A PILOT STUDY OF THE MORPHOLOGY AND GENESIS OF THE FERRUGINOUS PEDOTUBULE

by

DONALD A. GIER

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Approved by:

O. W. Bidwell

Major Professor

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I. INTRODUCTION

Ferruginous pedotubules are known to occur on the roots of certain plants. Theories advanced to explain formation appear to be mostly speculatory.

The term "pedotubule" has been adopted after Brewer and Sleeman (10) and Brewer (11). They define a pedotubule as:

> "a pedological feature consisting of soil material (skeleton grains plus plasma) and having a tubular external form, either single tubes or branching systems of tubes; the external boundaries are relatively sharp. Tubular form, in this context, means that the feature as a unit, or its impression in the enclosing soil material, has a relatively uniform cross-sectional size and shape, most commonly circular or elliptical. The impression of the pedotubule therefore conforms to the definition of channels."

The pedotubule appears as a tubular concretion, rusty red in color, with a considerably higher bulk density than the surrounding soil. The color may vary as concentric banding in cross sections from very dark reddish brown (2.5 YR 2/4)[1] to brownish yellow (10 YR 5/8-6/8) giving the appearance of growth rings.

The presence of a pedotubule on a root may present several problems in the realm of plant nutrition, physiology, and pathology, both by the formation of a physical barrier and by providing a high concentration of iron in the rhizosphere. Before such ramifications can be studied, it is necessary to understand the factors involved in the formation of the pedotubules.

II. PREVIOUS WORK

Previous work on ferruginous pedotubules and on factors directly related to pedotubules has been limited mostly to field observations, some quantitative analyses and limited qualitative work.

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[1]All colors are Munsell notations, from moist soil unless otherwise noted.

The classification of soil oxidation and leaching zones was introduced by Kay and Pearce (28) for glacial tills with further refinement by Kay and Apfel (26), Kay and Miller (29), and Kay and Graham (27). Ruhe and associates (41, 42) have used this system extensively in describing loesses and tills. Daniels, Simonson, and Handy (14) established criteria for determination of the different classes: deoxidized soil material with chroma of 2 or less; and oxidized soil material with chroma of 3 or greater; leached-unleached condition determined by pH, leached material below pH 7.

Allison and Scarseth (3) reported the finding of solutions of iron around plant roots in ferruginous sand. No particulars were given on the pH, moisture, or other pertinent factors. Swallow (47) reported finding numerous tubes or cylinders approximately the size of pipestems penetrating a "Bluff Formation" (loess deposit) in all directions, appearing on both the green and dry roots of white oak (Quercus alba). Hall (18) reported iron tubules in the C_1 Horizon of the Downs silt loam (gray substratum phase) in Tama County, Iowa. The soil material containing the tubules was a grayish brown (2.5 Y 5/2) silt loam, with a pH of 7.8-8.0, unleached and deoxidized. Few iron tubules were reported in the B_{31}, with soil colors dark yellowish brown (10 YR 4/4) to yellowish brown (10 YR 5/4) and a pH of 6.5. Kay and Graham (27) reported iron tubules in Peoria loess-derived soils in Scott County, Iowa. The soil material was described as gray and calcareous. Daniels (13) reported pedotubules in the B_2, B_{31}, and C_1 of Dow silty clay loam, in the B_{22} and B_3 of the Monona silt loam, and in the B_3, C_1, and C_2 of a Dow-Monona intergrade soil in Pottawattomie County, Iowa. The soils in which pedotubules were found were described as: 1. Dow-Monona intergrade soil: oxidized and unleached in the upper portion, deoxidized and unleached in the lower portion; 2. Monona silt loam: leached,

pH 6.2, and oxidized (chroma > 2); 3. Dow: leached, pH 6.6, in the upper part and unleached, pH 7.3, in the lower part and deoxidized (chroma < 2) throughout.

Blümel (8) reported depositions of iron in flake and root-tube form occurring in gley soils in Austria. No attempt was made to explain their genesis. Scholtes[1] reported pedotubules on the roots of horsetail (Equisetum sp.) in the soils of southwest Iowa.

A weathering or developmental sequence was indicated (14, 26, 27, 28, 29, 41, 42) as: 1. deposition of the parent material (deoxidized and unleached); 2. oxidation of soil material, particularly the iron; and 3. leaching. In deep profiles all combinations of these factors have been described, both in till and in loess. In the normal sequence as generally described, the uppermost material is oxidized-leached, the next layer oxidized-unleached, and the deeper layer, deoxidized-unleached. Oxidized-unleached and deoxidized-leached zones have also been reported.

Pedotubules have been reported as most abundant in the deoxidized-unleached zone, but a few have been reported in the oxidized-leached zone. Daniels (13) reported numerous pedotubules occurring in a deoxidized-leached zone and sparse pedotubules in the oxidized-unleached zone. No report was made of pedotubules in other oxidation-leaching zones.

Pedotubules have been reported to be an iron oxide concentration (13, 14, 18, 48). Daniels et al. (14) found that the pedotubules contained 6.7 to 11.4% free iron compared to the surrounding soil which contained 0.1 to 1.3% free iron and 1.9 to 3.1 total iron. They described the pedotubules as being dark

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[1]Personal Communication, Scholtes, W. H., Professor of soils, Iowa State University, Ames, Iowa.

reddish brown, 7.5 YR 5/8 and 5 YR 3/4 with loose to slightly hard consistencies and size range from 1/3" to 1" in diameter. The pedotubules were oriented mostly with the long axis vertical, with a few having horizontal orientation. A banding pattern of oxides was described in detail from thin section examination. Swallow (47) described pedotubules as being tubes or cylinders, about the size of a pipestem, composed of "argillo-calcareous oxide of iron or calcareous clay ironstone". Oades (34) and Bartlett (6) reported oxidation of iron around the roots of certain plants. Bartlett (6) found the following plants grown in submerged or moist soil in the laboratory to have the ability to oxidize iron around the plant roots: DuPuits alfalfa, Narragansett alfalfa, Pennscott red clover, common alsike clover, Empire trefoil, Mansfield trefoil, Climax timothy, Saratoga bromegrass, Cady rice, and Reed canarygrass.

According to Swallow's (47) interpretation, pedotubules are the result of decomposition of roots of white oak that had a high content of iron oxides, thus leaving the iron oxide tubules in the soil. The tubule being the result of the iron containing barks becoming incrusted and residual structure remaining after the rest of the root had decayed.

III. MATERIALS AND METHODS

This study of pedotubules was necessarily attacked from several angles: 1. field observations, 2. chemical analyses, 3. morphology, 4. bacteriological implications, and 5. iron movements and oxidation states under laboratory conditions.

Field observations were made on two areas, site 1 in northeastern Kansas near Hiawatha in Brown County and site 2 in north central Kansas near Scandia in Republic County. All soil descriptions were made in accordance with the Soil Survey Manual (45).

Chemical analyses of soils containing pedotubules and of the pedotubules were made specifically to determine the concentration of ferrous and total iron, by modifications of the technique described by Walker and Sherman (48).

Pedotubules and blocks of soil bearing pedotubules were collected from the observation areas and studied to determine general morphological characteristics, density, and relationships to soil and to roots. Density was determined by a modification of the kerosene displacement of Edwards et al. (15). Structure and relationships were studied from thin sections prepared by the Rudolph Von Huene Laboratories, Pasadena, California.

Studies of populations of iron-precipitating bacteria were made by plate counts of bacteria in and between the pedotubules with serial dilutions on ferric-ammonium-nitrate-citrate agar recommended by Allen (2).

Iron oxidation states and movement were studied from 1. a system of simulated roots in soils containing pedotubules, and 2. growth of Reed canarygrass (Phalaris arundinacea L.) in soils containing pedotubules, maintained under a variety of moisture conditions and iron concentrations.

A. Iron Analysis

Initial iron analyses conducted according to the procedure of Walker and Sherman (48) were not sufficiently consistent to produce desired results. Experiments were initiated to determine the reasons for wide variations in duplicate samples, and modifications were developed to eliminate the inconsistencies.

(1) Quantity of hydrofluoric acid (HF) used in the digestion process.

An additional 5 ml of saturated H_3BO_3, prior to the development of the color complex greatly increased the consistency, indicating the presence of uncomplexed fluoride ions in the solution. Correction was made by decreasing

the amount of HF used in the digestion from 10 ml to 3 ml. To compensate for the smaller quantity of HF and to keep the concentration approximately the same, the digestion was revised to use 20 ml distilled water, 10 ml of concentrated (98%) H_2SO_4, and 3 ml reagent grade (49.5%) HF. The cooling mixture was reduced to 30 ml distilled water and 3 ml 50% H_2SO_4, with the recommended 25 ml of saturated H_3BO_3. Times and procedures otherwise were maintained as described (48). Calculations and digestion trials using silica (SiO_2) as a basis, showed a large excess of HF, even at this reduced level.

(2) Adjustments of pH to critical value.

A test in initial experiments showed range from pH 2.9 to 3.7. A pH series was set up at 0.5 pH unit intervals, from 1.5 to 4.5, using a digestion solution from one of the soil samples and adjusting the pH with a saturated solution of dibasic ammonium citrate. Maximum optical density, indicating optimum pH, was determined to be between pH 2.0 and 3.0. (Table 1). Based on these results more critical series were determined with 0.2 pH unit intervals from pH 2.0 to 3.0 (Table 2).

Table 1. Initial determinations for maximum color development in iron determinations.

pH	Sample #1	Sample #2
	Optical Density	
1.5	.04	--
2.0	.05	.28
2.5	.15	.20
3.0	.145	.135
3.5	.08	.108
4.0	.04	--
4.5	.02	--

Table 2. Test series to determine maximum color development at 0.2 pH unit interval.

pH	Sample		
	1	2	3
	Optical Densities		
2.0	.042	.043	.26
2.2	.042	.048	.42
2.4	.041	.043	.38
2.6	.040	.043	.31
2.8	.038	.039	.27
3.0	.030	.037	.22

Based on the results, a pH value of 2.2 was chosen for the experimental standard for the color development in the iron determination.

Control and adjustment of pH was accomplished by 1. careful control of all acid and basic reagents added (washing of the filter after filtration of the digestion solution was accomplished with three 10 ml aliquots of 2% H_2SO_4). 2. Final adjustment of the pH was accomplished by adding sufficient quantity of a subsaturated solution of dibasic ammonium citrate to the digestion solution to bring the pH to 2.2. In the procedure (48) optical densities ranging from 0.01 to 2.0 were developed. Since maximum accuracy is obtained at optical densities between 0.1 and 0.7, samples containing 25 to 50 ppm ferrous iron were used, necessitating aliquots of digestion solutions ranging from 0.5 to 10.0 ml. An adjustment in the amount of buffer used, ranging from 0.2 to 3.5 ml, was the only consideration made when adjusting aliquot size.

Further improvement in the procedure could be achieved by using a

combination glass electrode to measure pH directly in the separatory funnels during titration.

The digestions were accomplished in 400 ml Teflon beakers covered with 100 mm Teflon watchglasses rather than in platinum dishes or the Vycor flasks as used and recommended by Walker and Sherman (48). For the quantity of liquid involved, 125 ml beakers with 75 mm covers would have been of adequate size. Digestion was accomplished on a sand bath at approximately 270°C. At the conclusion of the digestion, the solution was transferred quantitatively into the cooling solution to free the beakers for the next digestion.

Recovery of Iron. A series of tests was conducted to determine the percent iron recovery in the analytical procedure. Iron-free, white quartz sand was used as a base with sufficient $FeSO_4$ added to make each sample 5% in terms of Fe^{++}. Sufficient $FeCl_3$ was added to make the samples 5% in terms of Fe^{+++}. (Table 3A). The results (Table 3B) indicate that the best time lapse between digestion and analysis was 24 hours, with 48 hours almost as good. Other time lapses resulted in serious deviations from the known.

The obvious conclusion to be drawn from these tests is that the inconsistencies of earlier determinations were due to several factors, either singly or in combination: 1. inadequate control of pH in the determinations as there was a rapid decay of color with deviations from pH 2.2. (Tables 1, 2); 2. A chemical change in the digestion solution prior to the time of analysis, possibly a reduction of the Fe^{+++} ---> Fe^{++}; or 3. undetermined variation from temperature, climatic conditions, or investigator that made for slight daily fluctuation in the standard curve.

Table 3A. Recovery of iron after differing time lapses between digestion and analysis. Composition of samples.

Iron added	Sample 1	2	3	4
as	Percent Iron			
1. Sample for 1 hour determination				
$FeSO_4$	4.92	5.05	4.97	5.03
$FeCl_3$	5.72	4.97	5.02	5.02
Total	10.64	10.02	9.99	10.05
2. Sample for 24 hour and subsequent determination				
$FeSO_4$	5.22	4.98	4.98	5.05
$FeCl_3$	5.09	5.09	5.11	5.05
Total	10.31	10.07	10.09	10.10

B. Total Organic Carbon

Total organic carbon was determined by the dry combustion method. This method, originally designed for carbon determination in steel, was adapted for soil work by R. M. Salter (43).

The apparatus consisted of a bottle of oxygen for the oxidizer source; two potassium hydroxide scrubbers to remove any carbon dioxide from the oxygen stream; a mercury check valve to prevent blow back from the combustion oven; the oven; two sulfuric acid scrubbers for initial water removal; a zinc granule tube for acid removal; a calcium chloride tube for water removal; an ascarite bulb to collect the carbon dioxide; a bubble counter to prevent back flow and calibrate gas flow, and an aspirator to provide a vacuum at the end of the train.

One gram of air-dry soil was mixed with 2 grams of cupric oxide and placed

Table 3B. Recovery of iron after time lapses. Percent iron recovered and difference from known content.

	Sample 1		Sample 2		Sample 3		Sample 4			
Time Iron	%Fe	Diff.	%Fe	Diff.	%Fe	Diff.	%Fe	Diff.	Avg.Diff.	s^2
1 hr. Fe^{++}	5.80	+0.88	5.95	+0.90	6.05	+1.08	5.80	+0.77	+0.9075	0.0165
Total Fe	10.30	-0.34	9.40	-0.62	9.40	-0.59	9.60	-0.45	-0.5000	0.0169
24 hr. Fe^{++}	5.80	+0.58	5.90	+0.92	5.70	+0.72	5.90	+0.85	+0.7675	0.0225
Total Fe	11.20	+0.89	9.60	-0.47	10.30	+0.21	9.60	-0.50	+0.0325	0.0763
48 hr. Fe^{++}	5.38	+0.16	5.19	+0.21	5.19	+0.21	5.38	+0.33	+0.2275	0.0052
Total Fe	12.20	+1.89	11.40	+1.33	11.95	+1.86	10.85	+0.75	+1.4575	0.2886
72 hr. Fe^{++}	7.00	+1.78	6.75	+1.77	6.75	+1.77	6.86	+1.81	+1.7825	0.0004
Total Fe	13.90	+3.59	9.60	-0.47	10.00	-0.09	9.10	-1.00	+0.5075	2.4955
8 day Fe^{++}	9.45	+4.23	5.84	+0.86	6.10	+1.12	6.10	+1.05	+1.8150	2.6042
Total Fe	11.95	+1.64	11.65	+1.88	11.95	+1.86	10.85	+0.75	+1.5325	0.2840
20 day total Fe	10.50	+0.19	11.00	+0.93	10.70	+0.61	16.20	+6.10	+1.9575	7.7186
Avg. Diff. Fe^{++}		+1.5260		+0.9320		+0.9800		+0.9620		
s^2 Fe^{++}		2.6386		0.3075		0.3286		0.2939		
Avg. Diff. Fe Total		+1.3100		+0.4300		+0.6433		+0.9417		
s^2 Total Fe		1.5877		1.1770		0.6300		6.8934		

in a ceramic ignition boat. The oven was heated to 950°C. and the apparatus was swept out with approximately 500 ml of oxygen in 5 minutes, with the gas flow diverted from the collector by a bypass. The ascarite bottle, weighed to the nearest 0.1 mg was placed in the train and the sample in the combustion tube in the oven. The combustion tube was sealed immediately and the bypass closed, with the oxygen flow turned off. When the initial gas expulsion was completed, approximately 2 liters of oxygen were passed through the train in 20 minutes with the oven temperature maintained at 950°C.

At the completion of the determination, the ascarite bottle was removed from the train, allowed to cool and re-weighed. The difference in weight of the bottle prior to and after ignition was CO_2 resulting from oxidation of organic carbon and expressed (Table 4) as percent organic carbon in the soil.

Table 4. Summary of characteristics of pedotubules and soils containing pedotubules.

	Site 1		Site 2	
Characteristic	Soil	Pedotubule	Soil	Pedotubule
Fe^{++}	0.59	1.09	0.53	0.86
Fe (total)	3.32	25.00	3.65	25.50
Fe^{++}/Fe (total)	0.178	0.0436	0.145	0.0336
Particle Density	2.62gm/cc	2.59gm/cc	2.57gm/cc	2.55gm/cc
Bulk Density	1.85gm/cc	2.05gm/cc	1.76gm/cc	2.17gm/cc
Organic Carbon	0.4109%	0.6323%	0.3502%	0.5787%

C. Physical Determinations

Particle density was determined by an air pycnometer, according to the method of Jacobs (22) and Beckman Instruments, Inc. (7). Samples of oven-dried materials were weighed and placed in the sample cup of the pycnometer. The cup was sealed in place and the apparatus allowed to stand for several minutes to achieve equilibrium. The pistons were retracted completely, and the valves closed. Then the pistons were moved forward, maintaining equilibrium between the cylinders. When the index cylinder reached the stop, the volume of the material was read from the scale on the indicator cylinder. Particle density was calculated as grams per cubic centimeter.

Bulk density was determined by modification of the kerosene immersion procedure by Edwards, Fehrenbacher, and Vavra (15). This procedure was modified to the extent that no prolonged period of stabilization of kerosene level in the soil particles was used. Several larger aggregates of soil and pedotubules were selected, weighed, and placed in a vacuum desiccator in a container with sufficient kerosene to cover the sample. The desiccator was then evacuated by means of a water aspirator until no bubbles were observed coming from the soil. Evacuation was repeated three times. The vacuum was then reduced and the soil removed and spread on paper towels to remove excess kerosene. The aggregates were placed in a graduate cylinder partially filled with kerosene and the volume was determined by displacement of kerosene. Bulk density was calculated as grams per cubic centimeter.

IV. THE INVESTIGATIONS

A. Physical and Chemical Characteristics

Table 4 summarizes the chemical and physical determinations made on the pedotubules and the supporting soil.

The pedotubules have a more or less tubular structure, variously branched and fused (Figs. 1, 2, & 3), apparently arising from the encasement of the roots of plants. (Site 1, Bromus inermis; Site 2, Andropogon sp.) (Table 5). Figure 4 shows the encasement of the roots of Bromus japonicus, forming below the crown of the plant. This is apparently the first stage in the formation of pedotubules. Figures 5 and 6 show encasement of lower roots with soil material and cemented pedotubules respectively. Cross-sectional studies of blocks of soil (Fig. 7, 8, 9, 10) show that pedotubules penetrate the soil in all directions. In the sites studied, however, most of the penetration was vertical (Fig. 7, 8). Patterns of distribution of pedotubules were similar at both sites and compare to the distribution of primary roots. Structure would indicate similar mechanism of development.

The pedotubule in the center of Fig. 7 has been magnified in Fig. 9 to show what is apparently the unaltered or deoxidized soil with no iron enrichment filling the center of the pedotubule. A plant root (probably Bromus, sp.) can be seen in the lower portion of the center and a trace of white colored material can be seen on the inside surfaces of the pedotubule. This whitening is more pronounced in the pedotubules in the upper left portion of the block in Fig. 8.

This phenomena can be observed (Fig. 10) in the rhizosphere taken from the Bignell loess at Iowa Point, Kansas. Here a wide band of gray material surrounds the root before there is evidence of an accumulation of oxidized iron. There

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Fig. 1. Ferruginous pedotubules, cylindrical and broken sections showing cross-section and range of sizes. Scale: 1 division = 1 mm.

Fig. 2. Ferruginous pedotubules showing branching corresponding to the branched roots on which they developed. Scale: 1 division = 1 mm.

Fig. 3. Pedotubules that have developed in roots in close proximity to each other. Fusion has occurred in the outer perimeter of the pedotubules. Scale: 1 division = 1 mm.

Fig. 4. Crown and root system of Bromus sp. displaying rhizosphere effect, an encasement of soil clinging to the roots.

Fig. 5. Lower roots of plant (probably Bromus sp.) displaying encasement by uncemented soil particles.

Fig. 6. Plant roots encased by pedotubules.

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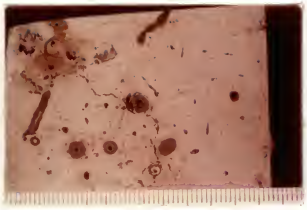
Fig. 7. Soil block from the Brown County, Kansas site containing pedotubules, illustrating predominant vertical orientation and iron-stain "accompanying" decomposition of pedotubule (upper left). Scale: 1 division = 1 mm.

Fig. 8. Soil block from Crete silty clay loam, Republic County, Kansas, (NE 1/4 section 12,T3S,R2W). Vertical orientation predominates. Iron stains are associated with the pedotubules. Pedotubule near upper edge of block displays extreme example of white inner surface. Scale: 1 division = 1 mm.

Fig. 9. Cross-section of a hard pedotubule from center of block shown in Fig. 7. Root is visible in upper part of core. Reduced core and white lining apparent on inside of pedotubule next to root. Scale: 1 division = 1 mm.

Fig. 10. Cross-section of rhizosphere from Bignell loess, Iowa Point, Kansas. Reduced zone surrounding root, with concentric bands of ferric oxide surrounding reduced zone. Little cementation of soil material by iron oxide. Scale: 1 division = 1 mm.



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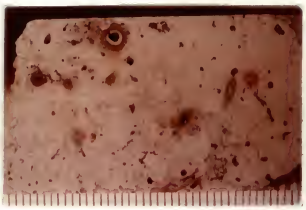

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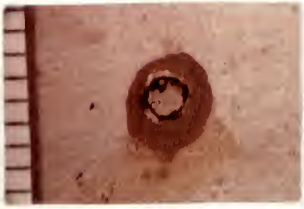

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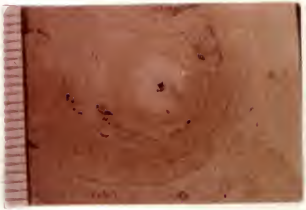

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Table 5. Partial list of flora on research sites, 11 September 1966.

Site 1

Plant	Common name
Tridens flavus	Purple top
Euphorbia marginata	Snow-on-the-mountain
Euphorbia sp.	Spurge
Kuhnia enpatonoides	False boneset
Helianthus sp.	Sunflower
Muhlenbergia sp.	Muhly grass
Bromus inermis	Smooth brome
Rosa sp.	Wild Rose
Setaria lutescens	Yellow foxtail
Setaria viridis	Green foxtail
Maclura ponifera	Osage orange
Cirsium sp.	Common thistle
Conyza canadensis	Horseweed

Site 2

Andropogon gerardii	Big bluestem
Sorghastrum nutans	Indian grass
Euphorbia marginata	Snow-on-the-mountain
Bouteloua curtipendula	Side-oats grama
B. gracilia	Blue grama
Elymus canadensis	Canada wild rye
Celtis sp.	Hackberry
Muhlenbergia sp.	Muhly grass
Conyza canadensis	Horseweed

was little apparent cementation in this rhizosphere, just an iron stain occurring in a more or less concentric band with the plant root as the center.

A study of thin sections, prepared by the Rudolph von Huene Laboratory, shows that the pedotubule is very dense, as determined by bulk density determinations. (Table 4) (Fig. 11, 12, 13, 14, 15).

In the overall view, a section of the pedotubule (Fig. 11), the zone of reduction, is quite pronounced. Several light spots show up at this magnification which upon closer examination under plane-polarized light with uncrossed and crossed nicols appear to be original soil material (Fig. 12, 13, 14, 15). This reinforces the contention that the pedotubule is a concretion of soil particles cemented by iron oxides.

B. Field Studies

Two sites were selected in which pedotubules were found in the soil profile. Both sites were inclusions in soil units as mapped for standard soil surveys (Fig. 17, 19) (4, 16).

Site 1 (Fig. 16) is in Brown County, Kansas, 8.5 miles east of Hiawatha and 0.5 miles north of U.S. Highway 36, in the center of section 23, T2S, R18E. The site is on a long south-facing slope in the Peorian loess hills of northeastern Kansas. Topography is rolling with maximum slopes from 6 to 8 percent. (Fig. 20). Flora is listed on Table 5. This site was reported to Dr. O. W. Bidwell by Robert W. Eikleberry Soil Convervation Service Soil Scientist, who made the Brown County Soil Survey.

Site 2 (Fig. 18) is in Republic County, Kansas, 7 miles south and 5 miles west of the U.S. Highway 36-81 junction in the SE 1/4 SW 1/4 of section 11, T4S, R4W. The Peorian loess mantle is not so thick as at site 1 and is

underlain with Loveland loess and in places by Greenhorn limestone. The site has a southwestern exposure, draining into an intermittent stream approximately 500 feet to the west. Slopes range from 6 to 8 percent (Fig. 21). Flora is listed on Table 5. This site was reported by Mr. Robert Raney of the Kansas State University Irrigation Experiment Field, Scandia, Kansas.

Pedotubule samples were obtained in two ways: 1. loose pedotubules were picked up from the surface for physical and chemical analysis; 2. large samples of fresh soil were obtained in March, 1965, from a depth of approximately 40 inches and placed in polyethylene bags for transport. Pedotubules with living-plant roots through them were selected for microbial analysis. These and all other material for laboratory biological experiments were utilized within 48 hours after collection. Samples for chemical and physical analysis were air-dried for later use.

Soil profile descriptions were made to a depth of 17 feet (Table 6). Color designations were determined by Munsell color charts, from moist samples unless otherwise stated; texture was determined by feel; and pH by bromocresol purple, bromothymol blue, and cresol red indicators.

The pedotubules were located in the C horizons of both soils: at site 1 at a depth of 15 in. in the lower B_3 horizon (Fig. 22), and at site 2 at an estimated depth of 6 ft. (Fig. 23). The color of the C horizon of the dominant soils indicated an oxidized state, yellowish-brown (10 YR 5/6) for site 1 and light yellowish-brown (10 YR 6/4) for site 2. The C horizon of the soils in the selected sites was much grayer, with pH neutral to basic: light gray (10 YR 6/1), pH 7.0, unleached-deoxidized at site 1, and light brownish-gray (10 YR 6/2), pH 7.6-8.2, unleached-deoxidized at site 2 (Table 6). Transition from the gray C horizon of the pedotubule sites to the brown C horizon of the

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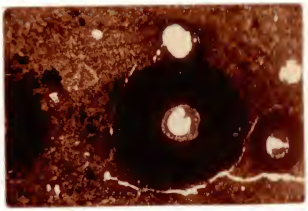
Fig. 11. Thin section (approximately 5μ) of pedotubule, approximate magnification, 7X. White lining of otherwise opaque pedotubule evident. Small light areas appearing in opaque ring are mostly quartz fragments.

Fig. 12. Thin section of pedotubule. Approximate magnification, 25X. Small light areas representing quartz and feldspar are numerous. White lining is made up of mineral fragments similar to soil outside pedotubule.

Fig. 13. Same thin section of pedotubule as Fig. 12, under cross-polarized light. Non-opaque minerals can be identified as quartz and feldspars.

Fig. 14. Thin section of pedotubule, approximate magnification 100X, under plane polarized light with uncrossed nicols.

Fig. 15. Same as Fig. 14, under cross-polarized light. Individual mineral fragments are will defined. Pedotubule appears to be a cementation of mineral fragments by ferric oxide.



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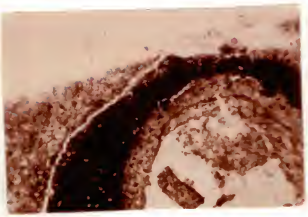

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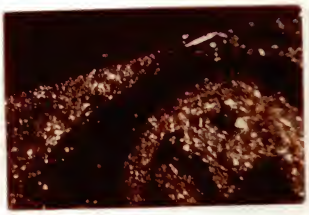

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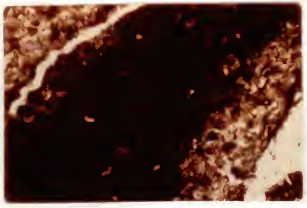

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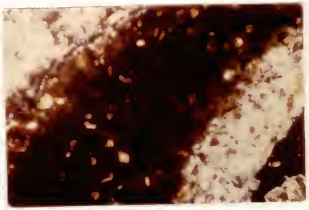

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EXPLANATION OF FIGURES

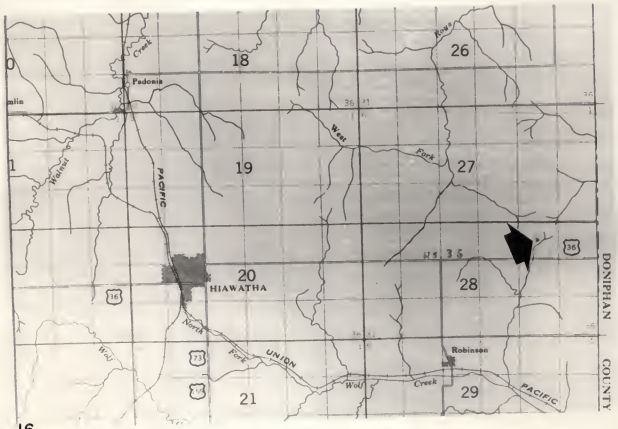
Fig. 16. Eastern section of Brown County, Kansas. Arrow indicates location of site 1.

Fig. 17. Soil survey map of area site 1, Brown County, Kansas. (16)

Legend

MaA	Marshall and Sharpsburg soils	0-2 %
MaB	Marshall and Sharpsburg soils	2-4 %
MaBB	Marshall and Sharpsburg soils	4-10 %
MaC	Marshall and Sharpsburg soils	10-18 %
JaA	Judson silt loam	0-3 %
SaB	Shelby clay loam	4-10 %
SaC	Shelby clay loam	10-18 %

Location of site 1 indicated by pointer.


Padonia
18
26
19
27
20
HIAWATHA
28
21
29
Robinson
PACIFIC
UNION
DONIPHAN COUNTY
US 36

16


MaBB
MaA
MaC
23
MaBB
MaC
MaB
JaA
Aa
SaB
MaB

17

EXPLANATION OF FIGURES

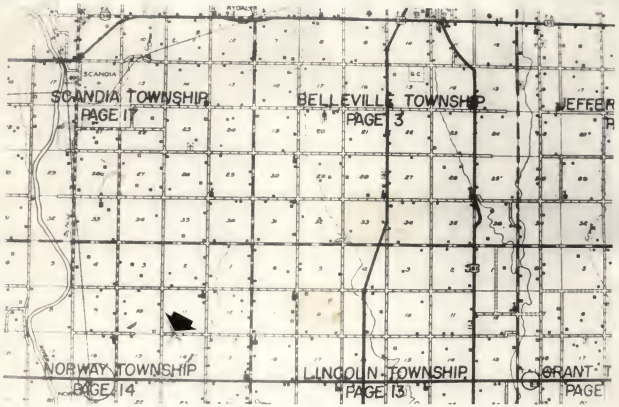
Fig. 18. Map of section of south central and southwestern Republic County, Kansas. Arrow indicates location of site 2.

Fig. 19. Soil survey map of site 2 area. (4)

Legend

F3B	Hastings SiCl	1-4 %
F3C	Hastings SiCl	4-8 %
F3C2	Hastings SiCl	4-8 % moderately eroded
2	Upland breaks and alluvial land	
5	Breaks and alluvial land	

Location of site 2 indicated by pointer.


SCANDIA TOWNSHIP
PAGE 17
BELLEVILLE TOWNSHIP
PAGE 3
NORWAY TOWNSHIP
PAGE 14
LINCOLN TOWNSHIP
PAGE 13

18


F3C
5
L
F3B
F3C2
F3
2
5
P

19

EXPLANATION OF FIGURES

Fig. 20. Site 1, Brown County, Kansas, facing north. Pedotubule site indicated by arrow.

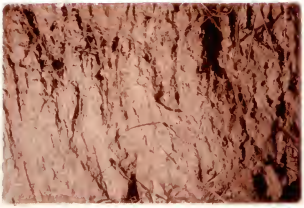
Fig. 21. Site 2, Republic County, Kansas, facing north-northwest. Pedotubule site located in road bank at rear of car in center of picture. Pedotubules at this site were scattered profusely in road.

Fig. 22. Pedotubules at site 1. Pedotubule located at depth of 3 feet. Pedotubule in center of figure, although broken, is still embedded in soil. Pedotubules are scattered on surface of soil in lower left of figure. Soil color is characteristic.

Fig. 23. Pedotubules at site 2. Embedded pedotubules protruding from soil. Light gray color is characteristic. Depth to pedotubules is approximately 7 feet.

20

21



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23

dominant soils surrounding the sites occurred at about 35 ft. from the edge of the areas in which the pedotubules were located.

Vertical distances from the surface to the brown parent material varied. At site 1 it occurred immediately beneath a strongly mottled, clayey layer approximately 10 feet deep. (Table 6). At site 2, a fine-textured layer (silty clay loam) occurred at a depth of 5 feet at the point of description (Table 6) and about 8 feet below the estimated original surface of the ground in the road cut. The underlying material is apparently loess, probably of Peorian age, although at site 2 it could be Loveland age.

Pedotubules have been generally observed in the C_2 horizon of the Crete silty clay loam in Republic County. In this soil the slowly pervious clayey B_2 horizon, characteristic of this soil series, prevents free oxygen diffusion to the horizon of pedotubule development.

In the Crete silty clay loam of Republic County and the research sites, a deoxidized condition was observed which appears to favor the development of pedotubules. Also, there was an iron-stain type of mottling observed that is apparently the product of decomposition of pedotubules. Neither the pedotubules nor the associated mottling has been observed in horizons with pH below 7.0.

Of the floral species found on both sites (Table 5) only three (Muhlenbergia sp., Euphorbia marginata and Conyza Canadensis) were common to both sites. Another pedotubule site north of site 1 reported by Dr. O.W. Bidwell and Mr. A.S. Hadimani contained Muhlenbergia sp. in the flora. At site 1 it was determined that pedotubules were developing on Bromus inermis, no determinations were made at site 2 as to plant involved in pedotubule development.

Table 6. Soil descriptions, sites 1 and 2.

Site 1

Brown County, Kansas. 50 feet North and 30 feet East of center of Section 23, T2S, R18E; south-facing 8-10% slope; crop - alfalfa.

A_p 0 to 4 inches, dark brown (10 YR 3/3); silty clay loam; medium moderate blocky; wet, slightly sticky; moist, very friable; dry, extremely hard; pH 6.8; extremely dense; abrupt lower boundary.

B_2 4 to 10 inches, dark yellowish-brown (10 YR 3/4); heavy silty clay loam; moderate medium sub-angular blocky; wet, slightly sticky; moist, firm; dry, hard; pH 6.8; gradual lower boundary.

B_3 10 to 16 inches, brown (10 YR 5/3); silty clay loam; weak medium sub-angular blocky; wet, slightly sticky; moist, firm; dry, slightly hard; iron stains and few small pedotubules; pH 6.8; clear lower boundary.

C_1 16 to 30 inches, grayish-brown (10 YR 5/2); light silty clay loam; weak coarse blocky structure; wet, non-sticky; moist, friable; dry, slightly hard; 5% small round yellowish-brown (10 YR 5/8) iron stains and numerous pedotubules; pH 6.8.

36 inches, gray (10 YR 6/1); light silty clay loam; massive; wet, non-sticky; moist, friable; dry, slightly hard; numerous iron stains (strong brown 7.5 YR 5/8) and hard pedotubules 1/8 to 1/4 inches in diameter; pH 6.8.

48 to 60 inches, gray (10 YR 6/1); silt loam; massive; wet, slightly sticky; moist, friable; iron stains (strong brown 7.5 YR 5/8) 10-25%; numerous to few small hard pedotubules; few small round ferro-manganese concretions at 5 feet; pH 6.8.

72 to 84 inches, light brownish-gray (10 YR 6/2); silt loam; massive; wet, slightly sticky; moist, friable; strong brown (7.5 YR 4/6) iron oxide precipitated in seams; 5% black (10 YR 2/1) mottles present.

96 inches, grayish-brown (10 YR 5/2), 45%; yellowish-brown (10 YR 5/4), 45% and strong brown (7.5 YR 5/8), 10%; few black mottles; silt loam; massive; wet, slightly sticky; moist, friable; pH 6.8.

108 inches, strongly mottled with gray (10 YR 6/1); dark gray (10 YR 4/1); yellowish-brown (10 YR 5/6); and strong brown (7.5 YR 5/8) in approximately equal proportions; 5% black (10 YR 2/1); heavy silty clay loam; moderate fine blocky; wet, plastic; moist, very firm; dry, very hard; pH 6.8.

120 to 132 inches, gray-light gray (10 YR 6/1); dark grayish-brown (10 YR 4/2); strong brown (7.5 YR 5/8); approximately equal proportions; silty clay loam; fine weak sub-angular blocky; wet, slightly sticky; moist, friable; dry, hard; pH 6.8.

144 to 180 inches, yellowish-brown (10 YR 5/4) mottled with 20% gray (10 YR 6/1) and 5% yellowish brown (10 YR 5/8); light silty clay loam; few small round iron-manganese concretions; massive; wet, slightly sticky; moist, friable; pH 7.2.

192 to 204 inches, yellowish-brown (10 YR 5/6) with light grayish-brown (10 YR 6/2) mottles; light silty clay loam; massive; wet, slightly sticky; moist, friable; pH 7.2.

Site 2

Republic County, Kansas. South edge SE1/4 SW1/4 Section 11, T4S, R4W; just above slope break; 6% west facing; crop, native grass.

A_1 0 to 6 inches, dark yellowish-brown (10 YR 3/4); silty clay loam; weak medium granular; wet, slightly sticky; moist, friable; pH 6.6; gradual lower boundary.

B_1 6 to 16 inches, dark yellowish-brown (10 YR 3/4); silty clay loam; moderate fine sub-angular blocky; wet, slightly sticky; moist, friable; dry, hard; pH 6.8; gradual lower boundary.

B_2 16 to 22 inches, brown (10 YR 5/3); silty clay loam; moderate fine blocky; wet, slightly sticky; moist, firm; dry, hard;pH 7.0; gradual lower boundary.

Cca 22 inches, light brownish-gray (10 YR 6/2); light silty clay loam; massive; wet, slightly sticky; moist, friable; dry, slightly hard; pH 8.0; calcium carbonate concretions.

36 inches, light yellowish-brown (10 YR 6/2); silt loam; massive; wet, slightly sticky; moist, very friable; dry, slightly hard; soft calcium carbonate concretions; few iron stains (10 YR 6/8) present; pH 8.0.

44 to 60 inches, dark brown (10 YR 3/3); silty clay loam; massive; wet, slightly plastic; moist, firm; dry, hard; pH 8.2.

72 inches, dark brown (10 YR 4/3); silty clay loam; fine weak blocky; pH 8.2; wet, more sticky, moist, friable; dry, slightly hard.

84 to 192 inches, dark yellowish brown (10 YR 4/4); silty clay loam; fine subangular blocky at 7 feet grading to massive at 8 feet and down; wet, non-sticky; moist, friable; dry, slightly hard; pH 8.2.

C. Greenhouse Studies

Twenty-four 5-pound glazed earthenware jars were each filled with 4 Kg of fresh soil on an oven-dry basis. Twelve pots were filled with soil from site 1 and twelve with soil from site 2. Commercial ammonium phosphate fertilizer (16-48-0) was added at the rate of 80 pounds of nitrogen per acre. Reed canary-grass (Phalaris arundinaceae L.) was sown on the soil and watered with distilled water.

The soils were kept moist until the plants were well established. Plants were thinned to 3 or 4 plants per pot. Four levels of moisture were established 4 March, 1964: 1. saturated (soil submerged); 2. semi-saturated (water content approximately half-way between saturated and field capacity); 3. field capacity; and 4. fluctuating between field capacity and wilting coefficient. Levels 1, 2, 3 were watered daily and 4 as needed to re-establish desired levels. The watering solutions were prepared by adding ferrous sulfate to distilled water to attain levels of iron of 0, 3, and 6 parts per million (ppm) ferrous iron. Pots were arranged on a greenhouse bench by water level and iron level by soil type (Fig. 24). Topgrowth was removed periodically in order to reduce root growth and water consumption. Plants were grown for approximately six months (Table 7), the topgrowth removed, the pots sealed with sheets of polyethylene and stored for future inspection. The moisture level at which the plants were grown was maintained during storage. The soil mass was removed from the pot when time permitted, cut once horizontally and inspected for evidence of iron accumulation and oxidation around the plant roots and for pedotubule formation.

During the growth period the pots were checked daily and watered as required to maintain the soils as closely as possible to the desired moisture level. The pots were removed from the bench, placed on a heavy duty triple-beam balance and

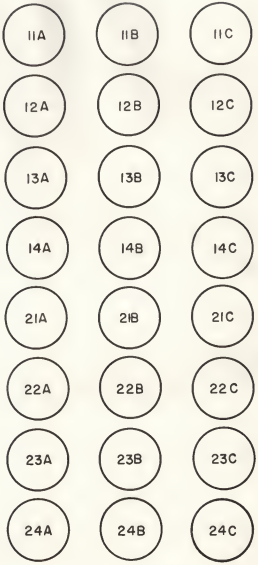

Fig. 24. Diagram of pot arrangement on greenhouse bench for grass experiment.

Key

1st digit	2nd digit	Letter
1 - Soil, site 1	1 - Soil saturated	A - No iron
2 - Soil, site 2	2 - Soil semi-saturated	B - 3 ppm. iron added to watering solution.
	3 - Soil at field capacity	
	4 - Moisture allowed to fluctuate from field capacity to wilting coefficient.	C - 6 ppm. iron added to watering solution.

Table 7. RECORD OF EVENTS (Greenhouse Study) 12 January to 1 August, 1964.

Coding

1st Digit	2nd Digit	Letter
Soil	Moisture Level	Iron In Watering Solution
1 Site 1	1 Saturated	A None
2 Site 2	2 Semi-saturated	B 3 ppm
	3 Field capacity	C 6 ppm
	4 Fluctuating field capacity to wilting coefficient	

Date (Day Month)	Event
12 - 1	Potting was completed and 1.3 gm commercial ammonium phosphate (16-48-0) fertilizer was added to each pot (80 pounds nitrogen per acre). Pots were seeded with Reed canarygrass and watered with distilled water to maintain adequate moisture.
14 - 2	All plants in pots 13A, 14A, 14B, and 14C were dead. Repotting was completed with 2.6 gm ammonium phosphate fertilizer added to each of these pots and 1.3 gm added to each of the other pots.
20 - 2	Plants were thinned to 3 or 4 per pot.
4 - 3	Iron and moisture levels established according to plan. Ammonium nitrate, (NH_4NO_3) fertilizer was added to watering solutions at the rate of 25 ppm nitrogen.
15 - 3	21A, 21B, and 21C showed strong chlorosis.
30 - 3	Chlorosis in 21B and 21C was no longer apparent, 21A was severe. Large differential in rate of water use between A series and B & C series noted.

Table 7. (cont.)

Date	Event
Day Month	
4 - 4	One percent ferrous sulfate ($FeSO_4$) in water with detergent (Tide) as foliar application added to plants in 21A.
5 - 4	Repeat application of ferrous sulphate to 21A.
8 - 4	Plants in all pots were clipped to 6-inch height.
6 - 5	Chlorotic condition of plants in 21A alleviated. All plants received foliar application of 1% ferrous sulfate.
16 - 5	Foliar application of 1% ferrous sulfate to all plants.
2 - 6	Plants watered by person unknown to investigator. Water source unknown.
5 - 7	Hail storm. Several panels broken out of greenhouse rendering air conditioning system useless for 3 days. Water consumption increased considerably.
10 - 7	All plants in pot 14C have died.
25 - 7	Eh-pH determinations were made.
1 - 8	Plants were clipped to below top of pots. Sheets of polyethylene were secured over tops of the pots.
Dec. 1964	Pots were opened and inspected.

water added to a pre-determined weight, to bring the soil to the desired moisture.

Determination of Moisture Relations.

To control the water content of the soil for growth trials, an accurate determination of soil moisture of the sample was necessary. Each bag of fresh soil material was randomly sampled by drawing handfuls of soil from different parts of the bag. The samples were composited by bag lots and aliquots were withdrawn for moisture determinations. Samples of 25 to 45 grams were placed in pre-weighed 2-inch moisture cans and weighed, dried for 48 hours at 110°C., and re-weighed. Percent moisture was computed on an oven-dry weight basis:

$$\left(\frac{\text{Weight of moisture}}{\text{Weight of oven dry soil}}\right) \times 100 = \% \text{ moisture in soil.}$$

The percent moisture determined on the aliquot was used in computing dry weight of soil when potting for the greenhouse experiment.

a. Field capacity determination. Field capacity is defined as the amount of water remaining in a well-drained soil when the velocity of downward flow into unsaturated soil has become small (17). For laboratory determination, field capacity was defined as 1/3 atmosphere tension on the soil moisture. This determination was a modification of a procedure by Jacobs (22) and Richards (36). The soil samples were placed in duplicate in plastic rings on a porous ceramic plate in a pressure container. All samples were wet thoroughly by capillary action by pouring water on the plate and allowing to stand overnight. The container was closed, the pressure raised to 25.3 cm Hg (1/3 atm) for 24 hours, samples were removed, placed in pre-weighed 2-inch moisture cans and weighed, dried for 48 hours at 110°C., re-weighed, and the moisture percent computed. The "field capacity" was computed as the water remaining in the soil after the pressure treatment expressed as a percent of the oven-dry weight.

b. Wilting coefficient determination. Wilting coefficient, the point at which a plant can no longer extract sufficient water from the soil to sustain metabolic processes and goes into a condition of permanent wilt, is approximately 15 atmospheres tension on the capillary water.

This determination was made in standard pressure plates (22, 35, 37, 38) using duplicate samples of the two soils. Prepared diaphrams were presoaked for several hours, placed on the pressure plate and the spacer ring put in place. Soil samples were placed in rubber rings directly on the diaphram, soaked by capillary action by pouring water on the diaphram and allowing it to stand overnight. A sheet of aluminum foil was placed over the samples and the pressure plate sealed with 15 lbs. torque on the bolts sealing the plate. Fifteen atmospheres (1140 cm Hg) air pressure was maintained for 24 hours. Samples were removed, placed in pre-weighed 2 inch moisture cans, weighed, dried for 48 hours at 110°C. and re-weighed. Wilting coefficient was computed as a percent of the oven-dry weight.

c. Saturation determination. The saturation point of the soil was determined in the greenhouse at the beginning of the experiment. The pots to be maintained in a saturated condition were filled with 4000 gm of soil (oven dry basis) and the weight of soil and pot determined. Water was then added until free water was apparent at the surface. The pots were weighed and the saturation percent determined. In the greenhouse, the saturated pots were maintained with free water on the surface of the soil.

Results and Discussion.

The intent of this study was to synthesize pedotubules and isolate and analyze rhizosphere material for iron. The entire experiment could be considered of limited success in that no pedotubules were produced and the root concentration in the soil mass was so great that sampling of individual rhizo-

spheres was impossible. Results can only be reported on a visual examination of the potted material.

In both soils in the saturated series under all iron treatments, the soil developed a highly reduced condition. Color of the soil was a light bluish-gray throughout (Fig. 25, 26). All the pedotubules that were in the original soil material were completely decomposed and the iron apparently reduced to the ferrous form. Odor, resembling sewer gas, typical of a saturated soil, was present. The bluish-gray color (No Munsell chips of this color available) of the moist soil changed to light gray (10 YR 6/1) upon drying. Analysis revealed no significant difference in iron level distribution between the pots on the same iron level treatment. (Table 8). The color change from bluish-gray to light gray (10 YR 6/1 dry) apparently resulted from the oxidation of the ferrous iron. (20).

The saturated pot of site 1 soil to which no iron was added showed evidence of iron accumulation around several roots in the soil mass (Fig. 27) and around roots in contact with the side of the crock (Fig. 26, 28), giving the roots (ordinarily white) a red color (Fig. 25).

In the semi-saturated pots, only the larger pieces of the residual pedotubules remained. The color of the soil was more nearly the color of the soil when potted, being quite gray but lacking the bluish cast. No newly developed pedotubules or accumulation of iron was apparent in any of these pots.

Pots maintained at field capacity showed no disintegration of residual pedotubules and color was similar to the soil color when potted. No accumulation of iron was detectable around the plant's roots.

In two of the pots (14B, 14C) in which the moisture content was allowed to fluctuate from field capacity to wilting coefficient the roots were coated with a thin layer of iron oxide imparting a red color to those roots. No

EXPLANATION OF FIGURES

Fig. 25. Bottom of soil mass from pots 11A (left) and 22C (right). Soil color (bluish-gray) of 11A was typical for soils under saturated conditions. Soil color of 22C was typical for all other pots. Roots on surface of pots illustrate accumulation of iron around roots. Pot 11A shows accumulation (reddish color of roots) and 22C shows no accumulation of iron.

Fig. 26. Close-up of bottom soil mass from pot 21A showing accumulation of iron around some roots and with no iron accumulation around others.

Fig. 27. Typical color variation of soils in moisture series.

Fig. 28. Pot 11A cut to expose interior of soil mass. Accumulation of iron around individual plant roots is evident.

Fig. 29. Construction of simulated roots. Left to right strips of polyurethane sponge, glass capillary tubes, completed "roots".

Fig. 30. Material utilized in simulated root experiment showing organization and dates of completion.

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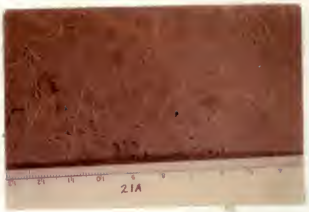

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Table 8. Iron analysis of soil at temination of greenhouse experiment.

Pot No.	$\%Fe^{++}$	% Total Fe
11A	.52	2.06
11B	.59	1.77
11C	.42	1.95
12A	.32	1.90
12B	.32	1.72
12C	.48	1.62
13A	.41	1.55
13B	.39	1.72
13C	.37	1.01
14A	.39	1.61
14B	.40	1.55
14C	.40	1.48
21A	.36	1.95
21B	.37	1.95
21C	.32	1.88
22A	.34	2.00
22B	.39	1.95
22C	.49	2.06
23A	.40	2.11
23B	.45	2.56
23C	.36	1.90
24A	.44	2.11
24B	.34	2.00
24C	.40	2.00

disintegration of residual pedotubules and no apparent color change occurred in the series.

On July 25, 1964, redox potentials (Eh) and pH determinations were made in all pots. The pH was determined on soil approximately 1/2 inch below the surface and the Eh, at a depth of 2 1/2 inches below the surface. The pH measurments were made with a Beckman pocket-model pH meter with a combination electrode, and the Eh measurements with a Beckman Model G potentiometer with a platinum electrode and a calomel reference electrode. A weak trend was found toward slightly higher acidity in the wet soils than in the drier soils and toward less acidity in iron-treated pots (Table 9).

There was a definite distinction in Eh between the saturated and the unsaturated pots. The electrochemical potential of the reaction $Fe^{+3} + e \rightarrow Fe^{+2}$ is -0.770 volts in water and -0.700 volts in 1N HCl (19).

In this experiment, the obviously "reduced" soils had redox potentials between 0.224 and 0.459 volts. Adjustment to pH 7, these extremes were 0.099 and 0.359 volts. Other "oxidized" soils in this experiment had potentials from 0.469 to 0.630 volts, with values corrected to pH 7 ranging from 0.403 to 0.598 volts. "Reduced"soils were correlated with a bluish-gray color and absence of residual pedotubules while the "oxidized" soils had gray to brownish colors and remnants of residual pedotubules.

Basically this experiment showed the susceptibility of the pedotubule to decomposition and dispersion under reducing conditions and the possibility of the accumulation of ferric iron around plant roots in otherwise reducing conditions.

One possible reason that pedotubules did not develop in the environment was the low pH. Only three pots (22A, B, and C) developed pH's above 7.0 and in these pots the Eh was apparently too high to permit development.

Table 9. Redox and pH values of soils at termination of greenhouse experiment 25 July 1964. Eh corrected to pH 7 using equation Jackson (17): E_{Redox} (pH7) = E red soil + .059 (soil pH-7)

Pot	Eh Volts	Eh Standard $Pt:H^+ = 0$	pH	Eh (Correct Volts to pH7)
11A	.465	.224	4.7	.099
B	.700	.459	4.8	.329
C	.522	.281	4.8	.151
12A	.830	.589	4.6	.447
B	.810	.569	4.9	.445
C	.794	.553	5.3	.453
13A	.840	.599	5.0	.481
B	.788	.547	5.6	.464
C	.785	.544	5.9	.479
14A	.855	.614	4.5	.466
B	.841	.600	5.8	.529
C	.871	.630	5.2	.524
21A	.630	.389	6.5	.359
B	.598	.357	7.0	.357
C	.495	.254	6.3	.213
22A	.780	.539	8.0	.598
B	.710	.469	7.4	.493
C	.782	.541	7.1	.547
23A	.750	.509	5.4	.415
B	.750	.509	5.2	.403
C	.752	.511	6.6	.487
24A	.724	.483	6.4	.448
B	.742	.501	6.2	.457
C	.722	.481	6.8	.469

Iron concentrations (Table 8) as determined on dry soils probably reflected more post-experimental oxidation and alteration than biologically-oriented activity.

D. Simulated Root Experiment

This experiment was designed as a pilot study of movement of iron and the effect of microbiological activity on the oxidation state of iron in the soil as affected by exudation by plant roots.

Twelve 4-inch flower pots were filled with soil from each site and treated: 1A, Brown County soil; 1C, Brown County soil with 0.5% Fe as ferrous sulfate added: 2A, Republic County soil; and 2C, Republic County soil with 0.5% Fe as ferrous sulfate added.

Simulated roots were constructed by inserting a 1/8 in. square by 1.5 inch polyurethane sponge strip to the end of a 6 inch section of thin-wall glass capillary tube (Fig. 29) allowing the sponge to protrude approximately 1.2 in. The "root" assembly was inserted into the potted soil using a probe to open a "channel".

Nutrient solutions were prepared using 0.5% a-dextrose as a sugar nutrient and 0.5% tryptophan as a protein nutrient. The pots were wet to approximately field capacity and the nutrient solutions added through the capillary tube by means of a syringe and hypodermic needle. The dextrose solution was added to half of the pots and the tryptophan solution to the other half.

Pots were treated twice weekly with the nutrient solution and watered frequently to maintain a favorable moisture condition for microbiological activity.

The treatment was conducted on all pots for two weeks, then one series

was terminated each week for six weeks until all series were completed (Fig. 30). Samples were taken by placing a 15 mm tube around the protruding capillary tube and taking a core to the bottom of the pot containing the "root" assembly. A second core was taken near the side of the pot as a check.

All samples were placed in test tubes, oven-dried, and stored in the dry condition until ferrous and total iron analysis could be made.

Iron analysis results and pertinent ratios are given in Table 10.

E. Microbiology

Determinative Bacteriology.

Initial isolation of higher iron bacteria from the soil samples was accomplished by using the ferric-ammonium-nitrate-citrate agar described by Allen (2). Preparation of the agar was as follows:

1. Mix

a.	Ammonium sulfate $(NH_4)_2SO_4$	0.5 gm
b.	Sodium nitrate $NaNO_3$	0.5 gm
c.	Dipotassium phosphate K_2HPO_4	0.5 gm
d.	Magnesium sulphate $MgSO_4.7H_2O$	0.5 gm
e.	Calcium chloride $CaCl_2.6H_2O$	0.2 gm
f.	Ferric ammonium citrate $NH_4FeC_6H_4O_7$ or $(NH_4)_3Fe(C_6H_5O_7)_2$ or $(NH_4)_6Fe(C_6H_5O_7)_3$	10.0 gm
g.	Water H_2O	1000.0 ml
h.	Agar (Difco)	15.0 gm

Adjust to pH 7 with NH_4OH or H_2SO_4

2. Heat until reagents are dissolved, sterilized and store until needed.

Table 10. Iron analysis of soils in simulated root experiment.

	Ferrous Iron %				Total Iron %				R:S Ratios		Fe^{++}:Total Fe	
Sample	Rhizo	s^2	Soil	s^2	Rhizo	s^2	Soil	s^2	Fe^{++}	Total Fe	Rhizo	Soil
1AP7	.469	.0021	.562	.003343	2.61	.0297	2.65	.04962	.844	.920	.180	.213
1AP8	.435	.0021	.443	.00113	2.52	.0000	2.17	.0013	.982	1.16	.172	.204
1AP	.450	.001109	.522	.005927	2.58	.009322	2.47	.1191	.862	1.04	.174	.211
1CP7	.440	.00156	.529	.00558	2.48	.0242	2.37	.0227	.832	1.05	.177	.223
1CP8	.365	.0063	.300	.0003	2.16	.0009	2.33	.0992	1.215	.928	.169	.128
1CP	.416	.002409	.4664	.02539	2.37	.0964	2.36	.04499	.895	1.01	.176	.198
1P	.437	.002378	.4960	.01078	2.48	.04125	2.41	.08038	.880*	1.03	.176	.206
2AP7	.448	.00023	.475	.00057	2.26	.00240	2.34	.00123	.944	.965	.198	.203
2AP8	.483	.00148	.585	.0199	2.47	.0213	2.41	.0676	.855	1.03	.195	.242
2AP	.470	.001960	.5173	.004560	2.40	.01742	2.36	.002289	.910	1.015	.196	.219
2CP7	.410	.00060	.400	.00400	2.31	.00393	2.38	.000900	1.02	.971	.178	.168
2CP8	.455	.00057	.485	.00063	2.54	.01403	2.24	.0437	.938	1.13	.179	.226
2CP	.434	.001537	.4425	.002514	2.42	.02249	2.37	.001012	.980	1.02	.179	.186
2P	.4542	.001494	.4857	.004950	2.41	.01796	2.36	.003639	.932**	1.02	1.88	.206
1AS7	.355	.00070	.410	.0002	2.31	.00383	2.41	.0229	.876	.958	.154	.170
1AS8	.416	.01354	.440	.00057	2.16	.2813	1.84	.01146	.946	1.16	.193	.239
1AS	.3958	.00790	.4300	.005873	2.22	.1648	2.03	.009092	.912	1.09	.178	.211
1CS7	.445	.00083	.494	.0022	2.53	.0282	3.18	1.8602	.901	.795	.176	.155
1CS8	.505	.00826	.518	.00326	2.21	.0469	2.21	.0780	.975	1.00	.228	.234
1CS	.4800	.004907	.5067	.002707	2.37	.09569	2.44	.1334	.945	.970	.202	.208
1S	.4440	.007440	.4726	.005865	2.30	.1320	2.25	.1446	.938**	.102	.193	.210
2AS7	.401	.00099	.480	.0770	2.30	.00653	2.41	.0137	.835	.955	.174	.199
2AS8	.442	.00010	.445	.00003	3.46	.0009	3.46	.0033	.995	1.00	.128	.129
2AS	.415	.001064	.4482	.002120	2.76	.2692	2.76	.2692	.916	1.00	.150	.162
2CS7	.372	.00097	.428	.00017	2.24	.0081	2.28	.0081	.870	.983	.166	.188
2CS8	.510	.000473	.542	.00017	3.44	.0000	3.42	.0009	.942	1.05	.148	.159
2CS	.441	.006014	.4850	.004057	2.85	.4074	2.85	.3817	.911	1.00	.155	.170
2S	.426	.003005	.4637	.003044	2.80	.2986	2.80	.2986	.919*	1.00	.152	.165
1			.48627	.007992			2.3294	.123418				.209
2			.4747	.004016			2.5865	.22849				.184

s^2 = variance. * Significant at 95% level. ** Significant at 80% level.

Culture plates were inoculated in a standard dilution series. The initial 10^{-2} dilution was prepared by thoroughly mixing 1.0 gram fresh soil with 99 ml of sterile water. Dilution plates were made by drawing 1 ml and .1 ml suspension from 10^{-2}, 10^{-4}, and 10^{-6} dilutions of the soil suspension.

The ferric-ammonium-nitrate-citrate agar was melted and cooled to 45°C. Approximately 12 ml of the agar was poured into the inoculated culture dishes and swirled to mix the inoculant and spread the agar over the bottom of the dish.

Plate counts were made from soil and rhizosphere material. Colonies, displaying the ability to precipitate iron in the colony, were isolated, recultured, and identified by criteria from Bergey's Manual (9) and Lord's Manual (33).

Test media used as described by Lord (33) were:

1. 0.1% potassium nitrate broth with fermentation tubes.
2. Litmus milk.
3. Frazier gelatin agar.
4. Ammonium phosphate glucose agar.
5. Urea glucose agar.
6. Motility test agar.
7. Mannitol B. T. B. agar.
8. Glucose B. T. B. agar.
9. 7% NaCl agar.
10. 12% NaCl agar.

Test reagents as described by Lord (33) were:

1. Frazier's gelatin developer.
2. Sufanilic acid reagent.
3. Dimethyl alpha-naphthylamine reagent.

Isolation of iron-precipitating bacteria and initial purification of the culture was done on ferric-ammonium-nitrate-citrate agar (2). Final purification of cultures were held on nutrient agar slants. Cultures were checked back on iron agar to see that the original iron precipitating capability had not been lost in purification. Nutrient agar slants were then inoculated in triplicate and the tubes incubated at 10, 23, and 37°C to determine the optimum growth temperature.

Determinative results and methods of inoculation and media are listed in Table 11 for 6 of the 12 colonies isolated.

Iron Bacteria.

This microbiological study was based on plate counts to determine relative numbers of higher iron bacteria in the soil and isolation and identification of the bacteria.

Plate counts of soils and pedotubule (rhizosphere) material disclosed wide variations in populations of higher iron bacteria in the two areas (Table 12).

The 2:1 R:S (rhizosphere:soil) ratio in site 1 material and 3:1 R:S ratio in site 2 material by plate counts indicate a definite increase in the higher iron bacteria in the pedotubules.

From the dilution plates, 12 colonies were isolated. Nine of the 12 colonies were identified as Micrococcus candidus. One colony was identified as Streptococcus sp., one colony was unidentified micrococcus and one colony an Actinomycete. The "actino" was determined not to have the ability to accumulate iron, so no further identification was made.

Table 11. Method of inoculation, media used and qualitative results of cultures for taxonomic determinations.

Inoculation	Medium	Culture 1	2	3	4	5	6
		Reaction					
Streak Plate	Frazier's Gelatin Agar	+	-	+	+	+	+
Slants	Ammonium Phosphate Glucose Agar	+	+	+	+	+	+
	Urea Glucose Agar						
	Urea used as N source	-	-	-	-	-	-
	Urea hydrolyzed	-	-	-	-	-	-
	7%NaCl Agar	+	-	+	+	+	+
	12%NaCl Agar	-	-	-	-	-	-
Stabs	Motility Agar	-	+	-	-	-	-
	Mannitol B.T.B. Agar	-	-	-	-	-	-
	Glucose B. T. B. Agar	-	-	-	-	-	-
Broth	0.1% KNO_3 Broth						
	KNO_3 ---> KNO_2	-	+	-	-	-	-
	Gas	-	-	-	-	-	-
	Litmus Milk Reaction	-	-	-	-	-	-
	Acid Curd	-	-	-	-	-	-
	Rennet Curd	-	-	-	-	-	-
	Peptonization	-	-	-	-	-	-
	Reduction of Litmus	-	-	-	-	-	-
	Gas Production	-	-	-	-	-	-
	Morphology	Micro coccus	Strepto coccus	Micro coccus	Micro coccus	Micro coccus	Actino-mycete
	Temp. optimum growth	23°C.	23°C.	23°C.	23°C.	23°C.	23°C.
	Gram Stain	-	-	-	-	-	-

Cultures 1, 3, 4, 5 Micrococcus candidus Cohn.

Table 12. Average number of higher iron bacteria per gram of soil material as determined by plate counts.

Site	Material	Number of Plates Counted	Average Number Bact./Gram X 10^4
1	Soil	10	527.8
1	Pedotubule	5	1032.0
2	Soil	4	8.72
2	Pedotubule	4	24.50

Note: Site 1, Brown County, Kansas. Site 2, Republic County, Kansas.

V. GENERAL DISCUSSION

A weathering sequence 1. deposition, 2. oxidation, and 3. leaching was explained in the work of Kay et al. (26, 27, 28, 29), Daniels (13), and Ruhe et al. (42). These workers and others (14, 16, 41)[1] found pedotubules to be most numerous in the deoxidized-unleached (young) soils. Blümel (8) found "root tubes" of iron oxide, interpreted to be essentially the same type structure as those observed in the present study, in gley soils. Scholtes[1] observed pedotubules on the roots of Equisetum sp. which typically inhabit moist to wet areas, indicating that perhaps the deoxidized or reducing situation is necessary for the development of pedotubules.

Pedotubules have not been located by the author in wet areas but have been found in areas of deoxidized loess that may possibly be saturated and anaerobic in wet weather.

[1]Scholtes, Loc. cit.

The greenhouse study and the simulated root experiment were attempts at studying causative factors for the development of pedotubules. In the simulated root experiment a possible movement and oxidation of iron through the soil into a "rhizosphere" was produced by the feeding in the nutrient solutions. Movement of the iron in this experiment could be only by diffusion. Barber (5) stated that three mechanisms by which nutrients become available to plants are: 1. root interception, 2. mass flow, and 3. diffusion. In natural environment, movement of iron would occur through the latter two mechanisms. At the high pH recorded in the soils in which the pedotubules are found in greatest abundance, the iron is at a low solubility. There is no way of explaining the enrichment of the rhizosphere with iron to a level of 25% except by the movement of iron in this environment and subsequent enrichment of the rhizosphere (Table 4) (13, 14). It has been tacitly assumed that the iron moves in the more soluble ferrous state in which form it would be relatively abundant under the deoxidized conditions which are apparently necessary for pedotubule formation.

In the greenhouse experiment, the necessary Redox potentials for the reduction of iron (19) did not occur. Areas of definite oxidation were observed in the rhizosphere in some treatments in an otherwise deoxidized soil. Eh measured in these pots ranged from well-oxidized to poorly oxidized (21). Apparently in the soils in this experiment, there was an insufficient supply of electron acceptors to permit rapid oxidation of the iron except in the rhizosphere. One possible explanation is that other elements with a lower Eh potential than $Fe^{++} + e^{-} \longrightarrow Fe^{+++}$ are present in the soil and are saturating the electron acceptors thus inhibiting the oxidation of the iron. Another possibility is that the ferrous iron is weathered out of the soil minerals at a rate faster than it can be oxidized by the oxidizers present and thus the

soil is maintained in a deoxidized state. In either case the rhizosphere has become a "sink" for electrons and a deposition area for the oxidized iron.

Action in the rhizosphere appears to hold the key to the formation of the pedotubule. The proposed mechanism is that the iron, moving into the rhizosphere in the ferrous form is oxidized and immobilized, and becomes the cementing "plasma" in the pedotubule.

The plant species involved influences the development of the pedotubule. Rovira (39a, 39b, 39c, 39d, 39e) showed conclusively that a large number of nutrients and metabolic products are exuded from the roots of plants and/or sloughed off by the roots, varying with the species. He also suggested that such factors as light, age of plant, nutrient levels, and microbial activity affect quantity and quality of the exudation, with definite effect by wetting and drying. These variables, in part, explain why pedotubules occur under some conditions and not in others.

Rovira (39a, b, c, d, e) found such compounds as aspartic acid, glutamic acid, phenylalanine, leucine, serine, asparagine, glucose, flavanones, nucleotides, fructose, and alpha-alanine in the exudates of peas, oats, tomato, clover, and wheat. Not all of these compounds were exuded by any one of the plants and not all plants exude the same number of compounds. Starkey (46) reported alanine, valine, glutamine, vitamins, other sugars, tartaric acid, d-xylose, malic acid, citric acid, and maltose and suggested the presence of indole or salicylic acid within the rhizosphere in addition to those listed. Katznelson et al. (24, 25, 40) reported cystine, lysine, and proline in addition.

Microorganisms requiring free amino acids receive the greatest benefit in the rhizosphere (30, 49). Those organisms that require B-vitamins or B-vitamins and amino acids displayed benefits from this environment but not the increase displayed by the group requiring amino acids only.

The rhizosphere:soil (R:S) ratio for the iron-precipitating bacteria studied in the research was approximately 2. The rhizosphere effect is best characterized by the R:S ratio, which has been shown to be nearly one to 230 for some groups of bacteria (1).

Several workers (1, 24, 25, 31, 32, 40, 47, 49) have shown a great increase in the microbial population and activity in the rhizosphere. The effect was assumed to be due, in part at least, to the increased supply of nutrients and energy source.

When metabolized by microorganisms, amino acids and sugars produce quite different redox potentials (12). When propagated in pure culture and in amino-acid substrate, the same strain of bacteria produce a much higher redox (Eh) potential than if it were grown in a sugar or starch substrate. It would, then, seem reasonable that in the soil around roots (the rhizosphere) into which amino acids were being exuded, the Eh would be higher than if sugars and/or starches were being exuded. Following the same line of reasoning, if the Eh differential is at the proper level and is sufficiently high, the iron in the rhizosphere in which amino acid predominates should be in the oxidized state (Fe^{+++}) and the iron in the rhizosphere in which sugar predominates would be in the reduced state (Fe^{++}). Therefore, the expected result from the simulated root experiment was that the ferrous iron:total iron ratio would increase with the sugar substrate and decrease with the amino acid (tryptophan) substrate, with a decrease of total iron in the sugar-supplemented situation. The expected result did not occur in all aspects.

The forms of iron in the rhizosphere and in the soil show some interesting trends (Table 10). The ratio of ferrous iron in the "rhizosphere" to ferrous iron in the soil was significant in all treatments at the 80% level. There was

no difference in the total iron (R:S) ratio between the protein-treated series and the sugar series. The cause of this difference is not clear, but there has apparently been some change in the system. From evidence shown in the ferrous iron to total iron ratios, the change is apparently in the rhizosphere. This is possibly due to bacterial action in the rhizosphere affecting the oxidation state of the iron.. The ferrous to total iron ratios were more consistent in the soil than in the rhizosphere.

Although the evidence obtained in the simulated root experiment was inconclusive, it was sufficiently strong to warrant further investigation with carefully designed experiments, both from the technical and statistical standpoints.

Burrows and Cordon (12) found that pure cultures of heterotrophic soil bacteria grown in sterile sand media on glucose and casein substrate demonstrated widely varying Eh potentials. Using Bacterium cereus, B. fluorescens, Trichoderma sp., Mucor sp., Actinomyces californicus, and A. bobili on both media, they found a low Eh of -85 mv. occurring in the Mucor sp. culture at 366 hours on glucose and the high Eh of +386 mv. in Mucor sp. culture at 144 hours on casein. Among the organisms growing on glucose, the low Eh was -85 mv. and the high Eh was +124 mv. in the Trichoderma sp. culture at 144 hours. Organisms grown on casein had a range of +30 mv. (A. californicus) at 24 hours to +386 mv. The group of organisms tested showed considerable variation with time, but very little overlap of Eh potentials between nutrient substrate. Eh determinations were made using a 1.5 inch bright platinum wire inserted directly into the soil with a saturated KCl salt bridge as reference (12).

Although the quoted values are of insufficient magnitude in themselves to cause spontaneous oxidation of Fe^{++} to Fe^{+++}, it should be emphasized that the

cultures were grown in sterile sand media and the fact that the Eh of this medium was changed would indicate that microorganisms metabolizing these different nutrient substrates may well vary the Eh of another media in a totally different range of Eh values.

There have been other reports (46) of Eh alteration by microorganisms metabolizing different substrates.

Allison and Scarseth (3) reported a reducing effect of molds on ferric compounds and also reported a solvent effect of sucrose on hydrated iron oxides.

Two other mechanisms, not investigated or discussed herein, may be complementary to the one discussed: 1. increased oxygen diffusion along or through plant roots (23) giving an isolated zone of higher 0_2 concentration and thus higher redox potential; and 2. the generation of electrical currents and potentials within the plant root (44).

The banding noted in the physical characteristics section and particularly Figs. 6, 7, 10, 11 appears to be due to alteration of redox potential by microorganisms. Although no experimental proof of the mechanism of sequence of zoning is presented, it seems that the soil microorganisms will metabolize the readily digestible material first, namely the sugars. This will create a lower redox potential near the root. While the sugars are being digested out of the "exudation solution", the proteins diffuse outward. Little of these substances are utilized by the microbes until the easily digested materials are gone. When the sugars are depleted and microbes start on the amino acids, iron precipitation and cementation of the soil particles begin. Thus, there occurs a zone of reduction immediately surrounding the plant root and a zone of oxidation (the pedotubule) surrounding the zone of reduction (Fig. 9, 10).

Zones of oxidation and reduction as they apparently occur around plant roots are depicted in Fig. 31 . This mechanism, of course, depends on the exudation solution containing both sugars and amino acids.

The difference in carbon content between the pedotubules and the matrix soil (Table 4) indicates accelerated biological activity in the pedotubule. Whether this increase in activity was due to microbiological activity or to exudation products from the plant root or a combination of the two factors was not determined specifically.

The iron difference is assumed to be due to a concentration in the rhizosphere of certain plants. The determination of the mechanism of this concentration was the purpose of this study, and was originally assumed to be an enrichment process.

Occurence of pedotubules in a soil series such as Crete silty clay loam would indicate that the agronomic complications due to pedotubules are not restricted to isolated areas as they were in the limited research sites.

VI. SUMMARY AND CONCLUSIONS

A natural phenomena consisting of iron-oxide cemented concretions on living plant roots was the object of the study. This structure was named the "ferruginous pedotubule" after Brewer's discussion on Fabric and Mineral Analysis of Soils (11). The morphology of the ferruginous pedotubule was determined from chemical analysis of iron and organic carbon, physical characteristics (bulk density, particle density, mineralogy by thin section) and a discussion of the location and basic relationships. Genesis was studied by way of experiment including an attempt to "grow" pedotubules in a greenhouse experiment, a study of iron movement and oxidation states with different nutrient substrates and a

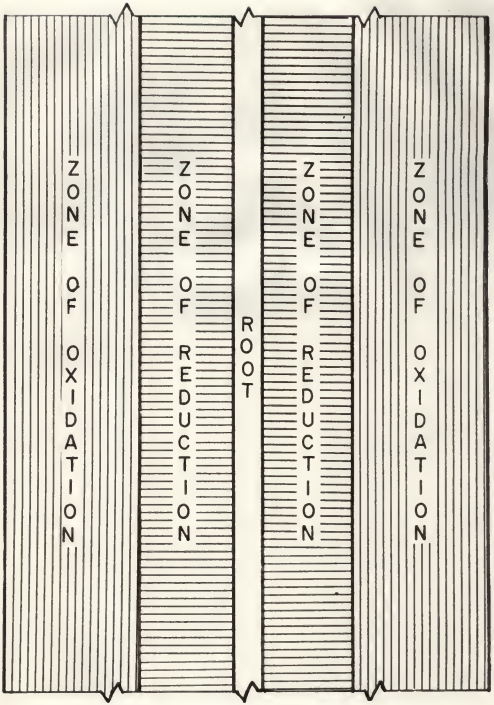

Fig. 31. Highly idealized sketch of rhizosphere depicting the zone of reduction immediately surrounding root which in turn is surrounded by a zone of oxidation.

study of rhizosphere effect on iron-precipitating bacteria. All other pertinent information, such as root exudation and bacterial effect on redox (Eh) potential was drawn from technical literature.

In the greenhouse experiment, iron accumulation was found adjacent to the roots in the saturated soils and in the soils where the moisture level was allowed to fluctuate between field capacity and wilting coefficient. The pedotubules present in the potting soil completely decomposed in the saturated soils. In one saturated pot, concentrations of iron were found around plant roots that would indicate incipient pedotubule development. The study of iron movement and oxidation states gave weak though recognizable trends. Probably causes of inconclusiveness were: 1. variability in chemical analysis and 2. oxidation of the ferrous iron to ferric iron during the interval between sampling and analysis.

The rhizosphere effect on iron-precipitating bacteria was determined to be a 2:1 to 3:1 Rhizosphere:Soil ratio. One iron-precipitating organism was identified as Micrococcus candidus. One other micrococcus and one streptococcus were unidentified.

The pedotubule is a physical phenomenon of the soil created by the enrichment with iron of the rhizosphere of certain plants with protein exudate, resulting in the precipitation of iron largely by biological influences, to form a tubular concretionary structure around the plant root by the cementing of soil particles with iron oxides. The iron moves into the rhizosphere in the soluble ferrous form by diffusion and mass flow, where it is immobilized as ferric iron by altered Eh.

For pedotubule formation, the Eh in the soil should therefore be in the reducing range for iron, normally occurring in a saturated soil.

Eh in the rhizosphere may be influenced by three agents: 1. microbiological activity, 2. increased oxygen difusion in the reducing soils along plant roots, and 3. electric charges set up in the root in conjunction with cations in the soil.

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APPENDIX

Appendix 1. Iron analysis for simulated root experiment.

Sample calculation:

$$\mu gm\ Fe \text{ in aliquot} \times \frac{250 \text{ ml. in sample}}{\text{aliquot size}} \times \frac{1 \text{ gm}}{.1 \text{ gm}} \times \frac{1 \text{ gm}}{1{,}000{,}000 \text{ ugm}} \times 100 = \%\ Fe \text{ in sample}$$

Designation of samples:

1st digit	1st letter	2nd letter	2nd digit
1 - soil site 1 2 - soil site 2	A - no iron added C - 5% $FeSO_4$ added to soil	P - 0.5% tryptophan utilized as nutrient sol'n. S - 0.5% dextrose utilized as nutrient sol'n.	7 - 7 week incubation 8 - 8 week incubation

IRON ANALYSIS

	Rhizosphere						Soil					
Sample	OD** x10^{-2}	µgm in aliq	%Fe^{++}	OD x10^{-2}	µgm in aliq	%Fe total	OD x10^{-2}	µgm in aliq	%Fe^{++}	OD x10^{-2}	µgm in aliq	%Fe total
	10 ml aliquots			2 ml aliquots			10 ml aliquots			2 ml aliquots		
1AP7	30	16.0	.40	39	20.7	2.59	43	22.8	.57	42	22.2	2.78
	33	17.6	.44	42	22.3	2.79	44	23.2	.58	42	22.2	2.78
	32	17.2	.43	39	20.7	2.59	39	20.8	.52	39	20.8	2.60
	36	19.2	.49	41	2.17	2.71	35	18.4	.46	42	22.2	2.78
	34	18.0	.45	38	20.2	2.52	51	26.4	.64	67	33.5	4.19*
	37	19.6	.49	34	18.1	2.26	44	23.2	.58	45	23.0	2.98
	38	20.2	.52	42	22.3	2.79	47	24.8	.62	35	15.9	1.99*
	40	21.2	.53	40	21.2	2.65	40	21.2	.53	40	21.2	2.65
1CP7	30	15.9	.40	38	20.2	2.52	40	21.2	.53	37	19.7	2.46
	31	16.5	.41	40	21.2	2.65	33	17.6	.44	38	20.2	2.52
	32	17.1	.43	37	19.7	2.46	36	19.1	.48	36	19.1	2.39
	30	15.9	.40	39	20.7	2.59	33	17.6	.44	38	20.2	2.52
	36	19.1	.48	37	19.7	2.46	50	26.5	.66	38	20.2	2.52
	35	18.6	.46	34	18.1	2.26	41	21.7	.54	34	18.1	2.26
	35	18.6	.46	34	18.1	2.26	42	22.3	.56	32	17.1	2.14
	37	19.7	.49	40	21.2	2.65	43	22.8	.58	32	17.1	2.14
1AS7	25	13.3	.33	35	18.6	2.32	31	16.5	.41	38	20.2	2.52
	26	13.8	.34	36	19.1	2.39	30	15.9	.40	38	20.2	2.52
	29	15.5	.39	34	18.1	2.26	32	17.1	.43	36	19.1	2.39
	27	14.4	.36	34	18.1	2.26	30	15.9	.40	33	17.6	2.20
1CS7	34	18.1	.45	40	21.2	2.65	44	23.3	.58	38	20.2	2.52
	35	18.6	.46	44	23.3	2.91	38	20.2	.50	38	20.2	2.52
	34	18.1	.45	40	21.2	2.65	40	21.2	.53	37	19.7	2.46
	39	20.7	.52	42	22.3	2.91	34	18.1	.45	38	20.2	2.52
	32	17.1	.43	38	20.2	2.52	48	25.5	.64*	98	52.0	6.50*
	32	17.1	.43	35	18.6	2.32	36	19.1	.48	44	23.2	2.12
	33	17.6	.44	35	18.6	2.32	35	18.6	.46	47	24.9	3.12
	35	18.6	.46	30	15.9	1.99	36	19.1	.46	43	22.8	2.86

* Values not used in figuring averages due to wide divergence.

** OD = optical density

Appendix 1. Iron analysis for simulated root experiment (cont.).

IRON ANALYSIS												
	Rhizosphere						Soil					
Sample	OD** x10^{-2}	µgm in aliq	%Fe^{++}	OD x10^{-2}	µgm in aliq	%Fe total	OD x10^{-2}	µgm in aliq	%Fe^{++}	OD x10^{-2}	µgm in aliq	%Fe total
	10 ml aliquots			2 ml aliquots			10 ml aliquots			2 ml aliquots		
2AP7	32	17.1	.43	34	18.1	2.26	37	19.7	.49	35	18.6	2.32
	33	17.6	.44	33	17.6	2.20	33	17.6	.44	35	18.6	2.32
	35	18.6	.46	34	18.1	2.26	37	19.7	.49	36	19.1	2.39
	35	18.6	.46	35	18.6	2.32	36	19.1	.48	35	18.6	2.32
2CP7	28	14.9	.38	36	19.1	2.39	31	16.5	.41	36	19.1	2.39
	30	15.9	.41	34	18.1	2.26	30	15.9	.41	36	19.1	2.39
	31	16.5	.41	34	18.1	2.26	30	15.9	.41	35	18.6	2.33
	32	17.1	.44	35	18.6	2.33	27	14.4	.37	36	19.1	2.39
2AS7	27	14.4	.37	35	18.6	2.32	39	20.7	.53	37	19.7	2.46
	28	14.9	.37	35	18.6	2.32	32	17.1	.44	38	20.2	2.52
	28	14.9	.37	33	17.6	2.20	40	21.2	.54	35	18.6	2.33
	29	15.5	.39	35	18.6	2.32	30	15.9	.41	37	19.7	2.46
	33	17.6	.44	35	18.6	2.32	51	27.0	.69*	36	19.1	2.39
	30	15.9	.40	34	18.1	2.26	30	15.9	.41	37	19.7	2.46
	32	17.1	.43	33	17.6	2.20	30	15.9	.41	35	18.6	2.33
	33	17.6	.44	37	19.7	2.45	31	16.5	.41	35	18.6	2.33
2CS7	29	15.5	.39	35	18.6	2.32	32	17.1	.43	35	18.6	2.32
	28	14.9	.37	32	17.1	2.14	31	16.5	.41	32	17.1	2.14
	25	13.3	.33	33	17.6	2.20	33	17.6	.44	35	18.6	2.32
	30	15.9	.40	35	18.6	2.32	32	17.1	.43	35	18.6	2.32
1AP8	32	17.1	.43	38	20.2	2.52	37	19.7	.49	33	17.6	2.20
	33	17.6	.44	38	20.2	2.52	32	17.1	.43	32	17.1	2.14
	33	17.6	.44	38	20.2	2.52	33	17.6	.44	33	17.6	2.20
	33	17.6	.44	38	20.2	2.52	31	16.5	.41	32	17.1	2.14
1CP8	27	14.4	.36	32	17.1	2.14	24	12.8	.32	30	15.9	1.99
	26	13.8	.34	32	17.1	2.14	22	11.7	.29	31	16.5	2.60
	30	15.9	.40	32	17.1	2.14	22	11.7	.29	32	17.1	2.14
	27	14.4	.36	33	17.6	2.20	--	----	---	31	16.5	2.60
1AS8	23	12.2	.31	39	20.7	2.59	32	17.1	.43	26	13.8	1.72
	23	12.2	.31	39	20.7	2.59	25	13.3	.33	29	15.5	1.94
	29	15.5	.39	39	20.7	2.59	30	15.9	.40	28	14.9	1.87
	22	11.7	.29	38	20.2	2.52	24	12.8	.32	28	14.9	1.87
	37	19.7	.49	26	13.8	1.73	42	22.3	.56	29	15.5	1.94
	36	19.1	.48	23	2.2	1.53	37	19.7	.49	25	13.3	1.66
	39	20.7	.52	35	18.6	2.32	38	20.2	.51	27	14.4	1.80
	41	21.7	.54	21	11.2	1.40	36	19.1	.48	29	15.5	1.94

* Values not used in figuring averages due to wide divergence.
** OD = Optical density

Appendix 1. Iron analysis for simulated root experiment (cont.).

IRON ANALYSIS

	Rhizosphere						Soil					
Sample	OD** x10^{-2}	µgm in aliq	%Fe^{++}	OD x10^{-2}	µgm in aliq	%Fe total	OD x10^{-2}	µgm in aliq	%Fe^{++}	OD x10^{-2}	µgm in aliq	%Fe total
	10 ml aliquots			2 ml aliquots			10 ml aliquots			2 ml aliquots		
1CS8	31	16.5	.41	31	16.5	2.06	38	20.2	.51	32	17.1	2.12
	32	17.1	.43	30	15.9	1.99	32	17.1	.43	31	16.5	2.06
	35	18.6	.46	31	16.5	2.06	42	22.3	.56	30	15.9	1.99
	31	16.5	.41	32	17.1	2.12	32	17.1	.43	31	16.5	2.06
	38	20.2	.51	40	21.2	2.65	42	22.3	.56	35	18.6	2.32
	44	23.3	.58	36	19.1	2.39	42	22.3	.56	43	22.8	2.85*
	47	24.9	.62	24	18.1	2.24	42	22.3	.56	33	17.6	2.20
	31	24.9	.62	33	17.6	2.20	40	21.2	.53	31	16.5	2.06
2AP8	32	17.1	.43	37	19.7	2.46	38	20.2	.51	35	18.6	2.33
	36	19.1	.49	39	20.7	2.58	34	18.1	.45	36	19.1	2.39
	34	18.1	.45	37	19.7	2.46	40	21.2	.53	35	18.6	2.33
	35	18.6	.46	38	20.2	2.52	35	18.6	.46	36	19.2	2.39
	46	24.1	.60*	34	18.1	2.26	67	35.6	.89*	45	23.9	2.99
	36	19.1	.49	34	20.2	2.52	45	23.9	.60	31	16.5	2.06
	40	21.2	.53	38	20.2	2.52	45	23.9	.60	32	17.1	2.39
	40	21.2	.53	37	19.7	2.46	48	25.5	.64	36	19.1	2.39
2CP8	33	17.6	.44	37	19.7	2.46	38	20.2	.51	35	18.6	2.33
	33	17.6	.44	39	20.7	2.71	36	19.1	.49	36	19.1	2.39
	34	18.1	.45	37	19.7	2.46	34	18.1	.45	35	18.6	2.33
	37	19.3	.49	38	20.2	2.52	37	19.7	.49	36	19.1	2.39
2AS8	33	17.6	.44	52	27.5	3.44	34	18.1	.45	51	27.0	3.38
	34	18.1	.45	52	27.5	3.44	33	17.6	.44	53	28.0	3.50
	32	17.1	.43	52	27.5	3.44	33	17.6	.44	58	27.5	3.44
	34	18.1	.45	53	28.0	3.50	34	18.1	.45	53	28.0	3.50
2CS8	36	19.1	.48	52	27.5	3.44	42	22.3	.56	52	27.5	3.44
	39	20.7	.52	52	27.5	3.44	41	21.7	.54	52	27.5	3.44
	38	20.2	.51	52	27.5	3.44	40	21.2	.53	52	27.5	3.44
	40	21.2	.53	52	27.5	3.44	41	21.7	.54	52	27.5	3.44

* Values not used in figuring averages due to wide divergence.
** OD = Optical density.

Appendix 2. Iron analysis data for soil and pedotubules from research sites.

	Opt. Den.	μgFe^{++}	$\%Fe^{++}$	Opt. Den.	μgFe^{++}	$\%Fe^{++}$
Site 1, Soil		10 ml aliquot			2 ml aliquot	
	45	23.9	.60	50	26.5	3.32
	46	24.4	.62	50	26.5	3.32
	43	22.8	.57	50	26.5	3.32
	42	22.3	.56	51	27.0	3.38
Site 1, Pedotubule		10 ml aliquot			0.5 ml aliquot	
	73	38.8	.97	96	51.0	25.5
	82	32.6	1.09	91	48.4	24.2
	84	44.6	1.11	96	51.0	25.5
	89	47.3	1.18	93	49.5	24.6
Site 2, Soil		10 ml aliquot			2 ml aliquot	
	44	23.3	.58	57	30.2	3.78
	36	19.1	.48	55	29.2	3.65
	42	22.3	.56	54	28.6	3.58
	39	20.7	.52	54	28.6	3.58
Site 2, Pedotubule		10 ml aliquot			0.5 ml aliquot	
	60	31.8	.80	96	51.0	25.5
	63	33.4	.84	96	51.0	25.5
	64	34.0	.85	96	51.0	25.5
	72	38.3	.96	90	47.8	23.9

Appendix 3. Carbon determinations.

Material	Wt.	CO_2 Produced (gm)	Carbon (%)	Avg.
Site 1				
Soil	2.0012	.0379	.5164	
	2.0009	.0315	.4293	.4109
	2.0003	.0274	.3736	
	2.0009	.0238	.3244	
Pedotubule	2.0006	.0589	.8029	
	1.9997	.0311	.4241	.6323
	2.0019	.0482	.6566	
	2.0005	.0474	.6457	
Site 2				
Soil	2.0005	.0970*	1.0496	
	2.0009	.0582*	.7932	.3502
	2.0007	.0262	.3571	
	2.0024	.0252	.3432	
Pedotubule	2.0011	.0354	.4824	
	2.0004	.0399	.5439	.5087
	2.0006	.0349	.4757	
	2.0017	.0391	.5327	

* Values not used in figuring averages due to wide divergence.

Appendix 4. Water, nitrogen and Fe^{++} added to individual pots in greenhouse experiment.

Pot	Days	Total Water (l)	Daily Avg.(ml)	N(gm)	Fe^{++}(gm)
11A	149	29.850	200.33	.62	0
B	149	25.020	167.91	.55	.75
C	149	31.860	213.82	.67	1.91
12A	149	32.160	215.83	.67	0
B	149	26.370	176.79	.59	.79
C	149	35.340	237.18	.74	2.12
13A	149	15.790	112.78	.29	0
B	149	23.340	156.64	.53	.70
C	149	27.905	187.28	.62	1.67
14A	149	13.980	93.82	.25	0
B	149	10.460	70.20	.21	.31
C	128	6.840	53.11	.17	.41
21A	149	29.560	198.38	.58	0
B	149	39.090	262.34	.85	1.17
C	149	27.045	184.22	.54	1.62
22A	149	31.320	210.20	.63	0
B	149	24.085	161.64	.51	.72
C	149	27.530	184.76	.55	1.65
23A	149	33.310	223.55	.67	0
B	149	27.400	183.86	.58	.82
C	149	28.350	190.26	.60	1.70
24A	149	20.360	136.64	.42	0
B	149	18.000	120.80	.39	.54
C	149	20.960	140.67	.42	1.26

Appendix 5. Plate counts of bacteria on fresh material from research sites.

Material	Dilution	Colonies	Avg. Count
Brown County			
Soil	10^{-4}	284*	
	10^{-4}	200*	
	10^{-4}	500*	
	10^{-4}	424*	
	10^{-4}	1200*	
	10^{-5}	97	
	10^{-5}	49	53.4×10^5
	10^{-5}	44	
	10^{-5}	34	
	10^{-5}	43	
Pedotubules	10^{-5}	137	
	10^{-5}	118	
	10^{-5}	98	103.2×10^5
	10^{-5}	60	
	10^{-5}	103	
Republic County			
Soil	10^{-2}	204	
	10^{-3}	109	
	10^{-3}	78	87.2×10^3
	10^{-3}	69	
	10^{-3}	93	
Pedotubules	10^{-3}	103*	
	10^{-3}	182*	
	10^{-3}	143*	
	10^{-4}	34	24.5×10^4
	10^{-3}	115	
	10^{-4}	10	
	10^{-4}	22	
	10^{-4}	32	

* Values not utilized in calculating averages.

A PILOT STUDY OF THE MORPHOLOGY AND GENESIS OF THE FERRUGINOUS PEDOTUBULE

by

DONALD A. GIER

B. S., Kansas State University, 1957

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY

Manhattan, Kansas

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The ferruginous pedotubule is a natural soil phenomenon that occurs only under specific condition, around growing plant roots. This study was conducted for the purpose of: (1) describing the pedotubule morphologically and (2) determining the factors responsible for its formation.

Pedotubule morphology was determined by means of (1) quantitative iron and organic carbon determination, (2) bulk and particle density determination, (3) thin-section examination, and (4) gross external form characterization. Genetic studies consisted of (1) field observations and soil relationships, (2) growth of Reed canarygrass (Phalaris arundinaceae L.) under different moisture conditions in the greenhouse and examination of resulting soil conditions, (3) study of iron movement and oxidation states in soils with artificial roots containing various nutrient compounds, and (4) studies of the higher iron-precipitating bacteria in pedotubules and soils collected from pedotubule-containing sites.

Pedotubules were found to be concretions of ferric-oxide-cemented soil minerals. Organic-carbon levels in the pedotubules were consistently higher than in the surrounding soil. Total iron content of the pedotubules was approximately 25%, compared to approximately 3% in the adjacent soil. Ferrous iron levels and ratios of ferrous to ferric iron varied considerably between pedotubules and soils.

Soil conditions under growing plants varied with moisture level. Saturated soils were more highly reduced than unsaturated soils. Redox values varied from 0.099 volts in the saturated soils to 0.598 volts in unsaturated soils. Zones of oxidized iron accumulation were observed around roots regardless of nutrient additive (sugar or amino acid). A 2:1 to 3:1 rhizosphere

to soil ratio was found with plate counts of iron-precipitating bacteria. Micrococcus candidus, Cohn, was identified as one iron-precipitating bacterium present is all samples.

A theory on the formation of ferruginous pedotubules was derived from field observations, laboratory analyses, and literature review:

1. Pedotubules form most frequestly in deoxidized-unleached soils.
2. Pedotubules form on plant roots as a result of cementation of soil materials in the rhizosphere by ferric oxides.
3. Ferrous iron migrates to the rhizosphere where an altered redox potential converts it to the less soluble ferric form.
4. Redox potential is altered by three complimentery mechanisms:
   a. bacterial metabolism of proteinaceous exudates from plant roots,
   b. increased oxygen diffusion along and/or through the plant root, and
   c. electric currents set up in conjunction with soil cations and plant roots.