CHAPTER 18

Phosphorus and Other Elements

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> The land belongs to itself. If anything, we belong to it . . . as much as earthworms or corn plants. We rise up a while and sink back in. We borrow our lives from it.
>
> —Nancy Paddock

We have seen that microorganisms are important in the cycling of carbon, nitrogen, and sulfur. Soil microorganisms are also actively involved in the cycling of many other elements. In fact, most of the nutrients required by living organisms and many other elements, including several potentially toxic materials, are biologically transformed in the environment (examples are shown in Table 18–1). The mechanisms by which soil microorganisms transform these elements include:

- **Mineralization** and **immobilization** reactions that mediate the transformation of an element from organic to inorganic and inorganic to organic forms, respectively.
- **Redox** reactions that involve the transfer of electrons from (oxidation) or to (reduction) the element in question.
- **Solubilization** by which some soil minerals, such as the relatively insoluble phosphate minerals, are made available to plants and other organisms.
- **Biomethylation** of elements, such as mercury, that results in increased mobility of the element in the environment.

In this chapter we discuss microbial transformations of several important elements. Phosphorus (P), iron (Fe), and manganese (Mn) are important nutrient elements, while arsenic (As), mercury (Hg), and selenium (Se) are elements that are associated with environmental problems. Our discussion of these elements is not meant to minimize the importance of the microbial community in the transformations of

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TABLE 18–1   Microbially Mediated Transformations of Several Essential and Nonessential Elements in Soil

| | Microbial Transformation | | |
Element	Mineralization-Immobilization	Oxidation-Reduction	Biomethylation
Phosphorus, potassium, calcium, magnesium, copper, zinc	yes	no	no
Iron, manganese	yes	yes	no
Arsenic, mercury, selenium	yes*	yes	yes

*Mercury is transformed between inorganic and organic forms; arsenic and selenium can act like phosphorus and sulfur in biological systems.

other elements. Many other elements are also subject to one or more of these processes. The elements discussed here serve only to illustrate the range of transformations that microorganisms mediate.

PHOSPHORUS

Forms of Phosphorus in Soil

Phosphorus is critical to all life-forms because of the role it plays in many important biomolecules such as DNA (deoxyribonucleic acid), phospholipids, and ATP (adenosine triphosphate). The amount of total phosphorus found in a surface soil can vary greatly, ranging from < 100 µg P g^{-1} ($\cong 200$ kg ha^{-1}) in sandy soils to $> 1,000$ µg g^{-1} ($\cong 2,000$ kg ha^{-1}) in soils derived from basic rocks (Stevenson and Cole, 1999). The primary mineral form of phosphorus is rock phosphate, or *apatite* (Table 18–2). Highly weathered soils, such as those of the southeastern United States, may have very little remaining apatite, while relatively unweathered and alkaline soils formed from basic parent materials, such as those of the northwestern United States, may have high contents. The chemical weathering of apatite results in the release of orthophosphate ($H_2PO_4^-$ is the dominant species at pH values below 7.2; HPO_4^{2-} dominates above pH 7.2). Very little orthophosphate is present in the soil solution at any one time, usually $< 1\%$ of the total phosphorus.

Solution phosphorus concentrations of 0.1 to 1 mg L^{-1} are common in soil. Of this, more than half may be in the form of soluble organic compounds released by dead cells or in colloidal organic compounds. Addition of soluble phosphate fertilizers or mineralization of organic phosphorus results in the release of orthophosphate into the soil solution, followed by precipitation as iron and aluminum phosphates in acid soils or calcium phosphates in alkaline soils (stable-inorganic phosphorus) or by adsorption to iron and aluminum oxides (labile-inorganic phosphorus). These reactions result in low orthophosphate concentrations in the soil solution (Fig. 18–1). The optimum availability of orthophosphate occurs at a soil pH

TABLE 18–2   **Examples of Inorganic Phosphorus Minerals in Soil and Their Solubilities with Respect to Dissolution of the Cation and PO_4^{3-}**

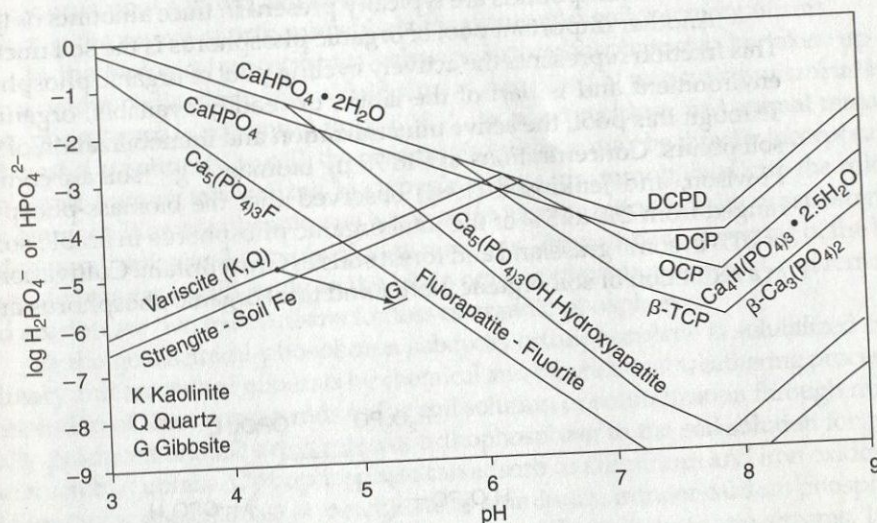| Name	Formula	Solubility Product (Log)
Fluorapatite	$Ca_5(PO_4)_3F$	−59
Hydroxyapatite	$Ca_5(PO_4)_3OH$	−57
Tricalcium phosphate	$Ca_3(PO_4)_2$	−29
Variscite	$AlPO_4 \cdot 2H_2O$	−21
Strengite	$FePO_4 \cdot 2H_2O$	−26

Calculated from equilibrium data in Lindsay (1979).

FIGURE 18–1
Solubility of orthophosphate from various calcium phosphates compared with variscite ($AlPO_4 \cdot 2H_2O$) and strengite ($FePO_4 \cdot 2H_2O$) and as a function of solution pH. These equilibria assume a Ca^{2+} activity of $10^{-2.5}$ M or equilibrium with $CaCO_3$ and CO_2 at 0.03 k Pa at high pH. *From Lindsay (1979), Used with permission.*

of about 6.5 because precipitation as both aluminum and calcium phosphates is minimized. One practical aspect of liming acid soils or acidifying alkaline soils is the improved availability of orthophosphate.

Many organic forms of phosphorus are also found in soils. As plant and animal remains or waste products are returned to the soil, readily mineralized organic phosphorus compounds are introduced. Microorganisms also produce organic phosphorus compounds as organic materials that are transformed in soil. Organic phosphorus in most soils may account for as little as 3% and as much as 90% or more of the total soil phosphorus but usually represents 30% to 50% of total phosphorus in most soils. The total amount of organic phosphorus in a soil is usually strongly correlated with total organic carbon; organic phosphorus decreases with depth in the soil profile, as does organic carbon.

The chemical nature of the soil organic phosphorus fraction is not well known. There are many different forms of organic phosphorus in soil; some common compounds are illustrated in Figure 18–2. Of these compounds, inositol phosphates, often called phytins or phytic acids, are typically present in the greatest quantity, comprising 10% to 50% of the total organic phosphorus. These inositol phosphates are often in a polymeric state in the soil and are relatively resistant to decomposition. The inositol hexaphosphates are found in the greatest amounts, followed by inositol compounds with one to five phosphate groups. Most of the inositol phosphates, and other polymeric forms of organic phosphorus in soil, are thought to be of microbial origin (Cosgrove, 1977). Phospholipids and nucleic acids may account for 1% to 5% of the organic phosphorus. Other identifiable organic phosphorus compounds are typically present in trace amounts only.

Another important pool of organic phosphorus is the soil microbial biomass. This fraction represents the actively cycling pool of organic phosphorus in the soil environment and is part of the labile, or readily available, organic phosphorus. Through this pool, the active mineralization and immobilization of phosphorus in soil occurs. Concentrations of 5 to 75 µg biomass P g^{-1} soil are common. Brookes, Powlson, and Jenkinson (1984) observed that the biomass-phosphorus fraction ranged from 2% to 5% of the total organic phosphorus in arable soils to upwards of 20% in some grassland and forest soils in Great Britain. Cultivation of soil results in a reduction of soil organic matter and total organic phosphorus and reduces the
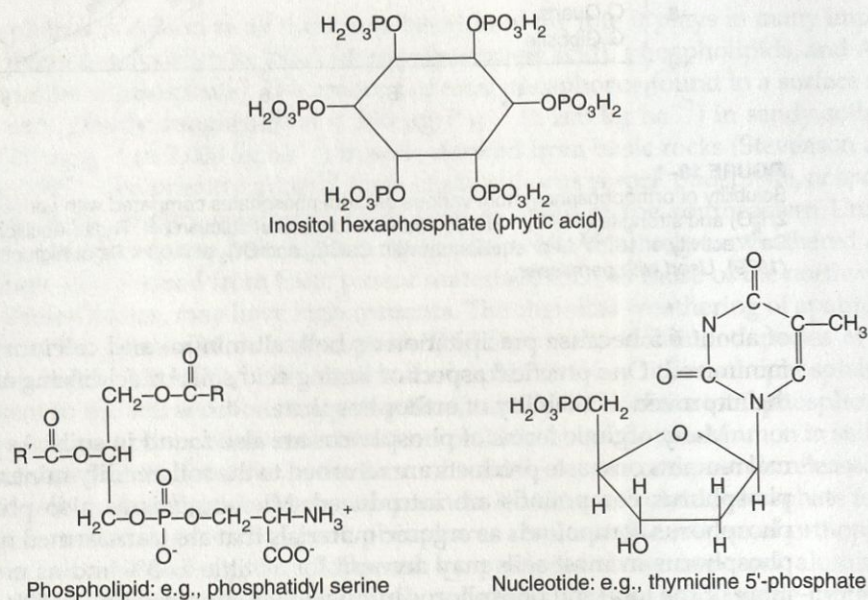

Inositol hexaphosphate (phytic acid)

Phospholipid: e.g., phosphatidyl serine

Nucleotide: e.g., thymidine 5'-phosphate

FIGURE 18–2
Representative forms of organic phosphorus inputs to the soil environment.

proportion of biomass phosphorus to total organic phosphorus. Therefore, tillage depletes the labile organic phosphorus pool more rapidly than the stable organic phosphorus fraction.

The Phosphorus Cycle

A model of the phosphorus cycle, illustrated in Figure 18–3, shows the various compartments of phosphorus in the terrestrial environment. Phosphorus is affected by both biological and chemical reactions. This model divides the phosphorus cycle into a geochemical subcycle and a biological subcycle, with the solution phosphorus pool serving as the central point in the overall cycle. Solution phosphorus is the source of orthophosphate for plants and soil microorganisms.

In the biological phosphorus subcycle, orthophosphate can be taken up by plants or immobilized into microbial biomass. A method of measuring microbial biomass phosphorus is presented in Box 18–1. As plant residues and animal remains and wastes are returned to soil, the organic phosphorus may be directly incorporated into stable humus, mineralized to orthophosphate, or immobilized into the microbial biomass. Biomass phosphorus is subject to incorporation into humic substances and mineralization and immobilization reactions. The turnover or cycling of the biomass contributes significantly to the labile organic phosphorus pool. Crop removal and erosion are two mechanisms for loss of organic phosphorus.

In the geochemical phosphorus subcycle, orthophosphate is solubilized from primary and secondary minerals by chemical and biochemical weathering processes. Dissolution of these compounds in the soil solution or solubilization through microbially produced organic acids releases orthophosphate to the soil solution for plant and microbial uptake. Orthophosphate can adsorb to aluminum and iron oxides (labile inorganic phosphorus) or precipitate as aluminum, iron, or calcium phosphates (secondary minerals). Through time, phosphorus finds its way into streams, lakes, rivers, and eventually the oceans where it ends up in sediments. In this sense the phosphorus cycle can be viewed as a sedimentary cycle.

There is limited evidence for microbial reduction of orthophosphate to phosphine gas (PH_3) and oxidation of orthophosphite ($H_2PO_3^-$) to orthophosphate. However, the thermodynamic state of soils and sediments would preclude the occurrence of these phosphorus species in such systems.

Mineralization and Immobilization of Phosphorus

Phosphorus availability is controlled by mineralization and immobilization through the organic fraction, and the solubilization and precipitation of phosphate in inorganic forms. As the remains of plants, animals, and microbes are returned to the soil, they are actively decomposed by soil microorganisms. Phosphorus in these organic residues must be released if it is to be available to plants and microorganisms.
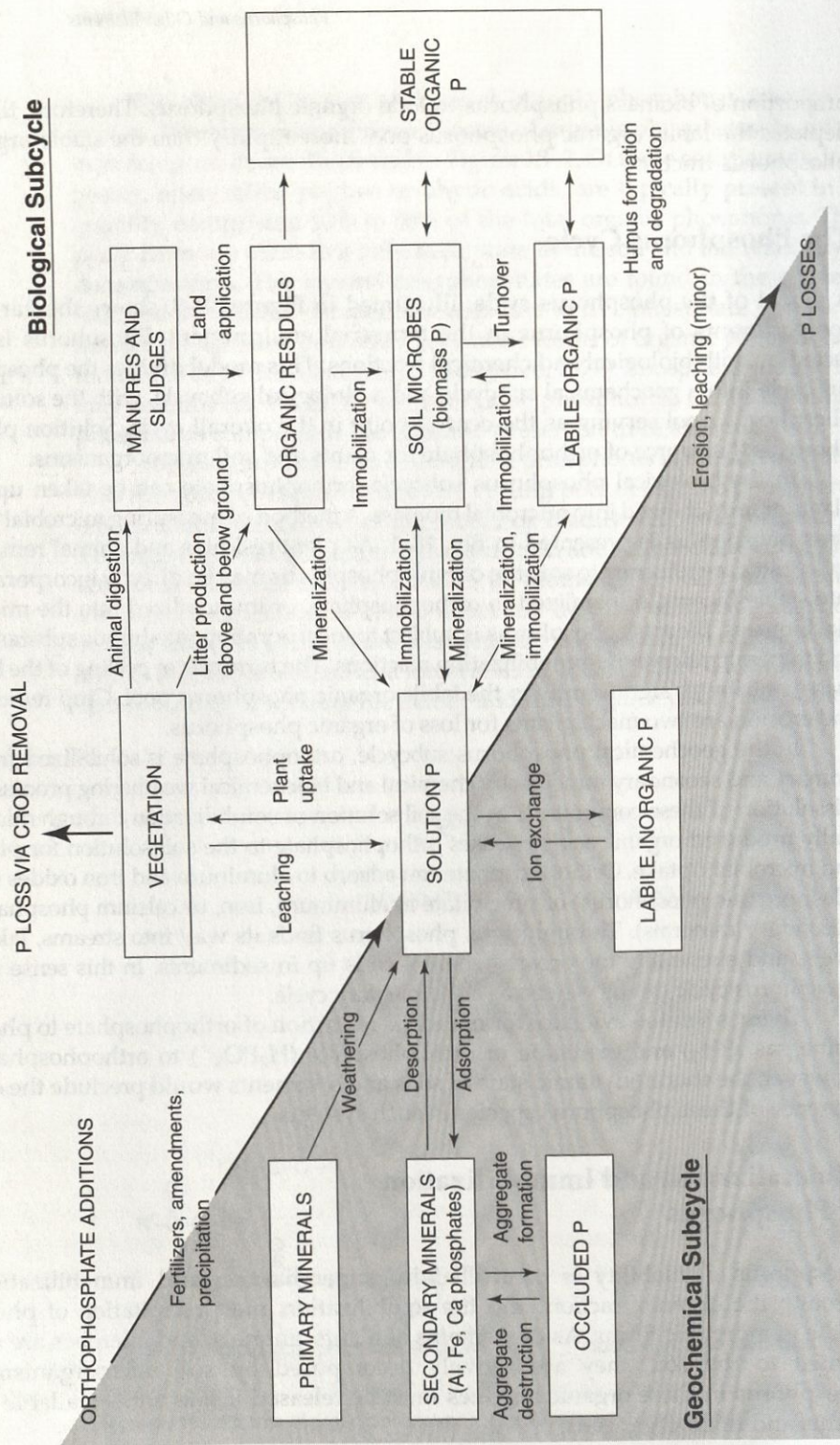
Biological Subcycle

STABLE ORGANIC P

MANURES AND SLUDGES

ORGANIC RESIDUES

SOIL MICROBES (biomass P)

LABILE ORGANIC P

Land application

Animal digestion

Litter production above and below ground

Immobilization

Mineralization

Immobilization

Mineralization

Mineralization, Immobilization

Turnover

Humus formation and degradation

Mineralization, immobilization

VEGETATION

SOLUTION P

LABILE INORGANIC P

Plant uptake

Leaching

Ion exchange

Weathering

Desorption

Adsorption

P LOSS VIA CROP REMOVAL

ORTHOPHOSPHATE ADDITIONS

Fertilizers, amendments, precipitation

Erosion, leaching (minor)

P LOSSES

PRIMARY MINERALS

SECONDARY MINERALS (Al, Fe, Ca phosphates)

OCCLUDED P

Aggregate formation

Aggregate destruction

Geochemical Subcycle

FIGURE 18–3
The phosphorus cycle, showing inputs, losses, and major transformations in the soil environment. The phosphorus cycle consists of two subcycles: one geochemical (lower left diagonal) and one biological (upper right diagonal). The boxes represent pools of phosphorus, and arrows indicate phosphorus inputs, transfers, and losses. *From Walbridge (1991). Used with permission.*

BOX 18–1

Measurement of Microbial Biomass Phosphorus

The method involves fumigating the soil with chloroform ($CHCl_3$) for 24 hours and extracting the soil with 0.5 M NaHCO$_3$. The chloroform extraction lyses microbial cells, releasing the contents of the cells to the soil. The inorganic orthophosphate (Pi) extracted from a nonfumigated control is subtracted from the Pi released in the fumigated soil. This Pi underestimates the total microbial phosphorus, and a correction factor, k_p, is used to account for this. A k_p of 0.4 is often used; this extraction efficiency was determined by evaluating the recovery of known amounts of phosphorus from microorganisms added to soil. However, this factor may actually be higher or lower, depending on soil properties. Because orthophosphate is readily adsorbed in soil, a correction for phosphorus fixation is also necessary. A known amount of $H_2PO_4^-$, typically 25 µg g^{-1} soil, is added to a third soil sample with the NaHCO$_3$ extracting solution. The difference between the Pi in this extract and the nonfumigated control represents the amount of added Pi recovered. Brookes, Powlson, and Jenkinson (1982) provide further details on this method. Microbial biomass phosphorus is calculated as follows:

$$\frac{\text{µg biomass P}}{\text{g soil}} = \left(\frac{25}{c-a}\right) \times \left(\frac{b-a}{0.4}\right)$$

where: a = µg Pi extracted from the nonfumigated control
b = µg Pi extracted from the fumigated soil
c = µg Pi extracted from a nonfumigated soil extracted with 0.5 M NaHCO$_3$ providing 25 µg Pi g^{-1} soil
25 = known concentration of Pi added to nonfumigated soil, in µg Pi g^{-1} soil
0.4 = k_p

Phosphorus mineralization is an enzymatic process. As a group, the enzymes involved, called *phosphatases*, catalyze a variety of reactions that release phosphate from organic phosphorus compounds to the soil solution. Phosphatases are released by microorganisms extracellularly into the soil solution to catalyze these hydrolytic mineralization reactions:

- *Phosphomonoesterases* hydrolyze the phosphate from monoester forms of phosphorus, such as those in nucleotides or phospholipids (Box 18–2).
- *Phosphodiesterases* hydrolyze phosphate from diester forms of phosphorus, such as in nucleic acids.
- *Phytases* hydrolyze phosphate from inositol phosphates.

BOX 18–2

The Phosphomonoesterase Assay

This assay measures the potential of a soil to mineralize orthophosphate by hydrolysis of phosphomonoester bonds in organic phosphorus sources. The assay includes an organic phosphate analog, p-nitrophenylphosphate, as a substrate (Tabatabai, 1994). The soil is treated with toluene to inhibit microbial activity and a buffer solution to maintain the reaction pH. As the phosphate-ester bond is hydrolyzed, p-nitrophenol is formed. After incubation, NaOH is added to the soil to stop the reaction and adjust the soil to an alkaline pH where p-nitrophenol forms a yellow color. Colorimetric determination of the p-nitrophenol concentration permits calculation of the rate of enzyme activity. The reaction is:

p-Nitrophenylphosphate

p-Nitrophenol
(yellow at alkaline pH)
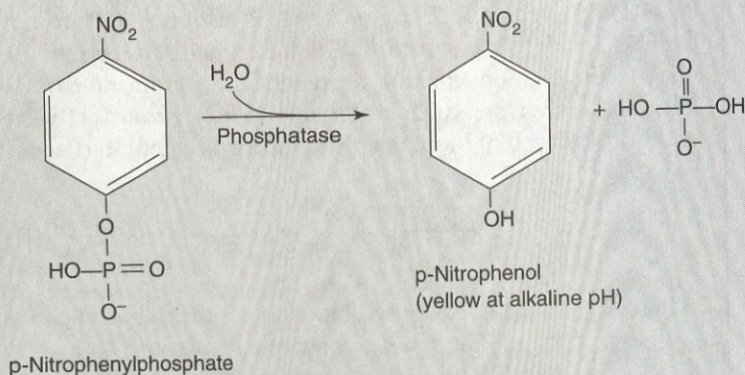
$$\text{and phosphatase activity} = \frac{\mu g \ p - \text{nitrophenol released}}{g \ \text{soil hour}}$$

Once phosphorus is mineralized, it can be taken up by plants or immobilized back into microbial cells, or it can form insoluble inorganic complexes. The microbial biomass can affect phosphorus availability through immobilization, the incorporation of orthophosphate ions into organically bound forms in the organism. For example, orthophosphate reacts with ADP (adenosine diphosphate) and a suitable input of energy to form ATP. The extent of immobilization is affected by the carbon to phosphorus ratio of the organic materials being decomposed and the amount of available phosphorus in the soil solution.

The carbon to phosphorus ratio of an added residue can determine the extent to which inorganic phosphorus is mineralized or immobilized. If insufficient phosphorus is available in the residue for assimilation of the added carbon, then inorganic phosphorus from the soil solution must be used and net immobilization occurs. Conversely, if more phosphorus is present in the residue than is needed for carbon assimilation, net mineralization of orthophosphate occurs. Generally, a

C/P ratio < 200/1 results in mineralization, while a C/P ratio > 300/1 results in immobilization. Ratios between 200 to 300 result in little net change in solution phosphorus concentrations. These processes are similar to those for nitrogen and sulfur mineralization and immobilization. In addition to the phosphorus content of a residue, other soil and environmental variables (e.g., pH, temperature, aeration, and soil moisture) affect microbial activity and phosphorus mineralization. The element that is most limiting controls the overall mineralization rate of a residue. If rapid carbon mineralization of a phosphorus-limited residue is occurring, then immobilization of phosphorus from the soil results. As mineralizable organic carbon disappears, a portion of the phosphorus-rich microbial biomass will be mineralized as well, resulting in the eventual release of the previously immobilized phosphorus.

Solubilization of Inorganic Phosphorus

Inorganic phosphorus minerals are generally found as aluminum and iron phosphates in acidic soils, while calcium phosphates dominate in alkaline soil. These slightly soluble compounds provide orthophosphate to soil solution to the extent allowed by the solubility of the compound. Solubility products (Table 18–2) provide a relative measure of solubility in pure water; however, these compounds are also affected by soil pH (Lindsay, 1979). Figure 18–1 illustuates the solubility of orthophosphate from a variety of phosphate compounds. It is evident from these data (Table 18–2 and Fig. 18–1) that phosphate is maintained at low concentrations in the soil solution. Orthophosphate is supplied to the roots primarily by diffusion. Thus chemical equilibria between orthophosphate, adsorbed orthophosphate, and inorganic phosphate minerals are important in supplying phosphorus to plants and microorganisms. As phosphorus is taken up, it is replenished from these sources.

Plant roots and soil microorganisms (Box 18–3) can enhance the dissolution of phosphate compounds by the release of carbon dioxide (carbonic acid), other


BOX 18–3

Phosphorus-Solubilizing Bacteria

Some bacteria are very effective in solubilizing phosphorus from rock phosphate. One example is *Bacillus megaterium* var. *phosphaticum*. This bacterium has been formulated into inocula referred to as *phosphobacterin* and applied to soils to enhance the solubilization of phosphorus minerals. This practice was reported to be successful in the former Soviet Union; however, it has had only limited success in the United States. Nonetheless, the incorporation of a readily mineralizable carbon source, such as manure, can enhance phosphorus solubilization through increased biological activity. The increased organic carbon also can serve to complex aluminum in acid soils, thereby reducing the extent to which aluminum phosphates precipitate.

FIGURE 18–4
Aluminum and phosphorus concentrations and pH (top) and production of organic acids (bottom) during the solubilization of $AlPO_4$ by the fungus *Aspergillus niger*. From Illmer et al. (1995). Used with permission.
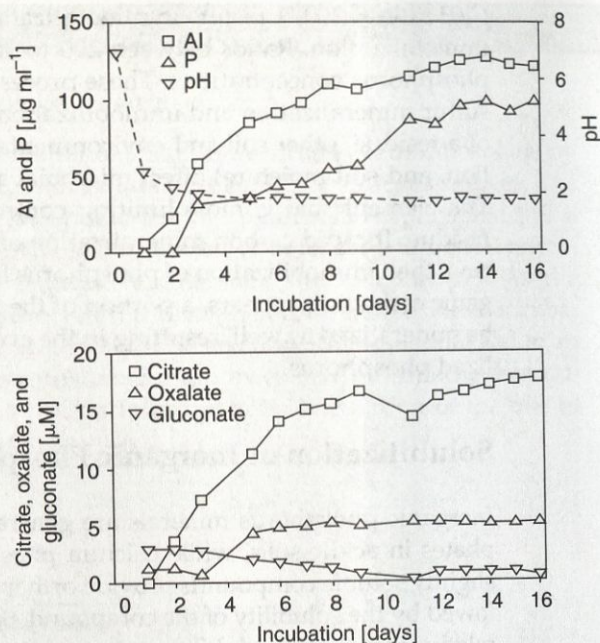
inorganic acids, and organic acids (Fig. 18–4) to the soil solution. Carbonic acid can promote the acid dissolution of calcium and magnesium phosphate compounds. Similarly, the acidity produced by the nitrifying bacteria and sulfur-oxidizing bacteria promotes solubilization of insoluble phosphate salts. A wide range of organic acids is produced by microorganisms and plants, and many act as **chelating agents** to sequester aluminum, iron, calcium, and magnesium phosphates, resulting in the release of orthophosphate into the soil solution (Fig. 18–5). One group of organisms that may be important in this regard is the mycorrhizal fungi, which form symbioses with plant roots and enhance the uptake of phosphorus and other nutrients (chapter 12). Under water-logged conditions, hydrogen sulfide, produced by sulfate-reducing bacteria or other processes, can also displace metal cations from insoluble phosphates, with the release of phosphate.

Environmental Aspects of Phosphorus Cycling

Phosphorus can cause environmental damage if applied in excess to soils. Phosphorus is the most limiting nutrient for primary productivity in many ecosystems, particularly aquatic systems. If a large amount of phosphorus is applied to soil, it may move to surface waters with runoff water and eroded soil particles that bind phosphorus. The major consequence is a process called **eutrophication.** Eutrophication can occur in surface waters when excessive phosphorus or nitrogen accumulates. The process occurs most noticeably in relatively still bodies of water such as ponds and lakes. If phosphorus is the limiting nutrient in the pond, excess phosphorus additions can re-
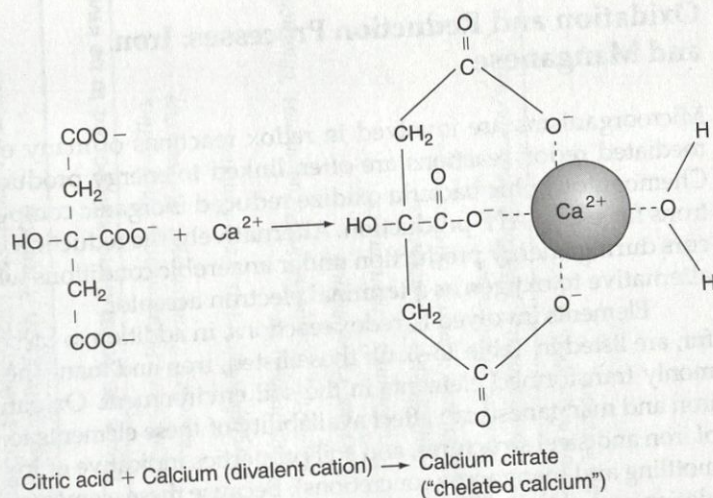
$$\text{Citric acid} + \text{Calcium (divalent cation)} \longrightarrow \text{Calcium citrate (``chelated calcium'')}$$

FIGURE 18–5
Example of Ca^{2+} chelation by citric acid.

sult in rapid growth of algae in the water. This algal bloom is often noticeable as a green "scum" or film on the water surface. As the algae die, they settle to the bottom, where bacterial decomposition of the nutrient-rich algae occurs. The decomposition of the algae results in oxygen depletion. If the process continues, the lake slowly becomes anaerobic, resulting in a poor environment for many forms of aquatic life.

Nonpoint sources of phosphorus, such as from agricultural activities, are often associated with increased phosphorus in surface water. For example, runoff from cropland and soils receiving animal manures can lead to significant phosphorus inputs into surface waters. In industrialized countries, nonpoint sources are the major source of phosphorus. In nonindustrialized countries with limited sewage-treatment capacity, raw sewage discharge into surface waters (a point source) is a significant source of phosphorus pollution.

The natural ability of soils to adsorb phosphorus from solution can be used to remove phosphorus from treated wastewater. Natural and constructed wetlands can serve as a sink for the removal of phosphorus from these wastewaters before their release to a river or lake.

IRON, MANGANESE, MERCURY, SELENIUM, AND ARSENIC

Many metallic and metalloid elements, some of them micronutrients and others that are environmental pollutants, are transformed by microorganisms during metabolic activities. These transformations may provide effective means for remediation of contaminated soils and sediments (Frankenberger and Losi, 1995). The transformations of iron, manganese, mercury, selenium, and arsenic can affect their solubility and availability to plants and other organisms, and their mobility in the environment.

Oxidation and Reduction Processes: Iron and Manganese

Microorganisms are involved in redox reactions of many elements. Biologically mediated redox reactions are often linked to energy production in an organism. Chemoautotrophic bacteria oxidize reduced inorganic compounds to extract electrons for use in ATP production. Alternatively, the reduction of many elements occurs during energy production under anaerobic conditions when the element is an alternative to oxygen as a terminal electron acceptor.

Elements involved in redox reactions, in addition to carbon, nitrogen, and sulfur, are listed in Table 18–3. Of those listed, iron and manganese are the most commonly transformed elements in the soil environment. Oxidation and reduction of iron and manganese can affect availability of these elements to plants, the corrosion of iron and steel structures, and soil properties indicative of imperfect drainage (i.e., mottling and manganese concretions). Because these elements are also subject to oxidation and reduction by chemical means, it is difficult to assess the extent to which these transformations are biotic or abiotic. As Ghiorse (1994) points out, the presence of organisms capable of iron and manganese oxidation and reduction in soils is often evidence for potential activity in the soil. However, the contribution of microorganisms to iron and manganese oxidation and reduction cannot be easily measured by direct means.

Iron is among the most abundant elements in the soil environment, but concentrations of soluble iron are typically very low in aerobic soil environments. Iron contents range from less than 0.05% in coarse-textured soils to more than 10% in highly weathered Oxisols found in the tropics. The iron cycle (Fig. 18–6) is characterized by the oxidation and reduction of iron compounds in soils and sediments. However, mineralization from organically bound iron and the solubilization of iron from inorganic compounds by microorganisms are also important processes. Manganese cycling is similar to that of iron. Total manganese concentrations in soil may be less than 0.01% to 0.3%. The form that is most available for plant growth is the reduced Mn^{2+} ion. Chemical and microbial oxidation results in the formation of relatively insoluble manganese oxides.

Chemical oxidation of Fe^{2+} occurs very rapidly under aerobic conditions at pH > 3 and is the major pathway of iron oxidation in most soil environments. Under acidic conditions, ferrous iron (Fe^{2+}) can be oxidized to ferric iron (Fe^{3+}) by chemoautotrophic bacteria such as *Acidithiobacillus ferrooxidans*. A representative reaction for ferrous iron oxidation by *A. ferrooxidans* is the oxidation of ferrous sulfate ($FeSO_4$) to ferric sulfate and ferric hydroxide:

$$12FeSO_4 + 3O_2 + 6H_2O \rightarrow 4Fe_2(SO_4)_3 + 4Fe(OH)_3$$
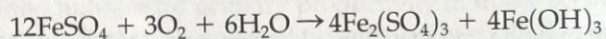
Manganese oxidation is also microbially mediated in soil at pH > 5. The oxidation of Mn^{2+} occurs as follows, and the rate increases with increasing alkalinity up to about pH 8:

$$Mn^{2+} + 2OH^- \rightarrow MnO_2 + H_2O$$

TABLE 18–3 Examples of Common Elements (Excluding Carbon, Nitrogen, Sulfur) Subject to Microbial Oxidation-Reduction Reactions in Soils and Sediments and Examples of Bacterial Genera Involved with Each Reaction

Element	Common Oxidation States	Reaction, Significance, and Redox Couple	Some Bacterial Genera Reported to be Involved
Arsenic (As)	As^{5+}, As^{3+}	Oxidation–E* $\quad As^{3+} \rightarrow As^{5+} + 2e^-$ Reduction–AR, D $\quad As^{5+} + 2e^- \rightarrow As^{3+}$	Alcaligenes, Pseudomonas, Thiobacillus Alcaligenes, Pseudomonas, Micrococcus
Chromium (Cr)	Cr^{6+}, Cr^{3+}	Oxidation–NR Reduction–AR, D $\quad Cr^{6+} + 3e^- \rightarrow Cr^{3+}$	Aeromonas, Bacillus, Pseudomonas
Iron (Fe)	Fe^{3+}, Fe^0	Oxidation–E $\quad 2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^-$ Reduction–AR $\quad 2\,Fe^{3+} + 2e^- \rightarrow 2Fe^{2+}$	Acidithiobacillus Geobacter, Desulfovibrio, Pseudomonas, Thiobacillus
Mercury (Hg)	Hg^{2+}, Hg^0	Oxidation–NE Reduction–D $\quad Hg^{2+} + 2e^- \rightarrow Hg^0$	Bacillus, Pseudomonas Pseudomonas, Streptomyces
Manganese (Mn)	Mn^{4+}, Mn^{2+}	Oxidation–E, D $\quad Mn^{2+} \rightarrow Mn^{4+} + 2e^-$ Reduction–AR $\quad Mn^{4+} + 2e^- \rightarrow Mn^{2+}$	Arthrobacter, Pseudomonas Bacillus, Geobacter, Pseudomonas
Selenium (Se)	Se^{6+}, Se^{4+}, Se^0, Se^{2-}	Oxidation–E $\quad Se^{2-} \rightarrow Se^0 + 2e^-$ Reduction–AR $\quad SeO^{2-} + 8e^- \rightarrow Se^{2-}$	Bacillus, Thiobacillus Clostridium, Desulfovibrio, Micrococcus

*NR, not reported to be biologically mediated; AR, element used as a terminal electron acceptor in anaerobic respiration; D, detoxification mechanism; E, energy source; NE, non-enzymatic reaction, microorganism alters the physicochemical environment. From Ehrlich (1990), Losi and Frankenberger (1997), Lovley (1993), and Newman et al. (1998).
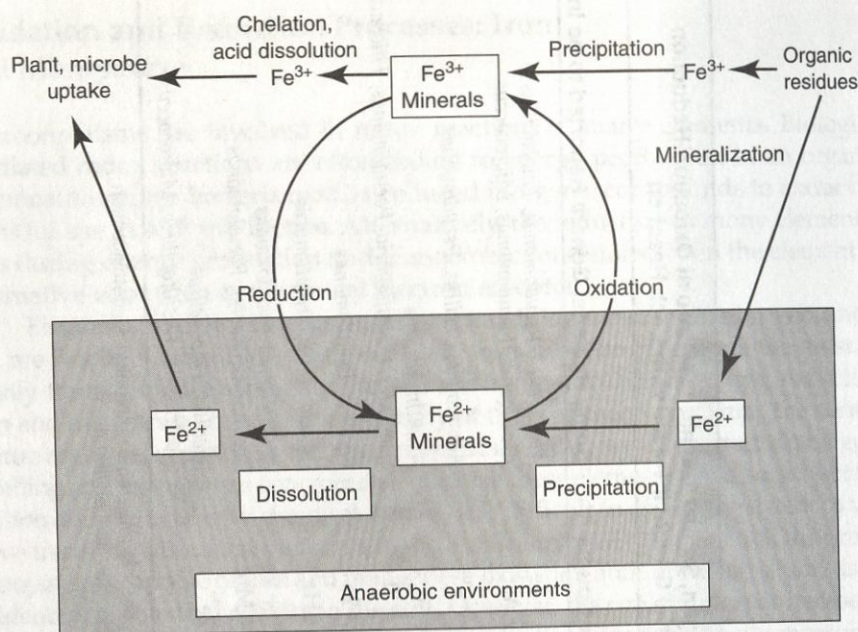
FIGURE 18–6
The iron cycle. Transformations include oxidation and reduction of iron minerals, precipitation of Fe^{2+} and Fe^{3+} minerals, dissolution of iron minerals to plant-available forms, and mineralization of organically bound iron.

The MnO_2 formed is very insoluble. Manganese concretions, or nodules, are often found in soils with cyclical oxidation and reduction processes, such as those with fluctuating water tables. Several bacteria enzymatically oxidize Mn^{2+}, although few are true chemoautotrophs. Some of the bacteria are **mixotrophs**; they derive some energy from the oxidation, but obtain carbon from organic carbon. Other organisms oxidize Mn^{2+} to MnO_2 with H_2O_2 via the *catalase* enzyme:

$$Mn^{2+} + H_2O_2 \rightarrow MnO_2 + 2H^+$$
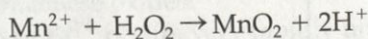
This reaction does not provide energy for the organism, but serves to remove potentially harmful H_2O_2. Manganese can also be oxidized nonenzymatically when microorganisms alter the pH of the microenvironment to 8 or above, where chemical oxidation occurs readily, or by producing compounds that can chemically oxidize Mn^{2+}. These reactions may serve to protect the cell from high concentrations of Mn^{2+}.

Microorganisms also carry out dissimilatory iron and manganese reduction (Lovley, 1993). Most organisms that reduce Mn^{4+} also reduce Fe^{3+}. Here we will discuss only iron reduction. Dissimilatory iron reduction is often coupled to the oxidation of fermentation products from other organisms. Complex organic matter, sugars, and amino acids can be partially oxidized by fermentation, resulting in the production of organic acids, alcohols, hydrogen, and methane. Bacteria from sev-

eral genera can oxidize these fermentation products with Fe^{3+} serving as the terminal electron acceptor. The reduction of Fe^{3+} by *Geobacter metallireducens* with the oxidation of acetate occurs as follows:

$$CH_3COO^- + 8Fe^{3+} + 4H_2O \rightarrow 2HCO_3^- + 8Fe^{2+} + 9H^+$$
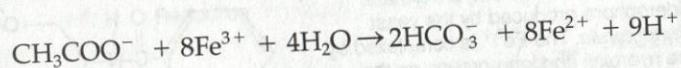
Some organisms can couple the complete oxidation of monoaromatic compounds with Fe^{3+} reduction. *Geobacter metallireducens* oxidizes environmental contaminants, such as toluene and phenol, to carbon dioxide in the presence of Fe^{3+}. These organisms may play important roles in the remediation of aquifers contaminated by polyaromatic hydrocarbons. There are also reports of bacteria that couple the oxidation of H_2 to H^+ with the reduction of Fe^{3+}.

Dissimilatory Fe^{3+} and Mn^{4+} reductions are important in the environment. For example:

- Fe^{3+} is an important electron acceptor in organic matter decomposition in aquatic sediments and saturated soils. This can lead to gleying of the soil (i.e., the characteristic gray to gray-blue colors associated with reduced iron compounds that indicate drainage problems).
- Fe^{3+} reduction in phosphate minerals can lead to the release of phosphate for subsequent uptake by plants and microbes.
- Fe^{3+} reduction can lead to steel corrosion, a problem of great concern to many industries.
- Mn^{4+} reduction can lead to the release of soluble Mn^{2+} into soil solution. Reoxidation of this manganese can result in the formation of manganese concretions in the soil, an indicator of fluctuating water tables in the soil profile.

Microbial Enhancement of Iron Activity

In well-aerated soils, Fe^{3+} is the dominant form of iron. The activity of Fe^{3+} in soil solution under aerobic conditions is low. For example, assume that $Fe(OH)_3$ is the solid phase controlling Fe^{3+} activities in soil solution. At pH 7, the calculated activity of Fe^{3+} in soil solution would be approximately 10^{-17} M (Lindsay, 1979). The activity continues to decrease as pH increases. The low solubility of Fe^{3+} in alkaline soils results in iron-deficient conditions for plants and microorganisms.

Many microorganisms produce Fe^{3+}-complexing compounds known as **siderophores.** These are low-molecular-weight, iron-transporting compounds with high Fe^{3+} affinity (Leong, 1986). The purpose of these compounds is to solubilize Fe^{3+} for uptake by the organism. Fungi and some bacteria produce siderophores with hydroxymate active sites, whereas other bacterial siderophores are catechol derivatives (Fig. 18–7). The organism has siderophore receptor proteins on its outer membrane that bind the siderophore-Fe^{3+} complex. The iron is released from the siderophore via reduction of Fe^{3+} to Fe^{2+}. The Fe^{2+} is then transported into the cell and the siderophore is released to complex more Fe^{3+}. Interestingly, the chelation of Fe^{3+} from iron phosphate minerals also results in the liberation of soluble orthophosphate.

FIGURE 18–7
Examples of microbial siderophores.
Enterochelin is a catechol derivative
produced by the bacterium *Salmonella*.
Rhodotorulic acid is a hydroxymate
siderophore produced by the yeast
Rhodotorula. The Fe^{3+} is complexed by
the hydroxyl and keto groups on the
molecules.

Enterochelin

Rhodotorulic acid
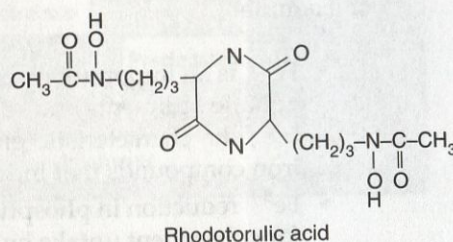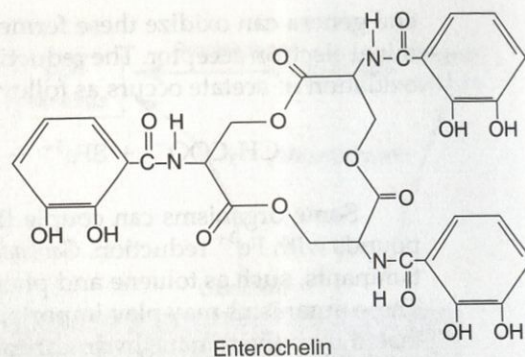
Some pseudomonads produce a yellow-green fluorescent siderophore, called *pseudobactin*, that binds iron tightly, prohibiting its use by other organisms. By this mechanism the pseudomonad can lower the availability of Fe^{3+} to other microbes and thereby control some fungal root pathogens in iron-limited environments.

Microbial Transformations of Mercury, Selenium, and Arsenic

Mercury, selenium, and arsenic are environmentally important elements that are affected by another interesting microbial transformation, **biomethylation**. Biomethylation is a detoxification mechanism for the organisms involved and results in the formation of volatile organic forms of these elements. Mercury can be microbially reduced, while selenium and arsenic can be microbially oxidized and reduced.

Mercury is used or produced in a variety of industries. It can enter the environment as a result of ore smelting, production of chlorine and caustic soda, agricultural practices, and other human activities. At one time, mercury compounds were common pesticides.

Mercury can cause defects in the central nervous system. In the 1950s, industrial discharges of methylmercury into Minamata Bay in Japan resulted in the contamination of fish with methylmercury, the poisoning of thousands of people, and several hundred deaths.
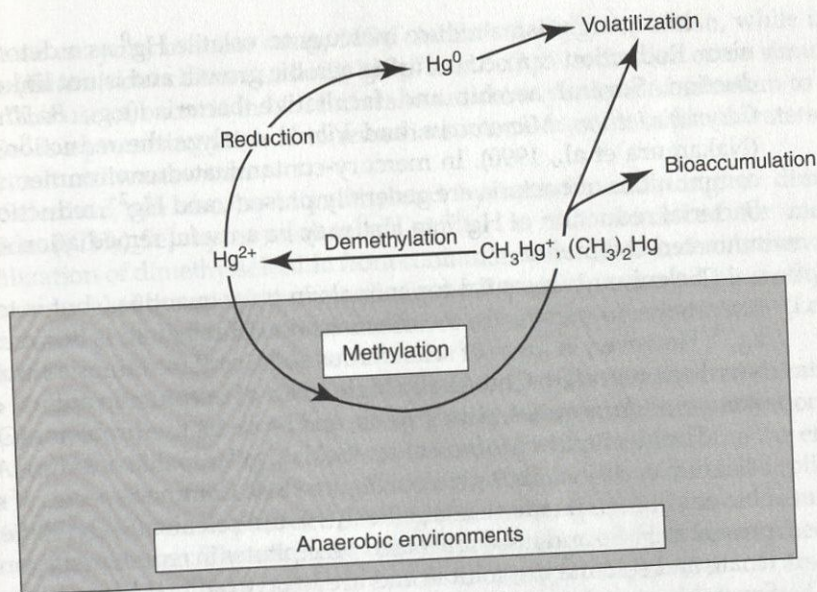
FIGURE 18–8
The mercury cycle. Transformations of mercury include reducing of ionic Hg^{2+} to elemental Hg^0, methylating of Hg^{2+} under anoxic conditions, and demethylating under aerobic conditions. Methylmercury and elemental mercury are volatile and can be lost to the atmosphere. Methylmercury is toxic and can accumulate in fatty tissues of animals, which is increasingly concentrated as methylmercury moves up the food chain.

Mercury undergoes several transformations by microorganisms in the environment (Fig. 18–8). It can be present in many forms, including elemental mercury (Hg^0), inorganic mercuric ion (Hg^{2+}), methylmercury (CH_3Hg^+), or dimethylmercury [$(CH_3)_2Hg$]. The methylation of mercury occurs under anaerobic conditions, such as those found in wetland soils and lake sediments (Zillioux, Porcella, and Benoit, 1993). This reaction increases the solubility and volatility of mercury, resulting in its increased movement into the food chain. Methylmercury is **lipophilic** and can be concentrated in fish in contaminated waters.

Recent evidence indicates that the methylation is mediated by sulfate-reducing bacteria. The methylation reaction occurs via the transfer of methyl groups from methylcobalamine, or methylated vitamin B_{12}, to Hg^{2+}.

$$Hg^{2+} + B_{12}-CH_3 \rightarrow CH_3H_g^+ + reduced - B_{12}$$
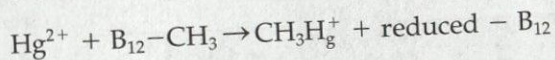
The methylcobalamine is produced by sulfate-reducing bacteria using fermentation products from other heterotrophs and methylation generally stops when fermentation ceases, although sulfate reduction may continue (Choi and Bartha, 1993). As methylmercury migrates into more aerobic zones, demethylation of the methylmercury can occur, followed by reduction of the Hg^{2+}, resulting in the formation of elemental mercury. Elemental mercury is volatile (though of very low vapor pressure) and can escape to the atmosphere.

Many organisms reduce mercury to volatile Hg^0 as a detoxification mechanism. Reduction can occur during aerobic growth and is not linked to energy production. Several aerobic and facultative bacteria (e.g., *Bacillus*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, and *Vibrio*) catalyze the reduction of Hg^{2+} to Hg^0 (Nakamura et al., 1990). In mercury-contaminated environments, Hg^{2+}-resistant communities of bacteria are generally present, and Hg^{2+} reduction is fairly rapid. Bacterial reduction of Hg^{2+} to Hg^0 may be a useful remediation strategy for contaminated sediments.

Selenium is essential for animals in trace quantities, but is toxic at high concentrations. In most soils, the concentration of selenium is between 0.01 and 2 mg kg^{-1}. However, in areas of seleniferous soils, concentrations as high as 38 mg kg^{-1} have been reported. Chronically high levels of selenium in animal diets can lead to embryonic deformities, poor growth, and reproductive problems (Ohlendorf, 1989).

The biochemistry of selenium (Se) is similar to that of sulfur. A diagram of the Selenium cycle in soils is given in Figure 18–9. Common species of selenium in aerobic environments are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). Selenide (Se^{2-}) is present as H_2Se and insoluble metal precipitates in reduced environments. Both selenate and selenite are soluble and are toxic to animal life in high concentrations. Several bacterial genera, including *Pseudomonas*, *Clostridium*, and *Flavobacterium*, use oxidized selenium, such as SeO_4^{2-}, SeO_3^{2-}, and Se^0, as terminal electron acceptors. The reduction of oxidized selenium to insoluble elemental Se^0 is a principal means for immobilizing soluble selenate and selenite from contaminated environments (Losi and Frankenberger, 1997). Interestingly, there is evidence that dissimilatory selenium reduction in some bacteria may be catalyzed by the nitrate
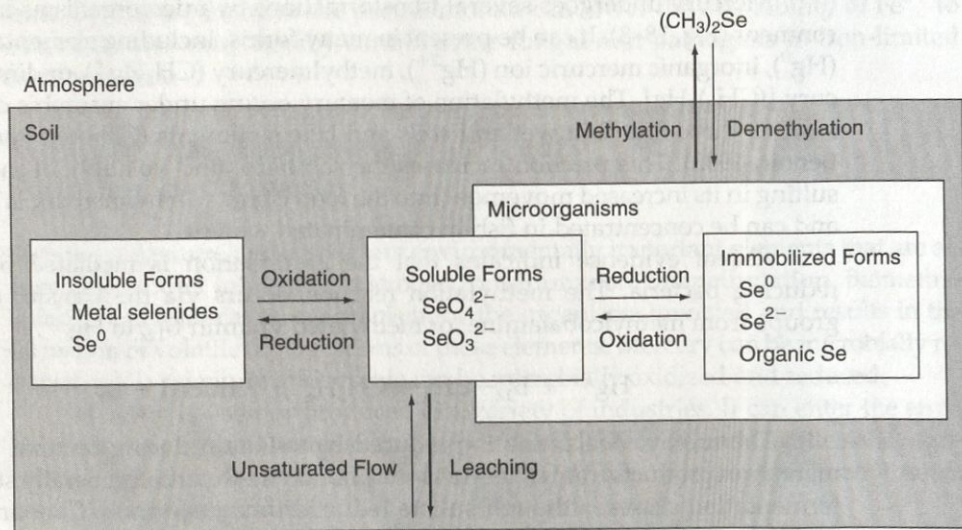

FIGURE 18–9
Selenium cycling in soils and sediments. Bold arrows indicate preferred direction of the process. *From Flury et al., 1997. Used with permission.*

reductase enzyme; in some cases, NO_3^- inhibits SeO_4^{2-} reduction, while in one isolate (*Enterobacter cloacae* SLD1a-1), NO_3^- and SeO_4^{2-} reduction occur simultaneously. The use of bacteria in bioreactors to reduce oxyanions of selenium to insoluble Se^0 is a promising method to remediate selenium-contaminated waters, such as agricultural wastewaters.

Selenium can also be biomethylated by bacteria and fungi to dimethylselenide $[(CH_3)_2Se]$ by a mechanism similar to that described for mercury. Volatilization of dimethylselenide from contaminated waters and soils then results in the removal of the selenium from the immediate environment. This methylation reaction has potential use for remediation of seleniferous environments (Losi and Frankenberger, 1997; Box 18–4).

Arsenic (As) in the environment is also of concern. Arsenic concentrations in soil vary from about 0.2 to 40 mg As kg^{-1}, with background concentrations typically less than 15 mg As kg^{-1}. Arsenic is introduced by humans into the environment by a number of mechanisms. Arsenic is used in many industrial applications and is therefore a waste product. Mining and smelting operations can result in arsenic contamination. Fly ash from coal-fired power plants can be enriched in arsenic. The wood preservative industry is also a major user of arsenic in manufacturing rot-resistant CCA (copper, chromium, arsenic)-treated wood.

BOX 18–4

An Example of Selenium Contamination

Kesterson Reservoir is comprised of a series of twelve ponds covering approximately 500 ha in the southern part of the Kesterson National Wildlife Refuge in California (Ohlendorf, 1989). The ponds were used as collection and evaporation basins for agricultural drainage water from the San Joaquin Valley. Between 1981 and 1986, the drainage waters were found to be high in selenium and salinity. Selenium concentrations approached 300 µg L^{-1} (the EPA drinking water standard for selenium is 10 µg L^{-1}), resulting in the transport of approximately 9 Mg of selenium into the reservoir during this time. **Bioaccumulation** and **biomagnification** of selenium in the food chain occurred, resulting in abnormal rates of mortality and deformity in waterfowl. These events resulted in the closure of the reservoir to drainage waters. Since the closure, many of the low-lying areas have been filled with soil, and management plans have been implemented to dissipate selenium through plant bioaccumulation and volatilization by microbial biomethylation. In one study, 68% to 88% of the total selenium in research plots at Kesterson was dissipated over a 100-month period (Flury, Frankenberger, and Jury, 1997). Thus, microbially mediated removal of selenium from contaminated sites may prove more cost effective than soil removal and disposal.

Agricultural use of arsenical pesticides was previously a major source of soil arsenic contamination. Arsenical pesticides were used for decades before the introduction of organochlorine compounds, such as DDT. These were applied heavily in orchards and in cattle operations, resulting in significant contamination of some soils. Herbicides such as monosodium methanearsonate (MSMA) have been widely applied in cotton and turf grass production. Additionally, phosphate fertilizers typically have significant amounts of arsenic in them.

An overview of the arsenic cycle in terrestrial systems is shown in Figure 18–10. In aerobic soils, inorganic arsenic is present as arsenate ($H_2AsO_4^-$ or $HAsO_4^{2-}$; As^{5+}). Under moderately reduced conditions, arsenite ($HAsO_2$; As^{3+}) is the dominant form. These forms are very soluble in water and are highly bioavailable. The oxyarsenates are adsorbed by iron oxides and hydroxides in soil; therefore, availability tends to be higher in sandy soils.

Bacterial reduction of arsenate under anaerobic conditions has been reported. In some cases, this may be a detoxification mechanism; however, arsenate respiration (i.e., use of arsenate as an electron acceptor) also occurs. Microorganisms can also ox-
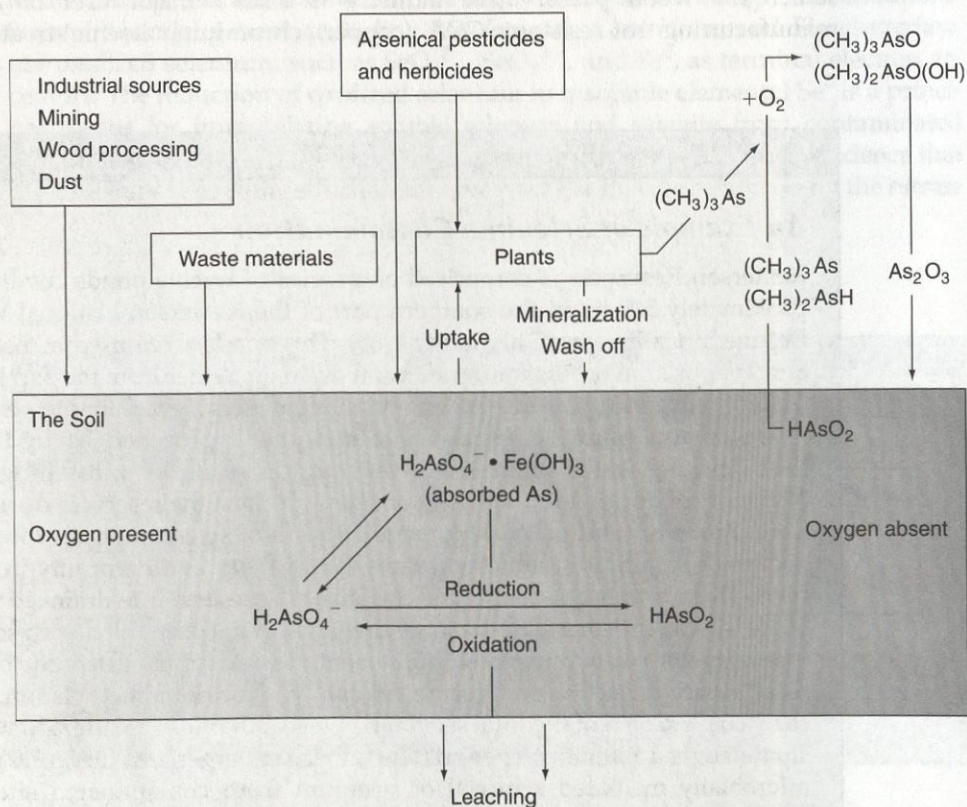

FIGURE 18–10
Arsenic cycling in the soil environment. Arsenate can be adsorbed by iron oxides in soil and reduced to arsenite in anaerobic environments. Bacteria and fungi can methylate arsenite, resulting in volatile loss of arsenic from the soil. *From World Health Organization, 1981. Used with permission.*

idize arsenite. In some cases, the arsenite is apparently used as an energy source by chemoautotrophic bacteria. As with mercury and selenium, biomethylation is also an important reaction with arsenic. Bacterial methylation produces dimethylarsine $[(CH_3)_2AsH]$, while fungi produce trimethylarsine $[(CH_3)_3As]$. Methylation occurs under aerobic or anaerobic conditions and is likely a detoxification mechanism.

Microbial mobilization of arsenic from contaminated soils and sediments can cause pollution of downstream areas. Zobrist, et al. (2000) showed that the anaerobe *Sulfurospirillum barnesii* reduces arsenate while oxidizing lactate, resulting in potential mobilization of the arsenite form. Ahmann, et al. (1997) reported significant solubilization from iron arsenates by dissimilatory arsenate reduction. They hypothesized that this activity was partially responsible for significant efflux of arsenic from anoxic sediments in the Aberjona Watershed near Boston, Massachusetts. This area was contaminated by sulfuric acid and pesticide production wastes in the late 1880s until 1929. Another, more widespread, problem with arsenic that has been related to microbial activities is the contamination of well water in Bangladesh (Box 18–5).

BOX 18–5

Arsenic Contamination of Well Water in Bangladesh

In the 1970s, the government of Bangladesh began a program, with the assistance of UNICEF and other aid organizations, to install millions of tube wells to provide groundwater for its population. This program greatly decreased the incidence of waterborne diseases. However, many of these wells were placed into groundwater containing significant levels of arsenic. Over 50,000 tube wells have been tested for arsenic contamination since the mid-1970s, with nearly 30% found to contain arsenic at concentrations greater than 50 µg L^{-1}, the maximum permissible concentration in drinking water in Bangladesh and many other countries. With approximately 4 million tube wells in place, a significant part of the population is exposed to high arsenic concentrations in their water. The arsenic in the water has resulted in a number of chronic health problems in large areas of Bangladesh over the past 25 years.

The mechanisms underlying this arsenic mobilization are not completely agreed upon. One mechanism that is generally agreed on is that microbial reduction of FeOOH in the aquifer sediments has resulted in the release of arsenic that is adsorbed to the iron oxyhydroxide. This reduction of FeOOH is coupled to the oxidation of sedimentary deposits of organic matter in the aquifer beds. Another recent hypothesis, fueled by observations of microbial arsenate reduction, is that As(V) is directly reduced by dissimilatory arsenate reduction coupled to oxidation of the organic matter. In either case, it is apparent that microbial activities have had a severe impact on human health in Bangladesh and surrounding areas.

For general information on this problem, go to *http://bicn.com/acic*, the Arsenic Crisis Information Centre web site.

## MICROORGANISMS AS CATALYSTS FOR MINERAL FORMATION

In this chapter, we have considered the microbial transformations of a number of elements. Another interesting area of research is the study of how microbial cells facilitate the formation of a variety of minerals in soils and sediments. Here, only a short introduction to the topic is possible. For an overview of bacterial mineral formation, refer to Douglas and Beveridge (1998).

Bacteria have a high surface-to-volume ratio and the presence of predominantly negatively charged carboxyl, phosphate, and amino groups on the cell wall surfaces. These properties result in the ability of microbial cell surfaces to act as nucleation points for mineral formation. Beveridge and Fyfe (1985) proposed a model for mineral formation by bacteria. These reactions occur predominantly on inactive cell surfaces, since actively metabolizing microorganisms produce protons which compete with the metals. Metal ions in solution interact with the charged cell-surface groups. This is essentially an electrostatic cation exchange mechanism resulting in the stoichiometric sorption of metal. This metal, which is bound in the matrix of the cell wall, then acts as a nucleation site for the further deposition of metal precipitates. The type of mineral formed depends on the types of negative counter-ions, such as carbonate, phosphate, sulfate, sulfide, or silicate, in the system.

Microbially mediated mineral formation has been shown to be important in many environments. For example, in sulfidic mine areas, in aerobic environments, thiobacilli catalyze the formation of iron oxides, whereas sulfate-reducing bacteria in anoxic soil and sediments can facilitate the formation of iron sulfides. Bacterial cells can also facilitate the formation of clay-like silicates, carbonate minerals, and a variety of elemental deposits, such as those of copper and gold. An example of iron-oxide precipitates forming on a bacterial cell wall is shown in Figure 18–11.

FIGURE 18–11
Transmission electron photomicrograph of an unstained thin section of an unidentified bacterium from a southern Ontario stream with high Fe^{2+} content. The cell has lysed, and the encrustations on the cell wall are iron-oxide precipitates. Bar = 1 μm. *Photo courtesy of T. J. Beveridge, University of Guelph. Used with permission.*
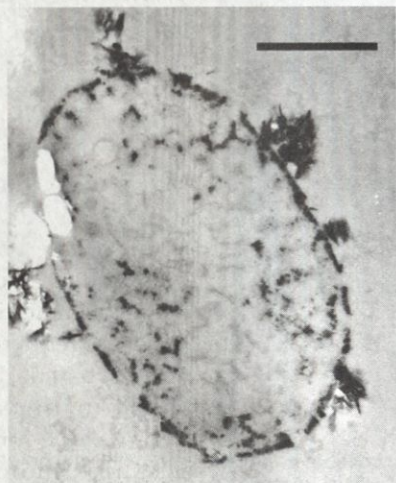
SUMMARY

The importance of microbial cycling of elements in soil extends beyond carbon, nitrogen, and sulfur. Most nutrients and other elements are actively transformed by a variety of mechanisms. Some of the nutrient elements include phosphorus, iron, and manganese, and elements of environmental significance including, but are not limited to, mercury, selenium, and arsenic. The phosphorus cycle includes mineralization and immobilization reactions through the microbial biomass pool, and the solubilization of inorganic phosphorus-containing minerals by organic acids, inorganic acids, and chelating agents produced by soil organisms. The mineralization of organic phosphorus is an important source of phosphorus for plant growth, particularly in nonagricultural ecosystems. Two other nutrients, iron and manganese, are also transformed in soils. Reduced forms (Fe^{2+} and Mn^{2+} minerals) can be oxidized by chemoautotrophic bacteria, providing a source of electrons for energy production. Oxidized forms (Fe^{3+} and Mn^{4+} minerals) can serve as terminal electron acceptors during dissimilatory reduction (i.e., a variety of respiratory processes). Reduction results in increased solubility of these elements. Reduced iron minerals also give rise to the characteristic gray or bluish colors present in soils with poor drainage. Mercury is a contaminant in many environments, particularly aquatic sediments. Ionic Hg^{2+} can be microbially methylated in sediments, resulting in the production of lipophilic methylmercury. Sulfate-reducing bacteria are thought to be primarily responsible for the methylation reaction. Methylmercury is highly toxic and can accumulate in the food chain. The ionic form can also be reduced to elemental mercury (Hg^0), which is volatile, resulting in removal of mercury from a given environment to the atmosphere. Selenium is an essential element for animals, but is toxic at high concentrations. The microbiology of selenium is very similar to that of sulfur; it can be oxidized and reduced, and can also undergo methylation reactions similar to those of mercury. Methylation of selenium results in decreased toxicity of selenium, however. Arsenic is widely distributed as a contaminant in the environment. Microorganisms have been shown to oxidize and reduce arsenic compounds, and to catalyze the biomethylation of arsenite to volatile methylated forms. Microorganisms also are important in the formation of soil and sediment minerals, serving as nucleation sites for the formation of a variety of minerals. Thus we see that microbially mediated transformations of a large number of elements are important in plant and microbial nutrition, in a variety of environmental concerns, and in biogeochemical processes such as mineral deposition.

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STUDY QUESTIONS

1. Why would tillage of soil result in a more rapid decrease in microbial biomass phosphorus relative to stable organic phosphorus?
2. Describe the importance of phosphorus mineralization to plant nutrition in undisturbed soil ecosystems. Why would this process be of primary importance to plant growth?
3. If a waste material were added to soil that had a C/N/P ratio of 500/20/1, would phosphorus likely be mineralized from the waste or immobilized from the soil solution? Why?
4. Why is a soil pH of approximately 6.5 important for plant and microbial growth?
5. You have measured total organic phosphorus and biomass phosphorus for two soils, one tilled and the other a long-term, no-till cropping system. For biomass determinations, you measured inorganic phosphorus (Pi) in a nonfumigated sample (a), a fumigated sample (b), and a nonfumigated sample extracted with $0.5\ M\ NaHCO_3$ containing KH_2PO_4 to provide 25 μg Pi g^{-1} soil (c). The data are as follows:

Sample	Soil 1	Soil 2
	(μg Pi g^{-1} Soil)	
a	5.8	4.3
b	20.9	7.9
c	25.2	23.0
Total organic P	262.4	212.6