A Survey of Pertinent Biochemical Literature

By EUGENE T. OBORN

CHEMISTRY OF IRON IN NATURAL WATER

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CHEMISTRY OF IRON IN NATURAL WATER

A SURVEY OF PERTINENT BIOCHEMICAL LITERATURE

By EUGENE T. OBORN

ABSTRACT

Biochemical factors relating to the occurrence of iron in natural water were studied by examining more than 800 published articles and abstracts. According to this literature iron is essential in normal metabolism of most of the fungi, bacteria, aquatic vegetation, and land plants studied as well as in many animals. Many organisms in diverse habitats may precipitate or dissolve iron. Aquatic plants seem to be important in the iron cycle in surface-water bodies. They take up iron from submerged soil or from the water and ultimately release it to water solution or return it to the bottom mud through rapid decay of dead plant material. Anaerobic bacteria in the absence of oxygen reduce precipitated ferric iron and return it to solution. Apparently, many of these bacteria in the presence of oxygen use the carbon that is so frequently fixed to iron in the form of humates, chelates or other complex compounds. Crustose lichens remove iron from bare rocks. Other life forms are also involved in the iron cycle.

Under oxidizing and alkaline conditions, mud layers in lake bottoms act as an adsorbent for the more common insoluble metallic ions. Mud layers reduced by anaerobic bacteria release iron and the more common metallic ions. The simpler aquatic plants use these metallic ions directly.

The redox potential of water is related to the content of dissolved ferrous and ferric iron. Iron in organic complexes may be difficult to determine by usual methods of chemical analysis. Special methods and techniques are suggested when the determination is to be performed in the presence of organic matter.

INTRODUCTION

This survey of published literature was made to aid further study and to summarize the knowledge of chemical and (or) biochemical factors that govern the solution, transport, and deposition of iron by natural water. No comprehensive summary of the subject has been published. However, many scientists have studied certain phases of the iron cycle in nature. Much of this work concerns the use of iron by plants and animals. This iron use is directly related to the occurrence of iron in water.

Iron is undesirable in water for human use. In amounts exceeding a few tenths of a part per million, iron in domestic water may stain porcelain fixtures, glassware, and laundered articles and may impart an unpleasant taste to water or to foods prepared with such water. In water for industrial use, iron may interfere with textile bleaching, dyeing or finishing, paper production, and manufacture of wines, beer, and other beverages; it may also impair the quality of dairy products or interfere with fish propagation. Therefore iron removal is essential in water treatment (Nordell, 1953).

Chemical-element cycles, particularly those of nitrogen and carbon, are presented in many elementary plant-science textbooks. These cycles emphasize the changes in elemental combination caused by microscopic and macroscopic organisms. Some phases of the iron cycle that affect iron content in natural water can be simply explained in diagrams.

More than 800 articles and abstracts were examined. These are listed in the bibliography, and many are summarized in this report. Common names used in the original articles are cited along with scientific names unless only one name was available.

In order to review thoroughly and systematically the factors affecting iron content in natural water, it was necessary to investigate iron utilization by all kinds of life, particularly soil and aquatic flora. Because other ions (calcium, copper, and manganese to name just a few) affect iron utilization or solubility, the literature on foreign ion interference to iron solution and availability was pertinent. Utilization of iron by many soil organisms affirms the importance of organisms in removing iron from the soil. Organic material in water interferes with the accuracy of certain methods of iron analysis. Accordingly, published articles on methods of detemining iron were examined. No attempt has been made to evaluate or classify the articles into basic or applied research.

Three works (Needham and Needham, 1935; Fassett, 1940; and Muenscher, 1944) are recommended for picture identification of the hydrophytes.

The interrelationship of water-soluble iron with other ions, pH, conductivity, redox potential, and dissolved oxygen is noted in many works and should be studied in any investigation concerning the solution of iron in and the removal of it from natural water.

The relationship of iron to chlorophyll, protein, ash, crude fiber, oxygen and carbon-dioxide production by both water-rooted and soil-rooted plants is important. Soil microflora leach iron from soils and affect the amount in natural water. The literature emphasizes the desirability of correlating and interpreting laboratory (greenhouse) findings with results obtained in the field (on a suitable lake).

NONTECHNICAL DEFINITION OF TERMS

The following selected biological terms are defined to facilitate the reading of this study:

Actinomycetes. Thread bacteria or fungi frequently breaking up into short rods.

Benthos. Organisms that inhabit the bottom of a lake or other water body.

Cambium. Perpetually young undifferentiated plant cells in perennial plants responsible for increased stem and root diameter giving rise to new tissue.

Chloroplast. An oval-shaped matrix in plant cells on which chlorophyll manufactured in the same plant cell is adsorbed.

Chromosome. Contains bent-rod appearing bodies made up of chromatin and probably present in all living cells.

Coenocyte. Organisms having cells with more than one nucleus.

Dicotyledon. Type of plant which may be identified in the field by presence of netted-veined leaves.

Gametophyte (haploid). Plant made up of cells having a single set of chromosomes. Conspicuous part of plant body as in many unicellular or filamentous algae, lichens and mosses.

Hydrophyte. Water plant.

Littoral. Inhabited water adjacent to the shore.

Meristem. Perpetually young undifferentiated plant cells responsible for differentiation of tissues in shoot, root, and leaf bases of plants.

Mesophyll. The green uniformly thin-walled plant cells between the epidermal layers of a foliage leaf.

Monocotyledon. Type of plant which may be identified in the field by presence of parallel-veined leaves.

Mycelium. Threadlike protoplasm containing filaments characteristic of fungi.

Nonvascular plant. Plant lacking a vessel or vessels for conveyance of sap through the plant. Mainly nonflowering plants such as the algae, fungi, lichen, bacteria, and moss groups.

Pelagic. Inhabiting open water.

Phage. Bacteria-destroying virus or agent normally present in sewage, the intestinal tracts of man and animals, and in blood, pus, and urine.

Phloem. Food-conducting tissue of flowering plants.

Plankton. Forms of life, frequently miscroscopic, near surface of open water.

Protozoa. Mostly microscopic one-celled animals.

Sporophyte (diploid). Plant made up of cells having a double set of chromosomes. Conspicuous part of plant body as in flowering plants and ferns.

Vascular plant. Plant having a vessel or vessels for conveyance of sap through the plant, mainly these are flowering plants, but include the ferns.

Virus. Submicroscopic infective substance capable of causing disease.

Xylem. Mineral-conducting tissue of flowering plants.

BIOCHEMICAL FACTORS IN THE IRON CYCLE

BIOCHEMICAL WEATHERING

Literature on the chemistry of iron in natural water emphasizes the widespread and continuous influence of biota on the amount and state of oxidation of iron in either moving or quiet water. All categories of fauna and flora, ranging in size from smaller than that seen through the regular biological microscope to tree-size growth, have many representatives which are vital in critical phases of the iron cycle.

Trees, shrubs, and garden crops are effective in removing iron from well-aerated soils. Fungi and particularly bacteria may remove or release iron from water-submersed or nonaerated soils. Rooted vascular hydrophytes are also prominent in the iron cycle and affect the solution of iron in natural water. The roots of this group of water plants generally grow in environments lacking oxygen. The plants are both emergent and submersed. They take oxygen to their own roots through special air tubes, and the roots absorb the relatively large amount of iron needed from the submerged mud. Unattached vascular hydrophytes, such as the surface floating duckweeds, are important in the iron cycle in natural water, particularly in ponds and in places protected from rapid waterflow, by (a) occluding normal air-water interchange of oxygen and (b) depleting the dissolved-oxygen supply of the water through respiration of rootlets projecting into and just below the water surface. Iron taken into solution in the resulting less-oxidizing medium is available for the needs of this plant. Mineral salts taken from the substratum are returned as organic mineral deposits upon death and decay of the aquatic organism (Butcher, 1933). Algae attached to bottom muds and sediments obtain most of the iron needed for metabolism directly from iron in the water in which the plants are submersed. The holdfast cells of fresh-water algae probably absorb some iron from the substratum to which they are attached; but in varieties lacking effective conduction tissue, most of the iron is absorbed by the individual generally haploid thallus cells of the filament as needed.

Some attached algae, such as the fresh-water stoneworts and the marine seaweeds, have functional conductive systems. These algae apparently absorb iron principally from the substratum rather than directly from the water.

Unattached or plankton algae obtain all the iron needed for metabolism directly from the water in which they are submerged. Apparently little or no iron is obtained directly from the substratum.

Crustose lichens are very active and effective in removing iron from barren rocks by physical and chemical methods. Further solution of iron from rocks is accomplished by successive lichen—foliose and fruticose—and moss growth. Fry (1924) believed that the rock substratum first was altered chemically by lichens and then the parts of the decomposed material were separated by the mechanical action of the growing hyphae. However, Fry has shown that these processes can be reversed, the initial stage being the mechanical disintegration of the unaltered rock by the lichen thalli, followed by the decomposition of the separated fragments. This mechanical action is illustrated in figure 12, which shows the tearing away of the surface of a piece of plate glass and of a petri dish by the drying contraction of 2- and 5-percent solutions of the plant gelatin agar-agar. Crustose and foliose lichens growing on schist are also illustrated. Lichens probably break up rock surfaces in a similar manner, one of the first steps in removing iron from the rock substratum to which they are attached. Gelatinizing agents, such as *d*-galactopyranose and *d*-galactose, through expansion-contraction wetting action bring about this mechanical disintegration of the rock substrata. Chemical disintegration comes about through carbonic and alginic acid solution and organic chelation processes (Schatz and others, 1956). It is noteworthy that the addition of gelatin to ferric solutions prevents (Taboury and Mangin, 1948) the precipitation of hydroxide when alkali is added.

IRON METABOLISM

Many have shown that iron in soluble or available form is indispensable for the synthesis of the chlorophyll-protein-lipoid complex in all green plants (Abadia, 1956; Samish, 1954; Singh, 1956). Organic-bound iron present as respiratory pigment in apparently all living protoplasm seems to act as a catalyst or oxygen carrier in oxidation-reduction processes occurring in living cells. Iron salts in any appreciable concentration, as a result of extreme soil acidity or great lack of aeration or both, are toxic to plants.

In spite of the absolute need of green plants for iron, the proportionate amount in plant tissues is very low and much of the iron pres-

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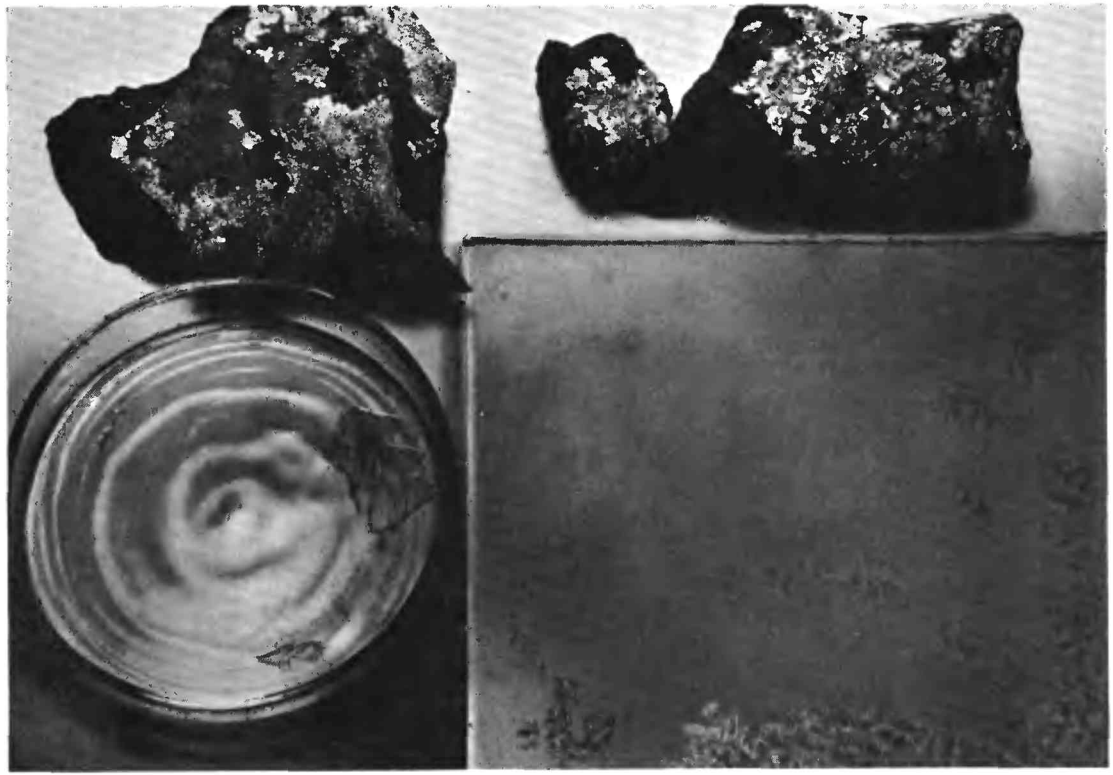

FIGURE 12.—Tearing away of glass surface by drying contraction of 2-percent solution (in petri dish, lower left) and 5-percent solution (on plate glass, lower right) of the plant gelatin agar-agar. In similar manner crustose lichens (upper left) and foliose lichens (upper right) are believed to break up rock surfaces.

ent is a constituent of organic compounds. Most of the literature indicates that iron is immobile within plants; no appreciable redistribution ever occurs from one tissue to another after the tissues have been built up. Therefore, during growth, aquatic as well as land plants must have a continuous source of iron; iron extraction from the soil is a continuous process throughout the growing season. Aquatic plants average 90 to 95 percent water. Because many have no woody parts, disintegration at the end of the growing season is rapid. The resulting gelatinous byproducts, tannins, and other plant acids released tend to reduce and hold iron in solution until used by bacteria or other aquatic organisms. Death and disintegration of aquatic fauna that feed on aquatic flora likewise bring organic-bound reduced iron into solution.

RELATION OF IRON TO LIFE PROCESSES

Several fundamental life processes seem to depend on an adequate supply of iron (Ellis, 1919; Guelbenzu, 1951; Guseva, 1939; Kaserer, 1910; Rankama and Sahama, 1952; Steward, 1948; Warburg, 1925). Iron is needed for plant photosynthesis (which involves alternate oxidation and reduction of iron), respiration, and protein synthesis. In the higher animals, the mammals, iron forms an integral part of the hemoglobin or red-blood molecule. Amounts of iron in plants and animals are nearly parallel with amounts of chlorophyll or protein. Relationships of protein and chlorophyll synthesis (and amount of iron) are discussed by Bennett, 1945; Bonner, 1950; De Boissezon, 1933; Escudero and others, 1944; Kliman, 1937; Peterson and Elvehjem, 1928; Randoin and Le Gallic, 1942; Sherman, 1907; Sisakyan and Melik-Sarkisyan, 1956; Williams, 1953; Wolken and Mellon, 1956.

Loss of iron from solution by precipitation as hydroxides and the effect of complexing in organic compounds normally limit the dissociated iron content of natural water to levels within tolerance limits for plant growth (Smith, 1950). If iron concentrations in water are excessive or when plant tissue comes directly in contact with certain iron compounds, as for example iron sulfate sprayed on young plants when they have their first 2 or 3 leaves, vegetation is destroyed by corrosive action (Duchaufour, 1953a). Before the advent of growth regulators, this principle was commonly applied in weed control (Adams, 1909; Beaumont and Holland, 1935; Blum, 1956; Blunno, 1911; Clark, N. A. 1936; Coste, 1896; De Jesus, 1937; Dusserre, 1900; Graftiau, 1900; Handke, 1933; Kharasch and others, 1936; Maze and Perrier, 1904; Ponnamperuma and others, 1955; Robinson 1930, 1951; Setter, 1930; Smith, 1950; Voicehovich, 1940; Willis, 1936; Yoshimura, 1936).

Most aquatic plants grow actively in the young tissue by rhizomes and die in the old tissue; therefore, they remain perpetually young (Arber, 1920). Thus, particularly in these plants, an extra large amount of perpetually young undifferentiated tissue requires iron for normal metabolism.

Because of the difference in methods of iron absorption, most but not all accumulation or concentration is accomplished by the lower algal forms of the hydrophytes. This process withdraws some of the dissolved matter of the water body.

Although plant life alters the iron content of natural water, animal life also alters the content through direct or indirect dependence on plants. More directly, mankind probably derives much of its daily iron need from culinary water (Asbury in Forman and Frink, 1950; Sebrell, written communication, 1942). The empirical chemical formulas of photosynthesis and respiration are as follows:

Photosynthesis (restricted to cells containing chlorophyll).

$$6CO_2+6H_2O+\text{light}\rightarrow C_6H_{12}O_6+6O_2$$

Aerobic respiration (living cells breathing atmospheric oxygen).

$$C_6H_{12}O_6+6O_2\rightarrow 6CO_2+6H_2O+\text{energy}$$

Anaerobic respiration (living cells not breathing atmospheric oxygen).

$$C_6H_{12}O_6\rightarrow 2C_2H_5OH+2CO_2 \text{ (fermentation)}$$

$$C_6H_{12}O_6\rightarrow 3CH_4+3CO_2 \text{ (methane formation) (Kufferath and Schmitz, 1955)}$$

or

$$R\cdot COOH\rightarrow CH_4+3CO_2+2H_2O \text{ (Steele, 1949)}$$

or

$$CO_2+4H_2\rightleftharpoons CH_4+2H_2O$$

Molecular hydrogen is released by the hydrogenase of the mud organism and (or) organisms as in the reaction

$$R\cdot COOH\rightarrow H_2+CO_2$$

Apparently, most of the carbon dioxide used in photosynthesis may be extracted from the soil (Dyakonova, 1957), and most of the carbon dioxide of underground carbonic acid water may originate in the atmosphere (Smirnov, 1955).

SOLUTION AND DEPOSITION OF IRON

Reducing and acid conditions (Goldschmidt, 1954), especially if pH is less than 3, generally promote iron solution in water. With the semiannual decrease of dissolved oxygen in lake bottoms, which in new reservoirs or in freshly inundated marshland may amount to total depletion, activity of anaerobic bacteria and other organisms

results in the solution of iron and manganese from the bottom deposits. Organic color in water represents the extract of soluble material contained in decaying vegetation, such as leaves and debris in peat bogs. Generally, the color is a brownish yellow, becoming deeper brown at higher concentrations. Iron may be an integral constituent in this organic color in water; and upon oxidation of the organic matter, the iron is precipitated and settles to the bottom of the body of water. If, during summer stagnation, conditions become anaerobic, the iron may be redissolved and later distributed throughout the water as colloidal ferric hydroxide. This change, however, is not a permanent condition; sedimentation and clarification soon follow.

The following biochemical processes or combination of processes aid solution of iron in natural water:

1. Formation (Rummeni, 1955) of citrate, tartrate, malate, and humate complexes of ferric iron (citric, malic, lactic, and tartaric acids, hemoglobin, chlorophyll, urine, and manure are chelating agents).
2. Reduction of iron to the ferrous state.
3. Processes releasing carbon dioxide or (other) sources of hydrogen ions.
4. Formation of complex ions, such as those with sodium hexametaphosphate.
5. Possible prevention by organic matter of formation of iron nuclei needed for crystallization.

Water draining from mines may be acidic, and being dependent on the state of oxidation of the iron (Braley, 1957), may contain large amounts of ferrous and ferric iron owing to oxidation of pyrite. Pyrite (FeS_2) deposited under conditions of oxygen deficiency (Murray, 1956), is oxidized (Segeberg, 1937) to ferric oxide and sulfuric acid in the presence of air and water.

Carbonic acid resulting from both aerobic and anaerobic respiration is an important aid in solution of iron in soil moisture and thus makes iron available, frequently as the bicarbonate, for absorption by rooted green plants (Shilova and Kreier, 1957). The preceding is true even though in well-drained and well-aerated soils oxygen is available in the plant root zone.

Many publications show organic matter to be an aid to iron solubility. Processes of altering iron solubility with the aid of organic matter are discussed by: Aiyar, 1946, 1948; Aleev and Mudretsova, 1937; Alexander and Walsh, 1952; Allyn, 1927; Betremieux, 1951; Beutelspacher, 1955; Bhaskaran and Pillai, 1937; Biddulph, 1948; Bloomfield, 1952, 1957; Boiret and Paturel, 1892; Boischot and Dur-

roux, 1949; Bould, 1957; Brewer and Carr, 1927; British Standards Institute, 1956; Broadbent, 1954; Carnes, 1954; Chaberek and Bersworth, 1953; Clark and others, 1957; Clark, N. A., 1936; Coldwell and De Long, 1950; Cooper, 1948; Correns, 1941; Datsko, 1955; De Kock, 1955a, 1955b; De Long and Schnitzer, 1955; Demolon and Bastisse, 1938; Doyne and Morison, 1926; Duchaufour, 1953a; Edelstein and Von Csonka, 1912; Eggleton, 1931; Falci, 1935; Fly, 1935; Gellerman, 1950; Gile and Carrero, 1916b; Guelbenzu, 1951; Hance, 1934; Hopkins and Wann, 1925, 1926; Hutchinson, 1941; Ichikawa, 1936; Iyengar, 1936; Iyengar and Subrahmanyan, 1935; Joffe and McLean, 1928; Kahler, 1956; Kapp, 1932a, 1932b, 1934a, 1934b; Kaserer, 1910; Kawamura, 1928; Kliman, 1937; Koeck, 1956; Kopal, 1940; Kuhnholtz-Lordat, 1928; Lackey, 1939; Leonard and Stewart, 1953; Lipmann, 1937; Loew, 1938; Lutwick and De Long, 1954; Lutwick and others, 1952; Malyuga, 1945; Mangenot, 1953; Martell and Calvin, 1952; Masoni, 1914; Mayer and Gorham, 1951; Maze and Perrier, 1904; Morison and Doyne, 1914; Nakabayashi, 1954; Narasinhamurty, 1940; Narasinhamurty and Subrahmanyan, 1953; Nierenstein, 1945; Ohle, 1937; Olivier and Le Peintre, 1955; Olsen, 1935; Onodera, 1928; Osugi and Aoki, 1936, 1938b; Osugi, Aoki and Morita, 1935; Osugi, Nishigaki, and Yoshimi, 1935; Pearsall, 1952; Pringsheim, 1949; Prozorovskaya, 1936; Ramaut, 1955; Rankama and Sahama, 1952; Reed and Haas, 1924; Robinson, 1930; Roelofs, 1944; Saint, 1935; Sarishvili and Bagaturiya, 1938; Saunders, 1938; Saunders, 1957; Schnitzer and De Long, 1954b, 1955; Schoeller and Webb, 1936; Seeberg, 1937; Segeberg, 1937; Siddappa and Subrahmanyan, 1935; Skeen, 1930; Skopintsev and Krylova, 1955; Smith, 1950; Steel and Gloyna, 1954; Stewart and Leonard, 1956; Tsujimura and Ikeda, 1957; Van Beneden, 1956b; Vialard-Goudon and Richard, 1955; Villeret, 1954a, 1954b; Vinberg, 1955; Wallace and Ashcroft, 1956; Wallace and others, 1955; Wallace, 1929; Wallace and Lunt, 1956; Weinstein and others, 1954; Weston, 1936; Whipple and others, 1927; Wiebosch, 1947; Willis, 1936; Yoshimura, 1931a.

Solubility of iron in soil affected by several interrelated biochemical phenomena, such as pH, adsorption, presence of calcium or sulfur, particle size, and different treatments of soil, is discussed by: Asana, 1946; Askinazi and Kheifets, 1938; Baker and Price, 1946; Bandt, 1938; Barbier, 1938a, 1938b; Barbier and Chabannes, 1949; Boischot and Durroux, 1949; Brown and Holmes, 1955; Brozek, 1949; Bruevich and Vinogradova, 1946; Byram, 1938; Calgon and Albright & Wilson, 1944; California State Water Pollution Board, 1952; Clarke, 1924; Cooper, 1935; Delecourt, 1947; De Wilkoszewski, 1917; Dienert, 1938; Dion, 1944; Edgerton, 1942; Fischer, 1950; Follett-Smith, 1938;

Follett-Smith and Robinson, 1936; Goldschmidt, 1954; Haase, 1940; Hance, 1934; Harvard, 1937; Henry, 1950; Hesse, 1956; Hoore, 1949; Hseung, 1941; Hylkema, 1939; Ignatieff, 1939, 1941; Ingleson and Thomas, 1946; Joffe, 1930; Jolivet, 1934; Kapp, 1932a, 1932b; Keller, 1945; Keller, 1955; Klut, 1937; Koczy, 1950; Kriventsov, 1954; Kurzweil and Exner, 1954; Lackey, 1939; Levin, 1947; Luhrig, 1927; Lutz, 1936; MacCarthy, 1925; Mackenzie, 1952; Mandl, 1949; Mason, 1952; Metzger, 1930; Mohler, 1951; Mukherjee and Chatterjee, 1945; Mukherjee and others, 1946, 1947; Mulwani and Pollard, 1939; Navellier, 1950; Navet, 1947; Nordell, 1953; Notarov, 1956; Ohle, 1937; Okura and Kayama, 1952; Osugi and Aoki, 1938a, 1938b; Osugi and others, 1934; Oudin, 1938; Parker, 1955; Patrick, 1953; Pearsall, 1950; Pronin, 1933; Puri and Sarup, 1938; Puustjarvi, 1952; Rakestraw and others, 1936; Rippel, 1941; Ritter and Christen, 1935; Rudge, 1936; Saruhashi, 1955; Schachtschabel, 1942; Schilling, 1936b; Solovev, 1940; Speller, 1922, 1951; Thompson, 1940; Todt, 1934; Toth, 1937; Urs and others, 1955; Valyashko, 1955; Van Beneden, 1947, 1948; Walker and others, 1907, 1908; Wheatly, 1946; Whipple and others, 1927; White, 1937; Winters, 1940; Zonn, 1945.

In submerged soils lacking organic matter, reduction of iron or increases in cation concentration are not likely. Chemosynthesis taking place under ice (Sorokin, 1955) and organic matter resulting from the death and decomposition of the aquatic plants (Arber, 1920) do affect the redox potential of soils and natural water.

Mortimer (1942) and others have shown relationships between increasing depth and the fall in redox potential and oxygen concentration in lake water and the rise of color, iron, and turbidity. Increase in turbidity and color resulted mainly from ferric hydroxide in suspension. Oxidation of ferrous iron and other processes using up oxygen accounted for an accelerated decrease in oxygen concentration with depth. At the point of zero oxygen content ferrous iron was detected in the water, and the redox potential had fallen to 0.15 corrected to a pH of 7. From this point to the lake bottom, the iron content of the water increased rapidly, and an increasing proportion consisted of ferrous iron. When seasonal changes in temperature brought this bottom water to the surface, the water absorbed oxygen from the air, and then the iron concentration decreased rapidly because of the precipitation of ferric hydroxide.

When the oxygen concentration and redox potential of the water in contact with the bottom mud dropped below a limiting value, ferric iron at the mud surface was reduced to the more readily soluble ferrous form. Freshly precipitated ferric hydroxide has some capacity for adsorption of cations. The oxidized layer at the mud surface with its

adsorbed ions constitutes a barrier to exchange between the water above and below the layer.

Mortimer (1941) cites the work of Ohle (1937), who demonstrated the ampholytic nature of the ferric hydroxide gel, which is electropositive in acid solution and electronegative in alkaline solution. Thus, in the presence of carbon dioxide at pH 4, negatively charged phosphate ions added to ferric hydroxide gel were strongly adsorbed, although some of the iron went into solution. After raising the pH to 9, a large part of the phosphate was liberated. These phenomena partly explain the fundamental and somewhat paradoxical influence of the presence or absence of bases (calcium) on the biochemical activity in fresh water. In the water of calcium-poor humus in lakes, pH generally will be below 7 and negatively charged phosphate and other plant nutrients are adsorbed on positively charged iron gels, which are themselves attached to electronegative humus colloids and are retained in the bottom muds; hence, few organisms can be supported.

An observation made by Mortimer (1941) and supported by many laboratory studies is that ferrous iron in water under natural conditions is not oxidized instantaneously. This fact was shown by conditions found during mixing preceding the overturn of lake water. At this time, ferrous iron was detected in water containing 8.4 milligrams per liter of oxygen. The rapid spread of ferrous ions in the water above the mud layer was believed by Mortimer to be the result of transition from molecular diffusion in the mud to turbulent diffusion in the water. The ferrous ions were oxidized and precipitated; thus the oxygen was depleted and an oxidized layer was built up at the surface of the bottom mud. When anaerobic conditions were reestablished at the bottom, the adsorbent oxidized surface mud layer became progressively thinner and disappeared about 40 days after oxygen was depleted. A rise in temperature accelerates greatly the rate of decomposition of organic matter. Manganous ions in the water before the appearance of ferrous ions indicates that manganic precipitates in the oxidized mud surface are reduced at a higher potential than the ferric compounds.

Oxidation and reduction in soils seem to be regulated by the prevailing conditions of air circulation and by the oxygen consumption of microorganisms. Waterlogging of a soil causes a decided decrease in the redox potential (Stobe, 1956). The change from an oxidizing to a reducing state is associated with the appearance of reduced, and more soluble, iron and manganese and a lower pH. Presumably, ferric iron is reduced to the ferrous state and dissolved in the soil solution. In the presence of atmospheric or dissolved oxygen, iron is oxidized from the ferrous to the ferric state. Microbiological action on organic

matter produces carbon dioxide. The solution of carbon dioxide in water liberates hydrogen ions and brings into the soil solution increased quantities of exchangeable iron, manganese, aluminum, calcium, and magnesium. The formation of iron-bearing organic complex ions is an important factor in stabilizing iron in solution under oxidizing conditions at concentrations of iron that would otherwise precipitate as ferric hydroxide.

The normal redox potential of the system $Fe^{+++}+e \rightleftharpoons Fe^{++}$ is given as 0.68 v in $\frac{1}{8}$ M H_2SO_4 by Smith and Richter (1944); the value 0.77 is generally accepted as standard at pH 0 at 25°C (Bray and Hershey, 1934).

Since Puri and Sarup (1938) found the relation of pH to Eh in soils to be nearly linear, they believe that the measurement of redox potentials in soils does not give results of any greater accuracy than could be obtained by pH measurements.

Knaysi and Dutky (1936) found that in a bacterial-culture medium of ordinary meat infusion broth, the strictly aerobic bacterium *Bacillus megatherium* grows almost entirely on and near the surface where oxygen is most plentiful; however, it may be induced to grow throughout the medium or even at the bottom by an increase in the redox potential of the medium. Redox potential thus may affect bacterial growth distribution and location in lake bottoms.

Ignatieff (1941), Lipmann (1937), and Moore (1914) point out that light affects the reduction of ferric iron.

Redox-potential effects are discussed by the following: Allison and Scarseth, 1942; Baas-Becking and Wood, 1955; Baas-Becking and others, 1956; Baas-Becking and others, 1957; Belcher and others, 1955; Blagovidov and others, 1957; Bloomfield, 1952; Broadbent, 1954; Copeland, 1957; Eristavi, 1953, 1955; Gantimurov, 1952; Gorbunov, 1957; Hewitt, 1957; Hutchinson, 1957; Kramli and others, 1954; Melnichenko, 1954; Prokhorova, 1957; Rabotnova and others, 1955; Remezov, 1929; Schoeller, 1955; Shkolnik and Steklova, 1954; Trojanowski, 1954; Varkov and others, 1950; Warburg and others, 1933; Weart and Margrave, 1957; Yamasaki, 1952.

The effect of other factors in the reduction of ferric iron, such as waterlogging of soil and formation of complex iron compounds, is discussed by: Bezier, 1943; Brown, 1934; Deevey, 1941; Hill and Michaelis, 1933; Hirsch and Ruter, 1926; Hutchinson and others, 1939; Izgarishev and Turkovskii, 1929; Limanowski, 1932; Mason, 1952; Nitschmann, 1938; O'Meara, 1937; Osugi and others, 1934; Pearsall, 1950; Puri and Sarup, 1938; Remezov, 1929, 1930; Robinson, 1930, 1951; Serdobolskii, 1950a, 1950b; Serdobolskii and Shavrygin, 1950; Shcherbina, 1939; Willis, 1936.

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ORGANISMS UTILIZING IRON IN METABOLISM

The following organisms apparently require iron for normal metabolism according to the literature:

FUNGI

[See also iron bacteria]

Iron-using fungi are as follows:

Aspergillus niger (Bertrand and De Wolf, 1956; Bortels, 1927; Gollmick, 1936; Javillier and Sauton, 1911; Linossier, 1910; Lutman, 1929; Steinberg, 1919, 1920, 1935a, 1936)
oryzeae (Nehira, 1955; Yamagata and others, 1955)
Cercospora nicotinae (Steinberg, 1950)
Fusarium lini (Saraswathi-Devi, 1955)
moniliforme (Saraswathi-Devi, 1955)
orthoceras (Saraswathi-Devi, 1955)
oxysporum (Saraswathi-Devi, 1955; Steinberg, 1950)
poae (Saraswathi-Devi, 1955)
scirpi (Saraswathi-Devi, 1955)
udum (Saraswathi-Devi, 1955)
vasinfectum (Mundkur, 1928; Saraswathi-Devi, 1955)
Fusarium sp. (Saraswathi-Devi, 1955)
Helminthosporium sativum (Peterson and Katznelson, 1954)
Neurospora crassa (Nicholas and Commissiong, 1957)
Pythium irregulare (Steinberg, 1950)
Rhizoctonia solani (Steinberg, 1950)
Sclerotium rolfsii (Steinberg, 1950)
Streptomyces griseus (Kramli and others, 1954)
Thielaviopsis basicola (Steinberg, 1950)
Trichophyton mentagrophytes (English and Barnard, 1955)
rubrum (English and Barnard, 1955)
Ustilago sphaerogena (Garibaldi and Neilands, 1955)
Molds (Harley, 1940; Humfield and Sugihara, 1952; Metz, 1930; Muth and Voigt, 1929; Sauton, 1911)

Some soil fungi grow at pH environments approaching 1.0 (Truog, 1946).

BACTERIA

[See also iron bacteria]

Iron-using bacteria are as follows:

Aerobacter aerogenes (Waring and Werkman, 1943)
indologenes (Waring and Werkman, 1942, 1943)
Azotobacter chroococcum (Greaves, 1933; Krzemieniewski and Kovats, 1937)
vinelandii (Burk and others, 1931; Horner and Burk, 1934)
sp. (Burk and others, 1932)
Bacillus prodigiosus (Bortels, 1927)
bruntzii (Marchal and Adams, 1956)
Brucella abortus (Evenson and Gerhardt, 1955)
Candida guilliermondia (Enari, 1955)

Chlorobium limicola (Larsen, 1953)
thiosulfatophilum (Larsen, 1953)
Clostridium tetani (Feeney and others, 1943; O'Meara, 1937)
Escherichia coli (Waring and Werkman, 1943)
Ferrobacillus ferrooxidans (Leathen and others, 1956)
Flavobacterium halobium (Brown and Gibbons, 1955)
Klebsiella pneumoniae (Waring and Werkman, 1942, 1943)
Mycobacterium phlei (Tysarowski, 1955)
tuberculosis (Cohn and others, 1954; Henley, 1925; Minami and others, 1955; Polster and others, 1953)
Pseudomonas aeruginosa (Waring and Werkman, 1942, 1943)
cattleyae (Marchal and Adams, 1956)
cutirubra (Brown and Gibbons, 1955)
salinaria (Brown and Gibbons, 1955)
Rhizobium meliloti (Thorne and Walker, 1936)
trifolii (Thorne and Walker, 1936)
Rhizobium spp. (Virtanen and others, 1947)
Rhodopseudomonas sp. (Lascelles and Cooper, 1955)
Sarcina littoralis (Brown and Gibbons, 1955)
Serratia marcescens (Novelli, 1953; Waring and Werkman, 1943; Williams and others, 1956)
Thiobacillus denitrificans (Baalsrud and Baalsrud, 1954)
ferrooxidans (Bryner and others, 1954; Temple and Colmer, 1951)
thiooxidans (Bryner and others, 1954)
Bacilliform bacterium (Brussoff, 1918)
Bacteria (Anderson, 1940; Kaserer, 1910; Lasseur and others, 1944; Waring and Werkman, 1942)
Bacteria producing blackbeet disease (Cameron, 1934)
Hemophilic bacteria (Lwoff, 1933b)
Intestinal flora (Stern and others, 1955)
Marine bacteria (MacLeod and Onofrey, 1956)
Marine sulfate-reducing bacteria (Kimata and others, 1955)
Soap-discoloring bacteria (Hollo and Gorog, 1954)

PROTOZOA

Iron-using protozoa are as follows:

Chilomonas paramecium (Bowen, 1940; Hutchens, 1940)
Paramecium candatum (Shoup and Boykin, 1931)
Pleurobrachia sp. (Cooper, 1939)
Trypanosoma sp. (Lwoff, 1933a)

PHAGES

The following workers have reported iron-using phages:

Bortels, 1953; Spizizen, 1943

VIRUSES

Iron utilization by virus is also known.

McIntire and others, 1941

YEASTS

Although yeasts are fungi they are listed separately in this report.

Bortels, 1927; Elvehjem, 1931; Kiene, 1940; Malkov, 1934

ALGAE

Iron-using algae are as follows:

Blue-green
- *Anabaena lemmermanni* (Guseva, 1937b)
- *Aphanizomenon flos-aquae* (Guseva, 1937b)
- *Coelosphacrium* sp. (Guseva, 1939)
- *Lyngbia confervoides* (Kinne-Diettrich, 1955)
  - *mainscula* (Kinne-Diettrich, 1955)
- *Microcystis aeruginosa* (Gerloff and Skoog, 1957)
- *Oscillatoria agarhdia* (Skadovskii and others, 1955)
  - *rubescens* (Edmondson and others, 1956)
- *Phormidium ambigaum* (Umemoto and Mifune, 1953)
- Blue-green algae in general (Slobodchikov and Stroikina, 1953; Vouk, 1936); (Gerloff, written communication, 1955)

Brown (ocean kelp)
- *Macrocystis integrifolia* (Wort, 1955)
- *Nereocystis luetkeana* (Wort, 1955)

Desmids
- Desmidiaceae (Hofler, 1925)

Diatoms
- *Asterionella formosa* (Cooper, 1935; Guseva, 1937b)
  - *japonica* (Goldberg, 1952)
- *Chaetoceras curvisetius* (Koblents-Mislnke, 1955)
- *Nitzschia closterium* (Harvey, 1933; Koblents-Mislnke, 1955)
- Diatoms in general (Correns, 1941; Gayral, 1954; Harvey, 1937, 1939; Thompson and others, 1932)

Green
- *Chlamydomonas* sp. (Lackey, 1939)
- *Chlorella pyrenoidosa* (Knauss and Porter, 1954; Myers and others, 1951; Walker, 1954)
  - *vulgaris* (Mayer, 1952; Mayer and Gorham, 1951)
  - sp. (Hopkins, 1930, 1935; Hopkins and Wann, 1926, 1927; Reisner and Thompson, 1956; Schwartz, 1956)
- *Cladophora glomerata* (Blum, 1953, 1956)
- *Euglena* sp. (Lackey, 1939)
- *Ulothrix* sp. (Lackey, 1939)
- Green algae in general (Roberg, 1932; Slobodchikov and Stroikina, 1953)

General
- (Blum, 1956; Cooper, 1935; Ellis, 1925; Fogg, 1952; Gayral, 1954; Gorham and Gorham, 1955; Goryunova and Nasonova, 1955; Guseva, 1937a; Hutchinson, 1941; Koblents-Mislnke, 1955; Lackey, 1956; Levring, 1957; Messikommer, 1956; Moore, 1950; Murray, 1908; Novozhilova, 1955; Ono, 1900; Pond, 1903; Prescott, 1956; Pringsheim, 1934; Riley, 1940; Roberg, 1932; Schroder, 1939; Slobodchikov and Stroikina, 1953; Smith, 1950; Uspenski, 1927; Villeret, 1954a, 1954b, 1954c, 1954d, 1954e; Voronkov 1953; Yoshimura, 1936)

MICRO-ORGANISMS, GENERAL

The following workers have reported use of iron by general micro-organisms:

Neilands, 1957; Osugi and Aoki, 1938b; Scouller, 1932

FLOWERING PLANTS

Iron-using flowering plants are as follows:

Aquatic, rooted (also see crop plants, rice)

Oryza sativa (Juliano and Aldama, 1937; Richter, 1926; Tullis and Cralley, 1936)

Potamogeton foliosus (Harper and Daniel, 1934–35)

spp. (Nierenstein, 1945)

Typha latifolia (Harper and Daniel, 1934–35; Lackey, 1939)

Watercress (Escudero and others, 1944; Gorham and Gorham, 1955)

General (Ward and Whipple, 1918)

Aquatic, unattached

Lemna major (Fly, 1935; Yoshimura, 1950)

minor (Steinberg, 1941, 1943, 1946)

polyrhiza (Olsen, 1930; Saeger, 1937)

sp., (Clark, N. A. 1936; Hopkins, 1935)

Aquatic, general

(Bowman, 1956; Butcher, 1933; Curry and Wilson, 1955; Dupont, 1954; Einsele, 1936a, 1936b; Lewis and Goldberg, 1954; Margalef, 1954; Mayer and Gorham, 1951; Moore, 1952; Olsen, 1950; Olson and others, 1941; Pond, 1903; Prescott, 1956; Roelofs, 1940; Rozenberg and Mefedova, 1956; Skadovskii and others, 1955; Villeret, 1954e)

Crop

Truog (1946) has shown that a soil pH of 6.5 is very favorable for absorption of iron by most crop plants.

Alfalfa (Dalle, 1946)

Apple (Milad, 1939)

Asparagus (Sheets and others, 1941)

Cabbage (MacGillivray and others, 1942; Sheets and others, 1941)

Carrots (MacGillivray and others, 1942)

Clover (Brewer and Carr, 1927)

Corn (Allyn, 1927; Arndt, 1922; Brewer and Carr, 1927; Brown and Holmes, 1955; Eckstein and Jacob, 1929; Hoffer and Carr, 1920; Jacobson, 1945; Maze and others, 1912; McGeorge, 1939; Olsen, 1938; Sayre, 1930; Scharrer and Schropp, 1933)

Lettuce (Harward and others, 1955; Sheets and others, 1941; Twyman, 1951)

Mustard greens (Abbott and Ahmann, 1940)

Oats (Mayer, 1892; Twyman, 1951)

Parsley (Randoin and Fournier, 1947)

Pea (Brown and Possingham, 1957; Possingham and Brown, 1957)

Pear (Bennett, 1928; Jacobson, 1945; Milad, 1939)

Pineapple (Gile and Carrero, 1916a; Sideris and Young, 1956)

Potatoes (MacGillivray and others, 1942)

Quince (Rodriguez and Abadia, 1956)

Rice[1] (Aiyar, 1946; Gile and Carrero, 1914, 1916a, 1916b; Kapp, 1934, 1938; Milad, 1939; Richter, 1926; Tullis and Cralley, 1936; Ueda, H., 1956; Ueda, K., and others, 1956; Willis and Carrero, 1921)

Soybean (Brown and Holmes, 1955; Marsh and Shive, 1925)

Spinach (MacGillivray and others, 1942)

Sugar beet (Whatley and others, 1951)

[1] Rice grows as an emergent aquatic plant. (Also see flowering plants, aquatic, rooted).

Sugarcane (Martin, 1935)
Tobacco (Jacobson, 1945; McMurtrey, 1933, 1952)
Tomato (Porter and Thorne, 1955; Twyman, 1951)
Tung (Dickey, 1942)
Turnip greens (Abbott and Ahmann, 1940)
Wheat (Barnette, 1921; Barnette and Shive, 1923; Brewer and Carr, 1927; Brown and Holmes, 1955; Greer and others, 1952; Singh, 1955, 1956)
Winter squash (MacGillivray and others, 1942)
General (Guelbenzu, 1951; Harris, 1952; Hewitt, 1950, 1953; Martin, 1935; McGeorge, 1951; Nicholas, 1949; Peterson and Elvehjem, 1928; Porter and Thorne, 1955; Randoin and Le Gallic, 1942; Remington and Shiver, 1930; Wallace, 1954; Wiebosch, 1947; Winsor, 1939)

Other than crop
Artemisia sp. (Susplugas and others, 1951)
Azalea spp. (Carnes, 1954)
Gardenia sp. (Baudenistel, 1957)
Hydrangea sp. (Atkins, 1923)
Rhododendron spp. (Carnes, 1954)
Xanthium pensylvanicum (Smith and others, 1957)
Roses (Carnes, 1954)
Sunflower (Carnes, 1954; Jacobson and Oertli, 1956)
Trees (Arnaud, 1919)

ANIMALS

The following workers have reported use of iron by animals:

Grazing on iron-deficient vegetation
Aston, 1934; Wind, 1951.

Nongrazing (other than man)
Davis and Loosli, 1954; Free, 1940; Galtsoff, 1934; Inoue, 1932; Kosugi and Umeda, 1931; Newell and McCollum, 1931; Parks and Rose, 1933; Supniewski, 1951; Unti, 1943.

Man
Adams and others, 1950; Chittenden, 1896; Elvehjem and others, 1927; Hendrych and Klimesch, 1935; Johnston and others, 1950; Lanyar and others, 1933; Lederer and Bogaert, 1939; Lindquist, 1949; Lintzel, 1929; McCance and Widdowson, 1943; Palit and others, 1944; Perosa and others, 1951; Piery and others, 1938; Sharpe, 1948; Steinkamp and others, 1955; Will and Vilter, 1954.

ORGANISMS, GENERAL

The following workers have reported use of iron by general organisms:

Antognini, 1954; Bear, 1954; Blunno, 1911; Boiret and Paturel, 1892; Bollmann and Schwanitz, 1957; Bonner, 1950; Frisbie, 1953; Granick, 1954; Ignatieff, 1941; Ingalls and Shive, 1931; Jenny and Overstreet, 1939; Keilin and Mann, 1944; Kliman, 1937; Loew, 1938; Masoni, 1914; Monnier and Kuczraski, 1917; New Jersey Agricultural Experiment Station, 1928; Nikitin, 1950; Olson, 1947; Parsche, 1940; Pirrie, 1950; Rankama and Sahama, 1952; Robinson, 1951; Stoklasa, 1898; Wallbach, 1932; Wann, 1930.

IRON BACTERIA

[See also bacteria and fungi]

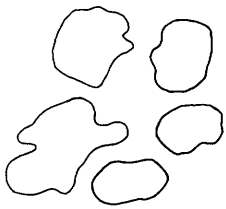
The iron bacteria constitutes a heterogenic group of organisms that are difficult to define. For example, Naumann (1928a) defined the group as all organisms that can precipitate or dissolve iron. However, the literature has described numerous organisms which dissolve and use iron in metabolism. Cholodnii (1928) has a definition more restrictive but apparently inadequate and even erroneous. Cholodnii believed the term "iron organism" should be restricted to organisms that respire ferrous oxide. The literature shows that on the spot, organic material rather than ferrous oxide is essential for the respiration of these organisms. The term "iron bacteria", as generally considered, includes many organisms that are not bacteria.

The following list of characteristics shows that the iron bacteria belong to a transition group, the actinomyces, which are fission fungi or thread fungi having both bacterial and fungal characteristics:

Bacterial characteristics	*Fungal characteristics*
Thallus width 0.5–1.2μ	Reproduction by aerial budding out of successive conidia by abstriction as for example, yeast of ascomycetes.
Some bacteria form only a rudimentary mycelium.	Ability to form a true coenocytic mycelium.
Relative primitive state of the nucleus	Normal ability or tendency to form a mycelium under conditions suitable for active vegetative growth.

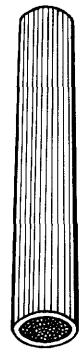
Lackey and Wattie (1940) were able to isolate only the iron bacterium *Sphaerotilus natans* from Ohio River sewage water although these field collections did show morphological differences. Pringsheim (1949) believed the many morphological field manifestations of the same iron bacterium to be responsible for the many synonyms in the literature. Thus, he found *Cladothrix dichotoma* Cohn, *Leptothrix ochracea* Kutzing, *Chlamydothrix ochracea* (Kutzing) Migula, *Chlamydothrix sideropons* Molisch, and *Clonothrix fusca* Roze to be synonyms of *Sphaerotilus natans* Kutzing. (See fig. 13 for diagrams of different iron bacteria.)

In spite of considerable overlap, classification according to morphological form is useful for a fast field identification. Accordingly, iron bacteria are classified by genera, using the original worker's nomenclature. However, in classifying these organisms as iron bacteria, it must be remembered that many precipitate or dissolve iron in addition to other elements, such as manganese and sulfur. Generally, ferric hydroxide is deposited only in the thick sheathlike envelope of living organisms (California State Water Pollution Control Board, 1952; Reilhes, 1944), but it may be deposited elsewhere. Iron bacteria



Fluffy bits of floating or immersed cottonlike material often may be found in sewage-contaminated streams in early summer or late autumn

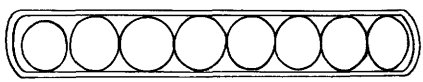
SPHAEROTILUS



Parallel threads with a common mucilaginous covering

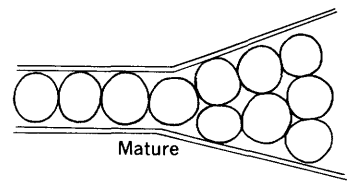
Most widely distributed of all iron bacteria. Predominate in open, shallow water exposed to direct sunlight. Stiff rods usually lack cell contents

LEPTOTHRIX



Semimature

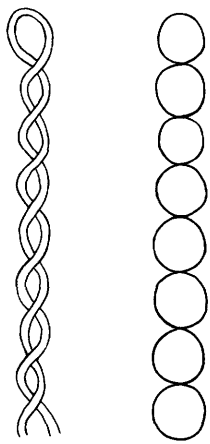
CLADOTHRIX



Mature

Normally attached at one end to some foreign object such as a particle of dirt or a stone granule

CRENOTHRIX



Young Old

A spirally twisted thread possessing no sheath or mucilaginous envelope; predominate in darkened waters in conduits and tunnels

GALLIONELLA

SPIROPHYLLUM

FIGURE 13.—Diagrams of different iron bacteria (after Ellis, 1919).

frequently act upon solutions of relatively low (less than 0.5 ppm) iron concentration. Deposits of ocher and bog ore made up of minute tubes of iron oxide are produced by them; the mucilaginous envelopes of the bacteria prevent the escape of the ferric hydroxide. In consequence, the iron begins to accumulate round the tubercle. The bacteria thus have an extraordinary capacity for storing iron and manganese on the sheath; the thickness of the encrusted sheath is sometimes 12 or more times that of the original filament (Ellis, 1919).

One of the earliest and most striking examples of the effect of iron bacteria is that recorded in the Book of Exodus (VII: 20–25) circa 500 B.C. In the account of 1 of the 7 plagues of Egypt, the water in the Nile River was "turned to blood," the fish died, the river became foul, and the Egyptians could not drink the water. These conditions probably were induced by the sudden growth of microscopic organisms. About 2,400 years later, in 1887, history repeated itself at Rotterdam, Holland, and the water became undrinkable (Yust, 1951); the walls of the reservoirs became thickly encrusted with mussels, which, in turn, became densely coated with ¼- to ½-inch covering of filamentous iron bacteria that had entered the service mains. Ten years earlier, in 1877, the service pipes of Berlin, Germany, were choked completely by iron-bacteria deposits (Yust, 1951), and all the water mains had to be renewed.

Because *Sphaerotilus natans* and other typical iron bacteria develop only in an approximately neutral environment (Stokes, 1954) in which chemical oxidation of ferrous iron is rapid and extensive, the function of the bacteria in oxidation becomes difficult to determine. (California State Water Pollution Control Board, 1952; Glicklhorn, 1920). Ferrous ions are readily oxidized in natural surface waters to insoluble ferric hydroxide. These precipitates tend to agglomerate, flocculate, and settle or be adsorbed on surfaces; hence, the concentration of iron in well-aerated waters is seldom high. Natural iron removal is fortunate as a concentration of more than 0.30 ppm is objectionable in waters for public supply. Probably many of the lower bacteria as well as thread bacteria function permanently or temporarily as iron bacteria (Ellis, 1919).

As cited in the literature the following organisms may be considered as iron bacteria according to popular usage of the phrase:

Manganese-metabolizing:

Anthophysa vegetans (Pringsheim, 1946)
Bacterium precipitatum (Kalinenko, 1946)
Bikosoeca petiolata (Pringsheim, 1946)
Crenothrix fusca (Thiele, 1928; Wasser, 1938)
sp. (Dienert, 1938)

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Gallionella ferruginea (Thiele, 1928; Wasser, 1938)
Leptothrix echinata (Beger, 1935)
ochracea (Lieske, 1919; Thiele, 1928; Wasser, 1938)
sp. (Dienert, 1938)
Pseudomonas ferrugineum (Pillai, 1938)
Siderocapsa sp. (Dienert, 1938; Wasser, 1938)
Siderodendron manganiferum (Pringsheim, 1946)
Siphomonas fritschii (Pringsheim, 1946)
Gel-forming bacteria: (Beger, 1935; Beger and Haase, 1941)
Manganese bacteria: (Baer, 1933; Hallsworth and Costin, 1953)

Sulfur-metabolizing:

Because sulfate-reducing bacteria seem prominent in causing the aqueous solution of otherwise insoluble iron, the environmental conditions favorable to the growth of this group of bacteria (Ivanov, 1956) are listed: (a) neutral pH (about 7); (b) absence of oxygen; (c) a source of organic nutrients; (d) a source of sulfates.

Chlorobium limicola (Larsen, 1953)
thiosulfatophilum (Larsen, 1953)
Spirophyllum sp. (Meehan and Baas-Becking, 1927)
Thiobacillus ferrooxidans (Temple and Colmer, 1951)
Toxothrix sp. (Meehan and Baas-Becking, 1927)
Vibrio desulphuricans (Butlin and others, 1952)
Anaerobe (Austen, 1931)
Marine bacteria (Francis-Boeuf, 1948)
Sulfur bacteria (Kimata and others, 1955; Rudolfs, 1922; Starkey, 1945; Starkey and Wight, 1943)

Organic iron metabolizing:

Cladothrix dichotoma (Ellis, 1921)
Crenothrix polyspora (Ellis, 1921)
sp. (Whipple and others, 1927)
Gallionella ferruginea (Ellis, 1921)
Leptothrix ochracea (Ellis, 1921; Kalinenko, 1940)
Pseudomonas ferrugineum (Pillai, 1938)
Spirophyllum ferrugineum (Ellis, 1921)
Microorganisms, general (Allison and Scarseth, 1942; Beger and Haase, 1941; Betremieux, 1951, 1954; Starkey, 1945; Thornton, 1956; Yoshimura, 1936)

Calcium-metabolizing:

Achromatium oxaliferum (Lackey, written communication 1959)
Bacterium precipitatum (Kalinenko, 1946)
Pseudomonas ferrugineum (Pillai, 1938)

Other-metabolizing:

Crenothrix fusca (Wasser, 1938)
polyspora (California State Water Pollution Control Board, 1952; Duchon and Miller, 1948)
Gallionella ferruginea (California State Water Pollution Control Board, 1952; Beger, 1937; Volkova, 1939; Wasser, 1938)
Leptothrix ochracea (California State Water Pollution Control Board, 1952; Beger, 1937; Glicklhorn, 1920; Volkova, 1939; Wasser, 1938)
trichogenes (Beger, 1937; Gleen, 1950)
Siderocapsa sp. (Wasser, 1938)

Sphaerotilus natans (Stokes, 1954; Wasser and others, 1937)
Trachelomonas sp. (Glicklhorn, 1920)
Iron bacteria, general (Banfi, 1952; Baylis, 1925; Betremieux, 1954; Dementiev, 1941; Fujihara, 1925; Haase, 1940; Harmsen, 1938; Hutchinson, 1941; Kliman, 1937; Kurzweil and Exner, 1954; Naumann, 1928a; Pringsheim, 1946; Scott and Brandly, 1933; Sorokina, 1938; Tillmans and Klarmann, 1924; Varkov, and others, 1950; Waring and Werkman, 1943; Winogradsky, 1922)

Control:

Methods of controlling iron bacteria have been suggested by the following investigators:

Anthophysa vegetans (Cataldi, 1937)
Asellus aquaticus (Holland, 1956)
Crenothrix polyspora (Duchon and Miller, 1948)
sp. (Grime, 1945; Hale, 1949)
Leptothrix ochracea (Cataldi, 1937)
Iron bacteria, general (Baer, 1933; Beger and Haase, 1941; Derby, 1956; Ellis, 1921; Gleen, 1950; Griffin, 1937; Haase, 1940; Harvey, 1939; Osugi and others, 1935; Pronin, 1933; Thiele, 1928; Tillmans and Klarmann, 1924; Volkova, 1939)
Fungus slimes (Hale, 1949)

Table 1 summarizes the many organisms that require iron for normal metabolism. Large forms of animal life are excluded from the table.

TABLE 1.—*Organisms that require iron for normal metabolism*

Organism	Number of genera	Number of species
Algae:		
Blue-green	6	7
Desmids	(1)	----------
Diatoms	3	4
Green	5	3
Other	3	4
Bacteria	20	25
Flowering plants (aquatic):		
Soil-rooted	3	3
Unattached	1	3
Flowering plants (land):		
Crop	25	----------
Noncrop	7	----------
Fungi	12	23
Iron bacteria:		
Calcium-metabolizing	2	2
Manganese-metabolizing	10	10
Organic iron metabolizing	6	6
Sulfur-metabolizing	5	4
Other-metabolizing	6	5
Phages	(1)	----------
Protozoa	4	4
Viruses	(1)	----------
Yeasts	(1)	----------

[1] 1 or more.

Thus, studies made by many investigators of the normal metabolism of numerous organisms affirm the essential function of iron in vital life processes.

MECHANICS OF IRON CONCENTRATION AND DEPLETION

LICHEN EFFECTS

Not only do lichens take certain chemical elements from the rocks, but after their death and decay, they release organic acids that increase the chelating or solvent power of the natural water (Schatz and others, 1956). The solubility of iron is much greater in the presence of these organic acids.

The brown or black shiny crust on rocks known as desert varnish consists mainly of oxides of iron and manganese. Lichens probably are important in contributing to such surface-crust deposition (Emmons and others, 1955). Also, lichens may be concentrators of iron (Yarilova, 1947).

HYDROPHYTE (OTHER THAN BACTERIA AND FUNGI) EFFECTS

The chlorophyll-bearing water plants are important in supplying iron to and removing iron from bodies of water. Algae, characteristically haploid (gametophyte) organisms generally lacking a mineral-conducting system, extract iron and other mineral salts directly from the water medium (Gessner, 1956; Glenck, 1954; Gloyna and others, 1955; Henderson and others, 1954; Smith, written communication, 1956; Steel and Gloyna, 1955; Walker, 1956; Yoshii and Watabe, 1956). Smith, 1950, has an excellent discussion of haploid-diploid variability in the algae. Soil-rooted aquatic plants, characteristically diploid (sporophyte) organisms generally having an excellent mineral-conducting system, extract iron and other mineral salts directly from the water-immersed soil medium; thus, they live aerobically in anaerobic growth situations (Crafts, 1954; Lowenhaupt, 1955; Marei, 1955; Pond, 1903; Prescott, 1956). Normally free-floating aquatic plants, whether haploid or diploid, absorb minerals directly from their water environment. A diagram of the relative importance of water-mineral and soil-mineral extraction during the life cycles of the four general types of plants is given in figure 14. Five features that characterize aquatic plants and emphasize their large use of iron are as follows:

1. Large proportional amount of chlorophyll-bearing tissue which results from (a) large expansion of plant body surface by lengthening and subdivision of plant parts, (b) assimilatory region, generally confined to the increased epidermal area. The work of Leibich (1941) cited by Bonner (1950) shows that as

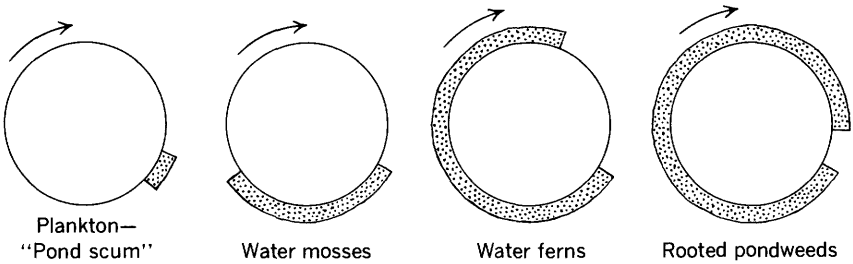

FIGURE 14.—Relative importance of the gametophyte (single line of the life-cycle circle representing water mineral absorbing) and sporophyte (stippled part of the life-cycle circle representing soil mineral absorbing) generations in the four major groups of aquatic plants (adapted from Milne and Milne, 1952).

much as 82 percent of the iron of spinach leaves occurs in the chloroplasts. Of this, at least four-fifths was found in organic combination. It seems logical to assume the same would be true for aquatic-plant chloroplasts.

2. Reduced organic iron compounds are easily extracted from soils devoid of oxygen. Large air-conducting spaces, through which oxygen for root respiration is sent from the leaves, in frequently cockscrew-shaped water roots are present. These air-conducting spaces enable the roots to penetrate far into water-saturated unaerated mud (Fujii and Tanaka, 1955). These anaerobic situations have a low redox potential. Land-plant soil roots would suffocate (Gausman and Struchtemeyer, 1957; Jackson, 1956) under similar undrained growth conditions because in land plants, oxygen for root respiration is supplied to the root directly from the air in the soil. The results is that aquatic plants readily and effectively, through growth and disintegration of their dead bodies, transfer or move iron from the soil to the water medium. Figure 15 illustrates the root and leaf structures of quatic and land plants.
3. The xylem which is usually considered to be tissue which conducts inorganic minerals frequently may be deficient as in Sago pondweed (Eames and MacDaniels, 1925). Aquatic plants have a well-developed phloem, or organic food-conducting tissue, through which reduced organic iron compounds may readily travel (Arber, 1920; Arisz and Schrender, 1956; Eames and MacDaniels, 1925; Kramer, 1957; Schimper, 1903).
4. The plants are characterized by (a) a high ash content, (b) a high protein content (Dubois, 1955), and (c) a very low crude fiber content. The legume hays are the only commonly used forages which can approximate aquatic plants in these constituents (Gortner, 1934).

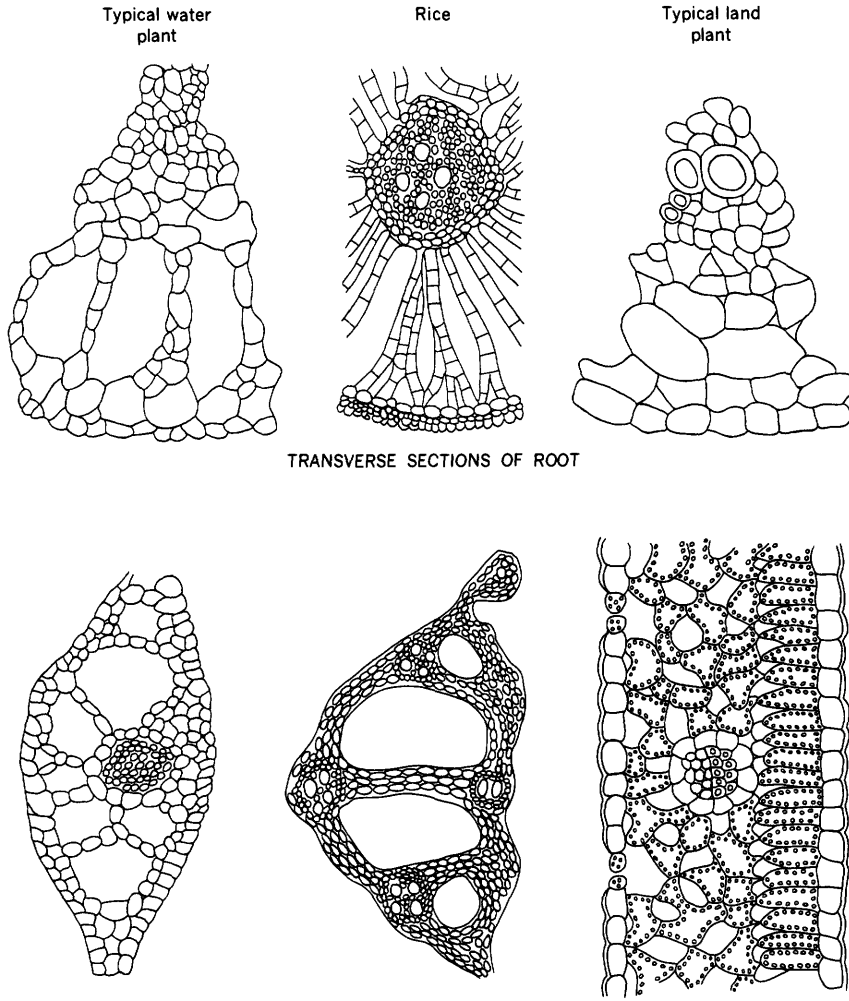

FIGURE 15.—Morphological (and therefore biochemical functional) comparison between leaf midrib and root structure of rice and comparable parts of typical aquatic and land plants. Rice plants have a typical aquatic plant structure as indicated by the large "air cells."

5. Percentage of iron in ash is high. For the common water plant *Elodea*, iron has been reported as 2.56 percent of the ash (Bonner, 1950).

Aquatic plants further reduce the quantity of suspended and (or) colloidal iron by (a) preventing mechanical silting and erosive wave action and (b) chemical clarifying action on water containing suspended matter (De Gruchy, 1938).

As excellent oxygenators of water during daylight hours, aquatic plants tend to remove the ferrous iron in aqueous solution.

$$4Fe^{++}+3O_2+6H_2O\rightarrow 4Fe(OH)_3$$

Thus, both aeration and photosynthesis in water-growth situations cause excess iron to flocculate and settle to the mud bottom. The principle of most iron-removal processes involves the neutralization of hydrogen ions and the increase in oxygen (Calise and Dietz, 1955; Klyachko, 1956; Weston, 1936). Conversely, during the night when photosynthesis is low and respiration is rapid, aquatic plants will return some iron to solution (Marcellin, 1956; Newell, 1957).

$$CO_2+H_2O\rightleftharpoons H_2CO_3\rightleftharpoons H^++HCO_3^-$$
$$3H^++Fe(OH)_3\rightleftharpoons Fe^{+++}+3H_2O$$

However, in open-water bodies such as streams and lakes, the hydrogen-ion concentration probably cannot be raised enough by this effect to greatly increase the iron concentration.

The removal of dissolved organic and inorganic constitutents in water by freezing has been investigated by Mortimer (1942). He found that 94 percent of dissolved salt, all humus coloring matter, and a large part of dissolved gas were removed from surface water by freezing. Water freezing thus resulted in an increase of the mineral content in the water immediately under the ice.

Tannins, which may form stable complexes with iron and other metallic ions are found in several parts of the aquatic plant, especially in epidermal cells of leaves, in the cortical tissues of stems, and in the walls or vacuoles of cells.

The plant *Enteromorpha*, which can exist in fresh water as well as in water more mineralized than sea water, has been found by Baas-Becking and Mackay (1956) on intertidal flats. It produces highly reducing acidic substances in the dark, such as dimethyl sulfonium compounds. This reducing environment probably causes some iron to go into solution. In the light, some of these compounds are either reabsorbed or destroyed; thus precipitation of iron results.

The simpler aquatic plants, the algae, may be attached or unattached to the substratum. (See p. 114 for discussion of their iron assimilation.) Some algae, such as the stoneworts among the fresh-water algae and some seaweeds in marine algae, apparently have effective conduction systems and, absorb iron from the mud substratum. However, this type is the exception rather than the rule.

The more complex flowering plants may be rooted or unattached to the substratum. Those unattached absorb iron directly from the water bathing the organism or from the water upon which the organ-

ism floats. (See p. 114 for discussion of the iron assimilation of attached flowering plants.)

Because aquatic plants generally occur in bodies of surface water, and frequently in great abundance their relationships to iron in water are emphasized. Because of proportionately high water and low cellulose contents, solid materials in the water-plant structure disintegrate rapidly upon death. Thus, when living, aquatic plants assimilate matter that may be used as food; and in death, they yield important salts and organic substances to the water. Tannin and other organic compounds that may be yielded by disintegration of dead plant material are important in holding the iron in solution.

Figure 16 shows some of the processes involved in the circulation of iron between soils and water, and the interrelationships between iron in dead organisms and iron used by the living. Both aerobic and anaerobic bacteria release and thereby make available iron to the new crop of plant growth. Animals either directly or indirectly feed on the plant organisms and by assimilation transfer iron to their bodies.

Iron must be present in plants for production of chlorophyll. Upon death and disintegration of the plant material, iron is released into the water. During the following growing season, which may be immediate, iron would again be taken from the substratum by the growing plant. Thus, there is an annual transfer of iron from the substratum to the water body. Under aerobic conditions, iron is oxidized and precipitated. Apparently, anaerobic bacterial disintegration is important in releasing iron from its organic complexes in dead plant tissue. Practically, anaerobic conditions in water are characterized by reduction of nitrogen and sulfur compounds and the appearance of methane as well as absence of oxygen (Baas-Becking and Kaplan, 1956; Baas-Becking and Mackay, 1956; Kufferath and Schmitz, 1955). In oxygen-deficient environments, sulfate may be reduced to hydrogen sulfide, which has an offensive odor of putrefaction, precipitates usually as black metallic sulfides, and may be toxic to some forms of life. In a strongly reducing environment, protein and its primary cleavage products are broken down by hydrolysis rather than by oxidation. Such breakdown causes foul-smelling mercaptans and amines such as accompany the decomposition of flesh.

Iron is transported in the phloem tissue of higher plants and is concentrated in the mesophyll or in the cambium (Kliman, 1937). Regions of plant tissue in which iron and other metallic salts tend to concentrate are illustrated in figure 17. Most active salt accumulation in the higher aquatic plants is in the meristem zone, for example,

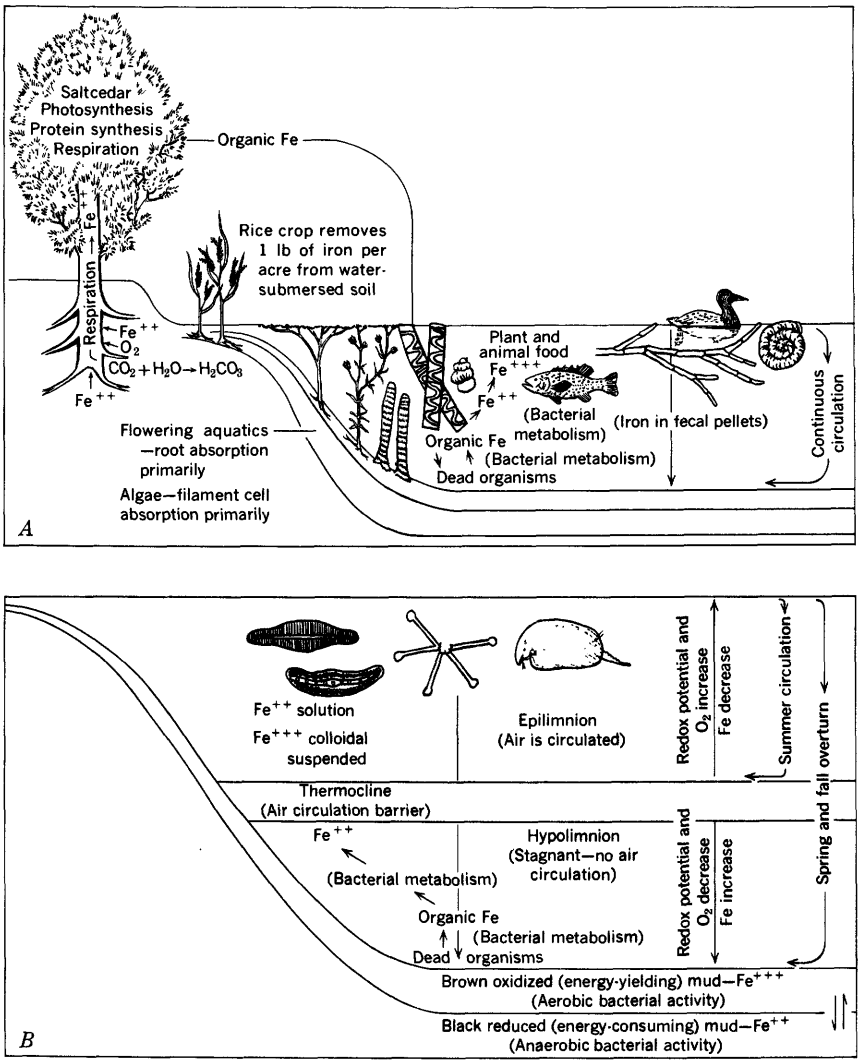

FIGURE 16.—Transverse portion of lake. Some phases of the iron cycle in *A*, by the macrobiota, and in *B*, by the microbiota, which affect the presence of iron in natural water.

root, shoot, and leaf base (Steward, 1948). Each lateral bud as a potential growing region is a potential region of salt accumulation.

Several hydrophytes are important concentrators of iron. Iron concentration of 26,000 times that of the immersing water medium has been recorded in some seaweeds (Oy, 1940). The following aquatic plants are reported to be concentrators of iron:

Hydrophyte concentrator and reference
Cladophora sp. (Whipple and others, 1927)
Chlorella sp. (Mayer and Gorham, 1951)
Crenothrix sp. (Whipple and others, 1927)
Fucus vesiculosus (Oy, 1940)
Iridaea spp. (Read and Gow, 1927)
Laminaria digitala (Oy, 1940)
L. japonica (Read and Gow, 1927)
L. religiosum
Microcystis aeruginosa (Gerloff, 1955)
Nostoc flagelliforme (Read and Gow, 1927)
Oedogonium sp. (Ellis, 1919)
Potamogeton spp. (Read and Gow, 1927)
Rhodymenia spp. (Read and Gow, 1927)
Sargassum siliquastrum (Read and Gow, 1927)
Algae in general (Smith, written communication, 1956)
Aquatic plants, general (Mayer and Gorham, 1951; Speirs, 1954)
Desmids (Ellis, 1919; Hofler, 1925)
Diatoms (Thompson and others, 1932)
Marine brown algae (Black and Mitchell, 1952)
Watercress (Escudero and others, 1944)
Zygnemaceae (Ellis, 1919)
General (Harder, 1919; Naumann, 1928b)

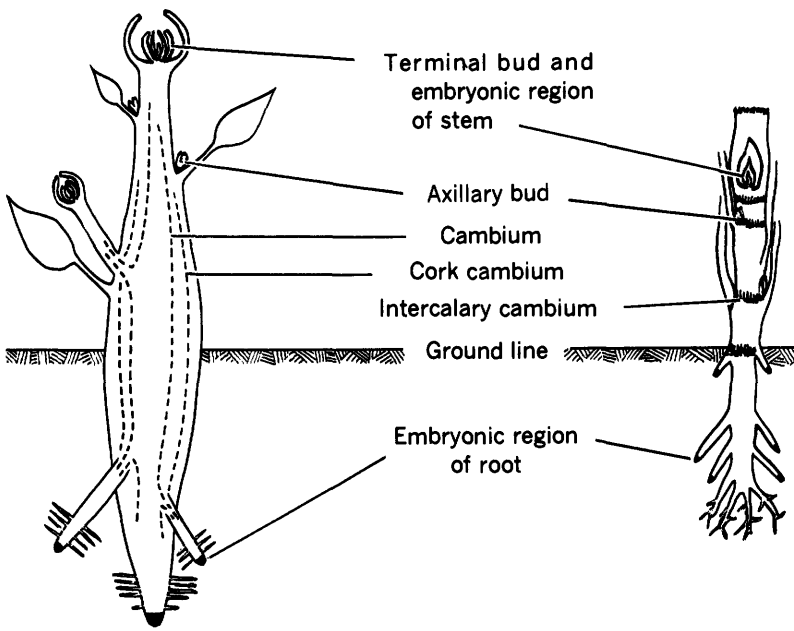

FIGURE 17.—Regions of dicotyledon (left) and monocotyledon (right) plants in which iron and other metallic salts tend to concentrate. These are regions of high metabolic activity.

A lush growth of *Cladophora* was recently found growing in a Colorado spring that yielded water high in iron content. Microscopic examination revealed that filaments of this alga had heavy pellicle deposits of ferric hydroxide similar to those commonly found on the iron bacteria *Leptothrix*. A similar phenomenon has been reported by Rackow (1957) for *Chlorella pyrenoidosa* and *Ulothrix zonata*. Gerloff (written communication, 1955) reported the iron content of algae ranges from 164 to 543 ppm.

Oxygen has been mentioned as one of the elements that lowers the solubility or alters the availability of iron in water. It also is true (Gerb, 1956; Speller, 1922, 1951; Walker and others, 1907, 1908) that the rate of corrosion of metallic iron in natural water is almost directly proportional to the oxygen content for concentrations up to about 6 cubic centimeters per liter (according to Stoltenberg, 1949, 2 cc per liter). According to Nietsch (1956), water containing a dissolved-oxygen content lower than 4 cubic centimeters per liter tends to dissolve iron. Oxygen, which increases galvanic corrosion, is an active depolarizer at all temperatures, and even in an alkaline solution. The processes of corrosion are not covered in this report.

CHEMICAL ELEMENTS INTERFERING WITH USE OF IRON BY PLANTS

Several chemical elements, when in water, interfere with the use of iron by plants. Some of these elements may decrease the solubility of iron or act as oxidizing agents in causing ferrous iron to be changed to insoluble and unavailable ferric iron. Others may react in different ways to prevent normal assimilation of iron. Calcium content, which affects lake and land productivity of plants, is mentioned by Aiyar, 1946, 1948; Allyn, 1927; Bennett, 1928; Boischot and Durroux, 1950; Brown and Gibbons, 1955; Crawford, 1939; De and Basu, 1949; De Kock, 1955c; Demolon and Bastisse, 1938; Doyne and Morison, 1926; Gayral, 1954; Gayral and Panouse, 1954; Gile and Carrero, 1914, 1916b; Hasler, 1951; Hopkins and Wann, 1927; Iyengar and Subrahmanyan, 1935; Kuhnholtz-Lordat, 1928; Masoni, 1914; Maze and others, 1912; McGeorge, 1951; McGeorge and Breazeale, 1956; Milad, 1939; Parsche, 1940; Robinson, 1951; Samish, 1954; Schachtschabel, 1942; Sidorin, 1914; Simakov and Isakova, 1933; Skeen, 1930; Steele, 1949; Stumm, 1956; Upadhyaya, 1955; Van der Spek, 1948; Vidal, 1938; Villeret, 1954e; Wallace and Ashcroft, 1956; Wallace and others, 1955; Wann, 1930; Wiebosch, 1947.

Copper in water acting as an oxidizing agent and causing ferrous hydroxide to form insoluble complexes (Puustjarvi, 1952) prevents

algae from using iron. These and other effects are mentioned by: Aiyar, 1946; Beaumont and Holland, 1935; Brown and Holmes, 1955; Cervenka, 1955; Coupin, 1898; Crabill, 1956; Curini-Galletti, 1937; De and Basu, 1949; Densch and Hunnius, 1924; Echave, 1937; Elvehjem, 1935; Erkama, 1949; Fattinger, 1950; Forster, 1953; Guseva, 1940; Hale, 1949; Harrison and Subrahmanyaayyar, 1917; Harward and others, 1955; Hood, 1948; Isizuka, 1940; Keilin and Mann, 1944; Koblents-Mislnke, 1955; Korinek, 1936; Lahey and others, 1952; Lazarev, 1939; Magie, 1951; Malyuga, 1945; Maquenne and Demoussy, 1920; Marsais and Segal, 1938; Moore and others, 1957; O'Meara, 1937; Ono, 1900; Ornstein, 1935; Pika, 1956; Posnjak, 1926; Puustjarvi and Juusela, 1952; Quartaroli, 1932; Riley, 1939; Rippel, 1941; Saeger, 1937; Schmidt-Nielsen, 1938; Schwaibold and Fischler, 1931; Shkol'nik and Makarova, 1950; Voznesenskii and Nagatkin, 1934; Whatley and others, 1951; Williams, 1953; Willis, 1936; Willis and Piland, 1936, 1937.

Watson and Bollen (1952) found a definite inhibition in bacterial growth in the presence of a large amount of organic matter when the total copper concentration ranged from 11 to 30 ppm. However, no deleterious effect on bacterial growth was observed in controls containing 4 to 7 ppm in concentration of total copper and low organic matter content. Conditions unfavorable for growth would no doubt decrease the amount of iron brought into solution by bacteria.

Magnesium is reported by Aiyar (1948), De and Basu (1949), and Schachtschabel (1942) to interfere in iron metabolism of plants.

Manganese is reported by the following writers to inhibit iron utilization by plants: Aiyar, 1946; Beger, 1935; De and Basu, 1949; Guseva, 1937a; Johnson, 1916; Kapp, 1932a, 1932b; Kimata and others, 1955; Kitano, 1955; Kuhnholtz-Lordat, 1928; Magie, 1951; Martin, 1935; Ouellette, 1951; Rippel, 1923; Somers and Shive, 1942; Willis and Piland, 1937; Yoshimura, 1931c; Zapffe, 1931, 1933. Excessive manganese can produce chlorotic symptoms which indicate iron deficiency of plants.

Water high in oxygen usually does not permit more than minor traces of iron in solution. Aspects of this are discussed by: Beger, 1937; Bochkarev and Yanko, 1953a, 1953b; Brozek, 1949; California State Water Pollution Control Board, 1952; Chernovskaya, 1954; Correns, 1941; Dementiev, 1941; Dienert, 1938; Einsele, 1940a, 1940b; Fabrin, 1948; Gayral and Panouse, 1954; Griffith, 1955; Haase, 1940; Hutchinson, 1941; Hutchinson and others, 1939; Ingleson and Thomas, 1946; Ivlev, 1938; Juday and others, 1938; Kriegsmann, 1938; Malyuga, 1945; Mancha, 1936; Martin, 1935; Muller, 1950; Nietsch, 1956; Pearsall, 1950; Pillai, 1938; Rankama and Sahama, 1952; Saunders,

1938; Seeberg, 1937; Stoltenberg, 1949; Strakhov, 1948; Sugawara, 1934; Tanaka, 1953, 1954; Thompson and others, 1932; Thompson and Bremner, 1935b; Todt, 1950; Urbain and Miller, 1930; Villeret, 1953; Weston, 1936; Whipple and others, 1927; Yoshimura, 1931a, 1931b, 1934, 1936.

Successful irrigation requires an adequate supply of water of suitable quality, available at the proper time and place, for agricultural crop production. As an aid in conserving water, a monomolecular layer of cetyl alcohol over the entire surface of water in storage reservoirs has been advocated to reduce water evaporation (Mansfield, 1953, 1955; Rideal, 1925). However, interference in water evaporation could cause interference in oxygen atmosphere-hydrosphere interchange (Downing and Truesdale, 1956). Also, evaporation-suppressing agents might indirectly alter the iron content of stored water.

The effect of phosphorous on iron utilization by plants is reported by: Aiyar, 1948; Biddulph, 1948; Bonner and Rombyn, 1931; Brock, 1937; Brown and others, 1955; Clark, H. W., 1936; Cooper, 1939; Curry and Wilson, 1955; Davis, 1943; De and Basu, 1949; De Kock, 1955b; Dell'Aquila and Bini, 1953; Demolon and Bastisse, 1938; Einsele, 1936a, 1936b; Franco and Loomis, 1947; Goldberg, 1952; Hester and Shelton, 1947; Hopkins and Wann, 1927; Hutchinson, 1941; Iyengar, 1936; Kahler, 1956; Kapp, 1938; McDonald, 1935; Midgley, 1940; Minami and others, 1955; Ohira, 1955; Pobeguin, 1954; Sarishvili and Bagaturiya, 1938; Sideris and Young, 1956; Willis, 1936; Zicker and others, 1956.

Potassium is reported by Aiyar (1948), Eckstein and Jacob (1929), Gouny and Mazoyer (1954), Schachtschabel (1942), Scharrer and Schropp (1933), and Willis (1936) to affect the use of iron by plants.

Silicon is reported by Holl (1938), Leaf (1948), and Pobeguin (1954) to interfere in the use of iron by plants.

Aluminum is reported by Avdonin and others (1957) to interfere in the use of iron by plants.

MEASUREMENT OF MATERIALS AFFECTING IRON SOLUBILITY

Selected references pertaining to analyses of substances that alter the solubility of iron in natural water are listed in table 2.

ANALYTICAL RAMIFICATIONS OF IRON BIOCHEMISTRY

Many have found that organic matter interferes with the determination of iron in natural water (Bezel, 1948; Bode, 1932; Briggs, 1930; Clark and Sieling, 1936; Egger, 1931; Ferrey, 1935; Hallinan, 1943; Kneer, 1939; Kryukov, 1933; Marsh, 1922; Nishida, 1954;

TABLE 2.—*Humic matter, dissolved oxygen, and tannin analyses*

Substance	Material tested	Author	Remarks
Humic matter.	Soil	Martin and Lavollay 1950.	Permanganate oxidation of an alkali extraction.
Oxygen	Water	Faber and others, 1955.	For samples high in iron, nitrites, or organic matter (Rideal-Stewart permanganate).
	do	Gad, 1938	Suitable for fieldwork.
	do	Muller, 1933	Do.
	do	Oesting, 1934	Modified Van Slyke.
Tannin	Watercress	Chuprovskaya and Golota, 1954.	
	Papilionaceous plants.	Foufas, 1955	Microchemical data are given.
		Mitchell, 1944	Matching made with Lovibond glasses and Osborn's comparison microscope.
	Spirogyra arcta	Nakabayashi, 1954	Titration with K-polyvinyl alcohol sulfonate.
	Potamogeton spp.	Nierenstein, 1945	Method of Mitchell used.
	Castanea sativa	Thomas and Brossard, 1956	Color formed by gallic acid and $FeCl_3$ stabilized by use of phthalic acid

Okura and Goto, 1955; Reed and Haas, 1924; Scheringa, 1931; Schnitzer and De Long, 1954a, Van Beneden, 1956a; Weston, 1936; Willcomb, 1936).

Accordingly, when organic matter is known or believed to occur in a water sample, suitable methods and techniques that take the organic matter into account should be selected for the analysis. Some methods of procedure in water sampling are discussed by Hermanowicz and Kelus (1955).

Of the methods listed in table 3, those of Bezel (1948), Briggs (1930), Clark and Sieling (1936), Hallinan (1943), Kryukov (1933), Nishida (1954), Pringsheim (1934), Scheringa (1931), Van Beneden (1956a), and Willcomb (1936) are pertinent in analysis of natural water containing organic matter.

Some iron in natural water apparently comes from atmospheric dust. Iron occurs in glacial ice and newly fallen snow (Correns, 1941; Sandstrom and Von Gegerfelt, 1945; Schenck and others, 1932; Silverman and Valenzuela, 1946; Woodcock, 1955), although the amount that could come from these sources would normally be very small. Perhaps this phenomenon is part of the reason why thorough washing of plant leaves with dilute acid is a prerequisite for valid

quantitative analyses of iron in the leaves (Jacobson, 1945; Kramer, 1957; Leonard and Stewart, 1953).

Selected references pertaining to methods for determining iron content in water, soil, and plant material are listed in table 3. They refer to likely useful analytical procedures particularly pertinent when interfering substances are present.

TABLE 3.—*Methods of analysis for iron in water, soil, and plant material*

Author	Materials tested	Method	Remarks
Aconsky and others, 1954.	Water	Spectrophotometric, $K_2Cr_2O_7$.	A permanent standard can be used.
Allison and Scarseth, 1942.	Soil	Incubate soil with sucrose under anaerobic environment.	Removes free iron oxides from soils and clays.
Am. Soc. Test. Mat., 1952.	Water		Also tests for iron- and sulfate-reducing bacteria.
Ashizawa, 1951	do	Thiocyanate	For determining small quantities in natural water.
Astruc and Castel, 1935.	Wine	Fe^{++} titrated with $KMnO_4$.	Fe^{+++} reduced by Cu_2O and a small amount of H_2SO_4.
Bashkirtseva and Yakimets, 1955.	Water	Titration with trilon B.	NH_4CNS is indicator.
Betremieux, 1949.	Soil	Glucose and NH_4–salt extraction.	Fermentation and exchange with NH_4^+ solubilized iron in soil.
Bezel, 1948	Water	Colorimetric using thiocyanate.	Baking destroys iron humates.
Briggs, 1930	Medicine	Titration with $Na_2S_2O_3$.	Iron preparations containing organic matter.
British Standards Institute, 1956.	Water		Industrial water testing.
Buydens and Muylle, 1952.	do	Thiocyanate, 0-phenanthroline, α,α'-bipyridyl.	Methods compared.
Buydens and Muylle, 1954.	do	Thiocyanate 2,2'-bipyridine.	Gave best reproducible results.
Carpenter and Pyle, 1934.	do	Fe^{++} titrated with $KMnO_4$.	
Clark and Sieling, 1936.	do	Colorimetric using iodohydroxyquinolinesulfonic acid.	Determination of humic iron.
Ekkert, 1931	do	Colorimetric using dimethylglyoxime.	Can be used in presence of lactate or oxalate.
Ferrey, 1935	do	Iodate titration for Fe^{++}	Method satisfactory in presence of some organic matter but not others.
Fodor and Rosenberg, 1928.	do		Talc and kaolin adsorption of $Fe(OH)_3$.

TABLE 3.—*Methods of analysis for iron in water, soil, and plant material*—Con.

Author	Materials tested	Method	Remarks
Gad and Knetsch, 1949.	-----do-------	Similar to usual macro methods.	Analysis of very small amounts of water.
Gad and Manthey, 1950.	-----do-------	Colorimetric using KCNS, HNO_3 and $KMnO_4$.	Method is rapid.
Goldberg and others, 1952.	Seawater-----	Microfilter followed by fixation, dehydration, and staining.	Size distribution of suspended iron particles can be studied.
Hakomori, 1931--	Water--------	Colorimetric using molybdic acid, and H_2O_2, intensity of color increased by organics.	Citric, tartaric, and succinic acids interfere with determination.
Hallinan, 1943---	-----do-------	Colorimetric using modified thiocyanate.	Merits are speed, ease of manipulation, and lack of interference by concentrations of organic matter.
Hetterschij, 1941-	Soil----------	Extraction from soil by 1 percent citric acid or HCl at 24°C.	More iron is extracted at 24°C than at 12°C.
Hirsch and Ruter, 1926.	Water--------	Potentiometric titration.	Method determines small quantities of ferrous iron in presence of ferric iron and vice versa.
Houlihan and Farina, 1953.	-----do-------	Thiocyanate method-	
Iyengar, 1937----	Soil----------	Fe^{++} titrated with $Ce(SO_4)_2$.	Diphenylaminesulfonic acid is internal indicator.
Jacobson, 1945---	Plant material.	----------	Thorough washing of the leaves of plants with dilute acid is prerequisite for valid quantitative analyses of iron.
Jane, 1942-------	-----do-------	Microscopic technique.	Microchemical detection.
Kato, 1934------	Water--------	Induced precipitation of FeS by presence of ZnS.	Due to adsorption.
Kraybill, 1930---	Plant material-	----------	Sample preparation.
Kretschmer, 1952-	Water--------	Hellige color comparator.	Good results obtained.
Kruse, 1949------	-----do-------	-----do----------	Sensitivity 0.01–0.001 mg iron per liter.
Lajoie and De Long, 1945.	Soil----------	Acid ammonium oxalate extraction.	
Lapin and Kill, 1931.	Water--------	Colorimetric using sulfosalicylic acid.	Both ferric iron and ferrous iron can be determined.
Lehmann and Reuss, 1927.	-----do-------	Colorimetric based on formation of $Fe(CNS)_3$	

TABLE 3.—*Methods of analysis for iron in water, soil, and plant material*—Con.

Author	Materials tested	Method	Remarks
Leonard and Stewart, 1953.	Plant material.		Citrus plant leaves should be washed before iron analysis is made.
Do	Water	Iron complexed by ethylenediaminetetraacetic acid for removal from soil.	Precipitation of iron in acid soils prevented.
Lieber, 1953	do	H_2SO_4, $KMnO_4$, KSCN and color comparison.	Simplified field test.
Lieffring and Buron, 1948.	do	Colorimetric using dimethylglyoxime.	Method is short. Limits 0–1 mg iron per liter.
Marsh, 1922	Nutrient solutions.		Organic iron compounds precipitate slowly from the nutrient solutions.
Meyer and Bunger, 1952.	Water	Pulfrich's photometer.	Determination of small amounts in a short time.
Mills, 1932	Sewage	Microanalytical using a microbalance.	Presence of sewage.
Morello, 1940	Plant material.	Sparteine sulfate and NH_4CNS.	Excellent reagent for direct detection of iron.
Morgan, 1941	Soil	0.05N CH_3COOH buffered at pH 4.8 with CH_3COONa for soil extraction.	Special tests provide indications relative to ferrous iron and ferric iron.
Morris, 1952	Water	Tripyridyl	Direct determination with degree of specificity not possible by other methods.
Moss and Mellon, 1942.	do	Colorimetric using 2,2′-bipyridyl and 2,2′,2″-terpyridyl.	
Muller, 1933	do		Field method.
New Process Rayon, Inc., 1938.	do	Separation of iron from copper.	Accomplished with H_3PO_4 and alkali addition.
Nishida, 1954	do	Colorimetric using KCNS.	Sample containing organic matter.
Olson, 1947	Soil		$NH_4C_2H_3O_2$ adjusted to pH 4.8 dissolved iron from soils.
Osugi and Aoki, 1938a.	do		At same pH more iron extracted by aqueous KCl than by HCl from soil.
Pomeroy, 1942	Sewage	Colorimetric using NH_4CNS.	Sample containing more than 20 ppm iron should be diluted.

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TABLE 3.—*Methods of analysis for iron in water, soil, and plant material*—Con.

Author	Materials tested	Method	Remarks
Pringsheim, 1934	Water	Fe^{+++} thiocyanate Fe^{++} $K_4Fe(CN)_6$ total iron.	Prussian-blue test to show iron bacteria sheaths contain ferric iron.
Puri and Sarup, 1938.	Soil		Difference in buffer solution pH makes a difference in the amount of iron extracted.
Rakestraw and others, 1936.	Seawater	Method depends on precipitation of the iron as sulfide.	
Runehjelm, 1930	Plant material	Colorimetric using KSCN.	
Scheringa, 1931	Water	do	Sample containing pharmacopoeial iron preparations.
Schilling, 1936a	do	Fe^{+++} with KSCN, total iron with H_2O_2.	Iron is immediately fixed in solution with 2 ml of 1:3 H_2SO_4.
Schnitzer and De Long, 1954a.	do	2,2′-dipyridyl	
Scholz, 1932	Plant material	Colorimetric using NH_4CNS.	Determination of iron in small amounts of plant ash.
Shidlovskaya Ovchinnikova, 1953.	Water	Equivalent amounts of 5.5N CH_3COOH and 1.0N CH_3COONa stabilize Fe^{++} in water.	Additions of acetate buffer at pH 4.0 prevented ferrous oxidation for as long as 30 days.
Simons and others, 1953.	Seawater	0-phenanthroline	Sensitive and rapid.
Smith and others, 1952.	Water	Colorimetric using 4,7-diphenyl-1,10-phenanthroline.	Determination of 1–10γ of iron in 100 ml of water.
Steigmann, 1946	Gelatin	Colorimetric using P-phenylenediamine-HCl or phenyl-P-phenylenediamine-HCl.	Test is rapid.
Steinberg, 1935b	Nutrient solution.	Coprecipitation with alkaline earth as phosphate, carbonate, or hydroxide.	Method gives good results.
Svedenius, 1929	Plant material.	Precipitation with 1-nitroso-β-naphthol.	
Thompson and others, 1932.	Seawater	Colorimetric using thiocyanate.	Interfering substances removed with H_2SO_4.
Thompson and Bremner, 1935a.	do	Colorimetric based on formation of $Fe(CNS)_3$.	
Urbach, 1934	Water	Step-photometric microanalysis.	
Van Beneden, 1956a.	do	Colorimetric using α,α′-dipyridyl.	Determination of humic iron.

TABLE 3.—*Methods of analysis for iron in water, soil, and plant material*—Con.

Author	Materials tested	Method	Remarks
Vanstone, 1951	Plant material.	Selection of interval standard with proper lines suppressed.	Flame photometric analysis.
Watkins, 1954	Water	Total and dissolved	
Werescagin and others, 1931.	do	Field method	
Wickert and Pilz, 1950–51.	do	Detection of iron	0-phenanthroline hydrochloride.
Wilcox, 1948	do	Irrigation water analysis.	
Willcomb, 1936	do	Problem of organic matter is discussed.	Separation of iron from manganese.
Winter, 1936	Plant material.	NH_4CNS is internal indicator.	Fe titrated with 0.02 N $TiCl_3$.
Yoshimura, 1936	Water		Colorimetric using KSCN.
Zhivopistsev and Minina, 1954.	do	CSN^- forms red precipitate solution in Me_2CO greatly increasing sensitivity of HCNS reaction.	Colorimetric using 2 furyl-diantipyrimylmethane.

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