decreased with increasing accumulation of nitric acid. The correlation is even more striking in the Clarion silt loam. A phenomenal increase of nearly 2 pH units occurred in the leguminous-treated samples during the first two weeks of incubation, and decreasing pH values were not noted until nitrates began to accumulate. A positive correlation is observed between the *rates* of nitrification and *rates* of decrease in pH also; more rapid nitrate accumulation and more rapid decrease in pH were noted with sesbania- than with the sour clover-treated samples.

An explanation for these phenomena is found in the following facts. Large amounts of nitrogen have been added to the soil in the leguminous plant material. This nitrogen, although present in complex nitrogenous or proteinaceous forms, is quickly released as ammonia by the ammonifying heterotrophes. The ammonium ion thus formed replaced sodium from the base-exchange complex of the Pima clay loam. The replaced sodium ion then combines with the carbonic acid resulting from the decomposition of the organic materials to form sodium carbonate. Hydrolysis of the sodium carbonate produces sodium hydroxide which imparts an alkaline reaction to the soil.

The exchange complex of the Clarion silt loam, on the other hand, is probably hydrogen saturated, so that the ammonium hydroxide reacts directly with the hydrogen clay to produce ammonium clay and water.

Since each of the leguminous materials contained approximately

1 per cent calcium, it is probable that large amounts of calcium, in addition to ammonia, were released during decomposition. Calcium ion thus released would tend to increase the basicity of the soil solution in the same manner that it does when added to an acid soil as lime.

Because of the heterotrophic nature of the majority of soil microorganisms, the ammonification process is rapid in both acid and alkaline soils. The oxidation of the ammonia thus formed to nitrous and nitric acids imparts an acid pH to the soil in direct contrast to the alkalizing action of the ammonia and calcium carbonate. It follows, then, that the relative rate of nitrification (or ammonia removal) determines whether the legume-treated samples become highly alkaline or acid—the magnitude of the effect depending, of course, on the buffer capacity of the soil.

The respective optimum pH values for the growth and activity of the nitritifiers and nitratifiers (37) are 7.8 and 7.1; therefore, the oxidation of the ammonia to nitrites and nitrates should be more rapid in alkaline and less rapid in acid soils. The surprisingly rapid formation of nitrates in the alkaline Pima clay loam in contrast to the sluggish formation in the acid Clarion silt loam has already been noted. It is believed, therefore, that the extremely high pH of the legume-treated Clarion silt loam samples prior to nitrate formation may be due to the accumulation of ammonia, and possibly, in part, due to the formation of calcium carbonate. The fact that the legume-treated samples of Pima clay loam never became as basic as the untreated samples probably results from the extremely rapid rate at which nitrates accumulated, thus rendering the soil less alkaline.

CHANGES IN AGGREGATION OF SOIL PARTICLES

The influence of the decomposition of organic matter on soil structure was measured by means of Gerdel's (15) method for determining water-stable aggregates. The results of these analyses are given in Tables 1 and 2; the data for Clarion silt loam, typical of those obtained on all the other soils, are plotted in Figure 4.

In all cases decomposition of the organic materials promoted the formation of larger soil aggregates; this is indicated by the decreasing percentage values for particles less than 0.05 mm. in diameter as incubation proceeded. The formation of larger aggregates continued steadily during the entire course of the experiment, but was most rapid during the first 7 to 15 days of incubation during which organic dissimilation likewise was most rapid. At the end of 133 days, the decrease in particles less than 0.05 mm. in diameter amounted to approximately 27 per cent of the initial values in the Pima clay loam and approximately 40 per cent in the Clarion silt loam.

The nonleguminous material caused greater aggregation of the soil particles than did the legumes in each soil. The order of decreasing effectiveness on aggregation for the four treatments was: Markton oats, hegari, sesbania, sour clover. These findings are in essential agreement

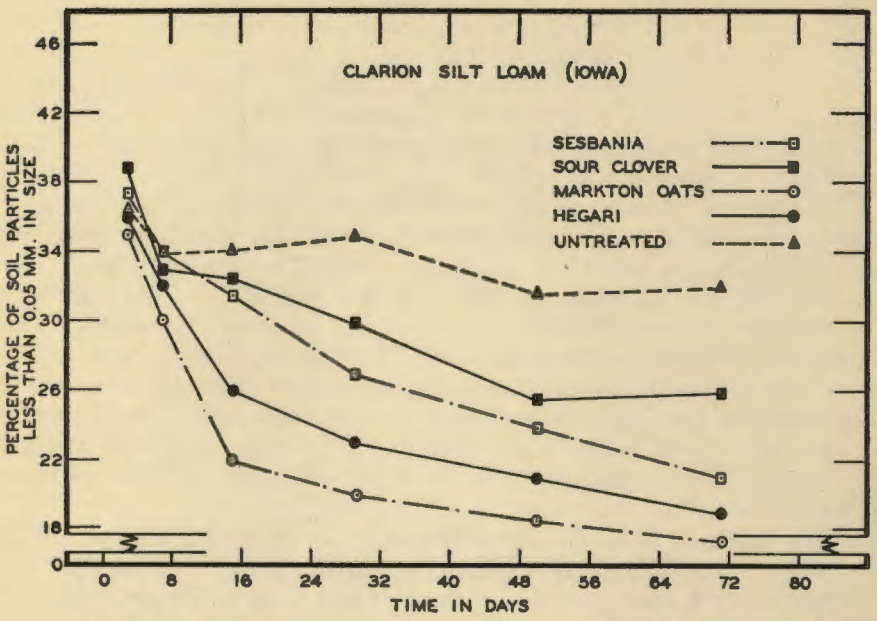

FIG. 4. Changes in size of particles of samples of Clarion silt loam during the decomposition of organic materials.

with those of Chapman (11), that only 10.8 per cent of the soil "crumbs" from a timothy-treated soil would pass through a sixteenth-inch screen, whereas 55.7 per cent of those from a clover-treated soil would do so.

Notwithstanding the normal differences in organic-matter content (Tables 1 and 2) and particle size in the Clarion *silt* loam and the Pima *clay* loam, the decomposition of the added organic materials caused a greater aggregation of the small particles in the Clarion than in the Pima-series soil. Moreover, the difference in influence between any two organic substances was greater in the Clarion than in the Pima soil; for example, after 70 days of incubation the difference in percentage values for particles less than 0.05 mm. in diameter in the sesbania- and in the Markton oats-treated samples was 5 per cent in the Clarion silt loam and only 1 per cent in the Pima clay loam.

No entirely logical explanation for the difference in effect of the legumes and nonlegumes on aggregation has been offered. The chief chemical difference of the two types of plant material is the higher protein content of the sesbania and sour clover (21.8 per cent) as compared with that of oats and hegari (8.5 per cent). Since the rapid conversion of protein-nitrogen to ammonia and then to nitric acid would tend to bring calcium into solution (28), one would expect greater aggregation effects in the legume-treated than in the nonlegume-treated samples of Pima clay loam. Similarly, the inherently higher calcium contents of the leguminous plant materials should show their influence on aggregation, especially in the calcium-deficient Clarion silt loam.

The mechanism of soil aggregation in all of its phases has never been clearly explained. It is known that organic binding materials play an important role in aggregate formation, however, and, according to Peele (31), may be divided into (a) the lyophobic colloids formed as part of the residues of plant materials added to the soil, and (b) microbial cells and their secretory products.

Martin and Waksman (19) showed that bacterial decomposition products are important in aggregate formation, and that microbial gums and mold mycelia are important binding agents. These investigators concluded that the aggregating effect of the various organic materials varied, however, not only with the organisms involved, but also with the nature of the organic material present. The latter appears to be in agreement with the results obtained in the present study. If this were not true, it is unlikely that the different organic materials would have similar aggregating effects in the highly alkaline Pima clay loam and the distinctly acid Clarion silt loam in which it may be assumed that markedly different microflora are present.

It is apparent from the above presentation of results that several of the findings are difficult to explain by existing knowledge in the field. Further study is consequently in prospect and shall be reported upon in subsequent communications.

SUMMARY

1. Data are reported on the influence of the decomposition of sesbania, sour clover, hegari, and Markton oats on the pH, nitrate-nitrogen content, and the degree of aggregation of Pima clay loam, an alkaline-calcareous soil from Arizona, and of Clarion silt loam, an acid soil from Iowa.

2. At a temperature of 30° C. and a moisture content of 65 per cent of the water-holding capacity, the rate of decomposition of each organic material was more rapid in the acid Clarion silt loam than in the alkaline Pima clay loam. At the end of the incubation period, a greater percentage of the nonleguminous than of the leguminous materials had been dissipated with the exception of sour clover; the latter decomposed quickly because of its unusually high content of water-soluble constituents.

3. A pronounced lowering of the pH of Pima clay loam occurred upon the addition of each organic material. Upon decomposition, however, the pH values of all the samples rose quickly and, in the case of the hegari- and oat-treated soils, assumed a position close to that of the untreated samples; this position was maintained essentially during the course of the decomposition. The pH values decreased sharply, coincident with the oxidation of ammonia to nitrates in the sesbania- and sour clover-treated samples.

4. The addition of organic materials was without initial effect on the pH value of Clarion silt loam. Upon decomposition, however, the pH

values of all the samples rose quickly and, in the case of the sesbania- and sour clover-treated samples, assumed a position some 2 pH units above that of the untreated samples. The pH values of the legume-treated samples did not decrease until nitrates began to accumulate and it was not until nearly 500 ppm of nitrate-nitrogen had accumulated (133 days of incubation) that the pH was again equal to that of the untreated samples. The pH values of the nonlegume-treated samples decreased more quickly than those treated with leguminous plant materials, but it was not until nearly 95 per cent of the carbon had been dissipated at the end of 133 days of incubation that the pH approached that of the control samples.

5. Nitrates disappeared quickly from both soils during the initial decomposition of the different organic materials. In the hegari- and oat-treated samples, nitrates were present in lesser amounts than in the untreated samples for an incubation period of approximately ten weeks. In the sesbania- and sour clover-treated samples of Pima clay loam, nitrates began to accumulate in one week and over 60 per cent of the nitrogen had been oxidized to nitrates in four weeks. In the sour clover-treated samples of Clarion silt loam, nitrates did not begin to accumulate for four weeks, and in the sesbania-treated, for nearly six weeks. At the end of eight weeks, the nitrate accumulation in the sour clover-treated samples of this soil amounted to only that in the untreated samples.

6. In all cases decomposition of the organic materials promoted the formation of larger soil aggregates; the nonleguminous materials were more effective in promoting aggregation in both soils than were the leguminous materials. The order of decreasing effectiveness in promoting aggregation for the four treatments was: Markton oats, hegari, sesbania, and sour clover. The plant substances had a greater aggregating effect in the *acid* Clarion silt loam than in the *alkaline* Pima clay loam. At the end of 133 days of incubation, the decrease in particles less than 0.05 mm. in diameter amounted to approximately 27 per cent of the initial values in the Pima-series soil, and approximately 40 per cent in the Clarion-series soil.

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A RAPID METHOD FOR THE DETERMINATION OF TOTAL PHOSPHORUS IN SOIL AND PLANT MATERIAL[1]

W. R. SHELTON[2] AND HORACE J. HARPER[3]

From the Agronomy Department, Oklahoma Agricultural Experiment Station

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Several colorimetric methods have been proposed for the determination of small quantities of phosphorus in biological material. Information concerning many of these procedures may be obtained from a textbook on "Colorimetric Methods of Analysis," by Snell and Snell (10). A recent article by Schricker and Dawson (9) contains a good review of the literature on this subject. A majority of these methods depend upon the partial reduction of molybdenum in phosphomolybdic acid which changes from colorless to blue (7), and the intensity of color is approximately proportional to the quantity of phosphorus in the solution.

One of the important problems in colorimetric determination is to obtain constant color values in solutions which vary in chemical composition and may contain compounds which interfere with normal color development. Many reducing agents which have been used to determine total phosphorus in biological material by the molybdenum blue method give inaccurate results in the presence of large quantities of ferric iron. Soil extracts from a soil digested with strong acid will frequently contain large quantities of this element. Since ferrous iron does not interfere with the partial reduction of hexavalent molybdenum in phosphomolybdic acid, heating phosphorus solutions containing ferric iron with sodium sulfite has been recommended to change ferric iron to the ferrous form. Truog and Meyer (11) recommend that solutions containing more than 6 ppm of ferric iron should be filtered through a Jones reductor containing cadmium before the molybdenum blue color is developed with stannous chloride. Since this reducing agent forms an unstable molybdenum blue which begins to decompose soon after a maximum intensity of color has developed, it is not as satisfactory for use in routine analysis as other reagents which produce a stable molybdenum blue colloid in solutions containing small quantities of phosphorus.

The upper and lower limits of acid concentration between which the intensity of the molybdenum blue color is relatively constant have been studied by several investigators. Some variation occurs in the quantity of acid recommended because molybdic acid is reduced at a lower acidity by some reducing agents than by others. Schricker and Dawson (9) found that a stable form of molybdenum blue is obtained using metol or

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[1] Contribution from the Oklahoma Agricultural Experiment Station.

[2] Formerly laboratory assistant, now employed by the Phillips Petroleum Co., Bartlesville, Okla.

[3] Professor of Soils, Oklahoma Agricultural Experiment Station, Stillwater, Okla.

unstable molybdenum blue as a reducing agent when the pH value of a sulfuric acid solution varies from .7 to .83. They recommend the use of quinaldine red as an indicator to aid in the accurate adjustment of acidity in a phosphorus solution before the reducing agent is added. Zinzadze (15) used 2,6-dinitrophenol for this purpose.

EXPERIMENTAL RESULTS

A study of several colorimetric methods recommended for the measurement of small quantities of phosphorus was made to discover or develop a rapid procedure which would be suitable for the determination of total phosphorus in soil and plant material. In the preparation of soil or forage samples for a total phosphorus determination, the organic matter may be destroyed by ignition with or without the addition of an oxidizing agent. Wet ashing with perchloric acid as recommended by King (6) or the use of a perchloric-nitric acid mixture as proposed by Gieseking *et al.* (4) has many advantages when total ash is not determined. One of the important advantages of the perchloric acid digestion is the elimination of silica without evaporating a solution to dryness, which is required when hydrochloric acid is used to dissolve the phosphorus from plant ash or a soil residue. Willard and Cake (13) found that silica was quickly dehydrated when boiled in concentrated perchloric acid for a short period of time. Volk and Jones (12) used a perchloric-nitric acid mixture for the destruction of organic matter in soil. It was found in this study that pretreatment with nitric acid was necessary only when soil samples containing a large quantity of active organic matter, such as the A_0 layer of a soil profile, a peat, or a muck, were analyzed.

Hillebrand and Lundell (5) report that phosphoric acid was lost by volatilization when a mixture of phosphoric and sulfuric acid was digested in an open platinum dish at temperatures between 200° and 260° for varying periods of time. Concentrated perchloric acid (70-72% $HClO_4$) boils at approximately 203° C. When 4 mg. of phosphorus as monopotassium phosphate was digested with 4 ml. of concentrated perchloric acid in a 30-ml. beaker covered with a watch glass for periods varying from 30 minutes to 4 hours, complete recovery of the phosphorus was obtained. This experiment indicates that perchloric acid may be used safely for the destruction of organic matter in the average soil or plant material without loss of phosphorus by volatilization.

A comparison of several reducing agents which have been used to develop the molybdenum blue color in phosphorus solutions by different investigators (1, 2, 3, 9, 10, 11, 15) indicated that hydrazine sulfate gave the best results when a considerable quantity of ferric iron was present. This reagent has been recommended for the reduction of the molybdenum in phosphomolybdic acid by Riegler (10, 14). Other investigators have used this method (8), and in all cases the ammonium phosphomolybdate precipitate was washed to remove the excess of precipitating reagent be-

fore the molybdenum blue colloid was formed. In this investigation it was found that hydrazine sulfate would not form a blue color with low concentrations of molybdic acid when the pH value of the solution was less than 1.0. If more than 6 ml. of 2 per cent solution of sodium molybdate is added to 150 ml. of solution containing no phosphorus, the pH value of that solution must be less than .85 to prevent color development when boiled with 5 ml. of 2 per cent hydrazine sulfate. Data on the recovery of phosphorus in solutions containing varying quantities of ferric iron using hydrazine sulfate as a reducing agent are given in Table 1. The molybdenum blue color was developed as recommended in the procedure for the analysis of soil. Color intensity comparisons were made with a Cenco-Sheard photelometer.

TABLE 1

THE EFFECT OF FERRIC IRON ON THE RECOVERY OF KNOWN QUANTITIES OF PHOSPHORUS DETERMINED BY THE MOLYBDENUM BLUE METHOD IN 200 ML. OF SOLUTION USING HYDRAZINE SULPHATE AS A REDUCING AGENT

Mg. of Phosphorus Added	Mg. of Ferric Iron Added	Mg. of Phosphorus Recovered
.04	0	.040
.10	0	.100
.20	0	.200
.40	0	.400
.50	0	.500
.04	10*	.041
.10	10	.106
.20	10	.200
.40	10	.404
.50	10	.500
.04	25†	.040
.10	25	.111
.20	25	.203
.40	25	.404
.50	25	.504

* Equivalent to 50 ppm of iron in the solution.
† Equivalent to 125 ppm of iron in the solution.

Additional studies were also made to determine the effect of 50 mg. of ferric iron on the recovery of phosphorus from solutions containing known quantities of monopotassium phosphate. A slight positive error occurred in the presence of large quantities of iron instead of a negative error which is usually obtained with other colorimetric phosphorus procedures which have been recommended. The positive error is not serious when the total iron content of a soil does not exceed 10 per cent. Under such conditions the total phosphorus in a soil would be increased approximately 32 pounds per acre. Separation of iron from phosphorus may be necessary before the molybdenum blue color is developed in a solution containing a high percentage of iron. Under such conditions the use of a volumetric procedure for total phosphorus would not be objectionable.

When hydrazine sulfate is added to a solution containing molybdic acid and no phosphorus, a pale yellow color will appear when this solution is heated to boiling. This color will interfere with the determination of small quantities of phosphorus if color comparisons are made in a visual colorimeter, because different shades of green are obtained before the blue color produced by increasing quantities of phosphorus will mask the effect of the yellow color appearing in a solution containing no phosphorus. Since a photelometer measures the quantity of light absorbed by a solution rather than the shade or intensity of color which may be present, a variation from the light blue to green colors which are obtained when less than .04 mg. of phosphorus is present in 200 ml. of solution is not objectionable.

Hydrazine sulfate reduces the molybdenum in phosphomolybdic acid very slowly at room temperatures. The rate of reduction increases rapidly between 50° and 70° C. It is necessary to heat a solution containing hydrazine sulfate to boiling in order to reduce ferric to ferrous iron in a short period of time. When a blue color does not appear in solutions which have been boiled for 1 minute and contain phosphorus and ferric iron, more hydrazine sulfate should be added to hasten the rate of reduction. No increase in color intensity occurs when phosphorus solutions are boiled longer than 1 minute after the blue color begins to appear. When green colors are obtained and the reading on the photelometer scale is high, a larger aliquot may be used to increase the accuracy of the determination if iron does not interfere with color development.

A second factor which affects the development of molybdenum blue color is the pH value of the solution in which reduction occurs. When the acidity of the solution is too low, many reducing agents will reduce molybdic acid not combined with phosphorus. When the acidity is too high, a decrease in the intensity of blue color for a known quantity of phosphorus is obtained. Data on the pH value of solutions containing .2 mg. of phosphorus and acidified with varying quantities of sulfuric,

TABLE 2

THE EFFECT OF ADDING VARYING QUANTITIES OF DIFFERENT ACIDS ON THE COLOR INTENSITY OF MOLYBDENUM BLUE PRODUCED BY THE REDUCTION OF .2 MG. OF PHOSPHORUS IN 200 ML. OF SOLUTION WITH HYDRAZINE SULFATE

pH Value of Sulfuric Acid Solutions	Photelometer Readings	pH Value of Hydrochloric Acid Solutions	Photelometer Readings	pH Value of Perchloric Acid Solutions	Photelometer Readings
1.29	58	1.34	58	1.30	57
1.17	58	1.20	59	1.15	61
1.07	59	1.08	60	1.05	60
1.00	58	1.00	60	.96	60
.92	59	.92	60	.90	60
.87	67	.86	60	.84	60
.84	79	.82	61	.80	59
.75	*	.74	72	.72	63
.69	*	.68	86	.66	71

* No blue color developed in these solutions.

hydrochloric, and perchloric acid before the molybdenum blue color was developed with hydrazine sulfate are given in Table 2.

The color values of stable molybdenum blue which are produced with hydrazine sulfate were approximately the same in solutions of similar pH values obtained by the addition of varying quantities of the different acids. Maximum color development appeared in a slightly higher pH range in the presence of sulfuric acid. No color was obtained at the higher intensities of acidity when this acid was used to acidify the phosphorus solutions before the hydrazine sulfate was added. No appreciable difference was observed in the hydrochloric and perchloric acid comparisons. Undesirable colors are obtained when hydrochloric acid is used to dissolve the phosphorus from soil samples which have been ignited to destroy organic matter due to the presence of iron; consequently, perchloric acid has been recommended for the determination of the total phosphorus in soil. Hydrochloric acid may be substituted for the perchloric acid in the analysis of forage.

Molybdic acid is reduced by hydrazine sulfate when boiled in a solution with a pH value above 1.1; consequently, it is necessary to carefully control the acid concentration to eliminate errors which may occur from the reduction of the precipitating reagent not combined with phosphorus. The photelometer readings of sodium and ammonium molybdate solutions (5 ml. of a 2 per cent solution in 200 ml.) boiled 1 minute with 5 ml. of a 2 per cent solution of hydrazine sulfate in the absence of phosphorus are recorded in Table 3. The pH values were controlled by adding increasing quantities of perchloric acid. The lower the photelometer reading, the greater the quantity of blue color. Only pale yellow colors appeared when the reading was 96 or higher. The blue color in the ammonium molybdate solutions at pH 1.2 and 1.1 was probably due to the slightly larger quantity of molybdic acid which they contained as compared with the sodium molybdate solutions.

TABLE 3

EFFECT OF pH VALUE ON THE REDUCTION OF MOLYBDIC ACID WITH HYDRAZINE SULFATE

	pH VALUE OF SOLUTIONS AND PHOTELOMETER READINGS							
REAGENT USED	1.30	1.20	1.10	1.00	.90	.80	.70	.65
Sodium molybdate ..	74	97	98	98	98	98	98	98
Ammonium molybdate	23	57	93	92	96	96	96	96

The data in Table 3 show that the acidity of a phosphorus solution should be below pH 1.0 in order that no blue color will be formed by the action of hydrazine sulfate on molybdic acid. Six ml. of 2 per cent sodium molybdate will not produce a blue color in the presence of 5 ml. of 2 per cent hydrazine sulfate and 150 ml. of distilled water when the pH of the solution is below 1.0. Since the accuracy of the photelometer is limited by the intensity of the blue color which did not change the light absorp-

tion readings appreciably when more than .6 mg. of phosphorus was present in 200 ml. of solution, more than 5 ml. of 2 per cent sodium molybdate would not be required to precipitate the phosphorus which can be measured by the proposed method. Ten ml. of sodium molybdate will not form a blue color when boiled with hydrazine sulfate if the pH of the solution is less than .85 and the volume is above 175 ml.; consequently, the precipitating reagent can be added rapidly from a pipette or a burette, keeping the volume as near 5 ml. as routine technique will permit.

REAGENTS FOR TOTAL PHOSPHORUS IN SOIL

1. Perchloric acid, 70-72% $HClO_4$
2. Beta dinitrophenol, .1% in 25% alcohol
3. Ammonium hydroxide, 1 part to 3 parts of distilled water
4. Sodium molybdate, 20 gms. of C. P. salt dissolved in 600 ml. of distilled water and add 400 ml. of concentrated perchloric acid (70-72% $HClO_4$) to this solution ($Na_2MoO_4 . 2 H_2O$)
5. Hydrazine sulfate, 20 gms. per liter

RECOMMENDED PROCEDURE FOR TOTAL PHOSPHORUS IN SOIL

Weigh 2 grams of 100-mesh soil into a 30 ml. Pyrex beaker. Add 4 ml. of concentratde perchloric acid (70-72% $HClO_4$) and cover beaker with a small watch glass. Digest on a gas or an electric hot plate covered with an asbestos pad for 30 minutes or until the residue is white. The solution should boil very slowly. When digestion is complete, remove beaker from hot plate, allow to cool a few minutes, wash lower surface of cover glass into the beaker with distilled water, and transfer the contents to a 200 ml. calibrated Erlenmeyer flask using a funnel with a short stem. Add distilled water to the mark, place a rubber stopper in the flask, shake thoroughly, and filter to remove the insoluble residue. Pipette 50 ml. of the filtrate which should contain from .04 to .6 mg. of phosphorus into another 200 ml. calibrated Erlenmeyer flask. Add approximately 100 ml. of distilled water and 5 drops of 2,6-dinitrophenol. Titrate the acidity in this solution with ammonium hydroxide until the yellow color of the indicator appears. Add 5 ml. of the sodium molybdate reagent, 5 ml. of hydrazine sulfate, place the flask on a hot plate, heat to boiling, and allow to boil about 1 minute after the blue color begins to appear. If a blue color does not appear within 1 minute after the solution starts to boil, add more hydrazine sulfate and continue heating. Remove flask from hot plate, cool to room temperature, add distilled water to the 200 ml. mark, mix solution thoroughly, and obtain a light absorption reading with a photelometer. The quantity of phosphorus in the soil is obtained from a curve prepared by plotting the photelometer readings of several phosphorus solutions containing from .025 to .750 mg. of phosphorus on cross-section paper. If a visual colorimeter is used, at least three standard solutions containing small, medium, and large quantities of phosphorus

should be prepared in order that more accurate color comparisons with the unknown solutions may be obtained.

In the analysis of peat, a .5-gram sample should be digested in a 200 ml. tall-form Pyrex beaker with 8 or 10 ml. of the nitric-perchloric acid mixture as recommended for forage and grain. Soils containing a high percentage of iron and/or manganese can be digested more rapidly by adding a small quantity of sodium chloride. Soils containing a high percentage of clay may require more than 4 ml. of perchloric acid for digestion.

RECOMMENDED PROCEDURE FOR TOTAL PHOSPHORUS IN FORAGE OR GRAIN

Place 1 gram of finely ground oven-dry forage or grain in a 200 ml. tall-form Pyrex beaker. Add 7 ml. of acid containing 1 part of perchloric (70-72% $HClO_4$) and 1 part of concentrated nitric acid. Cover beaker with a watch glass and digest on a steam plate or hot water bath for 20 minutes. Transfer beaker to a gas or electric hot plate covered with an asbestos pad, remove cover glass to permit nitric acid to escape, and heat gently until white fumes of perchloric acid have evolved for 1 minute. Replace cover glass and continue heating until the solution is colorless and the residue is white. Remove beaker from hot plate, cool, and transfer solution and residue to a calibrated 200 ml. Erlenmeyer flask, and add distilled water to the mark. Stopper flask, mix contents thoroughly, and filter to remove insoluble residue. Transfer 25 ml. of the filtrate to a calibrated 200 ml. Erlenmeyer flask and develop the molybdenum blue color as recommended for soil. A larger aliquot should be taken if the sample is low in total phosphorus. A smaller aliquot should be used if organic materials high in total phosphorus are being analyzed. If a larger sample is needed for the determination of other elements in the forage or grain, more acid should be added to destroy the organic matter. If the forage or grain is ignited to destroy organic matter, the ash may be treated with perchloric acid to remove silica and the phosphorus determined in a convenient aliquot by the recommended procedure. Hydrochloric acid may be used in place of the perchloric acid to extract the phosphorus from the ignited residue since the iron content of forage samples is usually low.

Molybdenum blue colors which are formed when phosphomolybdic acid is reduced with hydrazine sulfate are very stable and remain constant for a long period of time. In routine analyses, it is convenient to develop the color in an aliquot and allow the solution to cool overnight before the photelometer reading is obtained. A comparison of photelometer readings obtained at intervals of 2 and 16 hours after the blue color of phosphomolybdic acid had been developed is given in Table 4. These analyses were made in quadruplicate on two soils to show the variations that may be expected between individual analyses.

It will be observed that no appreciable change in the color of these solutions occurred during the 14-hour period as measured by the photelometer. The greatest variation between the photelometer readings for

TABLE 4

EFFECT OF TIME INTERVAL BETWEEN PHOTELOMETER READINGS ON THE STABILITY OF THE MOLYBDENUM BLUE COLOR PRODUCED BY REDUCTION OF PHOSPHOMOLYBDIC ACID WITH HYDRAZINE SULFATE AND THE VARIATION BETWEEN QUADRUPLICATE DETERMINATIONS ON TWO SAMPLES OF SOIL

Soil No.	Sample No.	Photelometer Readings	
		2 Hours After Color Development	16 Hours After Color Development
4193	1	57.0	56.8
	2	58.0	57.8
	3	57.9	57.9
	4	57.8	57.6
5174	1	71.0	71.0
	2	70.4	70.4
	3	70.0	70.0
	4	70.5	70.5

each analysis was one division on the photelometer scale, which is equivalent to 28 pounds of phosphorus per acre 6⅔ inches deep in sample 4193, and 24 pounds per acre in sample 5174. Since the readings between each division on the photelometer scale must be estimated, a difference of .2 cannot be considered significant.

A comparison of the total phosphorus in 206 soils analyzed by the colorimetric method described in this paper and by two volumetric procedures was made to determine whether similar results would be obtained. Eighty-four of these soils were analyzed in 1926 by the magnesium nitrate fusion method. One hundred and twenty-two samples were analyzed within the past three or four years by the method as recommended by Volk and Jones (12). The average phosphorus content of the 206 soil samples as determined by the two volumetric methods was 408 pounds per acre. The average phosphorus content of these samples when analyzed by the recommended colorimetric procedure was 407 pounds per acre. Only 27 of the 206 samples had a wider variation than 40 pounds of total phosphorus per 2 million pounds of soil. Of this number, 15 of the samples were higher and 12 samples were lower in total phosphorus content as determined by the volumetric procedures. Since different methods of digestion were used in these analyses, some difference in lack of agreement might be expected.

A comparison of the variation which might occur in the results obtained by different individuals using the colorimetric procedure as recommended was secured on 60 samples of soil analyzed by two individuals with a time interval between these analyses of approximately one year. The average total phosphorus content in these two sets of analyses varied less than .001 per cent. The greatest variation between individual samples of soil was .006 per cent or 120 pounds of phosphorus per acre 6⅔ inches deep. This variation would probably be greater if soils containing a larger quantity of total phosphorus were analyzed. The average total phosphorus content of the 60 soils was approximately .02 per cent and varied from .01 to .039.

During the past two years several thousand samples of soil, forage, and grain have been analyzed for total phosphorus by this method. The important advantage of this procedure as compared with a volumetric or a gravimetric determination is that less time is required to make the analyses. The cost of expendible material for each soil analysis is approximately 2 cents. The accuracy of the proposed colorimetric method is not the same at all points on a curve prepared by plotting photelometer readings against the quantity of phosphorus in standard phosphorus solutions. Less variation will occur between the photelometer readings of duplicate or triplicate analyses when smaller quantities of phosphorus are present. The standard phosphorus solutions which were used to prepare a curve from which the phosphorus in an unknown solution could be calculated were prepared from monopotassium phosphate diluted to a volume of 200 ml. before the readings were obtained. The phosphorus in the standard solutions varied from .025 to .75 mg. Photelometer readings obtained after the molybdenum blue color was developed in the phosphorus solutions are given in Table 4. A reading of 98 which was obtained when no phosphorus was added represents a blank determination on the reagents.

TABLE 5

PHOTELOMETER READINGS OF STANDARD PHOSPHORUS SOLUTIONS IN WHICH THE MOLYBDENUM BLUE COLOR WAS DEVELOPED WITH HYDRAZINE SULFATE

Mg. of Phosphorus in Standard Solutions	Photelometer Readings
.000	98.0
.025	92.0
.050	86.1
.100	76.0
.150	67.8
.200	60.0
.250	53.2
.300	47.5
.350	42.3
.400	38.5
.450	34.5
.500	31.0
.550	28.3
.600	26.5
.650	25.5
.700	25.6
.750	25.2

It is evident from the data presented in Table 4 that the accuracy of a phosphorus determination will decrease with increasing quantities of phosphorus in solution. When more than .6 mg. of phosphorus is present in 200 ml. of solution, a smaller aliquot should be used. Since very few soils contain more than 2,400 pounds of total phosphorus in the surface layer 6⅔ inches deep, no changes in the recommended procedure will be required for the average soil. If the logarithm of each photelometer reading is plotted on cross-section paper against the quantity of phosphorus in

the standard solutions as given in Table 4, a slight break will be observed in the curve obtained indicating that the intensity of color is not exactly proportional to the amount of phosphorus present. The tendency for the colloidal particles to aggregate as the concentration of phosphorus in a solution is increased may account for this condition, although studies on the dilution of standard phosphorus solutions with equal quantities of distilled water after color development has occurred has not had any appreciable effect on the recovery of phosphorus in them.

SUMMARY

A study was made of several colorimetric methods recommended for the determination of phosphorus in different types of organic materials to ascertain their value for the estimation of total phosphorus in soil. Large quantities of ferric iron retard the rate at which the molybdenum blue color is formed when reducing agents recommended for this purpose were used. Hydrazine sulfate will produce a stable molybdenum blue which does not change appreciably after color development has occurred. This reagent will reduce ferric iron at the boiling temperature, and reasonably accurate results for total phosphorus will be secured if the total iron in a soil does not exceed 10 per cent. Hydrazine sulfate does not produce a blue color when added to a solution containing less than .12 mg. of sodium molybdate in 200 ml. of distilled water if the pH value of the solution is properly controlled and no phosphorus is present. A colorimetric method using this reagent to form a stable molybdenum blue colloid from phosphomolybdic acid has been proposed for soils which do not contain more than 10 per cent of iron. The total phosphorus in 206 samples of soil determined by precipitation and subsequent titration of ammonium phosphomolybdate was compared with results obtained by the proposed colorimetric method. Only 27 of the 206 analyses varied more than .002 per cent in total phosphorus content by the volumetric and colorimetric procedures. The method for total phosphorus in soil can also be used for the determination of total phosphorus in forage and grain by using a mixture of nitric and perchloric acid to destroy the organic matter in these materials.

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STUDIES OF DIFFERENT CULTURES OF *RHIZOBIUM LEGUMINOSARUM* AND OF GYPSUM AND STRAW FOR SEED PEA PRODUCTION[1]

S. C. VANDECAVEYE AND W. H. FULLER[2]

From the Division of Agronomy, Soils Section, State College of Washington

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INTRODUCTION

The production of seed peas and wheat in alternate years is common practice in those parts of eastern Washington and northwestern Idaho where the annual precipitation is more than 18 inches. According to Rufener (12) this area, comprising approximately 960,000 acres, nearly two-thirds of which is situated in the State of Washington, produces more than half of the total amount of seed peas harvested in the United States. As a source of agricultural income, seed peas in this area rank second to wheat, the principal crop.

When peas were first introduced as a field crop on Palouse silt loam, seed inoculation with cultures of *R. leguminosarum* was necessary to obtain satisfactory growth and yields. As shown by earlier work (14), once this soil is thoroughly inoculated with *R. leguminosarum*, this organism is capable of surviving in the soil for 10 to 15 years in the absence of the host plant. Reinoculation, therefore, after one thoroughly inoculated crop of peas has been produced is unnecessary insofar as satisfactory production of root nodules is concerned. Since the variability in the ability of different strains or cultures of *Rhizobium* species to benefit their homologous host plants has been demonstrated convincingly by various investigators, notably Baldwin and co-workers (1, 2, 4, 5, 7) and others (8, 9, 11, 15), it was thought that this factor might influence the degree by which seed peas can be benefited from association with their homologous symbiotic nitrogen-fixing bacteria. Further development of this thought found additional support in the fact that in this area where soil moisture and available nitrogen are limiting factors in crop yields in annual cropping systems, seed peas fill a place which cannot be filled by nonleguminous crops. Although the yield of wheat following peas is less than that follow-

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[2] Professor of Soils and former research assistant in soils respectively. The authors gratefully acknowledge the valuable assistance of C. D. Moodie, research assistant in soils, in the field and greenhouse work in 1940, and of L. E. Dunn, research assistant in soils, in making the statistical analysis of the yield data. The authors express their gratitude, also, to I. L. Baldwin, Department of Agricultural Bacteriology, University of Wisconsin, Madison, Wisconsin, and to L. W. Erdman, the Nitragin Company, Incorporated, Milwaukee, Wisconsin, for their kindness and co-operation in supplying some of the cultures used in this study.

ing summer fallow, it is much larger than that following nonleguminous crops. Deficiency in available nitrogen is one of the principal factors causing reduction in yields in pea-wheat rotations. When both the straw and the stubble of the wheat crop are incorporated in the soil, the nitrogen deficiency is accentuated. Bjälfve (3) and Löhnis (9) noted, however, that symbiotic nitrogen fixation by the pea-nodule bacteria was enhanced in culture media that are poor in available nitrogen. Possibly the deficiency in available nitrogen prevailing in pea-wheat rotations can be reduced in intensity if symbiotic nitrogen fixation can be increased by more effective root-nodule bacteria.

Applications of sulfur or gypsum for alfalfa on Palouse silt loam have been known to result in better growth and larger yields. Neller (10) found evidence of this fact in pot cultures in the greenhouse, but obtained irregular results in plot experiments under field conditions. He attributed part of the beneficial effect of sulfur or gypsum on alfalfa to the stimulative action of sulfur on the activity of the nodule bacteria. The possibility that sulfur compounds may produce similar effects on nodule bacteria of peas is worthy of consideration.

The chief purpose of the studies reported in this paper was to determine the effect of different cultures of *R. leguminosarum* and of applications of gypsum and wheat straw on the growth and yield of seed peas on Palouse silt loam.

EXPERIMENTAL PLANS AND METHODS

Palouse silt loam is a fertile grassland soil. The topography of the seed pea-producing area is hilly and the slopes vary in productivity, depending in a large measure upon the depth of the dark brown topsoil. In virgin soils this depth ranges from less than 8 inches on certain hilltops to more than 20 inches on the lower slopes. The slope, size, and shape of the experimental field available for this study are such that the depth of the dark brown topsoil ranges from less than 6 inches to about 18 inches. This situation has the advantage of representing actual farming conditions and the disadvantage of presenting wide variations in soil productivity.

Insofar as is known, peas had never been produced in the experimental field, but occasional volunteer hairy vetch plants have been found growing there every year for the last 20 years. The inoculation experiment was repeated for three years in succession, and the experimental plots which varied from 1/150 to 1/200 acres in different years, depending upon the availability of suitable space, were located in a new area in the field each year. Plots with the same inoculation treatment were replicated three times and so distributed in the field that as near as possible one plot occupied an area where the depth of topsoil was less than 6 inches, another an area where the depth of topsoil was from 6 to 12 inches, and the third an area where the depth of topsoil exceeded 12 inches.

Alaska peas served as the experimental crop which was produced on

plowed wheat stubble land, except in 1939 when stubble land was not available and summer-fallow land was used instead. The peas were seeded with a "Planet Junior" seeder in rows 9 inches apart at the rate of 160 pounds per acre, a somewhat larger rate than the usual field rate, which is 120 pounds per acre. The seed was inoculated with the various cultures of *R. leguminosarum* in accordance with the methods recommended for field practice with the exception that the concentration of the suspensions was somewhat larger. Aseptic precautions were observed in the inoculation procedure, and those parts of the "Planet Junior" seeder which came in contact with the seed were washed thoroughly with 75 per cent ethyl alcohol each time a lot of peas inoculated with a different culture was introduced. Yields were obtained from three 1-square-yard quadrates harvested from each plot.

The effect of applications of gypsum and of wheat straw to the soil was determined in a pot culture experiment in the greenhouse. Wheat straw treatments were included because in many cases in field practice the straw and stubble of the previous wheat crop are incorporated in the soil before the peas are planted. The soil used for this experiment was taken from the surface 8-inch layer in a field where the topsoil was about 12 inches deep and which had been in a pea-wheat rotation for about 30 years. All the treatments were replicated twice. The wheat straw which was finely ground was mixed with the surface 3 inches of soil, and the gypsum was applied with the pea seed which was planted in two rows in each pot to simulate field planting. The variety of peas was the same as that used for the field plots.

EFFECT OF DIFFERENT CULTURES OF *R. LEGUMINOSARUM* ON NODULATION AND YIELD OF PEAS

The inoculation treatments of the small experimental plots of Alaska peas produced during 1938, 1939, and 1940, and the average yields obtained from three separate square-yard quadrates are given in Table 1.

The most striking characteristic of the yield data is their irregularity as shown in the columns from left to right by different plots planted to peas that received the same inoculation treatment. It is evident, also, that in general and regardless of inoculation treatments the plots where the dark brown topsoil was less than 6 inches in depth produced considerably lower yields than those where the topsoil layer was between 6 and 12 inches thick, and much lower than the plots where the topsoil layer was more than 12 inches thick. The extremely small yields on plots 4, 7, and 8 in 1939 are explainable by the fact that they occupied a typical clay hill knoll where the topsoil was very shallow or nonexistent. In interpreting the experimental results from the standpoint of the effectiveness of different cultures of pea-nodule bacteria, the significant difference in soil productivity based on thickness of the dark-colored topsoil layer and convincingly demonstrated by differences in yield of seed peas as indicated by analysis of variance introduced a disturbing factor. Soil variability

TABLE 1

YIELDS FROM THREE 1-SQUARE-YARD QUADRATES OF ALASKA PEAS INOCULATED WITH MIXED AND SINGLE PHYSIOLOGICAL STRAINS OF *R. LEGUMINOSARUM*

Treatment	*R. leguminosarum* Culture	Topsoil Depth Less Than 6 in.				Topsoil Depth 6 to 12 in.				Topsoil Depth More Than 12 in.				3-year Ave.	Increase Over Non-Inoculated Plots
		1938	1939	1940	Ave.	1938	1939	1940	Ave.	1938	1939	1940	Ave.		
		gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
1	No Inoculation	204	205	230	213	254	216	242	237	280	303	265	283	244	
2	S.C.W.*	279	201	245	242	307	252	260	273	313	296	325	311	275	31
3	L.A.*	263	169	242	225	281	290	287	286	290	297	380	322	278	34
4	N.X.*	271	82	225	193	304	240	317	287	354	415	337	369	283	39
5	W. 317†	238	132	250	207	250	249‡	335	278	298	353	345	332	272	28
6	W. 312†	219	111	220	183	261	296	342	300	316	417	345	359	281	37
7	3 HOQ 12†	233	82	280	198	253	213	305	257	306	357	332	332	262	18
8	3 HOQ 9†	269	91	260	207	272	235	290	266	287	302	325	305	259	15

* Commercial culture.
† Single physiological strain.
‡ Filled in by the method of Yates (13).

ANALYSIS OF VARIANCE*

Source of Variation	Degree of Freedom	Mean Square
Total—Soil groups	23	
Topsoil Depths	2	84,080†
Strain	7	1,551
Interaction (error)	14	1,500

* For significance (5 per cent level) the difference in values required between any two means of cultures or strains is .41.
† Highly significant.

in this case, however, should be considered as a logical and proper part of the experiment, for it represents normal field conditions on the farms in the seed pea-producing area.

Nitrogen deficiency is one of the chief causes of small crop yields on the hilltops and upper slopes. Good crops of wheat can be produced on them when adequate amounts of nitrogen are supplied in the form of nitrogen fertilizers. It might be assumed that with an adequate supply of soil moisture, thoroughly inoculated peas on these soils should be capable of fixing enough atmospheric nitrogen to produce optimum growth and yields, for plant nutrients other than nitrogen do not appear to be deficient, and in years of normal precipitation the supply of soil moisture is not seriously inadequate, as has been shown repeatedly by luxuriant growth of wheat. Bjälfve (3), using quartz sand and soil poor in nitrogen as media in pot cultures for field peas, found that well-inoculated peas grew excellently on the nitrogen they fixed symbiotically. Even though it is not likely that soil moisture or essential plant nutrients other than nitrogen were seriously deficient in the plots with shallow topsoil in our experiment, Bjälfve's results were not verified, for the growth of the peas was poor and the yields were low. Apparently, not enough nitrogen was fixed symbiotically to insure luxuriant growth.

Attempts have been made by various investigators to correlate the effectiveness of different strains or culture selections of rhizobia with the number, distribution, and size of the root nodules of the homologous host plants, but the results were not uniform. Ruf and Sarles (11) contended that effective strains of *R. japonicum* produced a few large nodules near the surface of soybean roots, whereas ineffective strains produced many scattered, small nodules. Erdman and Wilkins (6) found that the percentage of nitrogen fixed by soybeans increased with increasing nodulation of the plants. The work of Baldwin and Fred (2) with various strains of *R. trifolii* revealed that more nodules were formed by the poor strains, but the nodules were small and distributed over the entire root system. Later Baldwin (1) asserted that the number of nodules is not indicative of their beneficial effect on the host plant, although greenhouse experiments indicated that a few large nodules near the top of the taproot gave the greatest benefit, and parasitic strains produced many scattered nodules.

Careful examinations made each year at different growth stages of the Alaska peas in the various plots in our experiment disclosed that although the plants inoculated with different cultures of *R. leguminosarum* sometimes showed apparent differences in size, or distribution, or number of root nodules in the early growth stages, these differences were not stable, as they could not be verified by subsequent examinations at more advanced growth stages. The peas in all the plots, including those produced by noninoculated seed, had an abundance of root nodules and no significant permanent differences were exhibited with respect to size, distribution, and number of nodules.

As may be noted from the data in Table 1, seed inoculation had a tendency to result in increased yields of seed peas. Statistical treatment of the yield data as indicated by the analysis of variance shows that distinct differences in yields of seed peas resulted from variability in soil productivity, and that the beneficial effect caused by inoculation should be represented by a mean increase in yield of 41 grams in order to be significant. This value was closely approached by two of the seven cultures used, one, N. X., being a commercial culture probably composed of mixed strains of organisms, and the other, W. 312, a single physiological strain. The difference in effectiveness of the various cultures used for inoculation was not significant, but the *R. leguminosarum* organisms present in the soil and probably maintained in active state from year to year by the few scattered vetch plants proved to be less effective in benefiting Alaska seed peas.

EFFECT OF WHEAT STRAW AND GYPSUM ON THE GROWTH OF PEAS

The 1-gallon pots used in this experiment were filled with equal amounts of Palouse silt loam taken from the field described in the experimental plan. Excessively large applications of wheat straw were made in order to reduce the supply of available nitrogen in the soil to a minimum and determine the effect of this factor on the growth of the peas. Gypsum was applied at different rates and placed in contact with the seed in the seed rows to ascertain if larger quantities than those used and drilled in with the seed in field practice would affect germination. The same number of seeds which were inoculated with one of the commercial cultures used in the field plots were planted in each pot, and after emergence the plants were thinned to 12 per pot. The tops and roots were harvested separately at the time vegetative growth ceased. The rates of application of straw and gypsum, and the weights of dry matter of tops and roots of the peas are recorded in Table 2.

TABLE 2

YIELDS OF DRY MATTER OF TOPS AND ROOTS OF ALASKA PEAS GROWN IN 1-GALLON POTS ON PALOUSE SILT LOAM TREATED WITH WHEAT STRAW AND GYPSUM

Pot No.	Treatments	Rate per Acre	Tops	Ave.	Roots	Ave.
		lbs.	*gms.*	*gms.*	*gms.*	*gms.*
3 & 4	Check		28.1		3.1	
			13.7	20.9	2.3	2.7
5 & 6	Wheat straw ..	20,000	18.4		3.4	
			17.5	18.0	2.9	3.3
7 & 8	Wheat straw ..	20,000	13.6		2.1	
	Gypsum	200	16.6	15.1	2.7	2.4
9 & 10	Gypsum	100	16.9		2.7	
			15.4	16.2	2.0	2.4
11 & 12	Gypsum	200	19.4		2.6	
			11.8	15.6	2.0	2.3
13 & 14	Gypsum	400	20.0		2.4	
			21.2	20.6	2.7	2.6

Close observations made of the plants at progressive stages of maturity disclosed that germination was excellent and unaffected by any of the soil treatments. All plants, regardless of treatment, had an abundance of well-developed nodules on their roots. At the bloom stage the plants in the straw-treated pots were less vigorous and slightly less green in color than those in the other pots, particularly those in the pots treated with 200 and 400 pounds of gypsum per acre. At the time of harvest the plants in the straw-treated pots were more advanced in maturity than those in the other pots. The gypsum treatments had a tendency to prolong the period of active growth.

As may be noted from the yield data, the amount of dry matter produced by the tops and roots of the peas varied considerably regardless of treatments. Because of these variations the experimental data may not be conclusive, but it seems that the apparent differences in yield resulting from any of the treatments are insignificant. If stimulation of symbiotic nitrogen fixation occurred at all as a result of applications of gypsum or of reduced quantities of available soil nitrogen caused by the addition of large quantities of straw to the soil, it was insufficient in amount to effect significant increases in the production of vegetative matter of the peas.

Although apparently better yields of peas in the field plots were obtained from inoculated seed than from noninoculated seed, they were not significant statistically, and the nodulation and yield data did not indicate any significant difference in the effectiveness of seven selected cultures of *R. leguminosarum* to benefit the peas. Additions of straw and gypsum to the soil did not seem to be significant factors in influencing the effectiveness of one of these cultures. It would seem, therefore, that under the prevailing practice of growing peas and wheat in alternate years on Palouse silt loam in the seed pea-producing area, seed inoculation is not likely to be greatly beneficial once the soil has been thoroughly inoculated with an effective culture of *R. leguminosarum*, unless further investigation reveals that repeated passage of this organism through the same host plant reduces its beneficial effectiveness.

SUMMARY

A study was made to determine the effect of seven different cultures of *R. leguminosarum* on the nodulation and yield of Alaska seed peas grown three years in succession in small field plots on Palouse silt loam which had not produced peas before. One of these cultures was used to determine the influence of applications of large quantities of wheat straw and of different amounts of gypsum to the soil in pot cultures in the greenhouse on the effectiveness of the culture in benefiting the host plant.

The soil in the experimental field was found to contain a sufficient number of *R. leguminosarum* to effect thorough inoculation of the peas. Seed inoculation with seven different cultures had a tendency to cause increases in yields of seed peas. Two of these cultures, a commercial culture and a single physiological strain, resulted in increased yields that approached significance statistically.

Significant differences in yield of peas occurred as a result of variability in soil productivity, caused in a large measure by differences in depth of dark brown topsoil and in the supply of available nitrogen.

None of the cultures was sufficiently effective in symbiotic nitrogen fixation to insure vigorous growth of the peas on soil which was low in available nitrogen, and no significant difference was observed in the number, size, and distribution of the root nodules regardless of inoculation treatments.

The large quantities of straw applied to the soil in pot cultures in the greenhouse and the different amounts of gypsum placed in contact with the seed had no detrimental effect on seed germination. These treatments had no marked influence on the effectiveness of the *R. leguminosarum* culture used for seed inoculation either in producing root nodules or in benefiting the growth of the host plant.

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AN IDENTIFICATION SCHEME FOR NUMBERING CULTURES OF *RHIZOBIA*

LEWIS W. ERDMAN[1]

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The accumulation of large numbers of cultures of *Rhizobia* isolated from different genera, species, and varieties of legumes presents a real problem in the absence of a well-planned system for numbering individual cultures. Some scheme for labeling *Rhizobia* cultures to reveal their immediate identity with respect to source history and cross-inoculation group affiliation would seem to be highly desirable and almost necessary where large numbers of cultures are kept in stock. Such a scheme would prove especially valuable if adopted universally by the various laboratories and institutions working with these bacteria.

The purpose of this paper is to offer an identification scheme which the author believes would simplify and standardize the labeling of legume bacteria cultures. The idea for this scheme originated while working on the Iowa Agricultural Experiment Station project, "Studies on Rhizobium".[2] One phase of this project included the isolation and testing of cultures of *Rhizobia* from nodules of legumes widely separated botanically. An outline of the family *Leguminosae* was drafted with particular emphasis at that time being placed upon available species of the different cross-inoculation groups found growing in Iowa soils.[3] The books of Bailey (1), Britton and Brown (2), Engler and Prantl (3), Asa Gray (5), and Piper (6) were used as texts in working up the outline. The family *Leguminosae* is divided into three subfamilies, *Mimosoideae, Caesalpinioideae,* and *Papilionatae.* These subfamilies are subdivided into tribes, and many tribes in the *Papilionatae* are further divided into subtribes. Under the tribes and subtribes are listed the species of legumes. The scheme of numbering and lettering of the various subdivisions is shown in the outline given below. Where no subtribe is given the capital letter O is inserted.

FAMILY *LEGUMINOSAE*

1. Subfamily MIMOSOIDEAE
   - A. Tribe *Acacieae*
     - O. a. *Acacia filiculoides* (Prairie acacia)
       - b. *Acacia armata*
       - c. *Acacia linifolia*

[1] Formerly Assistant Chief in Soil Bacteriology, Iowa Agricultural Experiment Station. Now Director of Research and Production, The Nitragin Company, Inc., Milwaukee, Wisconsin.

[2] This Project was started by the late Dr. P. E. Brown, and under his capable guidance numerous scientific contributions were made in this highly specialized field. Everyone who was privileged to work with Doctor Brown considered this association a rare pleasure.

[3] Valuable assistance with this outline was gratefully received from Dean R. E. Buchanan, who has always shown a keen interest in the legume bacteria.

B. Tribe *Eumimoseae*
O. a. *Mimosa pudica* (Cultivated sensitive plant)
b. *Acuan illinoiensis (Desmanthus brachylobus)*
c. *Morongia uncinata (Schrankia uncinata)*

2. Subfamily CAESALPINIOIDEAE
A. Tribe *Bauhinieae*
O. a. *Cercis canadensis* (Red bud)
B. Tribe *Cassieae*
O. a. *Cassia chamaecrista (fasciculata)* (Partridge pea)
b. *Cassia marilandica* (Wild senna)
c. *Cassia nictitans*
C. Tribe *Kramerieae*
O. a. *Krameria secundiflora*
D. Tribe *Eucaesalpinieae*
O. a. *Gleditschia triacanthos* (Honey locust)
b. *Gleditschia aquatica*
c. *Gymnocladus dioeca* (Kentucky coffee tree)
d. *Hoffmanseggia jamesii*

3. Subfamily PAPILIONATAE
A. Tribe *Sophoreae*
O. a. *Sophora sericea* (Silky sophora)
b. *Cladrastis lutea* (American Yellow Wood)
B. Tribe *Podalyrieae*
O. a. *Thermopsis rhombifolia* (Prairie Thermopsis)
b. *Baptisia tinctoria* (Wild indigo)
c. *Baptisia bracteata* (Large bracted wild indigo)
d. *Baptisia leucantha*
e. *Baptisia australis*
f. *Baptisia alba*
C. Tribe *Genisteae*
1. Subtribe *Crotolariinae*
a. *Crotolaria sagittalis* (Rattle Box)
b. *Crotolaria juncea* (Sunn hemp)
c. *Crotolaria capensis*
d. *Crotolaria retusa* (Cherokee clover)
e. *Crotolaria candicans*
f. *Crotolaria spectabilis*
2. Subtribe *Spartiinae*
a. *Lupinus argenteus* (Silvery lupine)
b. *Lupinus albus* (White lupine)
c. *Lupinus perennis* (Wild lupine)
d. *Lupinus luteus* (Yellow lupine)
e. *Lupinus angustifolius* (Blue lupine)
f. *Lupinus polyphyllus*
g. *Lupinus hirsutus*
h. *Lupinus texensis* (Texas blue bonnet)

3. Subtribe *Cytisinae*
   a. *Ulex europaeus* (Thorn Broom)
   b. *Cytisus scoparius* (Scotch Broom)

D. Tribe *Trifolieae*

O. a. *Medicago sativa* (Alfalfa or Lucerne)
   b. *Medicago lupulina* (Black Medic)
   c. *Medicago arabica* (Southern Giant Bur clover)
   d. *Medicago hispida* (Bur clover)
   e. *Medicago arabica* var. (Manganese Bur clover)
   f. *Medicago orbicularis* (Button clover)
   g. *Melilotus indica* (Annual yellow sweet clover)
   h. *Melilotus alba* (Biennial white sweet clover)
   i. *Melilotus officinalis* (Yellow sweet clover)
   j. *Melilotus alba* (an.) (Hubam clover)
   k. *Trifolium pratense* (Red clover)
   l. *Trifolium hybridum* (Alsike clover)
   m. *Trifolium repens* (White clover)
   n. *Trifolium repens* var. (Ladino clover)
   o. *Trifolium agrarium* (Yellow or hop clover)
   p. *Trifolium procumbens* (Smaller hop clover)
   q. *Trifolium incarnatum* (Crimson clover)
   r. *Trifolium alexandrinum* (Berseem clover)
   s. *Trifolium reflexum* (Buffalo clover)
   t. *Trifolium glomeratum* (Cluster or McNeill clover)
   u. *Trifolium resupinatum* (Persian clover)
   v. *Trifolium fragiferum* (Strawberry clover)
   w. *Trifolium subterraneum* (Subterranean clover)
   x. *Trifolium dubium* (Little hop clover)

E. Tribe *Loteae*

O. a. *Lotus corniculatus* (Bird's-foot trefoil)
   b. *Lotus americanus (Trigonella Foenum-Graecum)* (Fenugreek)
   c. *Lotus uliginosus*
   d. *Lotus tetragonolobus* (Square pod pea)
   e. *Anthyllis vulneraria* (Kidney vetch)

F. Tribe *Galegeae*

1. Subtribe *Indigoferinae*
   a. *Cyamopsis tetragonoloba* (Guar)
   b. *Indigofera leptosepala* (Wild indigo)
   c. *Indigofera tinctoria*
2. *Subtribe Psoraliinae*
   a. *Parosela enneandra*
   b. *Parosela dalea (Dalea alopecuroides)*
   c. *Amorpha canescens* (Lead plant)
   d. *Amorpha fruticosa* (False or Bastard indigo)
   e. *Amorpha nana* (Fragrant false indigo)

f. *Kuhnistera (Petalostemom) candida*
g. *Kuhnistera purpurea*
h. *Psoralea tenuiflora* (Few-flowered psoralea)
i. *Psoralea floribunda* (Many-flowered psoralea)
j. *Psoralea argophylla* (Silver-leaf psoralea)

3. Subtribe *Tephrosiinae*
   a. *Galega officinalis* (Goat's rue)
   b. *Tephrosia (Cracca) virginiana*
   c. *Kraunhis (Wisteria) frutescens*
4. Subtribe *Robiniiae*
   a. *Sesbania macrocarpus* (Long-podded sesbania)
   b. *Robinia pseudacacia* (Black locust)
   c. *Robinia hispida* (Rose acacia)
   d. *Robinia viscosa*
5. Subtribe *Coluteinae*
   a. *Swainsona* sp.
6. Subtribe *Astragalinae*
   a. *Astragalus rubyi* (Green)
   b. *Astragalus falcatus*
   c. *Phaca neglecta* (Cooper's milk vetch)
   d. *Oxytropis (Spesia) (lamberti)* (Crazy weed)
   e. *Glycyrrhiza lepidota* (Wild liquorice)
   f. *Glycyrrhiza glabra*
   g. *Caragana arborescens* (Siberian pea tree)
   h. *Astragalus sinicus*
   i. *Astragalus canadensis* (Cow vetch)

G. Tribe *Hedysareae*

1. Subtribe *Coronillinae*
   a. *Ornithopus sativa* (Seradella)
   b. *Coronilla varia* (Axseed) (Crown vetch)
2. Subtribe *Euhedysarinae*
   a. *Hedysarum coronarium* (Spanish sanfoin)
   b. *Hedysarum americanum*
   c. *Onobrychis sativum* (Sanfoin)
   d. *Onobrychis viciaefolia*
3. Subtribe *Aeschynomeninae*
   a. *Aeschynomene virginica* (Sensitive joint vetch)
4. Subtribe *Stylosanthinae*
   a. *Stylosanthes biflora* (Pencil flower)
   b. *Arachis hypogaea* (Peanut)
5. Subtribe *Desmodiinae*
   a. *Desmodium purpureum* (Florida beggarweed)
   b. *Meibomia grandiflora*
   c. *Meibomia canescens*
   d. *Meibomia paniculata*
   e. *Meibomia illinoensis*

f. *Lespedeza hirta* (Hairy bush clover)
g. *Lespedeza striata* (Common Japan clover)
h. *Lespedeza capitata*
i. *Lespedeza stipulacea* (Korean)
j. *Lespedeza serecia*
k. *Lespedeza kobe*
l. *Lespedeza virginia*
m. *Lespedeza juncea*

H. Tribe *Vicieae*
O. a. *Cicer arietinum* (Chick pea)
b. *Vicia americana* (American vetch)
c. *Vicia hirsuta* (Hairy vetch)
d. *Vicia* sp. (Calcarata vetch)
e. *Vicia caroliniana* (Carolina vetch)
f. *Vicia sativa* (Common vetch)
g. *Vicia faba* (Broad bean)
h. *Vicia angustifolia* (Narrow-leaved vetch)
i. *Vicia atropurpurea* (Purple vetch)
j. *Vicia monantha* (Monantha vetch)
k. *Lathyrus odoratus* (Sweet pea)
l. *Lathyrus sativus* (Grass pea)
m. *Lathyrus tingitanus* (Tangier pea)
n. *Lathyrus latiofolius* (Perennial sweet pea)
o. *Lathyrus ochrus* (Ochrus)
p. *Lathyrus silvestris* (Flat pea)
q. *Pisum sativum*
r. *Pisum hortense* (Garden pea)
s. *Pisum sativa arvense* (Canada field pea)
t. *Pisum arvense* var. (Austrian winter pea)

I. Tribe *Phaseoleae*
1. Subtribe *Glycininae*
a. *Amphicarpa (monoica)* (Hog peanut)
b. *Glycine hispida* (Soybean)
c. *Clitoria mariana*
d. *Centrosema virginianum*
2. Subtribe *Erythrininae*
a. *Apios tuberosa* (Ground nut)
b. *Erythrina indica*
3. Subtribe *Galactiinae*
a. *Galactia regularis* (Milk pea)
4. Subtribe *Diocleinae*
a. *Pueraria thunbergibiana* (Kudzu)
b. *Canavalia eusiformis* (Jackbean)
5. Subtribe *Cajaninae*
a. *Rhynchosia latifolia* (Prairie rhyncosia)

6. Subtribe *Phaseolinae*
   a. *Phaseolus acutifolius*
   b. *Phaseolus polystachys* (Wild bean)
   c. *Phaseolus vulgaris* (Kidney or string bean)
   d. *Phaseolus lunatus* (limensis) (Lima bean)
   e. *Phaseolus multiflorus* (Scarlet runner)
   f. *Phaseolus angularis* (Adzuki bean)
   g. *Phaseolus acontifolius* (Moth bean)
   h. *Phaseolus aureus* (Mung bean)
   i. *Phaseolus mungo* (Urd)
   j. *Phaseolus vulgaris* var. (Field bean, Navy bean)
   k. *Phaseolus* sp.
   l. *Strophostyles helvola* (Trailing wild bean)
   m. *Strophostyles pauciflora* (Small wild bean)
   n. *Vigna sinensis* (Cow pea)
   o. *Dolichos lablab* (Egyptian or black bean)
   p. *Stizolobium deeringianum* (Florida velvet bean)

From a study of this outline it is obvious that relatively few of the vast number of legume species are included. An attempt was made, however, to list the more important cultivated and wild species which are readily available for study. Wilson and Sarles (7) have published a very complete list of all the legumes from the nodules of which the root nodule bacteria have been isolated and studied.

HOW THE CULTURES OF RHIZOBIA ARE IDENTIFIED

Assume that a pure culture of *Rhizobium* has been isolated from a red clover nodule. In the outline, red clover, *Trifolium pratense,* is found under 3 Subfamily *Papilionatae,* D Tribe *Trifolieae,* O no Subtribe, k *Trifolium pratense.* Its number would be 3DOk1; the figure 1 added after 3DOk would represent the first isolation or the first red clover culture to be numbered. A second isolation or the second culture to be numbered would be 3DOk2. If more than one colony is picked from a plate poured from the first nodule isolation, the second and third culture obtained in this manner would be numbered 3DOk1 (a) and 3DOk1 (b), respectively. Several other examples are given here to show how the scheme works. An isolation is made and a pure culture obtained from a Korean lespedeza nodule. Its designation would be 3G5i1, from 3 Subfamily, G Tribe *Hedysareae,* 5 Subtribe *Desmodiinae,* and i *Lespedeza stipulacea.* A pure culture of soybean bacteria would be 3I1b1, from 3 Subfamily, I Tribe *Phaseoleae,* 1 Subtribe *Glycininae,* and b *Glycine hispida.*

A record is kept on filing cards for each culture showing its identification number, name of the organism, scientific and common name of the host plant, source of nodule, date of isolation, growth characteristics on different media, nitrogen-fixation data and microscopic examination. A sample card for an alfalfa culture is shown below. This additional record contains the more specific data for reference when necessary.

Laboratory No. 3DOa2
Name of Organism: *Rhizobium meliloti*
Host plant: Scientific Name *Medicago sativa*
Common Name Alfalfa (Grimm)
Source of Nodules Waukesha Co. Wisconsin
Date of Isolation September 1938
Test On:
No. 79 Whitish, flaky medium heavy growth
L.M. Acid serum zone
A.M. Medium growth white smooth
Ca G.P.
B.T.B. 79 Acid
Host Plant Alfalfa 57 mg. N. 20 plants 6-13-40
Microscopic Examination O.K.

DISCUSSION

This scheme of numbering cultures of *Rhizobia* has been in successful operation in our laboratory for a number of years and has aroused considerable interest among other workers. The suggestion has been made that the use of Roman numerals be used to affiliate each culture with its proper cross-inoculation group. This might be desirable for those workers who do not work with *Rhizobia* exclusively. Fred, Baldwin and McCoy (4) have listed the various cross-inoculation groups designating each group by a Roman numeral, and these could easily be affixed to each culture number for this identification.

Some of the advantages of this identification scheme for numbering cultures of *Rhizobia* may be summarized as follows:

1. It would standardize the labeling of all legume cultures.
2. It immediately identifies the culture with respect to host plant source history and cross-inoculation group affiliation.
3. The increasing numbers of cultures isolated from any species of legume does not necessitate changing existing culture numbers regardless of how many may accumulate.
4. The identification number of any *Rhizobia* culture will be much shorter than actually writing the name of the legume and using a simple number afterward, such as Crimson clover 1, 2, etc., or Soybean 1, 2, etc.
5. The last figure in each culture number furnishes a ready chronological reference as to the approximate date of isolation. Exact dates are kept on the cards previously described.

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FACTORS INFLUENCING THE SOLUBILITY OF IRON AND PHOSPHORUS IN CHLOROTIC AND NONCHLOROTIC AREAS OF HYRUM CLAY LOAM

D. WYNNE THORNE

From the Department of Agronomy, Utah Agricultural Experiment Station

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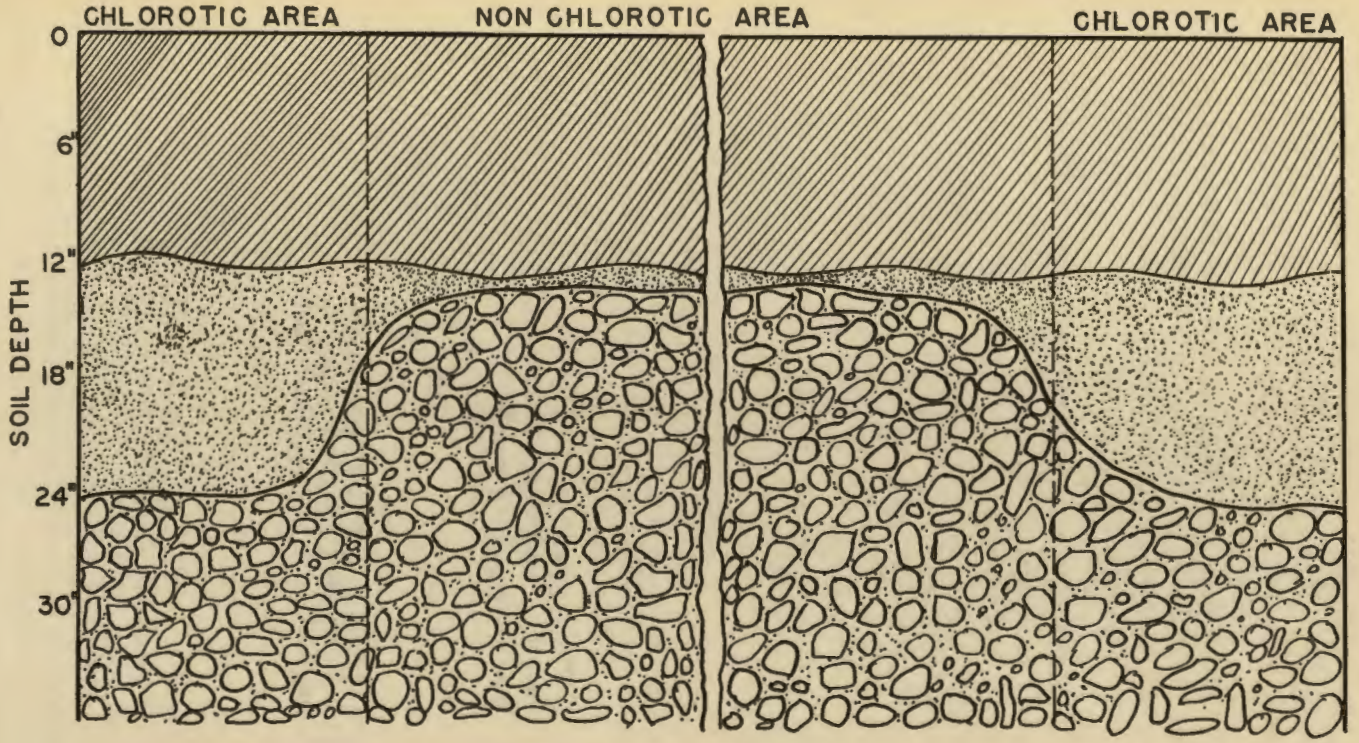
The soils of Utah and adjoining states are predominantly calcareous, and the pH is generally higher than considered optimum for most crops. Consequently, fertility problems of this area differ from those of humid regions. As in humid sections, phosphate unavailability is a problem, but the phosphate compounds in arid soils appear to be different from those in humid soils. Some phosphate fertilizers which are successful in humid areas bring about little plant response in the Rocky Mountain region. Probably the most extensive study of phosphate relations in calcareous soils has been made by McGeorge and associates in Arizona. According to their findings, the principal factors influencing phosphate availability under Arizona conditions are pH, soluble salts, drainage, aeration conditions, and organic matter content (9).

Another common problem associated with western calcareous soils is unavailability of iron and manganese, causing chlorosis. Chlorosis resulting from lack of iron is more marked in Utah than that resulting from manganese deficiency. In this state chlorosis attacks especially fruit trees, berries, roses, and ornamental shrubs. It occurs sporadically, and in some affected orchards badly chlorotic trees may be surrounded by healthy trees of the same variety.

Many studies have attempted to characterize soils on which chlorosis is prevalent. In general no relation has been found between chlorosis and the total iron content or the pH of soils. Ciferri (4) found greater quantities of lime in chlorotic soils than in closely adjacent nonchlorotic soils. Menchikousky and Preffeles (11) found that in the Jordan Valley chlorosis occurred on soils high in chlorides. Lipman (6) reported that in California chlorosis is associated with relatively high soil contents of phosphorus, potassium, and sodium, and with low total soluble salts, nitrates, calcium, and magnesium, as compared with normal soils.

No well-developed methods have been presented for estimating available iron in calcareous soils. Monnier and Kuczraski (12) demonstrated that in a calcareous soil, traces of iron could be brought into solution by leaching with solutions of acetic, oxalic, citric, and tartaric acids. Rigg (13) found that in New Zealand soils, iron soluble in 0.1 to 5 per cent oxalic acid was much lower in areas where animals suffered from bush sickness than in areas where animals were normal.



SKETCH SHOWING CROSS SECTION OF SOIL PROFILE CHARACTERISTICS OF ADJACENT CHLOROTIC AND NON-CHLOROTIC AREAS IN THE COLLEGE GRAPE TESTING PLOT. (HYRUM CLAY LOAM SOIL.)

INDICATES FRIABLE CLAY LOAM.
INDICATES COMPACT FINE SANDY CLAY TO FINE SANDY LOAM.
INDICATES COARSE GRAVEL AND COBBLESTONES

Fig. 1.

OUTLINE OF PROCEDURE

The present study was planned to investigate the interrelations of a number of characteristics of a highly calcareous soil, particularly in relation to the occurrence of chlorosis.

A survey of Utah County, Utah, indicated that about 85 per cent of chlorosis occurs on the Pleasant Grove, Orem, and Mapleton soils series. These soils are closely similar in profile characteristics to the Hyrum series which has a much wider distribution.

For several years a small plot of land on the Utah State Agricultural College campus, in an area of Hyrum clay loam, has been used as a testing plot to determine the susceptibility of plants to chlorosis. Concord grapes planted on the land became severely chlorotic except in a small area in the south central part of the field, where plants remained green and healthy. Injections and spraying with iron solutions have shown the chlorotic plants to be suffering from lack of available iron. (For convenience, soils producing chlorotic plants will be termed "chlorotic" soils, whereas those producing normally green plants will be termed "nonchlorotic" soils.)

A sewer pipe trench recently dug across the areas of chlorotic and nonchlorotic soils in the testing plot revealed a distinct difference in soil profile characteristics between the two areas. A sketch of the soil profile cutting across the field through the areas of chlorotic and nonchlorotic soils is shown in Figure 1.

This soil is very stratified, having been laid down as a delta by Logan River at the time Lake Bonneville covered much of northern and central Utah. The top soil of the field is about 12 inches deep and is a highly calcareous, dark brown, friable clay loam. It has a uniform appearance throughout the field. The top soil grades abruptly into a compact light gray layer varying in texture from a sandy clay at the top to a fine sand at greater depths. The depth of this second layer seems to be the principal profile difference between the chlorotic and nonchlorotic areas of soil in the field. The layer is much deeper in the chlorotic areas. The compact second layer grades into a third layer of coarse sand, gravel, and cobblestones. Near the top of the third layer is some lime accumulation on the lower sides of the cobblestones.

The initial soil samples were taken with an auger and shovel. Eight locations, four of chlorotic soil and four of nonchlorotic, were sampled at 6-inch intervals to depths of 3 feet. Later, when the trench was dug across the field, additional soil samples were taken from the first, second, third, and fourth feet of the soil at four points in the chlorotic area and at four points in closely adjacent nonchlorotic areas.

Unless otherwise specified, analyses are based on that fraction of soil passing a 20-mesh sieve. All unnecessary grinding was avoided because in some instances grinding greatly affects the determinations. This is somewhat in agreement with the statement of Chapman (3) that under

irrigation conditions soil particles frequently become coated with lime, making plant nutrients less available.

The pH values of the soil were determined with a Beckman glass electrode pH meter. Determinations were made with a soil paste (two parts soil, one part water) and with a 1:10 dilution. The determinations were repeated with tap water and with boiled distilled water. Total inorganic carbonates were determined by the method of Schollenberger (14).

Carbon dioxide-soluble calcium was determined by bubbling carbon dioxide through a 1:20 soil-distilled water suspension for 30 minutes, filtering, and determining calcium by the soap titration method. (1). Various lengths of time and rates of flow for passing the carbon dioxide through the soil suspension showed that reproducible results could be obtained with the conditions selected.

Available phosphorus was extracted by the pH 5.0 acetate buffer proposed by Dahlberg and Brown (5). This type of extraction has been found to correlate better with crop responses in Utah soils than other methods investigated.

Oxalic acid-soluble iron was determined by shaking the soil in a 1:5 dilution with the acid solution for 10 minutes, allowing to settle for 30 minutes, filtering, adding 5 ml. of 1-1 hydrochloric acid to 25 ml. of filtrate, oxidizing the oxalate with potassium permanganate, and developing color by the addition of potassium thiocyanate solution. Color intensity readings were taken with an Aminco Type F photo-electric colorimeter.

RESULTS

A general view of the textural characteristics of the soil profiles is shown by the percentage of soil, from each successive foot in depth, which could pass a 20-mesh sieve without having the larger sand and gravel particles broken up. The averages of these data for the chlorotic and nonchlorotic soils are shown in Table 1. These data are included in the statistical analyses as the only usable measurements indicating soil depth, texture, aeration, and drainage.

The data in Table 1 support the profile sketch of Figure 1. In the nonchlorotic soil, a predominance of particles larger than the holes of a

TABLE 1

AVERAGE PERCENTAGE OF CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL PASSING THROUGH A TWENTY-MESH SIEVE

DEPTH OF SOIL	SOIL PASSING THROUGH A 20-MESH SIEVE	
	CHLOROTIC SOIL	NONCHLOROTIC SOIL
	Percentage	*Percentage*
0-12″	97.2	95.1
12-24″	98.0	26.8
24-36″	99.0	15.3
36-48″	56.6	30.8

20-mesh screen occur in the second foot while in the chlorotic soil such a condition is not encountered until the fourth foot.

A 1:10 water extract of the soil samples was made, and the soluble calcium, magnesium, sodium, potassium, chlorides, sulfates, bicarbonates, nitrates, and phosphates in the extracts were determined. No consistent difference between samples or between chlorotic and nonchlorotic soils was found, and so the data are not reported.

With tap water the pH data were closely similar in the 2:1 and 1:10 soil-water mixtures. The data obtained with the 2:1 ratio with boiled distilled water were only slightly different from the determinations with tap water. The 1:10 dilution with boiled distilled water, however, gave much higher values. Since such a figure is described by McGeorge (7, 8) as representing the maximum pH brought about by the hydrolysis of basic soil constituents, it is termed "hydrolyzable" pH. The average pH data for the 2:1 ratio of soil and tap water and the 1:10 ratio of soil and boiled distilled water (hydrolyzable pH) are shown in Table 2.

TABLE 2

AVERAGE pH VALUES FOR CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL IN 2:1 RATIO WITH TAP WATER AND 1:10 RATIO WITH BOILED DISTILLED WATER

DEPTH OF SAMPLE	CHLOROTIC SOIL		NONCHLOROTIC SOIL	
	pH in Tap Water	Hydrolyzable pH	pH in Tap Water	Hydrolyzable pH
0-12"	8.19	8.81	8.31	8.57
12-24"	8.25	9.18	8.41	8.93
24-36"	8.33	9.25	8.39	9.31
36-48"	8.29	9.34	8.44	9.38

There was no significant difference in the pH of chlorotic and nonchlorotic soils moistened with tap water. The chlorotic soils did, however, have a significantly higher hydrolyzable pH. The difference was greater in the 0-12-inch and 12-24-inch depths than at lower depths.

The data for total inorganic carbonates and carbon dioxide-soluble calcium are presented in Table 3. The average values for both determinations are higher in the chlorotic soils at all depths. In the case of total

TABLE 3

AVERAGE PERCENTAGE TOTAL CALCIUM CARBONATE AND PERCENTAGE CARBON DIOXIDE-SOLUBLE CALCIUM IN CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM

DEPTH OF SAMPLE	CHLOROTIC SOIL		NONCHLOROTIC SOIL	
	Total $CaCO_3$	CO_2-soluble Ca	Total $CaCO_3$	CO_2-soluble Ca
	Percentage	*Percentage*	*Percentage*	*Percentage*
0-12"	22.7	0.481	5.20	0.208
12-24"	47.4	0.553	39.20	0.203
24-36"	53.4	0.534	47.40	0.381
36-48"	50.6	0.438	46.30	0.294

carbonates, however, the differences are only consistent in the 0-12, and 12-24-inch depths.

The carbon dioxide-soluble calcium and the percentage of total lime expressed as calcium carbonate in several samples of native limestone formations which serve as parent materials for many Utah soils are shown in Table 4. The samples were ground to pass a 40-mesh screen. The carbon dioxide-soluble calcium in the limestone formations was similar in all cases except for the marl which was definitely more soluble.

TABLE 4

TOTAL LIME AND CARBON DIOXIDE-SOLUBLE CALCIUM IN VARIOUS COMMON LIMESTONES OF UTAH

Type of Limestone	Source	Total $CaCO_3$	CO_2-soluble Ca.
		Percentage	*Percentage*
White Marl (Pleistocene Bonneville)	White Valley, Utah	59.6	0.816
Limestone	Cotton Wood Canyon	42.0	0.68
Fresh water algal Limestone	Thistle Canyon	37.9	0.718
Limestone, Salt Lake formation	Collinston area	31.0	0.696
Limestone, Madison formation	Providence Canyon	38.2	0.673
Limestone	San Juan County	42.8	0.696

Several solvents were tried in an attempt to distinguish between the solubility of iron in the chlorotic and nonchlorotic soil samples. Some differences were obtained with acetic, tartaric, and citric acids indicating a generally greater solubility of iron in the nonchlorotic soils, but much greater differences were obtained with oxalic acid. The differences obtained with oxalic acid, however, were somewhat dependent upon the proportion of acid to soil. With a 1:5 suspension, a strength of less than about 0.25 per cent usually extracted only traces of iron, while solutions more concentrated than 1.5 per cent extracted large quantities of iron. Differences between the chlorotic conditions of the soil were not distinct, however, with the more concentrated solutions of oxalic acid. Best results with this soil were obtained with 0.25 and 0.5 per cent oxalic acid. The data for these two strengths are reported in Table 5.

TABLE 5

AVERAGE OXALIC ACID-SOLUBLE IRON IN CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL

Depth of Sample	Soluble Iron in Chlorotic Soil		Soluble Iron in Nonchlorotic Soil	
	In 0.25% Oxalic Acid	In 0.5% Oxalic Acid	In 0.25% Oxalic Acid	In 0.5% Oxalic Acid
	ppm	*ppm*	*ppm*	*ppm*
0-12"	0.85	2.05	3.00	19.08
12-24"	0.45	1.01	2.33	22.50
24-36"	0.60	0.70	0.20	1.45
36-48"	1.03	1.15	0.44	8.96

The principal differences in iron solubility occurred in the upper 24 inches. Since most plants have a larger proportion of feeder roots concentrated above the 24-inch depth, the data give a clear distinction between the chlorotic and nonchlorotic soils.

To check the influence on some of the determinations of fineness of grinding the soil, some samples were divided into portions which were ground to pass through 20, 40, 60, 80, and 100-mesh sieves. Iron soluble in 0.5 per cent oxalic acid and carbon dioxide-soluble calcium in portions of one sample ground to the different degrees of fineness are shown in Figure 2. The data indicate that the grinding procedure increases carbon dioxide-soluble calcium relatively more than soluble iron.

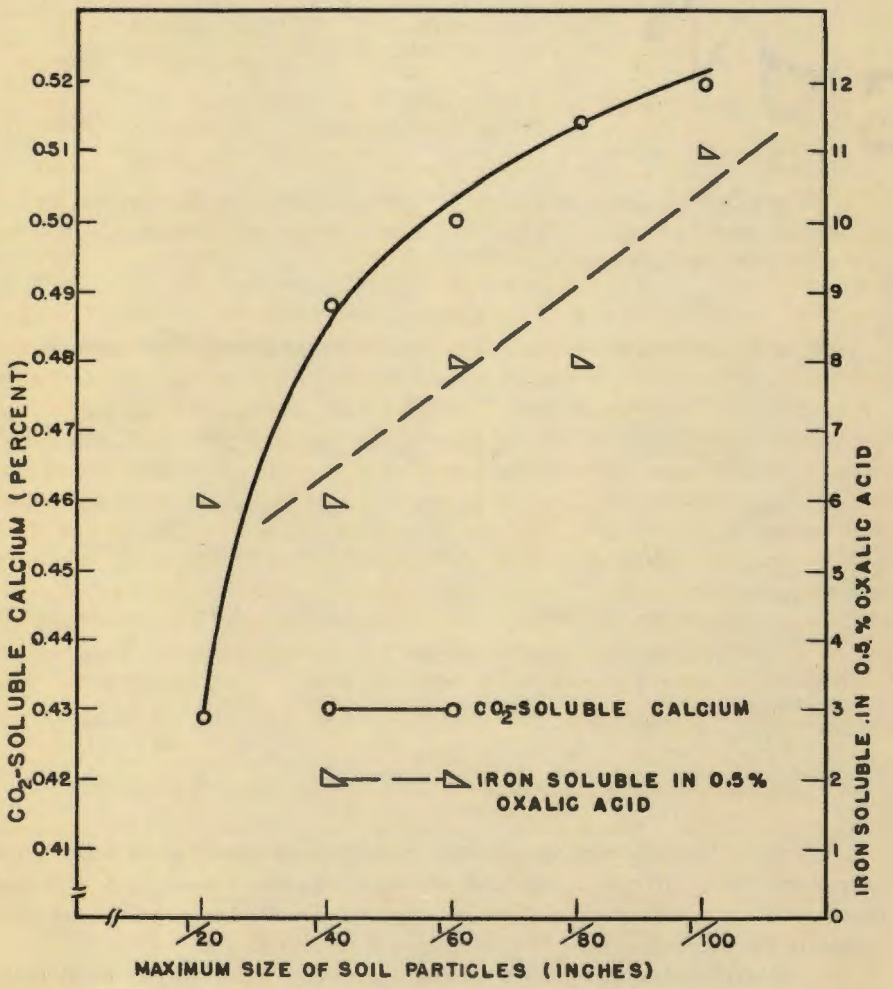


FIG. 2.—Influence of fineness of grinding of soil on carbon dioxide-soluble calcium and iron soluble in 0.5 per cent oxalic acid.

Since phosphate unavailability is also common in highly calcareous soils, and since its concentration has been reported related to the occurrence of chlorosis, phosphate determinations were included in the analyses. Averages of the results obtained are given in Table 6. Although average available phosphorus is slightly higher in the nonchlorotic soil, the differences are not significant.

TABLE 6

AVERAGE AVAILABLE PHOSPHORUS IN CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL

DEPTH OF SAMPLE	AVAILABLE PHOSPHORUS	
	Chlorotic Soil	Nonchlorotic Soil
	ppm	*ppm*
0–12"	2.01	2.33
12–24"	0.55	0.99
24–36"	0.09	1.18
36–48"	0.065	0.55

Simple correlation coefficients between the data for the various determinations were calculated (Table 7). From these calculations, the following relations are indicated:

TABLE 7

CORRELATION COEFFICIENTS BETWEEN VARIOUS VALUES FOR HYRUM CLAY LOAM SOIL

	Fe soluble in 0.5% Oxalic Acid	Available Phosphorus	Percentage Soil Passing 20-mesh Sieve	Percentage $CaCO_3$	CO_2-Soluble Ca	Hydrolyzable pH	pH in Tap Water
Fe soluble in 0.5% oxalic acid	.....	0.264	—0.245	—0.506	—0.705	—0.416	—0.099
Available phosphorus	0.264	.....	0.132	—0.869	—0.917	0.776	—0.202
Percentage soil passing 20-mesh sieve	—0.245	0.132	.....	—0.288	0.399	—0.376	—0.520
Percentage total $CaCO_3$	—0.506	—0.869	—0.288	.....	0.582	0.866	0.401
CO_2-soluble Ca	—0.705	—0.917	0.399	0.582	.....	0.477	—0.227
Hydrolyzable pH	—0.416	0.776	—0.376	0.866	0.477	.....	0.300
pH in tap water	—0.099	—0.202	—0.520	0.401	—0.227	0.300	.....

Least significant value equals 0.367
Least highly significant value equals 0.470

1. Soil pH with tap water was significantly correlated only with percentage lime. It had a highly significant negative correlation with the percentage of soil passing a 20-mesh screen. The latter relation appears owing to the tendency for pH to increase with soil depth.

2. Hydrolyzable pH had a highly significant correlation with total lime, carbon dioxide-soluble calcium, and available phosphorus. It had a significantly negative correlation with soluble iron and percentage of soil

passing a 20-mesh screen. The correlation coefficient between hydrolyzable pH and pH in tap water was just below the level of significance.

3. Percentage of total calcium carbonate had a highly significant correlation with all other determinations except percentage of soil passing a 20-mesh screen. The correlation with soluble iron was negative.

4. Of all factors investigated, carbon dioxide-soluble calcium showed the closest relation to soluble iron and available phosphorus. It also had a highly significant correlation with total lime and hydrolyzable pH, but was not significantly related to pH in tap water.

5. Oxalic acid-soluble iron was most closely related to carbon dioxide-soluble calcium and total calcium carbonate. The relationship between soluble iron and available phosphorus did not reach the level of significance.

6. Available phosphorus was highly correlated with total calcium carbonate, carbon dioxide-soluble calcium, and hydrolyzable pH.

On the basis of the close relations between oxalic acid-soluble iron, total calcium carbonate, and carbon dioxide-soluble calcium, a multiple correlation was calculated. The "R" value for this calculation was 0.714 which gave only a very slight decrease in the standard error of estimate for soluble iron over the use of the simple regression equation using carbon dioxide-soluble calcium alone for the estimation. An evaluation of the "β" values for this calculation showed that 80 per cent of the accounted-for variation in soluble iron could be based on carbon dioxide-soluble calcium and only 19 per cent on percentage of total calcium carbonate in the soil.

A similar calculation for the estimate of hydrolyzable pH based on total carbonates and carbon dioxide-soluble calcium indicated that carbon dioxide-soluble calcium had no value when used in addition to total lime for estimating hydrolyzable pH.

The multiple correlation coefficient between available phosphorus, total calcium carbonate, and carbon dioxide-soluble calcium was found to be 0.972. This represents a standard error of estimate for available phosphorus of 0.22 compared with 0.36 using carbon dioxide-soluble calcium alone and 0.45 using total calcium carbonate. Evaluation of the "β" values indicated that about 52 per cent of the accounted-for variation could be based on carbon dioxide-soluble calcium and 48 per cent on total calcium carbonate.

DISCUSSION

The results obtained in this investigation are not considered particularly representative of conditions in other calcareous or chlorotic soils. Soil conditions associated with chlorosis and phosphate unavailability are apparently so variable and complex that an introductory survey of many soils would probably lead to few specific conclusions. In some soils chlorosis may result largely from poor drainage, in other cases from alkali, but in many instances the only conditions noted to be associated with

chlorosis are a moderately high pH (usually 8.0 or above in Utah) and the presence of lime.

Accordingly, an intensive study of one soil producing chlorotic and green plants in well-outlined adjacent areas was made to determine some of the soil conditions closely associated with the disease. It is hoped that the results obtained with this one soil will be helpful in the study of iron and phosphate availability in other Utah soils.

The pH data are of interest in that pH of the soil moistened with tap water was not closely related with other soil characteristics investigated, while the maximum pH developed in a 1:10 soil suspension with boiled distilled water was significantly related to most of the factors. McGeorge (8) has pointed out that pH with tap water is close to the value existing under irrigated conditions, whereas hydrolyzable pH is much higher than exists under field conditions. The present study seems to indicate, however, that hydrolyzable pH may be more closely related to soil reactions than had been previously indicated.

The higher content of lime in the upper horizons of chlorotic soil compared with nearby nonchlorotic soil is in agreement with the findings of Ciferri (4). Since the soil studied was laid down by water and the topsoil has a similar depth and appearance over the entire field, the present differences in lime content are apparently owing to differences in internal drainage. Where the topsoil is closely underlain by coarse gravel, much of the lime has been leached, but where the compact sandy clay deposit underlies the topsoil, leaching has been retarded and the resulting soil is higher in lime and is chlorotic.

Except in such limited areas as that covered by the present study, it has not been possible to make any general distinction between chlorotic and nonchlorotic soils on the basis of percentage of total calcium carbonate alone. Chapman (3) has suggested that iron availability may be limited by the mobility of lime. He suggests that under irrigated conditions, lime coatings may be deposited around soil particles, thus limiting the nutrients available to many plants.

Attempts to distinguish a lime coating around soil particles by staining techniques were unsuccessful. Following these attempts, methods were sought to measure the mobility or solubility of lime in soil. Water-soluble calcium was determined on all samples, but the results showed no appreciable differences. Reaction of soil calcium with various oxalate solutions was also tried, and some differences were noted. This method is being further investigated.

The solubility of soil lime in carbon dioxide is an entirely arbitrary method for measuring mobility and gives somewhat different results under different conditions. Calcium carbonate may be brought entirely into solution upon prolonged agitation with water and a stream of carbon dioxide (Mellor (10)). Although the concentrations of carbon dioxide employed were much higher than occur in normal soils, the difference between the field and laboratory conditions is probably one of degree

rather than nature. Most studies on calcium carbonate indicate that there is only one predominating form in nature, and that under similar conditions the same reactions might be expected. The associations of calcium carbonate with other soil constituents appear very complex, however, and the relative activity is greatly altered by these relationships. This is indicated by the comparatively low pH of even highly calcareous soils compared with pure calcium carbonate or even limestones in similar states of division (Buehrer and Williams (2)).

Several methods for the measurement of iron solubility in soils were studied, but most of them were unsatisfactory. Soil extracts with distilled water or with water and carbon dioxide contained insufficient iron to give a test by the thiocyanate method. Extracts with 1 per cent solutions of citric and tartaric acid removed small amounts of iron from several of the soils, but the results were not as consistent as with oxalic acid. There is no evidence that iron soluble in 0.25 and 0.5 per cent oxalic acid represents that available to plants. But with the soil studied, good agreement was found between the iron brought into solution and the occurrence of chlorosis. A number of other soils have been subjected to iron extraction with oxalic acid solutions. In most cases chlorotic soils can be distinguished from closely adjacent nonchlorotic soils by the quantity of iron brought into solution. In some cases, however, the strength of oxalic acid must be altered. In one instance a strength of 1.5 per cent oxalic acid was required before a test for iron was obtained.

Acetate (pH 5.0) soluble phosphorus and oxalic acid soluble iron were related to the same soil characteristics but to a different degree. Both were most closely related to carbon dioxide-soluble calcium. But while the percentage of total lime in the soil accounted for almost as much variation in available phosphorus as carbon dioxide-soluble calcium, in the case of soluble iron it accounted for less than one-fourth as much variation as carbon dioxide-soluble calcium. These relations would seem to indicate that, while the availability of iron and phosphorus in the soil studied are influenced by many of the same factors, there must be factors not included in the present study which have different effects upon these two elements.

SUMMARY

1. The relations of a number of soil characteristics to oxalic acid-soluble iron and acetate (pH 5) soluble phosphorus in chlorotic and nonchlorotic areas of Hyrum clay loam soil have been investigated.

2. Chlorotic areas of the soil were characterized by a layer of compact sandy clay to fine sand ranging in depth from 2 to 3 feet and situated between the 12-inch layer of topsoil and the cobblestones and gravel below. In nonchlorotic areas of soil, this layer was not so compact and was only a few inches thick.

3. Iron was much more soluble in 0.5 per cent oxalic acid in nonchlorotic areas of soil than in chlorotic areas.

4. The pH values of the soils moistened with tap water were not significantly different in the chlorotic and nonchlorotic areas, but the pH values of 1:10 soil-boiled distilled water suspensions were higher in samples from chlorotic areas.

5. The percentage of total carbonates was much higher in the 0-12 and 12-24-inch depths of soil in the chlorotic areas as compared with the nonchlorotic areas.

6. Calcium soluble in a 1:20 suspension of water aerated with carbon dioxide for 30 minutes was employed as a measure of lime activity. The values obtained were higher for chlorotic than for nonchlorotic soil areas.

7. No appreciable differences in available phosphorus were found between the chlorotic and nonchlorotic soils.

8. The principal factors influencing oxalic acid-soluble iron were found to be carbon dioxide-soluble calcium and total calcium carbonate.

9. The principal factors influencing available phosphorus were carbon dioxide-soluble calcium, total calcium carbonate, and pH of a 1:10 suspension of soil in boiled distilled water.

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NUMBERS OF MICRO-ORGANISMS IN RELATION TO AGGREGATE SIZE[1]

G. G. Pohlman and R. J. Nottingham

From the Department of Agronomy and Genetics, Agricultural Experiment Station, Morgantown, West Va.

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The effect of organic matter in the formation of stable aggregates has recently been attributed to the binding action of soil micro-organisms. Waksman and Martin (6) and Martin and Waksman (2) found that micro-organisms caused a marked aggregation of soil particles, the extent of the effect varying with the nature of the organisms and with the nature of the organic matter added. Peele (5) states that "the mucus produced by bacteria was found to be an effective binding agent in the formation of water-stable granules." He also found that cultures of fungi were effective in producing water-stable aggregates. Recently Myers and McCalla (4) studied the relationship between bacterial numbers and aggregation in some prairie soils, and found that maximum aggregation usually occurred some time after maximum bacterial numbers. As a result of their studies they concluded that the metabolic products of bacterial action function as the cementing agents.

In addition, it has been noted that organic materials which decompose readily are more effective in producing aggregates than are materials which decompose slowly. It has also been noted by Metzger and Hide (1, 3) that the large aggregates have a higher content of organic matter than the small aggregates.

In view of these studies which emphasize the importance of micro-organisms in relation to soil aggregation it appeared desirable to determine if there is significant variation in numbers of bacteria and fungi in aggregates of various sizes.

METHODS

The soils used in this study were Gilpin silt loam, an upland soil derived from noncalcareous sandstone and shale; Elk silt loam, a terrace soil having some calcareous parent material; and Pope silt loam, a bottomland soil washed in from noncalcareous sandstones and shales.

Fresh soil samples representing each of the three types were taken from the field and passed through a 7-mm. screen while still moist. The samples were then air-dried and samples taken for aggregate analysis according to the method of Yoder (7). As soon as the separation of aggregates was completed the screens were removed from the water, allowed to drain, and air-dried overnight. The air-dry samples were then

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used for plating for numbers of bacteria and fungi. The soil samples were pulverized in a mortar to break up the aggregates before making dilutions. Plates were made in triplicate using sodium caseinate agar for bacteria and acid peptone glucose agar for fungi.

EXPERIMENTAL RESULTS

The numbers of bacteria and fungi in the various aggregates are given in Table 1. Although the largest numbers of bacteria in all three soils were found in the aggregates between 1 and 2 mm., and in two of the soil types the largest numbers of fungi appear in the same fraction, variations within triplicate plates were in most cases greater than variations between the numbers for the various aggregates. Consequently, no significance is attached to these differences.

TABLE 1

NUMBERS OF BACTERIA AND FUNGI PER GRAM IN SOIL AGGREGATES

SIZE OF AGGREGATES MM.	Gilpin		Elk		Pope	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
	millions	*thousands*	*millions*	*thousands*	*millions*	*thousands*
5.0	...	...	...	..	5.6	27
5.0 -2.0	10.8	190	8.9	38	6.0	25
2.0 -1.0	18.8	232	21.0	44	7.2	26
1.0 -0.5	14.6	174	8.6	35	6.0	19
0.5 -0.25	13.0	219	10.7	25	5.2	22
0.25-0.1	11.5	75	18.6	30	5.5	45

Numbers of actinomyces on the bacterial plates were determined in all three soils. These were found to represent 25 to 30 per cent of the colonies from the Gilpin, 15 to 25 per cent from the Elk, and 12 to 20 per cent from the Pope, but there were no variations which could be correlated with size of aggregates. Determinations of numbers of gum-producing bacteria on the Elk soil showed about the same number on all plates regardless of size of aggregates. Actual counts were not made on the other two soils, but no marked differences were evident.

The possibility that the washing method used in separating aggregates may have removed micro-organisms was recognized. There was also the possibility that during the process of wetting and drying there might have been changes in numbers. Consequently, a second sample of the Pope soil was dry-screened and numbers of micro-organisms determined on the dry-screened sample. The Pope soil was selected because over two-thirds of the large aggregates by dry-screening were found to be stable when wet-screened. The results are given in Table 2, together with results from wet-screening.

In the dry-screened sample, maximum numbers of bacteria and fungi were found in the 0.5 to 0.25-mm. aggregates, with a rapid decrease in the smaller fraction. Variations in bacterial numbers between plates were

TABLE 2

EFFECT OF DRY *vs.* WET-SCREENING ON NUMBERS OF MICRO-ORGANISMS PER GRAM IN SOIL AGGREGATES (POPE SOIL)

SIZE OF AGGREGATES MM.	BACTERIA *(millions)*		FUNGI *(thousands)*	
	Dry-Screened	Wet-Screened	Dry-Screened	Wet-Screened
5.0	4.3	5.6	19	27
5.0 -2.0	5.7	6.0	11	25
2.0 -1.0	5.7	7.2	18	26
1.0 -0.5	6.2	6.0	18	19
0.5 -0.25	7.5	5.2	23	26
0.25-0.1	3.6	5.5	13	45

greater than variations between aggregates except in the 5.0 and the 0.25 to 0.1 fractions, both of which were consistently lower than the other aggregates. Variations in number of fungi on triplicate plates were so high that the differences noted cannot be considered significant.

The numbers of bacteria and fungi were generally higher in the wet-screened soil, but the differences were in most cases within the limits of error. Although results from one sample cannot be considered as positive proof, it does not appear that the washing process materially influenced the numbers of organisms in the various aggregates.

DISCUSSION AND SUMMARY

The lack of correlation between numbers of bacteria and fungi and size of aggregates gives added support to the conclusions of Myers and McCalla (4) that the effect of micro-organisms in stabilizing soil aggregates is due to their products rather than to the organisms themselves. It is recognized that the type of organisms may be a more important factor than numbers, but in these studies there appeared to be little difference in types of organisms on the plates from various-sized aggregates. The products of decomposition or the gum produced by certain bacteria may account for the effect of bacteria which has been noted.

The effect of fungi does not appear to be related to the amount of mycelium, hence can only be accounted for either by its location with respect to the aggregate or by the products of decomposition. Both spores and fragments of mycelium were counted, but even the early counts which represent largely growth from pieces of mycelium did not show any significant differences.

It is recognized that this study is only preliminary and that the methods used are open to criticism. Further studies in which various types and amounts of organic matter are added and various micro-organisms used should be made to establish the relationship of micro-organisms to soil aggregation.

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