Microbiologic Factors in the Solution and Transport of Iron

By EUGENE T. OBORN *and* JOHN D. HEM

CHEMISTRY OF IRON IN NATURAL WATER

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Effects of soil microbiota on the concentration and leaching of iron from substrata under different time, admixture, and temperature conditions

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By EUGENE T. OBORN and JOHN D. HEM

ABSTRACT

Amounts of iron dissolved by distilled water from soil, sand, and mixtures of organic matter with soil and sand were determined before and after 2 weeks of incubation at temperatures from 35° to 100° F. Amounts of iron present in solution were from about 1 to 8 ppm (parts per million) after incubation at 72° or 100° F. After incubation at 35° or before incubation, only a few tenths of a part per million or less were present. Amounts of iron in solution were generally much larger when organic matter was mixed with the soil than when soil or sand alone was used. The amounts of iron brought into solution sometimes exceeded the amount available in the organic matter added. The organic matter consisted of pulverized dried plant leaves and stems, mostly aquatic species, and manures of three species of herbivorous animals.

The increase in amount of iron leached from soils after incubation is related to microbiologic activity. Organic complexes of iron aid in retaining in solution the iron made available by the microbiota. By helping to bring iron into solution, microbiota influence the iron content of surface and ground water in many areas.

INTRODUCTION

This report describes a study of the microbiochemical aspects of the solution of iron in soil moisture. The chemical factors that enter into the transport, solution, and deposition of iron in ground-water aquifers are published in other chapters of Water-Supply Paper 1459.

Published literature and experimental work in the study of biological aspects of the chemistry of iron in natural water (Oborn, 1960a) show conclusively that iron is present in comparatively large amounts in aquatic vegetation and that such vegetation is particularly important in adding iron to surface water and in removing dissolved or suspended iron from water. Iron combined with organic ions or molecules can form relatively stable and soluble complexes (Hem, 1960b). Aquatic vegetation seems to be an important source of iron for surface water, and iron from vegetable debris is likely to pass into ground water. Several steps are involved in the process: Iron is released from decaying organic matter by a multiplicity of microbes, it is dissolved in the soil solution, and then is carried down to the water table. Factors associated with removal of iron from water, such as adsorption or precipitation caused by changes in pH and redox potential, apparently have a minor effect.

RELATION TO PREVIOUS WORK

This study is intended to serve as a companion to another investigation, which pertains principally to the effects of the larger, more conspicuous types of aquatic vegetation on iron content in natural water (Oborn and Hem, in press). The iron content of 5 of the plants and the 4 soils used in the present study were reported in a previous investigation (Oborn, 1960b), but the iron content of stonewort and the three manures is here reported for the first time. Differences in both kind and amount of organic matter in the soils affect their iron content; for example, the organic-iron extraction effects from a given weight of organic matter high in fiber (cellulose) are not the same as those from the same weight of organic matter high in protoplasm (protein).

Unicellular and aquatic organisms are important to the presence of iron in water in two ways (Oborn, 1960b). First, they are high in protein and iron; and second, because of their high moisture content (and also the high moisture content of their natural environs), water-plant organisms disintegrate rapidly.

Differences in species, rather than lack of uniformity in the makeup of a particular organism, affect the concentration of iron. Therefore, the data here presented should be directly applicable to field conditions. Eaton (1954) and other workers have pointed out that soil solutions are more mineralized than the irrigation water from which they are derived and that soil-surface evaporation and water uptake (mostly transpiration) by plants in excess of their uptake of salts contributes to the mineralization.

Study (Oborn and Hem, in press) has shown that the larger water plants cause pronounced changes in iron content of natural water. When dense growths of aquatic plants cover all or nearly all the open-water surface, the natural interchange of oxygen between air and water is stopped or is greatly impeded. Aquatic plants, particularly algae, fungi, and bacteria, consume dissolved oxygen during the processes of respiration. Dull windless weather after the production of an algal bloom may cause catastrophic deoxygenation in shallow lakes (Hutchinson, 1957, p. 599). Although evaporation causes alkali slicks on western soils, it apparently contributes little or nothing to iron concentrations in marsh and lake bodies. Relations between oxygen and iron were reviewed in an earlier report (Oborn, 1960a).

Greenhouse experiments (Oborn and Hem, in press) have indicated the importance of the effects of aquatic vegetation in the transfer of iron from submerged soils to water bodies. The results of the greenhouse experiments, however, included effects of microbiota, which could not be practicably excluded. In experiments made for the present study, the larger forms of vegetation were absent; thus microbiota effects could be evaluated separately. Microorganisms have a profound effect upon the productivity of soils. In general, productive soils contain an abundance of microorganisms, whereas soils that are low in productivity support a small population. Fungi are more important in acid soils, where bacterial numbers tend to decrease, than in alkaline soils.

Microorganisms additional to those already in the soils used probably entered from the air or were present on the organic matter that was added. Distilled water was used exclusively and could have been an additional, although less likely, factor in microorganism entry.

Iron in soil can be in a form that is nearly insoluble in water. In most agricultural land, however, adequate solution of iron in soil moisture would be expected. Moore and Maynard (1929) discuss solution, transportation, and precipitation of iron in water. In the present study, iron entry into soil solution probably involves release from soil- or admixture-particle surface and solution in the aqueous gel or water film surrounding the soil or admixture particle. Obviously a close interrelationship exists between soil ion exchange reactions and soil microbiological activity (Stewart, written communication, 1960). Both are dependent on kind and amount of organic matter and clay colloidal complex in the soil.

Coagulation phenomena involving a polyvalent ion such as iron may be delayed by the presence of organic matter. This knowledge is daily used in the building trades in controlling the setting time of concrete.

NATURE AND BEHAVIOR OF SOIL MICROORGANISMS

Although a comprehensive taxonomic identification of the microbes involved in this study was probably not necessary two fungi, *Verticillium* sp. and *Cladosporium herbarum*, both spore formers, were identified by L. W. Durrell, Colorado State University (written communication, 1957). *Polyangium cellulosae*, *Pseudomonas flu-*

orescens, *Bacillus mycoides*, *Sarcina lutea*, and *Proteus vulgaris* are other common soil microbes.

Yust (1951a) has pointed out that soil composition and cultivation have remained almost constant through the centuries, resulting in the establishment of certain combinations or associations of microorganisms specially adapted to the soil habitat. Furthermore, these biochemically interrelated soil microbiota vary only slightly in different localities and at different times of the year.

Soil microorganisms decompose organic matter added to the soil such as manure and crop residue (Yust, 1951b). Different temperature ranges are optima for the development of different microorganisms (Greaves and Greaves, 1936). For example, about 60° to 70°F is optimum for water microorganisms, and about 70° to 95°F is optimum for soil microorganisms that live on decaying organic matter. Microorganisms involved in decomposition of manure and ensilage may multiply rapidly in the range of 160° to 175°F (Greaves and Greaves, 1936).

Although light is essential to the life of higher plants and animals, diffuse sunlight hinders the growth of microorganisms and direct sunlight destroys them. Most forms of artificial light, however, have little or no detrimental effect on nonchlorophyll-bearing microbe growth.

Microbes are also influenced by the reaction of the medium in which they are living. Bacteria predominate in neutral or slightly alkaline (normal) soils, and yeasts and molds predominate in either acidic or alkaline soils. Microorganisms are able to withstand great environmental variations and many apparently adapt themselves quickly to new conditions.

An important difference in the microorganism population of rice-paddy, ditch, lake-bottom, and sea-bed soils in comparison to forest and ordinary farm soils is the presence of large numbers of nonsulfur purple bacteria in the water-submersed soils (Okuda and others, 1957). Rudra (1956) associated higher soil nutrients with an increase in period of waterlogging.

Soil enzymes are important to soil productivity (Hoffmann, 1955; Katsnel'son and Ershov, 1958; Mikola, 1955) and facilitate solution of iron in water when organic matter is present.

RELATION OF TEMPERATURE TO MICROBIOLOGICAL ACTIVITY

Many inanimate organic reactions as well as animate protoplasmic growth and functions approximately double in rate with exposure to each 18°F (10°C) rise in temperature. This temperature effect seems to hold particularly well between 41° and 95°F (5° and 35°C). The question might arise in the present study as to whether organic extraction of iron in the soil was an animate or inanimate phenomenon or a combination of the two. Therefore substrata consisting of either sand containing relatively little iron and no organic matter, or soil containing a known amount of iron along with organic matter were mixed with pulverized organic matter of known iron content. The organic matter was either plant material or manure of one of the herbivorous animals; water was added, and the mixture was subjected to heat treatment.

Some mixtures of substrata, organic matter, and water were placed in tightly stoppered flasks and were pasteurized at 149°F (65°C) for 30 minutes in an oven; then the mixtures were immediately cooled to 50°F (10°C) or lower. Although a temperature of 149°F does not greatly change the chemical composition of the substrata, most ordinary (vegetative) cells are killed. Thus, a partial sterilization (except for spore formers) of the microbiota was attained.

Other mixtures of substrata, organic matter, and water were placed in unstoppered flasks and were sterilized by heating at 260°F (127°C) for 15 minutes in a pressure cooker at a pressure of 20 pounds per square inch. Sterilization at 260°F reduces the viable microbe population more than pasteurization at 149°F but still does not kill all spore formers.

Cooling to room temperature and immediate determination of iron in the previously heated mixtures would show the effect, if any, of heat alone in bringing iron into solution in the organic mixture, and incubation for 2 weeks before determination of iron would show the effect of microbe activity.

DESIGN OF EXPERIMENTAL WORK

Iron may be taken into solution in soil moisture as an organic complex. Apparently microbiologic activity in soils and in accumulated plant and animal debris greatly affects the rate at which iron is released from these organic compounds and is made available for complexing. Iron is an integral part of microorganism protoplasm. The microorganisms utilize iron in their metabolism and release it in waste products, and they, along with the higher forms of life, are capable of secreting wastes that form complexes with iron. Microorganisms such as the thiobacilleae are also active through sulfuric acid formation in bringing iron into solution from the inorganic part of the soil. For example in the complete absence of organic food (Greaves and Greaves, 1936) *Thiobacillus thio-oxidans*

$+2S+2H_2O+3O_2=2H_2SO_4$ (pH between 1 and 3, Raber, 1936). Insoluble metallic carbonates, silicates and phosphates in the soil are made soluble by the sulfuric acid.

Samples for microbiological experiments in the laboratory were prepared as follows: Natural soils (19.0 grams screened to pass a 2,000-micron sieve) containing known amounts of iron were spatula-mixed with 1.0 gram of dried pulverized plant parts or herbivorous animal manure containing known amounts of iron. All the organic admixture materials had been pulverized to pass a 500-micron sieve. Four identical samples of each mixture of soil and organic matter were placed in Erlenmeyer flasks of 250-milliliter capacity, 100 milliliters of distilled water was added, and the flasks were tightly corked (tables 2–7).

Animals digestive processes, including the different alimentary canal enzyme effects, seem to be very important in making iron available for solution from organic matter. Accordingly dried pulverized fecal matter from three herbivorous animal species—sheep, rabbit, and pigeon—was used instead of the pulverized dried-plant parts in one section of the experimental work. The six plants used in the study are shown in figures 21 and 22.

A comparable series was run on the soils alone in which no organic matter was added. The total iron content of the different components of the mixtures used, with a list and brief description of the soil and sand substrata and the organic-matter admixtures, is given in table 1.

The sample having no incubation was heated for 1 hour at 100°F, brought to room temperature, and immediately filtered with suction through quantitative low-retentivity filter paper. The pH and iron in the filtrate were determined. The ferrous and total iron contents were determined directly by using the 2,2′ bipyridine procedure and a spectrophotometer; the ferric iron content was computed by difference. Dissolved-iron measurements were also made after 2 weeks of incubation at 35°, 72°, and 100°F. Results of the pH and iron determinations for all samples are reported in tables 2 to 7. The iron

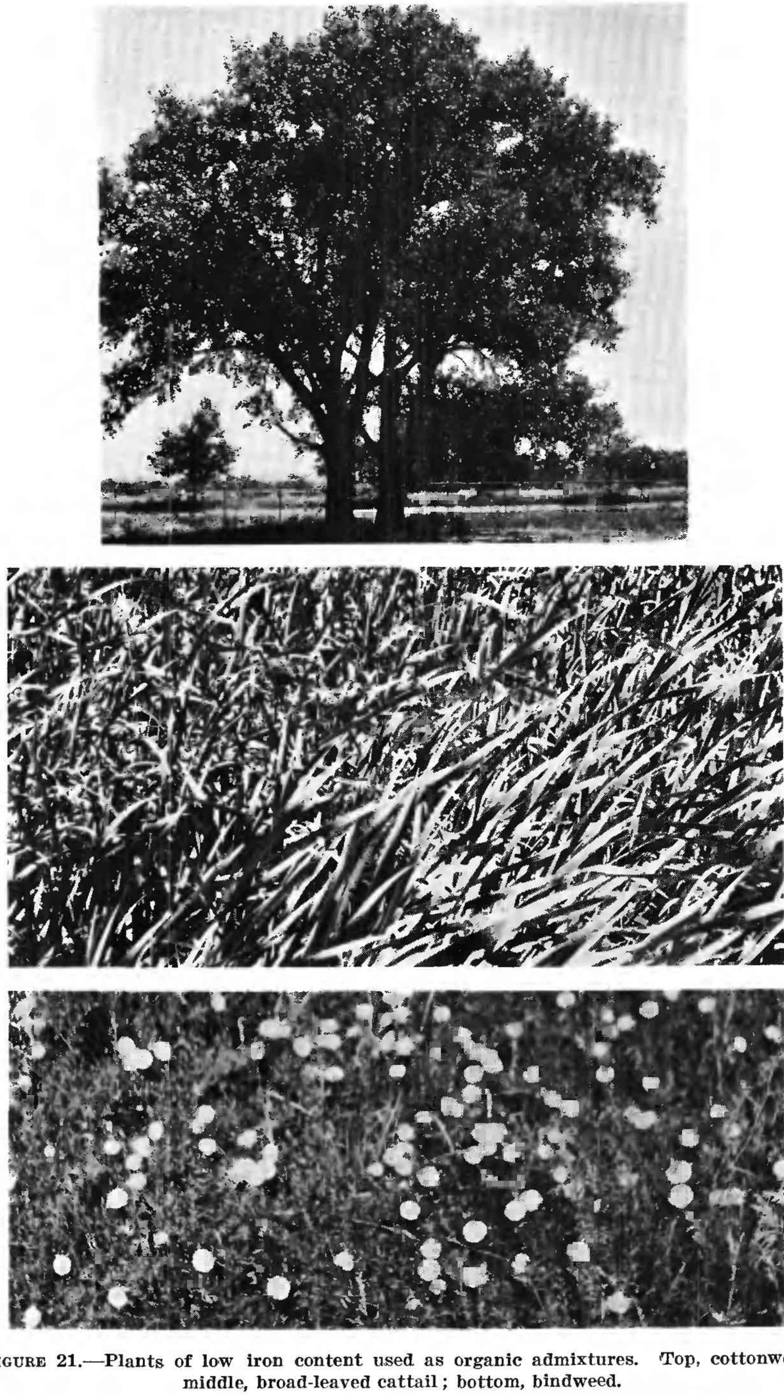

FIGURE 21.—Plants of low iron content used as organic admixtures. Top, cottonwood; middle, broad-leaved cattail; bottom, bindweed.

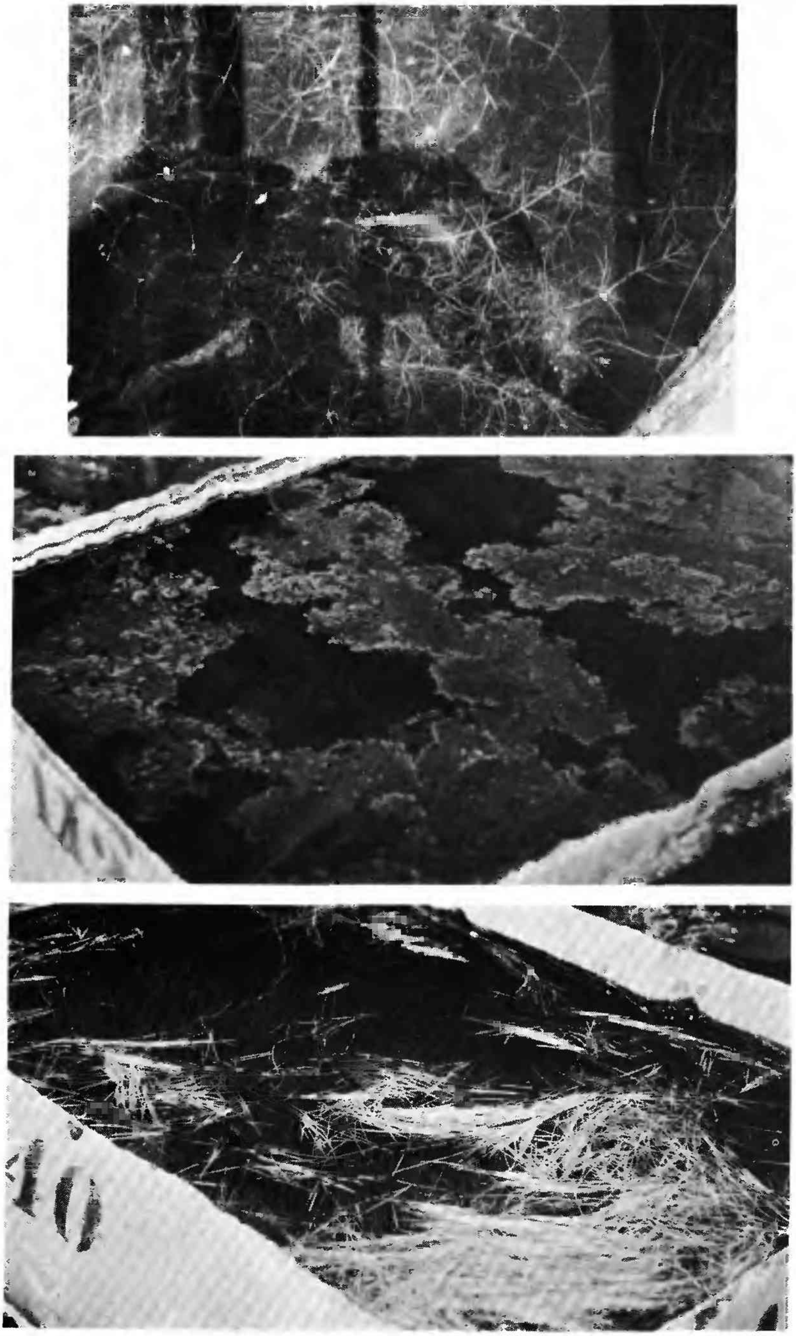

FIGURE 22.—Plants of high iron content used as organic admixtures. Top, stonewort; middle, pond scum; bottom, sago pondweed.

in the control sample was determined about 1½ hours after the water was added. The sample warming was performed to bring readily soluble iron into solution but at the same time did not permit incubation of the microbiota.

Analyses reruns were performed on identically processed random samples. These analyses showed consistent release of iron from substrata and admixture to the water solution.

TABLE 1.—*Iron content of sand, soil, and organic matter used in laboratory study*

[Data for soils and manures supplied by E. T. Oborn, except as indicated]

Material	Total iron content		Iron in sample (milligrams)
	Percent of dry weight	Milligrams per gram	
Soils (19.0 g in each sample):			
Ottawa sand (almost pure silica) [1]	0.007	0.07	1.3
Lake-bottom (organically rich)	3.5	35	660
Federal Center wheatgrass (regular cropland type)	3.4	34	650
John Martin saltcedar (intermediate in iron)	2.3	23	440
John Martin saltcedar (iron-rich)	4.2	42	800
Plant manures (total plant unless otherwise indicated):			
Chara sp. Valliant (stonewort)	.145	1.45	1.45
Cladophora sp. Kützing (pond scum)	.213	2.13	2.13
Convolvulus arvensis L. (bindweed)	.053	.53	.53
Populus deltoides Marsh. (cottonwood) [2]	.006	.06	.06
Typha latifolia L. (broad-leaved cattail) [2]	.005	.05	.05
Potamogeton pectinatus L. (sago pondweed)	1.5	15.1	15.1
Animal manures:			
Ovis aries L. (Columbia Suffolk cross sheep)	.138	1.38	1.38
Columba livia Gmelin var. *domestica* L. (domestic pigeon)	.106	1.06	1.06
Oryctolagus cuniculus L. (albino rabbit)	.094	.94	.94

[1] Data from Fuller (1926).
[2] Leaf only.

TABLE 2.—*Iron concentration in leachates from mixtures of Ottawa sand with organic matter*

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Temperature of incubation (° F)	Iron (parts per million)			pH
		Total	Fe^{+2}	Fe^{+3}	
None	----------	0.01	0.00	0.01	7.0
	35	.01	.00	.01	7.0
	72	.02	.01	.01	6.7
	100	.04	.04	.00	6.5
Plant (total plant unless otherwise indicated):					
Chara sp. Valliant (stonewort)	----------	.12	.00	.12	6.9
	35	.00	.00	.00	6.9
	72	2.88	.02	2.86	7.5
	100	2.77	.09	2.68	7.5
Cladophora sp. Kützing (pond scum)	----------	.10	.00	.10	6.6
	35	.00	.00	.00	7.0
	72	3.50	.45	3.05	7.4
	100	5.22	.19	5.03	7.4
Convolvulus arvensis L. (bindweed)	----------	.00	.00	.00	6.2
	35	.00	.00	.00	6.4
	72	.52	.23	.29	6.5
	100	.00	.00	.00	7.3
Populus deltoides Marsh. (cottonwood) [1]	----------	.00	.00	.00	6.5
	35	.00	.00	.00	6.6
	72	.05	.05	.00	6.0
	100	.00	.00	.00	7.4
Typha latifolia L. (broad-leaved cattail) [1]	----------	.00	.00	.00	6.4
	35	.00	.00	.00	6.5
	72	.10	.09	.01	6.8
	100	.00	.00	.00	7.1
Potamogeton pectinatus L. (sago pondweed)	----------	.12	.00	.12	6.7
	35	.00	.00	.00	6.9
	72	2.52	.93	1.59	6.7
	100	2.98	.09	2.89	7.5
Animal (manure):					
Ovis aries L. (Columbia Suffolk cross sheep)	----------	.00	.00	.00	7.8
	35	.27	.04	.23	7.6
	72	.36	.05	.31	7.8
	100	.00	.00	.00	7.9
Columba livia Gmelin var. *domestica* L. (domestic pigeon)	----------	.18	.00	.18	7.0
	35	1.22	1.05	.17	6.3
	72	2.59	2.59	.00	4.7
	100	3.92	3.90	.02	5.2
Oryctolagus cuniculus L. (albino rabbit)	----------	.00	.00	.00	7.8
	35	.16	.16	.00	7.6
	72	1.32	.75	.57	7.3
	100	.56	.31	.25	7.5

[1] Leaf only.

TABLE 3.—*Iron concentration in leachates from mixtures of lake-bottom soil with organic matter*

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Temperature of incubation (° F)	Iron (parts per million)			pH
		Total	Fe^{+2}	Fe^{+3}	
None	---	---	---	---	---
	35	0.00	0.00	0.00	7.3
	72	.10	.03	.07	6.1
	100	6.55	5.19	1.36	6.9
Plant (total plant unless otherwise indicated):					
Chara sp. Valliant (stonewort)	---	---	---	---	---
	35	---	---	---	---
	72	6.05	3.02	3.03	7.3
	100	8.40	6.40	2.00	6.9
Cladophora sp. Kützing (pondscum)	---	.11	.00	.11	6.1
	35	.15	.00	.15	7.3
	72	5.82	5.70	.12	7.2
	100	8.40	5.85	2.55	6.9
Convolvulus arvensis L. (bindweed)	---	.18	.00	.18	6.4
	35	.02	.00	.02	7.3
	72	7.40	6.40	1.00	7.1
	100	8.40	7.40	1.00	6.8
Populus deltoides Marsh. (cottonwood)[1]	---	.28	.00	.28	6.4
	35	.10	.00	.10	7.2
	72	7.10	5.00	2.10	7.1
	100	8.05	7.75	.30	6.9
Typha latifolia L. (broad-leaved cattail)[1]	---	.06	.00	.06	6.4
	35	.00	.00	.00	7.2
	72	4.28	2.81	1.47	7.2
	100	8.10	7.75	.35	6.9
Potamogeton pectinatus L. (sago pondweed)	---	.19	.00	.19	6.5
	35	.22	.00	.22	7.2
	72	6.45	3.28	3.17	7.4
	100	8.40	7.40	1.00	6.9
Animal (manure):					
Ovis aries L. (Columbia Suffolk cross sheep)	---	.07	.00	.07	6.6
	35	.05	.00	.05	7.2
	72	5.50	4.38	1.12	7.3
	100	8.40	2.62	5.78	7.5
Columba livia Gmelin var. *domestica* L. (domestic pigeon)	---	.00	.00	.00	6.0
	35	.00	.00	.00	7.0
	72	5.20	4.50	.70	6.2
	100	8.10	7.40	.70	5.9
Oryctolagus cuniculus L. (albino rabbit)	---	.06	.00	.06	6.4
	35	.11	.01	.10	7.1
	72	5.40	4.50	.90	7.0
	100	8.40	8.10	.30	7.2

[1] Leaf only.

TABLE 4.—*Iron concentration in leachates from mixtures of Federal Center wheatgrass soil with organic matter*

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Temperature of incubation (° F)	Iron (parts per million)			pH
		Total	Fe^{+2}	Fe^{+3}	
None	–	–	–	–	–
	35	–	–	–	–
	72	0.06	0.00	0.06	8.1
	100	1.34	.00	1.34	8.3
Plant (total plant unless otherwise indicated):					
Chara sp. Valliant (stonewort)	–	.12	.08	.04	7.6
	35	.00	.00	.00	8.1
	72	8.40	.59	7.81	7.9
	100	5.40	.00	5.40	7.9
Cladophora sp. Kützing (pond scum)	–	.13	.08	.05	7.6
	35	.05	.00	.05	8.2
	72	5.30	.06	5.24	8.2
	100	2.03	.00	2.03	8.2
Convolvulus arvensis L. (bindweed)	–	.00	.00	.00	7.5
	35	.02	.00	.02	8.1
	72	9.10	1.10	8.00	6.5
	100	4.97	.00	4.97	8.0
Populus deltoides Marsh. (cottonwood)[1]	–	.00	.00	.00	7.4
	35	.00	.00	.00	7.5
	72	8.40	.55	7.85	7.6
	100	5.10	.00	5.10	7.7
Typha latifolia L. (broad-leaved cattail)[1]	–	.00	.00	.00	7.6
	35	.00	.00	.00	7.7
	72	3.37	.04	3.33	7.9
	100	3.06	.00	3.06	8.1
Potamogeton pectinatus L. (sago pondweed)	–	.11	.00	.11	7.7
	35	.00	.00	.00	7.7
	72	6.05	.00	6.05	7.9
	100	2.92	.00	2.92	8.1
Animal (manure):					
Ovis aries L. (Columbia Suffolk cross sheep)	–	.11	.00	.11	8.0
	35	.07	.00	.07	7.8
	72	6.40	.08	6.32	7.9
	100	3.52	.00	3.52	8.0
Columba livia Gmelin var. *domestica* L. (domestic pigeon)	–	.17	.08	.09	7.5
	35	.00	.00	.00	7.4
	72	7.60	3.31	4.29	7.6
	100	6.40	5.10	1.30	5.7
Oryctolagus cuniculus L. (albino rabbit)	–	.05	.00	.05	8.1
	35	.00	.00	.00	7.7
	72	8.40	.32	8.08	7.6
	100	7.15	.30	6.85	7.7

[1] Leaf only.

The remaining three unheated identical samples were incubated for 14 days before being analyzed for iron in solution: One sample was incubated in a refrigerator at 35°F; the second was incubated on the laboratory bench, where room temperatures varied within a few degrees of 72°F; the third was incubated in an oven at 100°F. After the 2-week incubation period, the water was filtered with a Büchner funnel through low-retentivity filter paper.

Microbe growths were readily detected with the naked eye in many of the samples incubated at 72° and 100°F. Frequently, fungus growth was accompanied by both frothing and massing of filaments, and bacterial development by a fluorescence on the surface of the culture medium. Bacterial growth also was sometimes evident through evolution of gas. Some of the samples incubated at 100°F encouraged growth of anaerobic microbes such as the sulfur-reducing organisms and gave a strong odor of H_2S when opened.

Results from mixtures of sand with the same organic matter on page 222 are shown for comparison with the soil data. Concentration of iron in leachates from mixtures of Ottawa sand and organic-matter additives but with no preliminary pasteurization or sterilization heat treatments are shown in table 2.

Pasteurizing or sterilizing the substrata (sand or soil) and (or) admixture (plant or animal manures) was intended to accomplish two objectives: First, any dissolved-iron increase during incubation would be due principally to activity of the more heat-resistant spore-forming microbes; second, progressive preliminary heat treatments of 100°, 149°, or 260°F might feasibly bring inanimate organic or inorganic iron into solution, which, without the heat treatment, might remain insoluble. Throughout the study, concentration on the effects (or lack of effects) of soil microbes in releasing otherwise insoluble iron to soil-water solutes has been stressed. Water quality with respect to iron content might thus be profoundly altered.

Iron-leach results for the samples of Ottawa sand substrata that received preliminary heat treatment are shown in table 7. Pasteurization and sterilization heat treatments were not intended to establish aseptic conditions with any of the flask mixtures. If aseptic conditions were to be established, the more effective discontinuous or intermittent heating techniques would have been used (Greaves and Greaves, 1936).

TABLE 5.—*Iron concentration in leachates from mixtures of John Martin Reservoir saltcedar soil (low in iron) with organic matter*

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Temperature of incubation (° F.)	Iron (parts per million)			pH
		Total	Fe^{+2}	Fe^{+3}	
None	----------	0.02	0.01	0.01	7.4
	35	.00	.00	.00	7.6
	72	.05	.00	.05	7.9
	100	.15	.02	.13	7.8
Plant (total plant unless otherwise indicated):					
Chara sp. Valliant (stonewort)	----------	----------	----------	----------	----------
	35	----------	----------	----------	----------
	72	3.85	.04	3.81	7.9
	100	.37	.00	.37	8.0
Cladophora sp. Kützing (pond scum)	----------	.00	.00	.00	7.5
	35	.00	.00	.00	7.3
	72	5.40	.00	5.40	7.8
	100	.23	.00	.23	8.0
Convolvulus arvensis L. (bindweed)	----------	.00	.00	.00	7.5
	35	.00	.00	.00	7.3
	72	1.11	.04	1.07	8.0
	100	.06	.00	.06	8.0
Populus deltoides Marsh. (cottonwood) [1]	----------	.00	.00	.00	7.3
	35	.00	.00	.00	7.4
	72	7.10	2.07	5.03	7.8
	100	.78	.03	.75	7.9
Typha latifolia L. (broad-leaved cattail) [1]	----------	.00	.00	.00	7.5
	35	.00	.00	.00	7.4
	72	3.80	.00	3.80	7.9
	100	1.80	.20	1.60	7.9
Potamogeton pectinatus L. (sago pondweed)	----------	.00	.00	.00	7.5
	35	.00	.00	.00	7.6
	72	2.45	.05	2.40	8.0
	100	2.11	.04	2.07	7.6
Animal (manure):					
Ovis aries L. (Columbia Suffolk cross sheep)	----------	.00	.00	.00	7.8
	35	.00	.00	.00	7.6
	72	5.85	.06	5.79	8.0
	100	.98	.02	.96	8.0
Columba livia Gmelin var. *domestica* L. (domestic pigeon)	----------	.00	.00	.00	7.3
	35	1.63	.00	1.63	6.7
	72	7.10	7.10	.00	6.5
	100	6.40	6.40	.00	5.5
Oryctolagus cuniculus L. (albino rabbit)	----------	.00	.00	.00	7.6
	35	.00	.00	.00	7.8
	72	.63	.00	.63	8.1
	100	.54	.03	.51	7.7

[1] Leaf only.

TABLE 6.—*Iron concentration in leachates from mixtures of John Martin Reservoir saltcedar soil (high in iron) with organic matter*

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Temperature of incubation (° F)	Iron (parts per million)			pH
		Total	Fe^{+2}	Fe^{+3}	
Plant (total):					
Cladophora sp. Kützing (pond scum)					
	35				
	72	8.80	0.00	8.80	8.0
	100	6.05	.28	5.77	7.9
Potamogeton pectinatus L. (sago pondweed)					
	35				
	72	4.45	.06	4.39	7.9
	100	8.45	.55	7.90	7.8
Animal (manure):					
Columba livia Gmelin var. *domestica* L. (domestic pigeon)					
	35				
	72	7.75	7.75	.00	7.2
	100	9.87	3.77	6.10	6.4

TABLE 7.—*Iron concentration in leachates from mixtures of Ottawa sand with organic matter, subjected to preliminary heat treatment*

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Heat treatment	Temperature of incubation (° F)	Iron (parts per million)			pH
			Total	Fe^{+2}	Fe^{+3}	
None	Pasteurization		0.03	0.00	0.03	6.9
		35	.02	.00	.02	6.7
		72	.02	.01	.01	6.8
		100	.02	.00	.02	6.5
	Sterilization		.01	.00	.01	7.0
		35	.00	.00	.00	6.8
		72	.02	.00	.02	6.9
		100	.02	.01	.01	6.7
Plant (total plant unless otherwise indicated):						
Chara sp. Valliant (stonewort)	Pasteurization		.14	.00	.14	6.8
		35	.02	.02	.00	7.2
		72	2.98	.40	2.58	7.4
		100	5.52	1.22	4.30	6.5
	Sterilization		.28	.00	.28	6.6
		35	.01	.01	.00	7.1
		72	1.21	.44	.77	7.3
		100	4.40	.10	4.30	7.6
Cladophora sp. Kützing (pond scum)	Pasteurization		.00	.00	.00	6 7
		35	.00	.00	.00	7.2
		72	7.09	1.12	5.97	7.1
		100	5.85	.47	5.38	7.2
	Sterilization		.28	.00	.28	6.2
		35	.01	.00	.01	7.0
		72	.89	.33	.56	7.3
		100	4.23	.52	3.71	7.4
Convolvulus arvensis L. (bindweed)	Pasteurization		.04	.00	.04	5.8
		35	.00	.00	.00	6.8
		72	.00	.00	.00	6.2
		100	.00	.00	.00	7.6
	Sterilization		.00	.00	.00	5.7
		35	.00	.00	.00	6.0
		72	.00	.00	.00	6.4
		100	.00	.00	.00	6.2

TABLE 7.—*Iron concentration in leachates from mixtures of Ottawa sand with organic matter, subjected to preliminary heat treatment*—Continued

[Measurements of four samples after 2 weeks' incubation at indicated temperature]

Organic matter	Heat treatment	Temperature of incubation (° F)	Iron (parts per million)			pH
			Total	Fe^{+2}	Fe^{+3}	
Plant (total plant unless otherwise indicated)—Continued						
Populus deltoides Marsh. (cottonwood) [1]	Pasteurization		0.00	0.00	0.00	6.2
		35	.00	.00	.00	6.2
		72	.00	.00	.00	6.3
		100	.00	.00	.00	7.1
	Sterilization		.00	.00	.00	5.8
		35	.00	.00	.00	6.5
		72	.00	.00	.00	6.8
		100	.00	.00	.00	6.6
Typha latifolia L. (broad-leaved cattail).[1]	Pasteurization		.04	.00	.04	6.2
		35	.00	.00	.00	6.3
		72	.00	.00	.00	6.8
		100	.00	.00	.00	6.8
	Sterilization		.08	.00	.08	5.9
		35	.00	.00	.00	6.5
		72	.00	.00	.00	6.7
		100	.00	.00	.00	6.2
Potamogeton pectinatus L. (sago pondweed).	Pasteurization		.00	.00	.00	6.5
		35	.00	.00	.00	7.2
		72	2.40	1.74	.66	5.7
		100	3.42	2.90	.52	5.7
	Sterilization		.00	.00	.00	6.4
		35	.00	00	.00	6.9
		72	.00	.00	.00	6.9
		100	.00	.00	.00	7.1
Animal (manure):						
Ovis aries L. (Columbia Suffolk cross sheep).	Pasteurization		.00	.00	.00	7.9
		35	.00	.00	.00	7.9
		72	.45	.00	.45	7.8
		100	.00	.00	.00	7.9
	Sterilization		.05	.00	.05	7.8
		35	.00	.00	.00	8.0
		72	.00	.00	.00	7.7
		100	.00	.00	.00	7.7
Columba livia Gmelin var. *domestica* L. (domestic pigeon).	Pasteurization		.05	.00	.05	6.8
		35	.08	.00	.08	7.6
		72	.64	.12	.52	4.9
		100	2.98	2.56	.42	4.5
	Sterilization		1.08	.00	1.08	7.1
		35	.00	.00	.00	7.1
		72	.21	.00	.21	7.2
		100	1.68	1.68	.00	4.9
Oryctolagus cuniculus L. (albino rabbit).	Pasteurization		.09	.00	.09	7.9
		35	.00	.00	.00	7.9
		72	2.32	.02	2.30	7.4
		100	5.70	3.58	2.12	5.7
	Sterilization		.05	.00	.05	7.9
		35	.00	.00	.00	7.9
		72	.01	.00	.01	7.0
		100	.00	.00	.00	7.6

[1] Leaf only.

DISCUSSION OF RESULTS

Data in tables 2 to 7 show that a few tenths or hundredths of a part per million of iron may be readily leached from ordinary soils, manures, or mixtures of soil and organic matter by contact with distilled water brought to 100°F. However, amounts 10 to 100 times greater can be extracted when water remains in contact with soil and organic matter for 2 weeks under conditions favorable for the growth of micro-organisms. Generally, the amount of iron extracted from samples incubated at 35°F was small and invariably less than the amount that was extracted following incubation at 72° or 100°F for the same period. The differences are most readily explained by the fact that most microbiological activity at 35°F is minimal compared with that at the higher temperatures (Greaves and Greaves, 1936).

The microbiota cause extraction of iron by several mechanisms. Additional information both qualitative (identification) and quantitative (actual microbe counts) would be necessary to understand more thoroughly the relative importance of each. Respiration and fermentation consume oxygen and produce carbon dioxide; thus pH and redox potential (Eh) decrease. As pH and Eh decrease the solubility of iron increases. Iron brought into solution in such reactions is an indirect but important result of the microbiologic activity. In attacking organic matter to obtain food, micro-organisms release organic complexes containing iron. Iron is required for the synthesis of microbe protoplasm. Iron involved in this synthesis would, upon death, be released in soluble form as a byproduct of the growth of micro-organism colonies.

Data in table 7 indicate that large amounts of iron were dissolved from the mixtures of Ottawa sand and organic matter even after heat treatment. Visual examination of the culture mixtures showed that in most samples the microbiologic activity was not stopped by the heat treatment.

A summary of the effectiveness of different kinds of leach treatment caused by mixtures of soil and organic manures is presented in table 8. This table gives the total amounts of iron contained in the soil and organic admixtures and the amount of iron that was leached by microbes when incubated for 2 weeks at 72° and 100°F. The "leaching coefficient" is the amount of iron in solution divided by the amount in the solid material at the beginning of the test.

TABLE 8.—*Effectiveness of leaching of iron from soil and plant materials at 72° and 100° F*

[Measurements after 2 weeks' incubation at indicated temperature; 19.0 g of soil and 1.0 g of organic matter used in each sample]

Organic matter	Fe in soil (milligrams)	Fe in organic matter (milligrams)	Total Fe in solid (milligrams)	Leaching at 72° F: Fe in solution (milligrams)	Leaching at 72° F: Leaching coefficient (× 10⁴)	Leaching at 100° F: Fe in solution (milligrams)	Leaching at 100° F: Leaching coefficient (× 10⁴)
Ottawa sand							
None	1.3	--------	1.3	0.002	20	0.004	30
Plant (total plant unless otherwise indicated):							
Stonewort	1.3	1.4	2.7	.29	1,000	.28	1,000
Pond scum	1.3	2.1	3.4	.35	1,000	.52	1,500
Bindweed	1.3	.5	1.8	.05	300	0	0
Cottonwood [1]	1.3	.1	1.4	.005	40	0	0
Cattail [1]	1.3	.1	1.4	.01	70	0	0
Sago pondweed	1.3	15	16	.25	200	.30	200
Animal (manure):							
Sheep	1.3	1.4	2.7	.04	300	0	0
Pigeon	1.3	1.1	2.4	.26	1,000	.39	2,000
Rabbit	1.3	.9	2.2	.13	600	.06	300
Lake-botton soil							
None	660	--------	660	0.01	0.2	0.66	10
Plant (total plant unless otherwise indicated):							
Stonewort	660	1.4	661	.60	9	.84	10
Pond scum	660	2.1	662	.58	9	.84	10
Bindweed	660	.5	660	.74	10	.84	10
Cottonwood [1]	660	.1	660	.71	10	.80	10
Cattail [1]	660	.1	660	.43	7	.81	10
Sago pondweed	660	15	675	.64	9	.84	10
Animal (manure):							
Sheep	660	1.4	661	.55	8	.84	10
Pigeon	660	1.1	661	.52	8	.81	10
Rabbit	660	.9	661	.54	8	.84	10
Federal Center wheatgrass soil							
None	650	--------	650	0.006	0.009	0.13	2
Plant (total plant unless otherwise indicated):							
Stonewort	650	1.4	651	.84	10	.54	8
Pond scum	650	2.1	652	.53	8	.20	3
Bindweed	650	.5	650	.91	10	.50	8
Cottonwood [1]	650	.1	650	.84	10	.51	8
Cattail [1]	650	.1	650	.34	5	.31	5
Sago pondweed	650	15	665	.60	9	.29	4
Animal (manure):							
Sheep	650	1.4	651	.64	10	.35	5
Pigeon	650	1.1	651	.76	10	.64	10
Rabbit	650	.9	651	.84	10	.72	10
John Martin Reservoir saltcedar soil (low in iron)							
None	440	--------	440	0.005	0.01	0.015	0.3
Plant (total plant unless otherwise indicated):							
Stonewort	440	1.4	441	.38	9	.04	.9
Pond scum	440	2.1	442	.54	10	.02	.5
Bindweed	440	.5	440	.11	2	.01	.2
Cottonwood [1]	440	.1	440	.71	20	.08	2
Cattail [1]	440	.1	440	.38	9	.18	4
Sago pondweed	440	15	455	.24	5	.21	5
Animal (manure):							
Sheep	440	1.4	441	.58	10	.10	2
Pigeon	440	1.1	441	.71	20	.64	10
Rabbit	440	.9	441	.06	1	.05	1

[1] Leaf only.

The iron leached from the mixtures of sand and organic matter primarily would have to come from the organic admixture. Although amounts of iron recovered were less than the amounts added, more iron was leached from the samples that contained organic matter high in iron than from the samples containing organic material low in iron.

Evidently, iron dissolved from samples containing the soils was derived mainly from the soil fraction. Iron content of the soil was so great that the amount added from the organic matter was only a minor part of the total in each sample. The total amount of iron removed from most samples containing bindweed, cottonwood leaves, or cattail leaves exceeded the amount added as organic matter. This fact is the most conclusive evidence presented in the study that iron was removed from the soils themselves. Also, a large amount of iron was dissolved from the nonaerated lake-bottom soil and smaller amounts from the other aerated soils containing less organic matter when the soil alone, without the addition of organic matter, was subjected to incubation leaching at 100°F for 2 weeks.

Tokai and others (1957) reported that pH and ferrous iron content of water in contact with rice-paddy soils increased during incubation at 86°F. In the present study, however, the pH of solutions in contact with the lake-bottom and other soils shows no special trend, partly because of the differences in kinds and amounts of microbes present. Nevertheless, increase of iron content is obvious.

The form in which iron occurred in the soil samples was not known with certainty. Because the nonaerated lake-bottom soil is conducive to a reducing environment and is rich in organic debris, a large part of the iron in this soil probably was in reduced form or combined with organic matter. The other aerated soils contained almost as much iron as the lake-bottom soil, but aeration would favor the oxidation of the iron to the less soluble ferric form. Consequently, if other factors (including ingredients) are constant, lake-bottom soil should be the better source of iron.

Data in table 8 show that of the soil samples, without the addition of organic matter, the lake-bottom soil allowed the most iron to come into solution. This was to be expected. However, the addition of organic matter to the ordinary cropland-type surface soil from the Federal Center and to the soil from the periphery of the John Martin Reservoir, followed by incubation, resulted in extraction of amounts of iron that generally were similar to amounts obtained in the same treatment of the lake-bottom soil. The availability of similar amounts of iron by manure additions to different soils is an important aid in successful farming. Only small amounts of dissolved iron were detected in some samples after incubation at 100°F for 2 weeks.

Lesser amounts of dissolved iron in these leachates may result from anaerobic conditions being established in the solutions, and sulfur-reducing bacteria in the reducing environments producing sulfide ions. These ions would cause some of the iron to precipitate as ferrous sulfide. Ferrous sulfides have very low solubilities at the pH levels observed in the solutions (Hem, 1960a).

Both ferrous and ferric iron were determined in all the samples. Although results were variable, some general trends were evident. Iron in leachates from the nonaerated lake-bottom soil was mostly in the ferrous form in the samples that yielded the largest concentrations of iron; in most leachates from the other aerated soils ferric iron predominated. Many concentrations of ferric iron were much greater than the concentration possible in dissociated form at the pH levels of the extracts. The two principal alternative forms in which the ferric iron could be present are as a colloidal suspension or as an organic or other complex.

Organic matter in the extracts could be an important factor in forming complexes or stabilizing a colloidal suspension of ferric hydroxide. These two actions might easily overlap, for example in the combination of large organic molecules with ferric ions to produce a complex whose particles would be of colloidal size or larger.

Most of the organic coloring materials derived by water passing through soil and organic debris have properties characteristic of negatively charged colloids. At the pH values attained in the samples, any ferric hydroxide particles that might be released would have a positive charge (Hem and Skougstad, 1960). Such positively charged particles could become attached to organic molecules having negative charges and possibly may have passed through the filter used to clarify the samples before analysis. In the distilled-water extracts of the experiments, the colloidal suspension of ferric hydroxide could even be accomplished without much aid from organic materials. In solutions having relatively high concentrations of electrolytes at or near neutral pH, however, the amounts of any form of colloidal iron that could be present would probably be small because of the coagulating effect of the electrolytes.

Ferrous complexes with tannic acid have been studied by Hem (1960b). When iron was present in the reduced form and a large excess of tannic acid was available, a red ferrous complex was formed which resisted oxidation in aerated water to a much greater degree than uncomplexed ferrous iron. The complex had an apparent oxidation half life of about 30 days at pH 8. In the samples organic matter capable of forming complexes with ferrous iron was available. In many of the extracts from lake-bottom soil, therefore, the presence of ferrous complexes may explain the rather large amounts of ferrous iron. The pink color observed in some of the extracts further suggests the presence of complexes.

The proportion of ferrous iron in extracts from certain mixtures of the soil and fecal matter was also high. The excretory system of birds differs from that of mammals in having a cloaca, which probably accounts for the lower pH in some of the extracts from samples containing pigeon excrement. Fortunately for agriculturists, iron contained in the manures is usually not all available for immediate soil solution, but becomes available in appreciable amounts only after incubation in soil at temperatures suitable for microbe growth.

APPLICATIONS OF RESULTS TO NATURAL-WATER CHEMISTRY

The microbiological experiments indicate that the activities of microorganisms are of extreme importance in bringing iron into solution from soils and from mixtures of soils and organic matter. Under some circumstances the iron carried by the water in contact with soil and organic matter may be in a colloidal form; however, in this study the iron was brought to a fairly stable state where it could be transported in water.

For dissolved or suspended iron to be present in ground or surface water, the iron must first have been dissolved in the soil from the solid state. Although vegetation utilizes part of the iron from the soil solution, some of the iron enters the soil circulation system and leaves the area in surface runoff or percolates to the ground-water reservoir. Whether the iron carried by the soil moisture travels long distances or is shortly redeposited depends on several chemical factors that have been evaluated in other reports of this series on the chemistry of iron in natural water. In a system where the pH and Eh are low, for example, iron once brought into solution will remain, and additional amounts may be picked up. If, however, the Eh becomes sufficiently low and sulfate-reducing bacteria are present, the iron may be precipitated as sulfide (Hem, 1960a).

In surface water, oxygen from the air generally maintains an adequately high Eh to oxidize ferrous iron. If the iron has been released in the soil as a ferrous complex, however, the rate of oxidation is retarded, and the reduced product may still be retained by the stream as a relatively stable suspension.

Chemical analyses of surface waters published by the U.S. Geological Survey (1959a, 1959b) show that several tenths of a part per million of iron generally occur in streams of New England, often accompanied by at least moderate amounts of organic coloring matter. The highly colored water of swamp areas such as the Everglades of Florida may also contain as much as 1 ppm of iron, but the amounts of iron do not correlate necessarily with the amount of organic color. Both the New England and southern Florida regions have abundant precipitation and vegetation. In streams of the Western States (west of the 100th meridian) iron content generally is low. In these areas rainfall and vegetation are less abundant, and iron in the soil is more likely to be in the oxidized form and is less readily transported by water. Many microbiological variables, including kinds, amounts and proportions of soil and stream bacteria, fungi, and algae, affect the iron content in these streams.

Iron is leached much more rapidly from soil and organic-matter mixtures at warm temperatures than at cold ones, suggesting that iron-bearing streams more likely would show seasonal differences in iron content where winters are cold, with the lowest amounts occuring during the winter.

Iron may be brought into solution in ground water after the water leaves the upper soil zone. Biochemical activity in the soil evidently is a very important source of the iron in many areas. Conditions are probably most favorable in humid and subhumid regions where soils are more likely to provide the essential ingredients—moisture, temperature, organic matter—for optimum microbe activity. Also, the ground water is intermittently recharged by surplus soil moisture penetrating beyond the plant-root zone. On the way down to the water table, complexed iron might easily be metabolized by microbes or otherwise may be lost by oxidation. Conditions for providing a supply of iron to ground water from reactions in the soil likely are favorable in a swampy region where the water table occasionally stands above the land surface but is more often a short distance below the surface.

Differences in the chemistry of iron and in the environments of ground water are many. In some parts of the United States, for example, the glaciated regions of North-Central United States, the Atlantic Coastal Plain, or the Mississippi embayment, both soil and underlying materials may be rich in organic debris, either in local small areas or in extensive areas covering many square miles. The amount of iron in ground water, especially at shallow depth, is sufficient to create a serious problem in many places in these regions. The combination of favorable soil conditions (supporting a microbiota population of between 1 and 50 million per gram of soil), Eh, and pH would certainly result in high iron concentrations, particularly if the rock and soil minerals contain much iron.

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