Chapter 18

Microorganisms and Metal Pollutants

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18.1 METALS IN THE ENVIRONMENT

Metals pose a very different pollution problem than organics (see Chapter 17). Metals cannot be degraded through biological, chemical or physical means to an innocuous by-product. More specifically, while the chemical nature of a metal can be changed through oxidation or reduction, the elemental nature of a metal remains the same. Consequently, metals are persistent and more difficult to remove from the environment.

An important concept to define with respect to metals is bioavailability (see Information Box 18.1). A bioavailable metal is one that can be taken up by a microorganism, plant or animal. Bioavailable metal usually consists of the ionic species that can be readily transformed into free ionic species in solution. Given this definition, metals can clearly exist in both bioavailable and unavailable forms in the environment, and it is only the bioavailable portion that can exert toxicity on microbes, plants or animals. As a result, the total metal in a sample does not necessarily reflect the degree of biological metal toxicity, making it difficult to accurately assess the extent of risk posed by metal contamination. Only recently have investigators begun to try to elucidate the ecological significance of bioavailable metal concentrations.

Because of the toxicity and the ubiquity of metals in the environment, microorganisms have developed multiple ways of dealing with both essential and unwanted toxic metals. Levels of essential metals have to be carefully regulated to ensure sufficient supply while avoiding toxicity. This process is often referred to as metal homeostasis, as opposed to resistance. All organisms need to maintain homeostasis of different essential metals such as copper, iron, manganese and zinc to maintain cell functioning. The most common way microorganisms deal with excess metal is to pump the metal ions out of their cells while

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Information Box 18.1 Total versus Soluble Metal

Normally in environmental samples the soluble metal is a small fraction of the total metal. For example, Kong (1998) found that the soluble metal concentration in sediment slurries initially amended with 20 mg/L cadmium, copper or chromium were below detection limits of 0.03–0.04 mg/L. Furthermore, at 100 mg/L added metal, only 1 mg/L cadmium and 0.12 mg/L copper and chromium were found in the aqueous phase. An even more dramatic example is a site that was contaminated with lead-based paint in Camp Navajo, Colorado. The total amount of lead in the soil was 17,520 mg/kg. This can be compared to background levels of 2–200 mg/kg! Extraction of the soil with a 10 mM KNO_3 solution yielded only 7 mg/kg lead. Extraction with a metal chelator (DTPA) yielded 1289 mg/kg, and finally, extraction with mild acid yielded 13,209 mg/kg. This example illustrates first the large difference between total and soluble metal concentrations and, second, how important it is to define the extraction procedure used to determine soluble metal.

simultaneously restricting metal uptake. In addition, some microorganisms have mechanisms to sequester and immobilize metals, whereas others actually enhance metal solubility in the environment. The goal of this chapter is to demonstrate how the presence of metals influences both the degree and type of metal resistance mechanisms expressed, and how microbial resistance, in turn, can influence the fate of metals in the environment. The first part of the chapter introduces metals and their interaction with the physicochemical components of the environment. The remainder of the chapter then focuses on specific metal–microbe interactions including mechanisms of metal resistance, positive and negative effects of metal–microbe interactions, and applications of microorganisms in metal mining and remediation of metal-contaminated sites.

18.2 CAUSE FOR CONCERN

Concern over metal pollution was once primarily related to mining activity and industrial waste. Now reports of metal-related contamination can be found almost daily in the news, including reports on mercury in fish and arsenic in drinking water. The environmental levels of metals in many locations around the world continue to increase, in some cases to toxic levels, due to contributions from a wide variety of industrial and domestic sources. For example, anthropogenic emissions of lead, cadmium, vanadium and zinc exceed those from natural sources by up to 100-fold.

Metal-contaminated environments pose serious health and ecological risks. Metals, such as aluminum, antimony, arsenic, cadmium, lead, mercury and silver, cause adverse effects including heart disease, liver damage, cancer, neurological and cardiovascular disease, central nervous system damage, encephalopathy, hypophosphatemia and sensory disturbances. The problem of mercury pollution came into focus in Minamata Bay, Japan, after the discovery of high levels of methylmercury in fish and shellfish that resulted in thousands of poisonings and hundreds of deaths (Kudo *et al.*, 1998). The mercury contamination originated from a chemical factory that generated small amounts of highly toxic and bioavailable methylmercury during its manufacturing process, which was disposed of into Minamata Bay, and ultimately accumulated in fish. It is also likely that microbial activity in the sediment converted elemental mercury that was disposed of into the bay into methylmercury. Assignment of responsibility and compensation for this tragedy continues to this day.

Lead is a second metal of concern because lead poisoning of children is common and leads to behavioral problems resulting from impaired mental function and even semipermanent brain damage. The Centers for Disease Control (CDC) and the Agency for Toxic Substances and Disease Registry (ATSDR) estimate that 10% of children in the United States have blood lead levels greater than 10 μg/dl, a potentially toxic level. Historians have speculated that the decline of the Roman Empire may have been due in part a decrease in the mental skills of the ruling class as a result of lead poisoning from wine stored in pottery lined with lead and from lead water pipes. Although contamination of drinking water supplies and concentration of metals in edible fish are of particular concern, soils and sediments are the major sinks for metal-containing wastes as the production of domestic and industrial wastes increases.

18.3 METALS DEFINED

There are three classes of metals: metals; metalloids; and heavy metals. Metals, in general, are a class of chemical elements that form lustrous solids that are good conductors of heat and electricity. However, not all metals fit this definition; for example, mercury is a liquid. Elements such as arsenic, boron, germanium and tellurium are generally considered metalloids or semimetals because their properties are intermediate between those of metals and those of nonmetals. Heavy metals are defined in a number of ways based on cationic-hydroxide formation, a specific gravity greater than 5 g/ml, complex formation, hard–soft acids and bases, and, more recently, association with biological and environmental toxicity. This chapter will focus on the metals most commonly associated with metal pollution: arsenic (As); cadmium (Cd); copper (Cu); chromium (Cr); mercury (Hg); lead (Pb); and zinc (Zn). The metals most commonly associated with severe pollution at

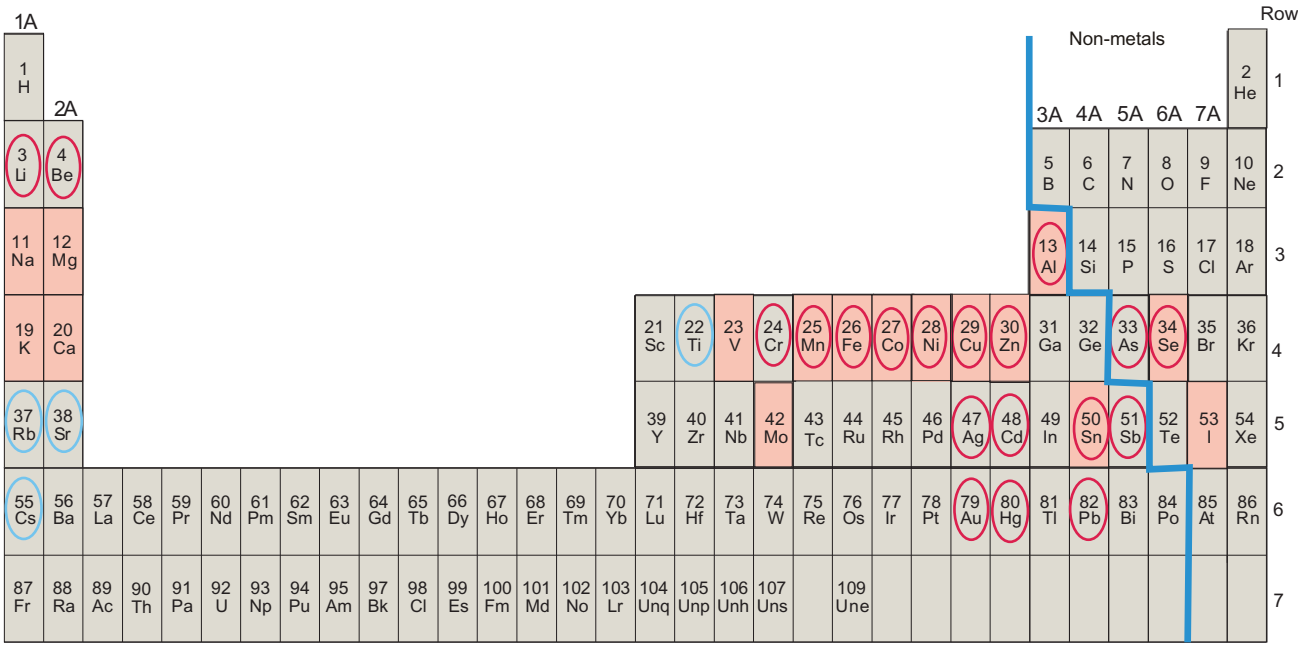


FIGURE 18.1 Periodic table showing the essential metals (pink squares), the toxic metals (circled in red) and the nonessential, nontoxic metals (circled in blue). Essential metals at high concentrations can be toxic; while some limited biological function has been associated with some toxic metals, e.g., chromium and cadmium. Many of the radionuclides, such as uranium (U) and radium (Ra), are thought to be toxic but the associated mechanisms are not well understood.

Superfund sites throughout the United States include arsenic, barium, cadmium, nickel, lead and zinc.

As a result of the complexity of the chemical definitions, metals have also been classified into three additional classes on the basis of their biological functions and effects: (1) the essential metals with known biological functions; (2) the toxic metals and metalloids; and (3) the nonessential metals with no known biological effects.

18.3.1 The Essential Metals

Metals currently known to have essential functions in microorganisms include Al, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Sn, V, W and Zn (Figure 18.1). Chromium is also thought to be essential, although this is still in dispute. Metals such as Na, K, Mg and Ca are required by all organisms. Tungsten (W), on the other hand, appears to be essential only in hyperthermophilic bacteria, such as *Pyrococcus furiosus*, found in hydrothermal vents (Holden and Adams, 2003). Tungstate is thought to replace molybdate in these environments. In general, essential metals are required for enzyme catalysis, molecule transport, protein structure, charge neutralization and the control of osmotic pressure. Required metals are usually transported into the cell via membrane transport systems. It is important to note that although these metals are essential for microbial growth and metabolism, at high concentrations even essential metals can become toxic, e.g., Cu and Se.

18.3.2 The Toxic Metals

Although the toxic metals and metalloids, such as Ag, Al, As, Au, Ge, Cd, Cr, Hg, Pb, Sb and Sn, have been thought to have no known biological function, new data suggest some limited physiological need. For example, while Cd is considered nonessential, marine phytoplankton can functionally substitute cadmium, cobalt and zinc for one another to maintain the activity of enzymes when the needed element is limiting.

Metals are predominantly present as cationic species, and metalloids are predominantly present as anionic species. Radionuclide metals like uranium are also toxic, and are of increasing concern as a result of their enrichment and careless disposal in the past. Metal toxicity can occur in a number of ways including the displacement of essential metals from their normal binding sites on biological molecules (e.g., arsenic and cadmium compete with phosphate and zinc, respectively), inhibition of enzymatic functioning and disruption of nucleic acid structure. Intuitively, one would expect the toxicity of these metals to increase as concentration increases; however, for microorganisms, recent studies have found that, in some cases, higher metal concentrations activate aggressive resistance mechanisms that increase microbial tolerance

to these metals. Overall, the toxicity of a metal depends to a large extent on its speciation, which in turn influences metal bioavailability.

18.3.3 The Nontoxic Nonessential Metals

The nontoxic nonessential metals include Rb, Cs, Sr and Ti. These metals sometimes accumulate in cells as a result of nonspecific sequestration and transport. Cation replacement is the general biological effect; for example, Cs^+ replacement of K^+, but there seems to be no apparent effect on the cell (Avery, 1995). In general, the appearance of nontoxic metals in the cell results from elevated environmental levels of the metals.

18.4 METAL SOURCES

18.4.1 Anthropogenic Sources

Metal pollution results when human activity disrupts normal biogeochemical activities, or results in disposal of concentrated metal wastes. Sometimes a single metal is involved, but more often mixtures of metals are present. Mining; ore refinement; nuclear processing; and the industrial manufacture of batteries, metal alloys, electrical components, paints, preservatives and insecticides are examples of processes that produce metal by-products. Examples of specific metal contaminants include arsenic, copper and zinc salts that have been used extensively as pesticides in agricultural settings; silver salts that are used to treat skin burns; and lead, which is utilized in the production of batteries, cable sheathing, pigments and alloys. Other examples include mercury compounds that are used in electrical equipment, paints, thermometers, and fungicides and as preservatives in pharmaceuticals and cosmetics. Triorganotin compounds, such as tributyltin chloride and triphenyltin chloride, can be used as antifouling agents in marine paints because of their toxicity to plankton and bacteria. The extent of metal pollution becomes even more obvious when one considers the amount of waste generated in metal processing. For example, for every kilogram of copper produced in the United States, 198 kg of copper-laden waste is produced (Debus, 1990).

Thus, while metals are ubiquitous in nature (Table 18.1), human activities have caused metals to accumulate in soil. Such contaminated soils provide a metal sink from which surface waters, groundwaters and the vadose zone can become contaminated. Metal contamination has occurred for centuries since metals have been mined and used extensively throughout human history. Archeological evidence unearthed in Timma, Israel, indicates that mining and smelting of ores has been carried out in Western civilization since at least 4500 B.C.E. (Debus, 1990). Roman lead and zinc mines in Wales are still a source of contamination nearly 2000 years after they were first used. Atmospheric metal concentrations have also increased. Contaminated soil contributes to high metal concentrations in the air through metal volatilization and creation of windborne dust particles. In addition, industrial emissions and smelting activities cause release of substantial amounts of metals into the atmosphere. For example, in 1973, a lead smelter in northern Idaho in the United States released an estimated 27,215 kg of lead per 1.6 km^2 within a 6-month period (Keely *et al.*, 1976).

TABLE 18.1 Typical Background Levels of Metals in Soil and Aquatic Systems

Metal	Fresh water[a] (μm)	Seawater[b] (μm)	Soil[c] (μm)
Gold (Au)	ND[d]	0.0028	ND
Aluminum (Al)	Trace[e]	0.37	2.63×10^7
Arsenic (As)	Trace	0.040	660
Barium (Ba)	ND	0.22	31,623
Cadmium (Cd)	0.00053	0.00098	5.37
Cobalt (Co)	0.012	0.0068	1349
Chromium (Cr)	Trace	0.00096	19,054
Cesium (Cs)	Trace	0.0023	447
Copper (Cu)	0.010	0.047	4.667
Mercury (Hg)	Trace	0.0010	1.48
Manganese (Mn)	0.18	0.036	1.10×10^5
Nickel (Ni)	ND	0.12	6.761
Lead (Pb)	0.00029	0.00014	478
Tin (Sn)	Trace	0.0067	851
Zinc (Zn)	0.30	0.153	7.585

[a]*From Goldman and Horne (1983), Leppard (1981) and Sigg (1985).*
[b]*From Bidwell and Spotte (1985).*
[c]*From Lindsay (1979).*
[d]*ND, no data reported.*
[e]*Trace, levels below detection.*

18.4.2 Natural Sources

Naturally occurring high metal concentrations can also be found as a result of the weathering of parent materials that contain high levels of metals. For example, Stone and Timmer (1975) found a natural copper concentration as high as 10% in surface peat that was filtering copper-rich spring water in New Brunswick, Canada; Forgeron (1971) described a natural surface soil with up to 3% lead and zinc at a site on Baffin Island, Canada; and Warren *et al.*

Information Box 18.2 Arsenic in Drinking Water

Arsenic is naturally widely distributed throughout Earth's crust and arsenic poisoning, particularly from groundwater, affects millions of people worldwide. Arsenic in drinking water can cause bladder, lung and kidney cancer, as well as skin lesions, hyperkeratosis (skin discoloration and thickening) and weakening of the blood vessels leading to gangrene. Concern over arsenic toxicity in the United States resulted in January 2006 in a change in the maximum concentration limit (MCL) from 50 to 10 parts per billion in drinking water. There are severe drinking water crises in some parts of the world including Bangladesh, India, Mexico and several countries in Southeast Asia. In Bangladesh alone, it is estimated that 28–62% of the 125 million inhabitants are exposed to toxic levels of arsenic ranging up to 300 parts per billion or higher (Smith *et al.*, 2000). These exposures are due in large part to the installation of tube wells that were constructed in the 1970s to replace surface water supplies that were often contaminated with pathogens. These wells are up to 200 m in depth and underlie parent media that are naturally high in arsenic. Unfortunately, the water was not tested for arsenic and thus the problem went unnoticed for many years until arsenic toxicity symptoms became increasingly observed in the population. While arsenic-containing water can be safely used for washing and bathing, it is not suitable for drinking or food preparation and thus, for the latter, safe water sources must be identified. This arsenic problem has elevated global interest in processes controlling the fate, mobility and ecotoxicology of arsenic in soil and water.

(1966) reported a mercury concentration of 1–10 mg/kg in soil overlying a cinnabar (HgS) deposit in British Columbia. One metalloid currently receiving attention in countries around the world is arsenic. The concern is contamination of groundwater which serves as the source of drinking water and, in some cases, irrigation water. This contamination is most often from naturally occurring arsenic in the parent minerals that make up the soils and subsurface in these areas (Information Box 18.2). Regardless of the source, metals are of concern because they cannot be degraded and therefore accumulate in the environment, which results in the potential for increased exposure and toxicity over time.

18.5 METAL SOLUBILITY, BIOAVAILABILITY AND SPECIATION

The aqueous phase or soluble metal in a soil is usually a small fraction of the total metal present. Most metal is found:

- An inorganic precipitates (e.g., oxides, carbonates) that are either part of or form surface coatings on the solid mineral phase
- Sorbed to inorganic/organic soil colloids
- Sorbed to or complexed with organic matter that is bound to mineral surfaces

For example, soluble cadmium was measured in two soils that were amended with cadmium nitrate. The first was Brazito sandy loam with 7% total organic carbon (TOC), and a cation exchange capacity (CEC) of 6.8 milliequivalents/100 g. The second was a Gila loam soil with 0.2% TOC and CEC of 15 milliequivalents/100 g. In the Brazito soil, 650 mg/kg of cadmium were required to obtain a soluble concentration of 10 mg/L (1.5% of the metal was soluble). For the Gila soil, 1050 mg/kg were required to obtain a similar soluble concentration (in this case 1% of the metal was soluble) (Maslin and Maier, 2000). In both cases, small amounts of metal were soluble, but the actual solubility was dependent on soil properties such as CEC and TOC.

In addition to total and soluble metal, the term bioavailable metal has been defined. Bioavailable metal is the concentration that can be taken up by plants or microbes. Although the soluble metal in a system can provide an approximation of bioavailable metal, this approximation is not entirely accurate. In some cases, metals that are loosely associated with organic matter, colloidal particles or even mineral surfaces can be accessed by plants or microbes, and thus, while not soluble, are considered bioavailable. Even so, the bioavailable concentration of a metal (like the soluble concentration) is generally very low compared to the total metal present. However, approaches have been developed to measure bioavailable metal (Section 18.8.2). For example, one can measure metal bioavailability in a soil or water sample using a microbial biosensor (Chapter 13)—an organism that responds in a measureable way to the amount of metal it can take up from a given system.

Much of the research on metal solubility and bioavailability has been conducted in soil systems because understanding the fate of metals in soils and sediments is crucial to determining metal effects on biota, metal leaching to groundwater and metal transfer up the food chain (Figure 18.2). The environmental hazards posed by metals are directly linked to their concentrations in the soil solution. High bioavailable metal concentrations in the soil solution result in greater plant uptake and/or leaching of metals. In contrast, metals that are precipitated or complexed in the soil solid phase pose a greatly reduced environmental hazard.

The speciation of a metal involves the identification of its specific forms. This is important because each specific form of a metal may have different solubility, bioavailability and toxicity. A good example of this pertains to the Minamata Bay mercury poisonings described in Section 18.2. The same amount of methylmercury (CH_3Hg^+), dimethylmercury ($(CH_3)_2Hg$) and inorganic

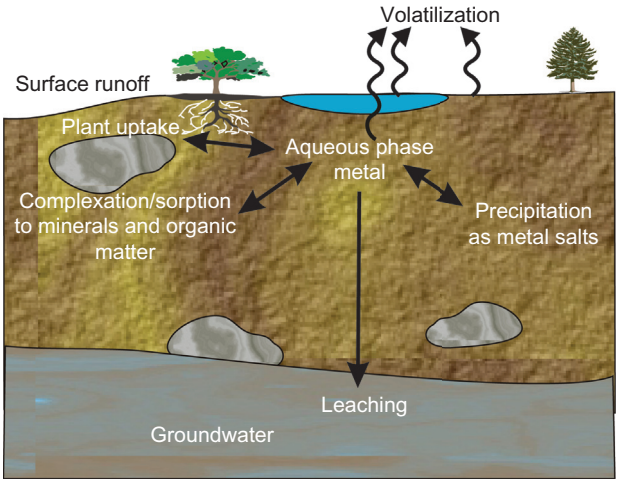


FIGURE 18.2 Potential fates and transformations of metals in the soil environment. The ultimate fate of metals in soil may to be dissolved in groundwater. Metal transformations may also occur at the surface of a soil particle or colloid or in the soil solution.

mercury (Hg^0) in fish tissue exerts very different toxicities to a human consuming the fish since the toxicity of $(CH_3)_2Hg > CH_3Hg^+ > Hg^0$ (Figure 18.3). In fact, a tragic event unfolded in 1997, when an internationally respected professor of chemistry at Dartmouth College (New Hampshire, U.S.) died following exposure to one drop of dimethylmercury that fell on her gloved hand.

Several abiotic and biotic factors can affect the chemical speciation and bioavailability of metals in the environment. These factors (discussed below) include metal chemistry, sorption to clay minerals and organic matter, pH, redox potential and the microorganisms present, e.g., some solubilize metals while others precipitate metals. All of these factors interact to influence metal speciation, solubility, bioavailability and the overall toxicity of metals in the environment. Thus, it must be emphasized that determination of the total concentration of a metal in a soil is not enough to predict toxicity in biological systems. It is the bioavailable amount that is most important. As such, the biosensors described above as well as environmental biological indicators or biomarkers for metal toxicity are being developed and studied. Indicators or biomarkers are populations that can be either microbial or arthropod, whose presence or absence provides information on the toxicity of metals in an environmental sample. It is hoped that such biomarkers will be of use in predicting the effects of metal bioavailability on environmental quality.

18.5.1 Metal Chemistry

Whether a metal is cationic or anionic in nature helps determine its fate and bioavailability in an environment. Most metals are cationic which means they exhibit a positive charge when in their free ionic state, and are most reactive with negatively charged surfaces. Thus, in soil, cationic metals such as Pb^{2+} or Ca^{2+} strongly interact with the negative charges on clay minerals and with anionic salts, such as phosphates and sulfates. Positively charged metals are additionally attracted to negatively charged functional groups such as hydroxides and thiols on humic residues. Unfortunately, cationic metals are also attracted to negatively charged cell surfaces where they can be taken up and cause toxicity. Cationic metals sorb to both soil particles and cell surfaces with varying strengths or adsorption affinities. For example, of the common soil cations, aluminum binds more strongly than calcium or magnesium:

$$Al^{3+} > Ca^{2+} = Mg^{2+} > K^+ > Na^+$$

As this affinity series shows, the size and charge of the cationic metal helps to determine the strength of adsorption. When in excess, metal cations will compete for the limited number of cation exchange sites present in a soil with the larger multivalent cations replacing smaller monovalent cations such as Na^+ (see Figure 4.11). For example, Al^{3+} has such strong affinity for clay surfaces that it is primarily found as $Al(OH)_3$ which has extremely low bioavailability. Since many of the toxic metals are large, divalent cations, they have high adsorption affinities, and thus are not readily exchanged for common soil cations such as Na^+, K^+ or Ca^{2+}. Components in the soil solution also affect metal solubility. Specifically, phosphates, sulfates and carbonates in the soil solution form sparingly soluble metal-salt compounds.

Negatively charged or anionic metals, such as AsO_4^{3-} (arsenate), are attracted to positively charged surfaces. In soils, anions can be sorbed to negatively charged clays by divalent cation bridging, using cations such as Ca^{2+} or Mg^{2+}. In summary, the mobility of a metal in the environment is strongly influenced by the nature and intensity of its charge.

18.5.2 Cation Exchange Capacity

One of the most important factors affecting metal bioavailability is the soil cation exchange capacity (CEC), which is dependent on both the organic matter and clay content of the soil (see Section 4.2.2.4). Cation exchange reflects the capacity for a soil to sorb metals. Thus, the toxicity of metals in soils with high CEC (organic and clay soils) is often low even at high total metal concentrations. In contrast, sandy soils with low CEC, and therefore low metal binding capacity, show decreased microbial activity at comparatively low total metal concentrations, indicative of higher metal bioavailability.

Clay minerals provide a wide range of negative adsorption sites for cationic metals. These include planar sites associated with isomorphous replacements, edge sites derived from partly dissociated Si−OH groups at the

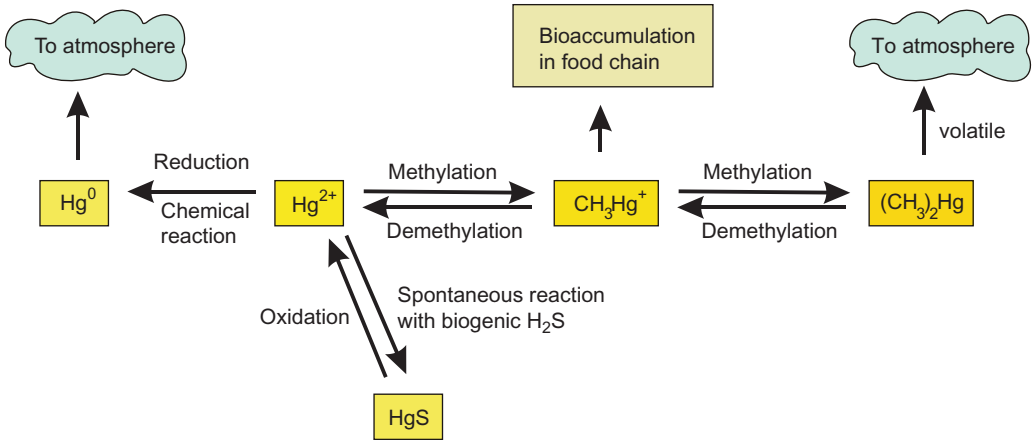


FIGURE 18.3 Microbially-mediated reactions with Hg^{2+} in the environment. Hg^{2+} can be reduced to elemental Hg^0 by chemical reaction with humic acids or by microbially-mediated reactions which are believed to be a detoxification mechanism. Hg^{2+} can be precipitated by reaction with H_2S produced under sulfate-reducing conditions but can also be released by microbial oxidation of HgS. Methylation of Hg^{2+} produces organometals, which can accumulate in the tissue of living organisms. The production of organometals may to some extent be balanced by demethylation reactions occurring in both aerobic and anaerobic environments. Based on Gadd (1993) and Ehrlich (1996).

edges of clay minerals, and interlayer sites located between clay platelets. Permanently charged sites on clay minerals interact with metallic ions by means of nonspecific electrostatic forces. Metallic oxides and hydrated metal oxides offer surface sites for the sorption of metals. Iron, aluminum and manganese oxides are an important group of minerals that form colloidal size particles, which, in the presence of water, assume various hydrated forms able to strongly retain most metals in the soil. Soil organic matter contains both humic and nonhumic substances such as carbohydrates, proteins and nucleic acids, which are normally quickly degraded, as well as less readily degraded substances including lignin, cellulose and hemicellulose. The nonhumic organics in soil are relatively short-lived, and have little influence on the long-term fate of metals.

Humic substances are relatively stable and patchily coat the particle surfaces of natural soils. Humic substances contain a variety of organic functional groups that are able to interact with metals. These functional groups include carboxyl, carbonyl, phenyl, hydroxyl, amino, imidazole, sulfhydryl and sulfonic groups. Metals complexed with humic substances are generally not bioavailable, and therefore less toxic to biological systems.

18.5.3 Redox Potential

Metal bioavailability changes in response to changing redox conditions. Under oxidizing or aerobic conditions (+800 to 0 mV), many metals are often found as soluble cationic forms, e.g., Cu^{2+}, Cd^{2+}, Fe^{3+}, Pb^{2+} and Ca^{2+}. In contrast, reduced or anaerobic conditions (0 to −400 mV), such as those commonly found in sediments or saturated soils, often result in metal precipitation. For example, in areas rich in sulfur and sulfate-reducing bacteria, the sulfide that is generated is available to form nontoxic, insoluble sulfide deposits, e.g., CuS and PbS. As another example, in soils rich in carbonates, metals are precipitated as metal carbonates, e.g., $CdCO_3$. Conversely, reducing conditions generally increase the bioavailability of arsenic by stimulating microbial reduction and dissolution of iron oxides (with which arsenate is often associated) thus releasing previously bound arsenate. Under reducing conditions, the arsenate can also be microbially reduced to arsenite, which is more soluble and bioavailable (Information Box 18.3).

18.5.4 pH

For cationic metals, the pH of a system can have an appreciable effect on metal solubility, and, hence, metal bioavailability. At high pH, metals are predominantly found as insoluble metal mineral phosphates and carbonates, while at low pH they are more commonly found as free ionic species or as soluble organometals. The pH of a system also affects metal sorption to soil surfaces. The effect of pH on metal sorption is principally the result of changes in the net charge on soil and organic particles. As the pH increases, the electrostatic attraction between a metal and soil constituents is enhanced by increased pH-dependent CEC. This is in addition to the decrease in metal solubility that occurs as pH is increased. Accordingly, the net effect of increased soil pH is to decrease metal bioavailability. In contrast, as soil pH decreases, metal solubility increases while pH-dependent charge decreases, and metal bioavailability is increased.

Information Box 18.3 Impact of Soil Microorganisms on Arsenic in Rice

When grown in soil containing arsenic, rice tends to accumulate higher concentrations of arsenic in comparison to most other food crops. This is due to the continuous flooding practices commonly used in rice cultivation. Under aerobic conditions, most of the arsenic in soil occurs as arsenate (bound to iron oxides) and has limited bioavailability. Once flooded, the soil becomes anaerobic with iron-reducing bacteria reducing and dissolving the iron oxides, thus releasing the arsenate which is also microbially reduced to arsenite. In addition, other soil microorganisms can convert the arsenic to methylated arsenic species. The reduced and methylated arsenic species are much more bioavailable and are accumulated by rice. One management practice that has promise for decreasing rice uptake of arsenic is the use of intermittent flooding, and other less water-intensive management systems, that maintain the soil at a higher redox potential, thus decreasing arsenic bioavailability. In fact, studies have shown that these practices can decrease rice grain arsenic concentrations by over 40% compared to traditional, continuous flooding practices (Somenahally *et al.*, 2011). These systems also have the potential benefits of requiring less water for rice production and generating lower amounts of greenhouse gases such as methane. Research is ongoing to determine the optimal water management systems for maintaining high rice yields yet minimizing these negative aspects of production.

Metalloids such as chromium, arsenic and selenium are usually found in the oxyanion form, e.g., chromate (CrO_4^{2-}), arsenate (AsO_4^{3-}) and selenate (SeO_4^{2-}). In a soil with low pH, these anionic species may become increasingly sorbed as the overall positive charge on soil particles increases. As the pH increases, the fate of these anionic species becomes highly dependent on other environmental factors including redox and metal speciation.

The influence of pH, redox and organic and inorganic minerals on the chemical form and nature of metals demonstrates how important and difficult it is to clearly define metal speciation and bioavailability. Metal bioavailability is further complicated by the complexity of microbiological interactions with metals.

18.6 METAL TOXICITY EFFECTS ON THE MICROBIAL CELL

Due to ionic interactions, metals bind to many cellular ligands and displace essential metals from their normal binding sites (Figure 18.4). For example, cadmium can replace zinc in a cell. Metals also disrupt proteins by binding to sulfhydryl groups, and nucleic acids by binding to phosphate or hydroxyl groups. As a result, protein and DNA conformation are changed and function is disrupted. For example, cadmium often outcompetes catalytic zinc within enzymes, rendering the enzyme inactive, and also nonspecifically binds to DNA, inducing single-strand breaks. Metals may also affect oxidative phosphorylation and membrane permeability, as seen with vanadate and mercury. Metals, such as copper and iron, can exist in different redox states and may generate reactive oxygen species that damage proteins and nucleic acids. Microorganisms often use specific transport pathways to bring essential metals across the cell membrane into the cytoplasm. Unfortunately, toxic metals can also cross membranes, via diffusion, nonspecific uptake systems, or pathways designed for other metals. For instance, Cd^{2+} uptake occurs via the active uptake system for Mn^{2+} in many bacteria. Figure 18.5 illustrates various mechanisms used to transport metals into the cell.

These metal−microbe interactions result in decreased growth, abnormal morphological changes and inhibition of biochemical processes in individual cells. The toxic effects of metals can also be seen at the community level. In response to metal toxicity, overall community numbers and diversity can both decrease. However, few studies have addressed community resistance. While individual microbial populations may be quite metal resistant, how do microbial populations interact with each other when toxic concentrations of metals are present? Further, is it possible for metal-resistant populations to interact in such a way as to confer resistance on a consortium of organisms? Likewise, are there symbiotic relationships between metal-resistant and metal-sensitive populations such that the metal-sensitive organism receives protection from metal toxicity while providing the metal-resistant organism with some essential nutrient or carbon source? The answers to these questions are currently being sought through research of microbial metal resistance.

18.7 MECHANISMS OF MICROBIAL METAL RESISTANCE AND DETOXIFICATION

Microorganisms are believed to have evolved metal resistance because of their exposure to toxic metals on early Earth (Information Box 18.4). Additional development of metal resistance in response to recent exposure to metal pollution over the past 50 years has also been observed. Anthropogenic contamination of the environment with metals has motivated the need for research concerning microbial metal toxicity and resistance, to better understand the fate of metals in the environment, and to develop new remediation techniques for metal-contaminated sites. Microorganisms directly influence the fate of metals in the environment, and so may provide the key to decreasing current contamination.

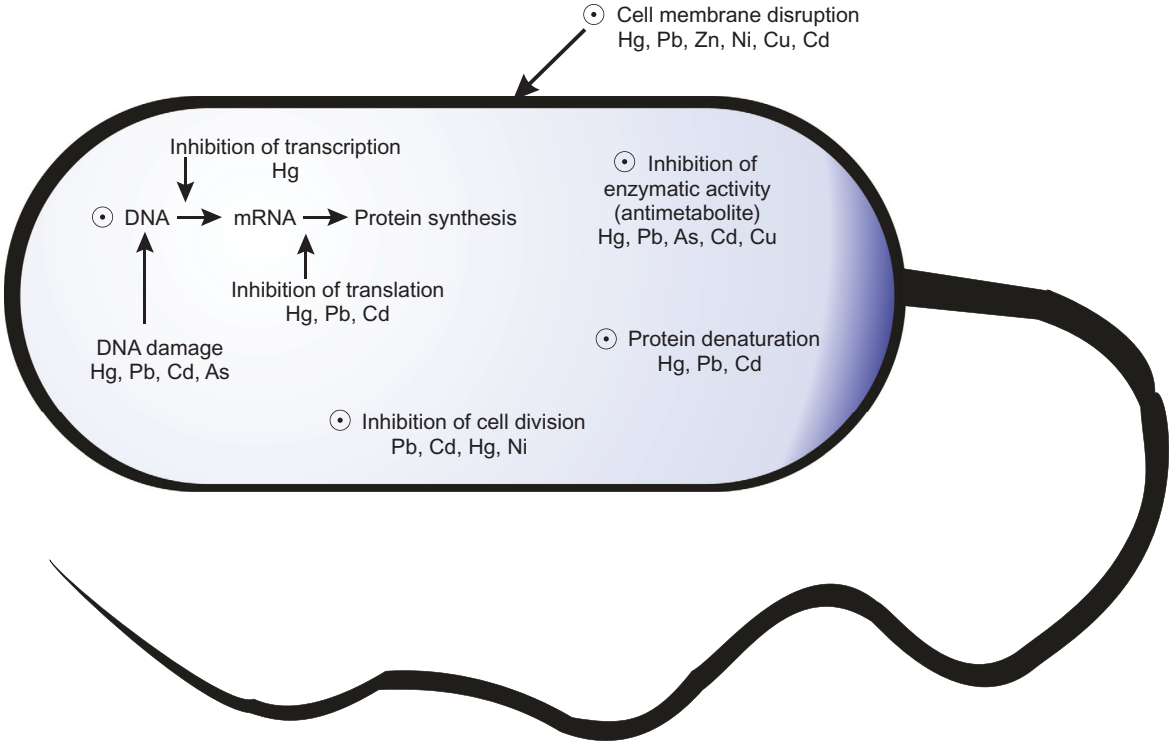


FIGURE 18.4 Summary of the various toxic influences of metals on the microbial cell, demonstrating the ubiquity of metal toxicity. Metal toxicity generally inhibits cell division and metabolism. As a result of this ubiquity, microorganisms have to develop "global" mechanisms of resistance that protect the entire cell from metal toxicity.

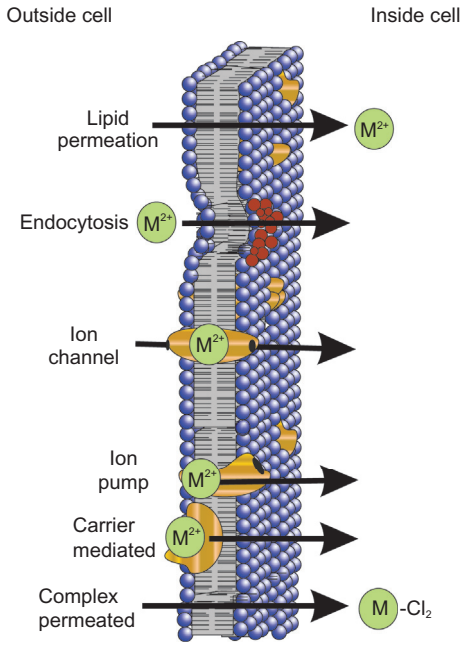


FIGURE 18.5 Mechanisms of metal (M) flux across the microbial cell membrane. Adapted from Simkiss and Taylor (1989).

Microorganisms have evolved ingenious mechanisms of metal resistance and detoxification in response to metals in the environment (Figure 18.6). Many of the resistance determinants are encoded on the chromosome, but some are encoded on mobile genetic elements such as plasmids and transposons. Microbial metal resistance may be divided into three categories. These include:

- General resistance mechanisms that do not require metal stress
- General resistance mechanisms that are activated by metal stress
- Resistance mechanisms that are dependent on a specific metal for activation

General mechanisms of metal resistance often serve other functions. For example, slime layer production, while effectively providing a barrier against metal entry into the cell, also serves in surface adhesion and protection against desiccation and predation. The sole purpose of metal-dependent mechanisms, both specific and general, is cell protection from metal toxicity.

18.7.1 General Mechanisms of Metal Resistance

Binding of metals to extracellular materials immobilizes the metal and prevents its entry into the cell. Metal binding to anionic functional groups on cell surfaces occurs with a large number of cationic metals, including cadmium, lead, zinc and iron. For example, sulfhydryl,

carboxyl, hydroxyl, sulfonate, amine, amide and phosphonate groups are examples of functional groups that strongly bind to metals. Binding of metals by microbial cells is important ecologically since the binding of metals by cell surfaces plays a dominant role in the distribution of metals, especially in aquatic environments. In practical terms, the ability of cells to sorb metals has been developed into a technology used to remove metals from contaminated waste streams.

Extracellular binding usually occurs on slime layers or exopolymers composed of carbohydrates, polysaccharides and sometimes nucleic and fatty acids (Schiewer and Volesky, 2000). These exopolymers or extracellular polymeric substances (EPS) are common in natural environments and provide microbial protection against desiccation, phagocytosis and parasitism. Microbial exopolymers are particularly efficient in binding heavy metals, such as lead, cadmium and uranium. Exopolymer functional groups are generally negatively charged, and consequently, the efficiency of metal:exopolymer binding is pH dependent. Metal detoxification through EPS production results in metal immobilization and prevention of metal entry into the cell. For example, the immobilization of lead by exopolymers has been demonstrated in several bacterial genera, including *Staphylococcus aureus*, *Micrococcus luteus* and *Azotobacter* spp. In fact, extracellular polymeric metal binding is a common resistance mechanism against lead.

A second extracellular molecule produced microbially that complexes metals is the siderophore. Siderophores are iron-complexing, low-molecular-weight organic compounds. Their biological function is to harvest iron in environments where concentration is low, and to facilitate its transport into the cell. Siderophores may interact with other metals that have chemistry similar to that of iron, such as aluminum, gallium and chromium (which form trivalent ions similar in size to iron). By binding metals, siderophores can reduce metal bioavailability and thereby metal toxicity. For example, siderophore complexation reduces copper toxicity in cyanobacteria.

Information Box 18.4 Evolution of Metal Resistance

A question plaguing environmental microbiologists is how do microorganisms become metal resistant? Earth is estimated to be 4.7 billion years old and microbial life is thought to have appeared approximately 4 billion years ago. Since microorganisms were the first life forms on Earth, early resistance mechanisms developed in response to the toxic metals that existed early on in Earth when life began. Genetic sequencing of hundreds of microbial genomes provides evidence supporting the early development of metal resistance. However, another possible scenario is that microorganisms have recently developed metal resistance in response to increasing anthropogenic pollution with various metals. Metal concentrations in the atmosphere, in surface soils and in both surface and groundwaters have increased with industrialization. On average, a mutation occurs once in every 10^6 base pairs, so microorganisms have the capacity to rapidly respond to metal-contaminated environments and evolve metal resistance. The answer to the origins of microbial metal resistance is probably a combination of both early and recent exposure to toxic metals. Future study of the physiological and the genetic diversity in microbial metal resistance mechanisms will provide intriguing insights into how microorganisms adapt to and maintain responses to environmental pressures.

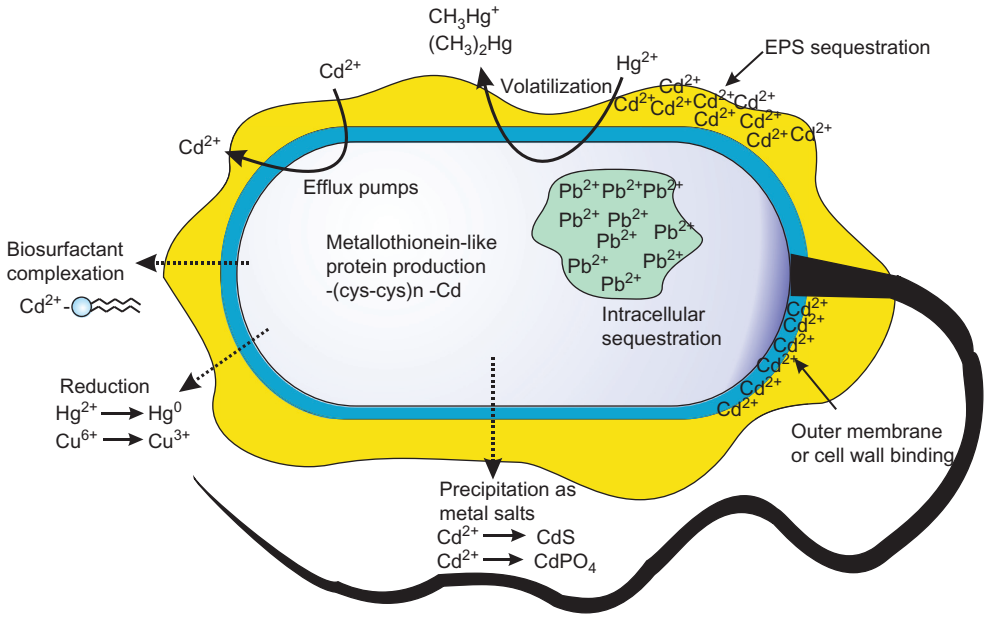


FIGURE 18.6 In response to metal toxicity, microorganisms use a variety of mechanisms to resist and detoxify harmful metals. These mechanisms of resistance may be intracellular or extracellular and may be specific to a particular metal, or a general mechanism able to interact with a variety of metals.

Biosurfactants are a class of compounds produced by many microorganisms that in many cases are excreted from the cell. Biosurfactants have been investigated for their ability to complex metals such as cadmium, lead and zinc (Maier and Soberon-Chavez, 2000). Biosurfactant complexation can actually increase the apparent solubility of metals; however, the biosurfactant-complexed metal is not toxic to cells. It is not yet clear whether biosurfactants are produced specifically to reduce metal toxicity. However, evidence shows that biosurfactant-producing microorganisms can be isolated in greater diversity from metal-contaminated environments than from uncontaminated ones. More information on biosurfactants is available in Chapter 17.

Finally, metal bioavailability can be influenced by common metabolic by-products that result in metal reduction. In this case, soluble metals are reduced to less-soluble metal salts, including sulfide and phosphate precipitates. For example, under aerobic conditions *Citrobacter* spp. can enzymatically produce phosphate, which results in the precipitation of lead and copper. Under anaerobic conditions, high H_2S concentrations from sulfate-reducing bacteria, such as *Desulfovibrio* spp., readily cause metal precipitation as metal sulfides.

18.7.2 Metal-Dependent Mechanisms of Resistance

Free-living, nonsymbiotic microorganisms use energy-dependent metal efflux systems to remove metals from the cell. These mechanisms effectively pump toxic ions that have entered the cell back out of the cell via ATPase pumps or chemiosmotic ion/proton pumps. Arsenic resistance in a number of bacteria involves the enzymatic reduction of arsenate to arsenite followed by arsenite efflux, either by a transporter protein called ArsB or by an unrelated ACR3 transporter. Arsenate reduction and subsequent arsenite efflux are common in archaea, bacteria and fungi.

There are also some plasmid-encoded genes that can confer even higher (and more complex) levels of resistance (Figure 18.7). For example, plasmid R773 contains genes encoding the regulator ArsR, the transporter ArsB, the arsenate reductase ArsC, the ATPase ArsA and an arsenite chaperone ArsD. Arsenate enters the cell through proteins involved in phosphate-specific transport (Pst) with initial binding by the phosphate binding protein PhoS. The reduction of arsenate to arsenite is then mediated by the ArsC enzyme, an NADPH-dependent cytoplasmic protein (Mukhopadhyay *et al.*, 2002). Finally, together, the ArsA and ArsB proteins form a complex that uses ATP for the active efflux of arsenite from the cell. This complex can confer much higher levels of resistance than ArsB alone.

Another well-studied example of efflux-based resistance is cadmium resistance (Figure 18.8). The toxicity of Cd is primarily due to its binding to sulfhydryl groups of proteins and causing single-stranded DNA breaks. Several cadmium-resistant organisms have been studied, including what was then known as *Alcaligenes eutrophus*, and *Bacillus subtilis*, *Escherichia coli*, *Listeria* spp., *Pseudomonas putida*, *Staphylococcus aureus*, some cyanobacteria, fungi and algae. Most microorganisms possess a chromosomally encoded P-type ATPase termed CadA or ZntA, which pumps Cd^{2+} out of the cytoplasm using ATP hydrolysis as an energy source. Most of these pumps can also transport other cations such as Pb^{2+} and Zn^{2+}. In addition to these P-type ATPases, there are systems in Gram-negative bacteria responsible for transport of Cd^{2+} and other metals from the periplasm across the outer membrane. The best-studied system includes the *czc* genes from *Cupriavidus metallidurans* CH34, where *czc* stands for cobalt, zinc and cadmium resistance (Nies, 2003).

Either in place of or in addition to metal efflux, bacteria can use intracellular metal resistance mechanisms. Possibly the best-known mechanism involves metal binding or sequestration by metallothioneins or similar proteins. Primarily documented in higher microorganisms, plants, algae, yeast and some fungi, metallothioneins are low-molecular-weight, cysteine-rich proteins with a high affinity for cadmium, zinc, copper, silver and mercury metals. Their production is induced by the presence of metals, and their primary function is metal detoxification. Metallothioneins are being found in an increasing number of microorganisms, including bacteria. Metal binding by metallothioneins can result in cellular accumulations visible as electron dense areas within the cell matrix. Suspected deposits are confirmed using electron dispersive spectroscopy that can identify the metal.

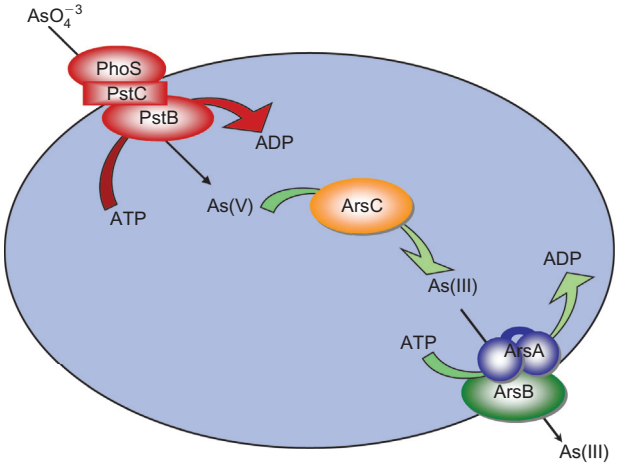


FIGURE 18.7 Schematic of arsenic resistance demonstrating both the influx and efflux systems for arsenic. Arsenate enters the cell via a phosphate-specific transport pathway. Once in the cell, arsenate is reduced to arsenite, and an efflux mechanism then pumps arsenite out of the cell via an anion pump that is fueled by ATP. Note that arsenic is not detoxified by this mechanism because arsenite can still be toxic.

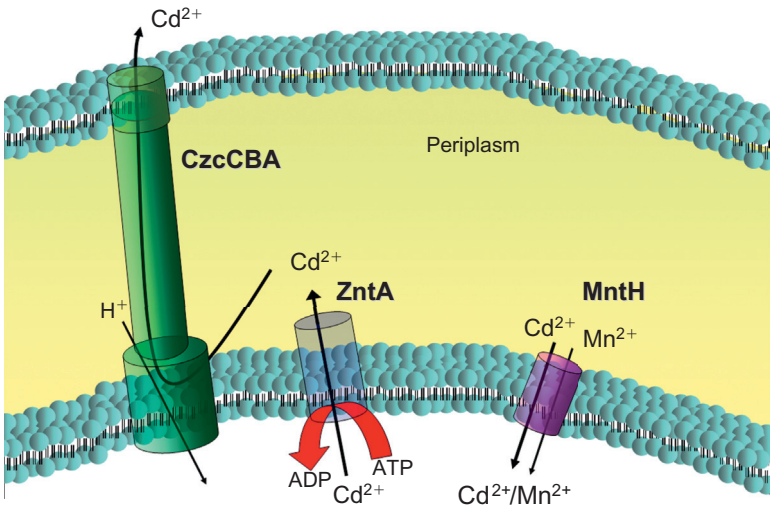


FIGURE 18.8 Cadmium influx and efflux in a Gram-negative bacterial cell. Cadmium crosses the cytoplasmic membrane via a manganese transport pathway (MntH), which relies on membrane potential (see purple structure). Cadmium can also enter the cell via the zinc transporter ZntA (see red arrow). The cadmium efflux system (CzcCBA) excretes cadmium via a $Cd^{2+}/2H^{+}$ antiport protein (see green structure).

The methylation of metals is considered to be a metal-dependent mechanism of resistance because only some metals are methylated. Methylation involving the addition of methyl or ethyl groups (e.g., conversion of Hg^{2+} to CH_3Hg^{+}) increases metal volatility, and can increase metal toxicity as a result of increased lipophilicity, thus increasing permeation across cell membranes. However, methylation of some metals, such as selenium, decreases their toxicity. Methylation has also been observed with arsenic, lead and tin. Methylation facilitates metal diffusion away from the cell, and in this way effectively decreases overall metal toxicity. In this manner, methylation has been known to remove significant amounts of metal from contaminated surface waters, sewage and soils.

Mercury is unusual in that it can additionally be volatilized through reduction. Mercury resistance may involve the enzymatic reduction of Hg^{2+} to elemental mercury (Hg^{0}) in both Gram-positive and Gram-negative bacteria. Often plasmid mediated, two additional pathways of mercury resistance involve the detoxification of organomercurial compounds via cleavage of C−Hg bonds by an organomercurial lyase (MerB), followed by reduction of Hg^{2+} to Hg^{0} by a flavin adenine dinucleotide (FAD)-containing, NADPH-dependent mercuric reductase (MerA). Specific to inorganic mercury, the MerP protein in the periplasmic space shuttles Hg^{2+} to the membrane-bound MerT protein, which releases Hg^{2+} to the cytoplasm. Once in the cytoplasm, Hg^{2+} is reduced to Hg^{0} by mercuric reductase (Figure 18.9).

18.8 METHODS FOR STUDYING METAL−MICROBIAL INTERACTIONS

Unique considerations apply when studying metal−microorganism interactions. Metal concentrations on a macroscale poorly reflect the toxic influences of metals at the microscale. Recall that total metal concentrations do not accurately assess the biologically toxic concentration. Because metals are not biodegradable, it is difficult to determine whether and how a metal is being detoxified when the total metal concentration does not change. However, new and exciting technical and analytical developments in metal chemistry, microbiology and molecular biology are now making it possible to expand our understanding of how microorganisms influence metal fates in the environment. A few of these approaches are highlighted here.

18.8.1 Culture Medium

The culturing of metal-resistant microorganisms in the laboratory often occurs in either nutrient-rich or chemically defined media, which may contain yeast extract, phosphate buffers and amino acids that bind metal ions. A neutral medium pH is an additional factor that may increase metal binding in culture media. The presence and amount of these reagents strongly influence metal bioavailability, thereby influencing metal toxicity to microorganisms. Thus, depending on the growth medium, metal toxicity will vary. For example, it has been shown that the alga *Chlamydomonas reinhardtii* accumulates more metal (Cd^{2+} or Cu^{2+}) and shows less metal toxicity when grown on a medium containing high levels of phosphate (Wang and Dei, 2006). Similarly, for the bacterium *Comamonas testosteroni*, cadmium toxicity followed a dose-dependent pattern in minimal chemically defined media, but was not dose dependent in an organically rich medium (Hoffman *et al.*, 2005). Consequently, several factors need to be taken into consideration when choosing a culture medium to assess microbial metal resistance. Most importantly, medium components must be defined and chosen in such a way as to minimize metal binding. This applies to both

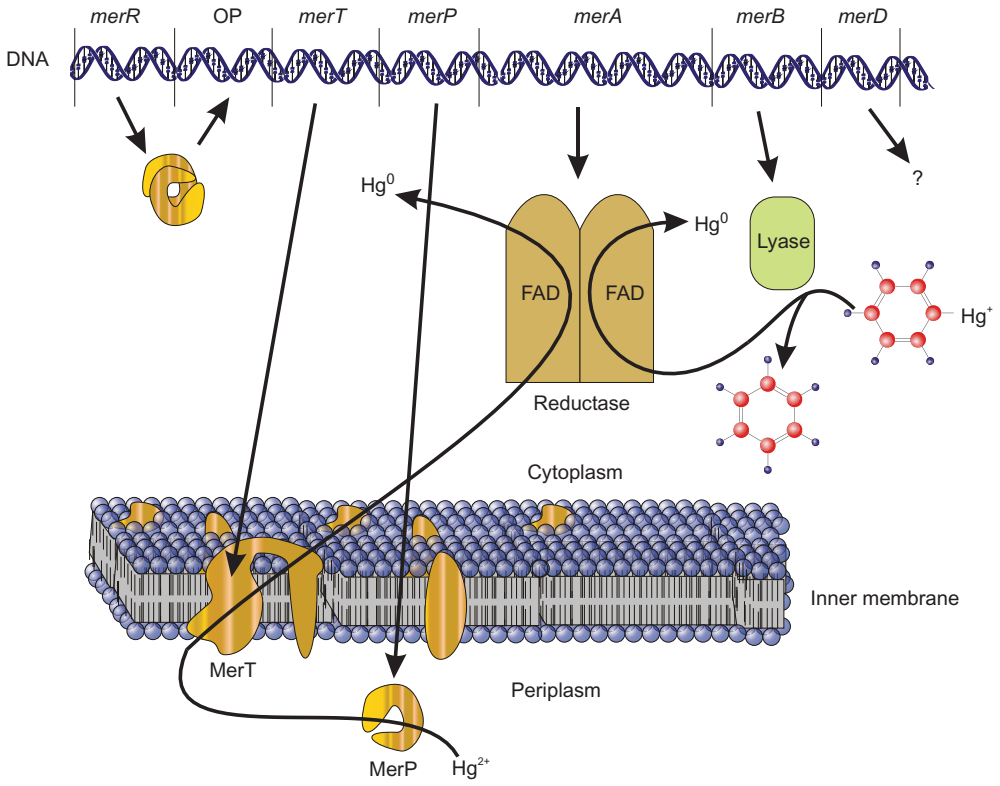


FIGURE 18.9 Proposed model for bacterial mercury resistance encoded by the *mer* operon. Adapted with permission from Silver *et al.* (1986).

carbon substrates and to buffers. For example, phosphate buffers strongly precipitate metals. Recall that phosphate production in some microorganisms confers protection from certain metals. Nonmetal-binding buffers, including the sulfonic acids such as MES [2-(*N*-morpholino)ethanesulfonic acid; $C_6H_{13}NO_4SH_2O$], p*K*a = 6.15, and PIPES (1,4-piperazinediethanesulfonic acid; $C_8H_{18}N_2O_6S_2$), p*K*a = 6.80, optimize metal bioavailability in culture media (although even these buffers can alter the toxicity of metals). Finally, pH strongly influences metal bioavailability. Metals readily precipitate as carbonic salts at pH >7.0. Therefore, the pH should be kept slightly acidic ($\approx$ pH 6.0) to maintain metal solubility.

18.8.2 Measurement of Total, Soluble and Bioavailable Metal

To experimentally determine the relationship between metal bioavailability and toxicity, and to determine the rate and extent that microorganisms sequester metals, one must be able to determine both total and soluble metal concentrations. Total metal concentration is determined by digestion of the sample with acids such as nitric or perchloric acid. This process dissolves soil particles and releases even tightly bound metals. Soluble metal determination often involves extraction with the weak acid DTPA (diethylenetriamine pentaacetic acid), or extraction with deionized water to release loosely bound, readily exchangeable metals. In either case, metal concentrations in the extract are determined using atomic absorption spectroscopy or inductively coupled plasma atomic emission spectroscopy.

When studying metal resistance, it is assumed that a decrease in the soluble metal concentration corresponds to the amount of metal sequestered by the cell. However, care must be taken in interpreting these data since metals often precipitate with culture medium components. In addition, metals may bind to the walls of flasks and test tubes. Controls with no inoculum are therefore crucial in distinguishing between biological and chemical metal removal. It should be noted that while it is relatively easy to determine a macroscale estimate of bioavailability in the environment, such an estimate does not necessarily reflect microscale metal concentrations. So even if very low soluble levels of metal are measured, it is likely that in some micropores (where most soil microorganisms live) substantial levels of bioavailable metal may be encountered. This would explain why microorganisms in metal-contaminated environments with no detectable soluble metal may exhibit extreme resistance.

Flame or flameless atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICPAES) and inductively coupled plasma mass spectroscopy (ICPMS) are efficient techniques for the determination of metal ions in solution. For AAS, metal is determined by aspirating a metal solution into an air–acetylene

flame to atomize the metal. A metal-specific lamp is placed into the AAS and is used to determine the difference in light absorbance between a reference source and the metal solution. This difference reflects the amount of metal present. ICPAES determination is based on light emitted from metal atom electrons in the excited state. An argon plasma is used to produce the excited state atoms. Matrix interference can be a significant problem with these techniques, and some samples need to be acid digested before analysis. Spectroscopy can be used for any metal; however, detection limits vary for each metal. For example, for AAS, the limit of detection is 1 μg/L (8.9×10^{-3} μM) for cadmium and 700 μg/L (2.9 μM) for uranium. For comparison, the detection limits for ICPAES are 1 μg/L and 75 μg/L (0.32 μM) for cadmium and uranium, respectively.

A newer technique is ICPMS, which can speciate as well as quantify metals. The ability to speciate metals yields valuable information regarding metal transformations and ratios of toxic to nontoxic metals important in risk management and environmental assessment. ICPMS detects an element's unique mass-to-charge ratio through ionization in an argon plasma. The use of ICPMS is increasing due to its greater sensitivity (parts per trillion level) than either AAS or ICPAES.

Ion-selective electrodes (ISEs), which are available for some metals such as cadmium, lead and arsenic, provide a quick way to determine metal concentrations in solution to approximately 10^{-6} M. The electrodes are easy to use, relatively inexpensive and are not strongly influenced by sample color or turbidity. They are, however, influenced by the presence of other ions in solution. ISEs are different from AAS and ICPAES in that they allow the determination of extracellular free metal ion in solution following cellular interactions. In this case, the ISE will only measure free metal ions, and does not measure complexed metal even if the complexed metal is soluble. However, this can be considered an advantage since the ISE measures only metal that would likely be bioavailable.

One of the only methods available to truly measure metal bioavailability is through the use of microbial biosensors (see also Chapter 13). These biosensors have been developed to report bioavailable metal concentration in environmental samples. The biosensor is created using recombinant DNA technology (see Chapter 13) to construct a plasmid in which a strictly regulated promoter is connected to a sensitive reporter gene. The best studied example is the mercury resistance (*mer*) operon which causes the reduction of Hg^{2+} to Hg^0 (by the *merA* gene product, the mercuric reductase) and degradation of methylmercury (by the *merB* gene product, the organomercurial lyase). This is beneficial because it reduces the toxicity of mercury to the bacterial cell (Figure 18.9). The *mer* promoter is activated when Hg^{2+} binds to the regulatory protein MerR. Indicator bacteria that contain gene fusions between the promoter of the *mer* operon and a reporter gene (such as luminescence) are able to detect Hg^{2+} (Selifnova *et al.*, 1993). This promoter–reporter gene concept has also been used with other metals including arsenic, cadmium, zinc, lead, lead ions and also xenobiotic compounds (Rensing and Maier, 2003).

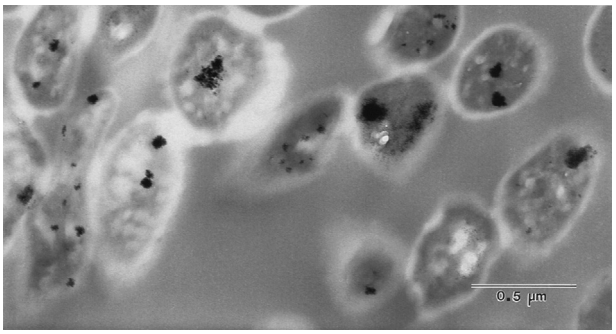


FIGURE 18.10 Transmission electron micrograph of a bacillus exhibiting an intracellular accumulation (dark material) of lead in response to the production of a metallothionein-like protein. Courtesy T.M. Roane.

Whereas the methods discussed so far can detect the presence of a metal, transmission electron microscopy (TEM) provides an effective means of locating and visualizing suspected metal deposits associated with microorganisms (Figure 18.10). This technique is particularly useful for determining whether microorganisms sequester metals inside or outside of the cell. When TEM is coupled with energy-dispersive X-ray spectroscopy (EDS), the metal element can be identified. Metals emit characteristic X-rays as the electron beam interacts with deposits. Elements can be identified by signature spectral lines (Figure 18.11). High- and low-magnification TEM micrographs can help distinguish between intracellular and extracellular metal interactions, depending on the location of metal deposition.

Newer X-ray-based techniques, such as X-ray absorption spectroscopy (XAS) and X-ray fluorescence (XRF) are gaining popularity due to their ability to detect and even speciate metals *in situ* within samples. One of the most used absorption-based approaches is X-ray absorption near-edge structure (XANES) spectroscopy. This method uses an X-ray source, such as a synchrotron, to excite and shift the target's photoabsorption cross-section thus revealing elemental composition and valence states. In contrast, the fluorescence-based approaches, such as micro X-ray fluorescence (μXRF) and related imaging and spectroscopy approaches, instead use the emission following X-ray excitation to spatially observe, quantify and even speciate metals within various types of samples (Figures 18.12 and 18.13). Although access to these approaches is currently limited due to the specialized equipment needed, they have the major analytical advantage of enabling *in situ* determination of elemental composition and speciation within environmental samples (Ginder-Vogel and Sparks, 2010).

18.9 MICROBIAL METAL TRANSFORMATIONS

18.9.1 Oxidation-Reduction

Many microbial transformations of metals occur due to their use as terminal electron acceptors in anaerobic respiration (metal reduction), or their use as an energy substrate in which the metal is oxidized. A number of metals and metalloids are subject to redox cycling in the environment including iron, manganese, selenium and arsenic. For example, the oxidation of arsenite (As(III)) to arsenate (As(V)) can be described by the following chemical reaction:

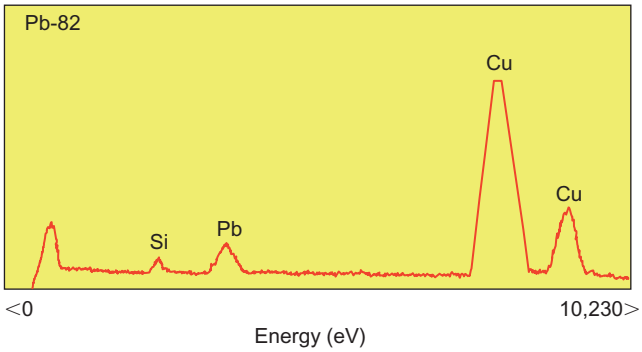


FIGURE 18.11 X-ray analysis of the suspected lead (Pb) deposit in Figure 18.10. Copper and silica were present from the copper grid and embedding medium, respectively, used in mounting the sample for analysis. Courtesy T.M. Roane.

$$2H_3AsO_3 + O_2 \rightarrow HAsO_4^{2-} + 3H^+$$
$$\Delta G^{o'} = -256 \text{ kJ/mole}$$

Arsenite oxidation can occur as an abiotic process, but microorganisms play an important role in arsenite oxidation in natural systems. As(III) oxidation can also serve as a detoxification reaction since As(III) is up to 50 times more toxic to bacterial cells than As(V) in most biological systems (Silver *et al.*, 2002). A variety of arsenite oxidizing bacteria have been identified including both chemoautotrophs and heterotrophs (Ehrlich, 2002).

Reduction of arsenate to arsenite occurs by one of two mechanisms—dissimilatory reduction or detoxification. In detoxification, reduction of arsenate is not coupled to respiration and does not provide energy for the bacterium—it is simply reduced to arsenite and then exported out of the cell. In dissimilatory reduction, As(V) is used as the terminal electron acceptor during anaerobic respiration. An example of such a reaction is the growth of *Bacillus arsenicoselenatis* using lactate as the electron donor and As(V) as the TEA. The reaction can be described by the following equation (Oremland *et al.*, 2002):

$$\text{Lactate} + 2HAsO_4^- + H^+ \rightarrow \text{acetate}^- + 2H_2AsO_3^- + HCO_3^-$$
$$\Delta G_f^o = -23.4 \text{ kJ/mole}$$

Studies of the rates of arsenate reduction by this mechanism show half-lives averaging around 30 hours (Inskeep *et al.*, 2002). For dissimilatory reduction of As(V) to occur, however, there must be high enough arsenic concentrations to support growth, and strict anaerobic

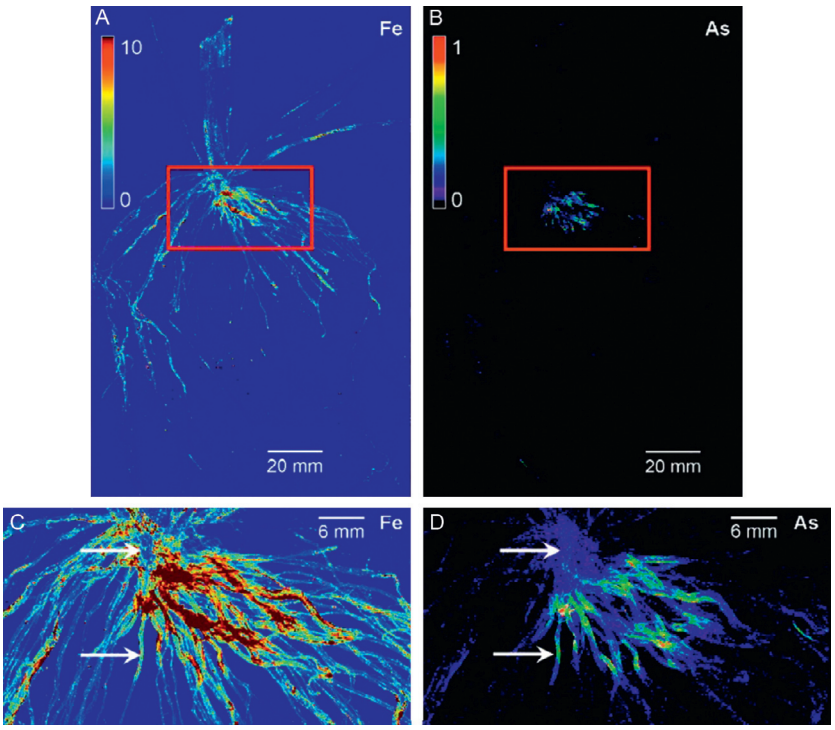


FIGURE 18.12 X-ray fluorescence images of iron (A, C) and arsenic (B, D) distributed within the root system of rice grown in contaminated soil. Images C and D represent magnified images of boxes in A and B, and the arrows represent areas where arsenic is abundant while iron is not. Note the spatial variability in iron and arsenic with highest concentrations occurring on the older roots near the shoot base. From Seyfferth *et al.* (2010).

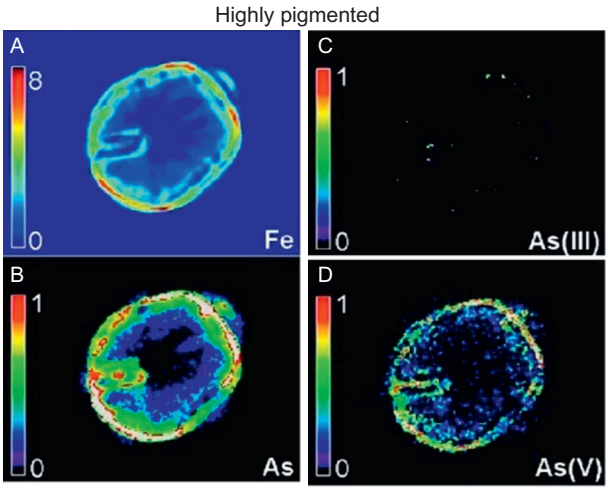


FIGURE 18.13 Cross-sectional computed X-ray tomography of a rice root grown in contaminated soil showing the spatial distribution of total iron (A), total arsenic (B), arsenite (C) and arsenate (D). Note the colocalization of iron and arsenic and that the majority of arsenic on and in the root is oxidized [As(V)]. From Seyfferth *et al.* (2010).

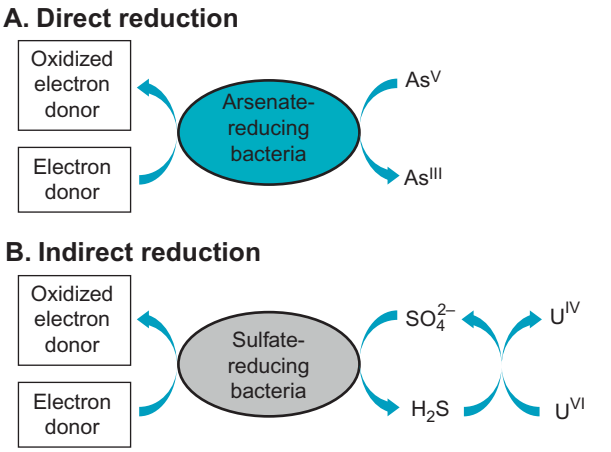


FIGURE 18.14 Examples of mechanisms for bacterial reduction of metals: (A) direct reduction through use as a terminal electron acceptor in anaerobic respiration and (B) indirect reduction following abiotic reactions with reduced chemical species such as sulfides.

conditions may be required. Environments such as sediments, hot springs and freshwater and marine systems can support such conditions.

In addition to some metals being directly reduced during anaerobic respiration, metals can also be reduced indirectly through reaction with other reduced products such as sulfides. In fact, for some metals, such as uranium, this may actually be a major mechanism for their reduction in the environment (Figure 18.14).

The oxidation and reduction of metals can have profound practical implications. For example, in subsurface geological formations, metals are often found in a reduced state as, for example, pyrite (FeS_2). Pyrite is often associated with metal ore deposits. Pyrite is stable until it is exposed to oxygen by mining activities, e.g., strip mining. Upon the introduction of oxygen, a combination of autoxidation and chemoautotrophic microbial oxidation of iron and sulfur results in the production of large amounts of acid. Acid, in turn, facilitates metal solubilization, resulting in a metal-rich acidic leachate called acid mine drainage (Information Box 18.5). Contaminating groundwater and over 10,000 miles of rivers in the United States alone, acid mine drainage is highly toxic to plants and animals, often resulting in widespread fish kills. Acid mine drainage is a problem associated with many types of mining activity including subsurface mining, where metal deposits become exposed to atmospheric oxygen; strip mining, where large expanses of land are exposed to oxygen; and mine tailing wastes, which are large deposits of processed or spent ore.

Microbially induced corrosion of metal pipes and fuel and storage tanks is a second significant problem of concern. Corrosion occurs due to cooperation between two groups of bacteria, the anaerobic chemoheterotrophic sulfate-reducing bacteria (SRB) and the aerobic chemoautotrophic iron-oxidizing bacteria. These two groups of bacteria work together to create an environmental niche on pipe surfaces that is favorable for their simultaneous activity, even though one group requires oxygen and the other does not.

Information Box 18.5 Formation of Acid Mine Drainage

The formation of acid from pyrite ore is a complex mechanism that involves the oxidation of both iron and sulfur. The initial reaction leading to the formation of acid mine drainage (AMD) is the spontaneous chemical oxidation of pyrite (FeS_2):

$$4FeS_2 + 14O_2 + 4H_2O \rightarrow 4Fe_2 + (OH)_3^- + 8SO_4^{2-} + 8H^+$$

As the local pH decreases due to the formation of acid, the sulfur- and iron-oxidizing bacterium, *Acidithiobacillus ferrooxidans*, further acidifies the environment. An acidophilic chemoautotroph, *A. ferrooxidans*, derives energy for carbon fixation, and growth from the oxidation of inorganic sulfur- and iron-containing compounds, such as pyrite. Because of the acidophilic nature of *A. ferrooxidans*, as the pH decreases, the microbially-facilitated oxidation of iron increases. Once oxidized, the iron can contribute to the formation of more acid:

$$Fe_3^+ + 3H_2O \rightarrow Fe(OH)_{3ppt} + 3H^+$$

Or the ferric iron can aid in the further chemical oxidation of pyrite:

$$FeS_2 + 14Fe_3^+ + 8H_2O \rightarrow 15Fe_2^+ + 2SO_4^{2-} + 16H^+$$

Note that this reaction produces acid and regenerates reduced or ferrous iron, which can then be reoxidized by *A. ferrooxidans*. Acid mine drainage can have a pH as low as 2. The produced leachate dissolves metal-containing ore, resulting in high concentrations of soluble and bioavailable toxic metals.

18.9.2 Methylation

The microbial methylation of metals not only results in increased metal mobility because some organometals are volatile, but also because in some cases it can change the toxicity of the metal. Methylation involves the transfer of methyl groups (CH_3) to metals and metalloids, e.g., lead, mercury, arsenic and selenium. The resulting organometal is more lipophilic than the metal species. This results in the potential of bioaccumulation and biomagnification in food webs (Figure 18.3).

Methylation of mercury occurs in the sediments of lakes, rivers and estuaries, where organic matter concentrations are high and redox conditions are favorable for the activity of sulfate-reducing bacteria, the primary generators of methylmercury (Drott *et al.*, 2007). The most important intracellular agent of mercury methylation is believed to be methylcobalamine (CH_3CoB_{12}), a derivative of vitamin B_{12}. Methylation reactions can be summarized as follows:

$$\underset{\text{methylcobalamine}}{CH_3CoB_{12}} + Hg^{2+} + H_2O \rightarrow \underset{\text{methylmercury}}{CH_3Hg^+} + H_2OCoB_{12}^+$$

$$\underset{\text{methylcobalamine}}{CH_3CoB_{12}} + CH_3Hg^+ + H_2O \rightarrow \underset{\text{dimethylmercury}}{(CH3)_2Hg} + H_2OCoB_{12}^+$$

The dominant product formed is the salt of the methylmercuric ion, CH_3Hg^+ (methylmercury), because the volatile dimethylmercury ($(CH_3)_2Hg$) forms at a much slower rate. Microbially mediated reactions affecting the fate of Hg^{2+} are shown in Figure 18.3. Since methylation actually increases the toxicity of mercury, methylation of mercury may facilitate diffusion of both methylmercury and dimethylmercury from the cell more easily than Hg^{2+}. In contrast, methylation of selenium directly decreases its toxicity of selenium (see Case Study 18.1).

Mercury is used extensively in the electrical industry, instrument manufacturing, electrolytic processes and chemical catalysis. Mercury salts and phenylmercury compounds are also used as fungicides and disinfectants. Approximately 10,000 metric tons of mercury are produced worldwide annually. Fossil fuel burning releases an additional 3000 metric tons. Methylmercury compounds are highly lipophilic and neurotoxic. Several outbreaks of mercury poisoning have occurred throughout history. In Minimata Bay, Japan, release of mercury-containing effluents by a chemical processing plant resulted in serious illness in people who consumed fish with elevated levels of mercury. Another example is the Great Lakes in the United States, which until the 1970s had relatively uncontrolled releases of polychlorinated biphenyls (PCBs), dioxins and mercury. For a period of time, parts of the lakes were closed to fishing, but the problem has improved due to restricted use and release of the organic and metal pollutants. At this time, health advisories are in effect that make recommendations about the type and amounts of fish that can be safely consumed.

Arsenic is another example of a metal that is methylated as a resistance mechanism. It is methylated by some bacteria, such as *Rhodopseudomonas palustris*, and many fungi, such as *Scopulariopsis brevicaulis* to mono-, di- and trimethylarsine, volatile forms of arsenic (Information Box 18.6). Arsenic poisonings have occurred in the past when fungi growing on damp wallpaper converted and volatilized arsenate (used as a coloring agent) in the wallpaper. Illness occurred upon inhalation of the resulting methylated arsenic species. There is also growing evidence that microbial methylation of soil arsenic has led to elevated levels of methylated arsenic in rice from some parts of the world (Somenahally *et al.*, 2011).

18.10 PHYSICOCHEMICAL METHODS OF METAL REMEDIATION

The remedial methods used to treat contaminated soil or sediments may be broadly divided into two main categories:

- Methods aimed at preventing movement of metals to the immediate surroundings, also called immobilization
- Methods aimed at metal removal

The goal in metal immobilization is to reduce metal solubility. Two immobilization strategies include pH alteration and addition of organic matter. Since metal solubility decreases with increasing pH, metal solubility should be reduced when site pH is raised. Liming is sometimes used to increase soil pH causing precipitation of contaminating metals as calcic and phosphoric metal-containing minerals. Amendment with organic matter can also aid in metal immobilization as a result of the electrostatic attraction between metals and organic particles. The addition of organic matter may involve the addition of highly organic waste material, such as biosolids. Often sites containing high levels of toxic metals have little or no vegetation. Revegetation of such sites, while sometimes difficult to achieve, is a good way to increase organic matter content.

Metal removal from soils or sediments can be achieved by excavation (which simply moves the problem to another location) or by using soil washing techniques. Soil washing methods rely on chemicals to facilitate metal removal. Washing with acidic solutions or chelating agents, e.g., ethylenediaminetetraacetic acid (EDTA) or nitrilotriacetic acid (NTA), solubilizes metals, enhancing removal from the system. One problem with the use of these chemical agents is the residual toxicity left by the washing agent after treatment. Newer biodegradable chelating agents show promise for metal removal. For example, 84% of nickel in a spent catalyst was removed with the biodegradable chelating agent [S,S]-ethylenediaminedisuccinic acid (EDDS) (Chauhan *et al.*, 2012), while studies show the degree of metal removal by chelating agents can be metal specific (Tandy *et al.*, 2004). Researchers are also looking at biological alternatives to these chemicals. For example,

Case Study 18.1 Selenium Bioremediation in San Joaquin Valley, California

Selenium is known to bioaccumulate and can cause death and deformities in waterfowl. Agriculture is the primary cause of selenium contamination in the San Joaquin Valley, but other anthropogenic sources of selenium include petroleum refining, mining and fossil fuel combustion. Once in soil, selenium exists as selenate (SeO_4^{2-}, Se_6^{+}), selenite (SeO_3^{2-} Se_4^{+}), elemental selenium (Se^0), dimethylselenide [DMSe; $(CH_3)_2Se$], and/or dimethyldiselenide [DMDSe; $(CH_3)_2Se_2$]. The most toxic forms of selenium are selenate and selenite; elemental selenium is considered insoluble and is least toxic of the selenium species. For selenium, the methylated forms of selenium are 500–700 times less toxic than the inorganic forms.

As a result of agricultural practices, largely irrigation, soils found in the San Joaquin Valley have elevated selenium levels (400 to 1000 mg/kg soil). Drainage waters in the valley can contain up to 4200 mg/L. Consequently, in some areas, selenium has concentrated to hazardous levels in evaporation ponds and in soils. The Kesterson Reservoir, located within the valley, is one such area where selenium contamination has resulted in extensive bird kills.

The bioremediation of the San Joaquin Valley and specifically the Kesterson Reservoir is based on the ability of a large number of microorganisms to reduce selenium oxyanions to the insoluble, Se^0 or to the volatile, methylated forms, e.g., DMSe. Under anaerobic conditions, organisms such as *Wolinella succinogenes* and *Desulfovibrio desulfuricans* can reduce SeO_4^{2-} and SeO_3^{2-} to elemental selenium. However, reduction to insoluble forms of selenium is not sufficient to stabilize the selenium pool within the soil matrix. Both microbial reoxidation and resolubilization are facilitated by organisms including *Acidithiobacillus ferrooxidans* and *Bacillus megaterium*. Consequently, long-term remediation of contaminated soils requires selenium removal, hence the role of selenium -volatilizing microorganisms becomes important.

Laboratory experiments initially conducted with Kesterson sediments found that certain environmental conditions influence microbial selenium volatilization. Researchers found that increased soil moisture (− 33 kPa), soil mixing, increased temperature (35°C) and application of an organic amendment increased selenium volatilization. In greenhouse experiments, sediment samples containing 60.7 mg/kg sediment were treated with various carbon sources to enhance microbial selenium methylation (Karlson and Frankenberger, 1990). The highest amount of selenium volatilization was seen upon the addition of citrus peel (≈44%), compared with manure (19.5%), pectin (16.4%) and straw plus nitrogen (8.8%). Without amendments, selenium volatilization was 6.1%.

On the basis of promising laboratory and greenhouse results, field plots (3.7×3.7 m^2) were set up at the Kesterson Reservoir. Plots were treated with different carbon amendments, including manure, gluten, citrus and casein, in an attempt to stimulate microbial activity and selenium methylation. With periodic tilling and irrigation, approximately 68–88% of the total amount of selenium was removed from the top 15 cm of soil within 100 months (Flury *et al.*, 1997). The highest rates of selenium removal were in soils amended with casein.

The remediation of selenium-contaminated soils is a successful example of how metal-transforming microorganisms can be used to detoxify and remove metals from affected systems. Microbial selenium remediation is also an excellent example of how laboratory experimentation has led to a viable approach for remediating selenium-contaminated environments. The effective microbial remediation of other metals will need development of strategies similar to those used in this case study.

biosurfactants have been explored as alternative "green" soil washing agents for metal-contaminated soils (Maier and Soberon-Chavez, 2000).

While metal removal by excavation may be appropriate when the area of contamination is small or there is immediate risk to human health, increasing cost and shrinking landfill space emphasize the need for cheaper, environmentally friendly alternatives. Following excavation, contaminated soils must be stored in a hazardous waste containment facility or incinerated. In some situations, however, excavation can exacerbate the problem. For example, excavation of sediments, called dredging, can actually result in increased metal toxicity. Metal sediments are often anaerobic and the metals within the sediment exist in an immobile, reduced state. Exposure to oxidizing conditions results in metal oxidation and increased metal solubility, increasing both bioavailability and transport. There is current discussion about whether physically removing metal-containing sediments is more detrimental than leaving them in place.

Incineration of soils can be used to remove metals from soils. However, incineration is not only expensive and impractical for large volumes of soil, but also releases metals to the atmosphere only to be deposited elsewhere. In addition, such thermal treatment of soil also destroys important soil properties, destroying soil structure and soil biota.

The nonbiological remediation of aquatic systems, including surface water, groundwater and wastewater, is fairly straightforward, albeit costly. Metals are removed and concentrated from contaminated waters through flocculation, complexation and/or precipitation. Lime addition precipitates metals as metal hydroxides. Chelating agents complex metals and can be recovered with a change in pH. Electroreclamation methods include ion exchange, reverse osmosis and electrochemical recovery of metals. At an estimated operating cost of $1.1 million per year, chemical treatment of the acid mine water discharging from the Argo

Tunnel in the Clear Creek Superfund site in Colorado involves precipitation of metals using sodium hydroxide to increase the pH to 10 or above. The metal precipitate is then earmarked for landfill disposal. Plans are under way to replace the sodium hydroxide neutralization with lime neutralization due to expected cost savings. This can be compared to a biological approach used to treat mine waste effluents from the Homestake Mine in Lead, South Dakota, in the United States (see Case Study 6.1).

A developing approach for the removal of organic and metal contaminants from water is the use of permeable reactive barriers (PRB) containing materials such as zero valent iron. In the case of metal contaminants, the goal is to convert the metal into a less-toxic form and/or immobilize it within the PRB as the contaminated water passes through. For example, a zero valent- or oxidized-iron barrier would promote the sorption and precipitation of arsenic within the barrier, resulting in the water exiting the PRB having decreased levels of arsenic. This approach has tremendous potential for containment and/or remediation; however, in practice, it has faced major challenges including microbial and chemical fouling of the PRBs that prematurely shorten their effective lifetime. Continued advances in PRB materials and coatings may improve their longevity and applications in the future.

Information Box 18.6 Discovery of Bacterial Genes for Arsenic Methylation

It has been recognized for years that some bacteria could methylate arsenic; however, it was not known what mechanism was used to do this and how widespread this was in the environment. In 2006, Qin *et al*. took advantage of the explosion in the number of sequenced bacterial genomes and scoured these data to find homologues to eukaryotic genes known to encode for methylation of arsenic. They found a subset of these genes that appeared to be under the control of an arsenic regulatory gene. In order to prove that these genes encoded the ability to methylate arsenic, Qin and colleagues cloned the putative gene, dubbed *arsM*, from *Rhodopseudomonas palustris*, into an arsenic hypersensitive *E. coli* strain. This enabled the *E. coli* to convert arsenite into various methylated species with trimethylarsine as the end product. Additional research indicates that this gene occurs in a large variety of microorganisms, and is widespread in nature, thus likely having a major impact on the global arsenic cycle.

18.11 MICROBIAL APPROACHES IN THE REMEDIATION OF METAL-CONTAMINATED SOILS AND SEDIMENTS

The goals of microbial remediation of metal-contaminated soils and sediments are to:

- Immobilize the metal *in situ* to reduce metal bioavailability and mobility
- Remove the metal from the soil (Figure 18.15)

There are several proposed methods for microbial remediation of metal-contaminated soils including: microbial leaching; microbial surfactants; microbially induced metal volatilization; and microbial immobilization and complexation.

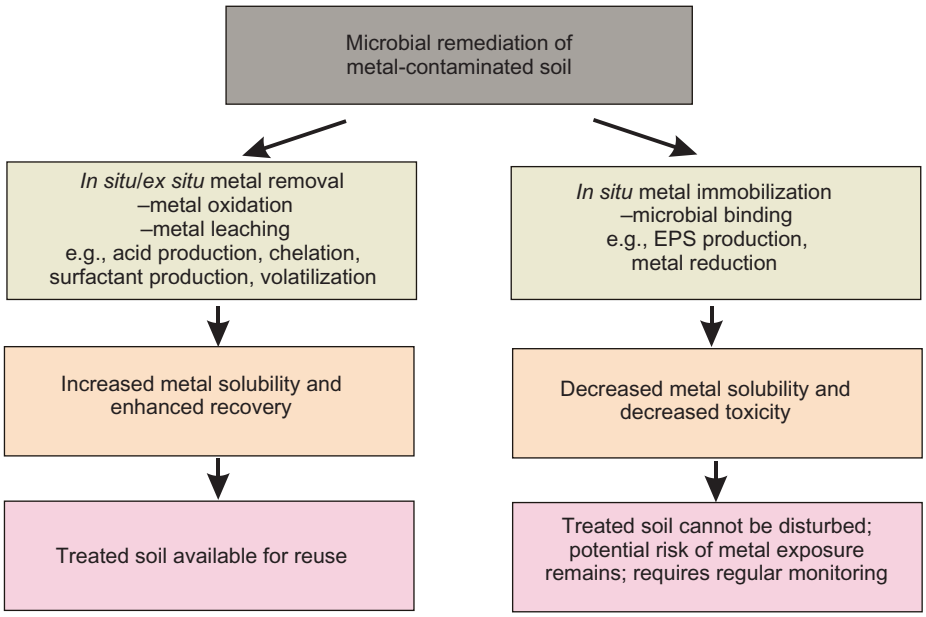


FIGURE 18.15 Microbial metal remediation in metal-contaminated soils relies on either metal removal or, more commonly, metal immobilization. Metal removal is generally more expensive but is ideal because following treatment the soil is available for reuse. In metal immobilization, soil reuse may be limited because of the continued potential risk of exposure.

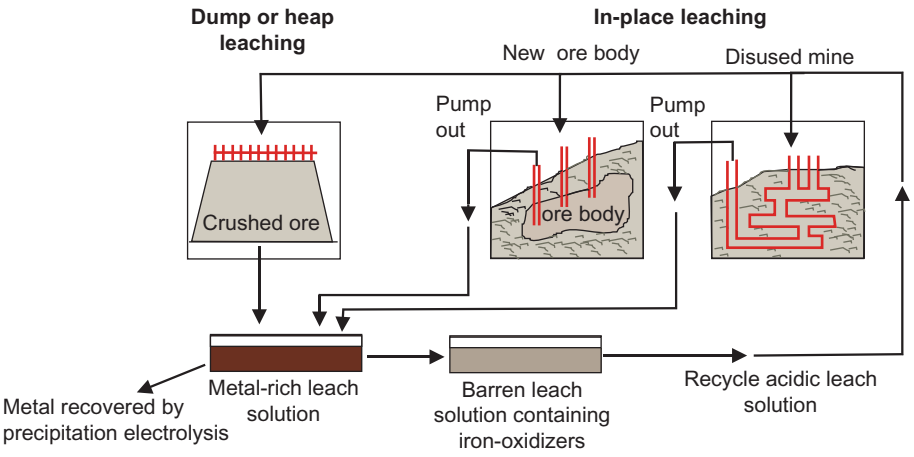


FIGURE 18.16 Various approaches to bioleaching. Metals can be recovered from ores that are in place in the ground if the hydrological conditions permit, or in dumps or heaps on the ground. Some of these heaps can be hundreds of feet high. In each case, an acidic leach solution created and maintained by iron-oxidizers is flushed through the ore, dissolving the metals. The metal-laden leachate is subjected to a precipitation or electrolysis process to remove the metal and then the spent leach solution is recycled back onto the ore body.

Certain microorganisms, such as *Acidithiobacillus ferrooxidans*, can facilitate the removal of metals from soil through metal solubilization or leaching via the same acidification process as seen with the formation of acid mine drainage. Generally used in the recovery of economically valuable metals from ores, bioleaching has also been used to recover copper, lead, zinc and uranium from tailings. The process uses acidophilic iron- and sulfur-oxidizers (e.g., *Acidithiobacillus*, *Leptospirillum*) and is considered to be environmentally friendly (Rawlings, 2002). These microorganisms can participate in both direct bioleaching and indirect bioleaching of metals from a variety of ores. Copper is the major metal recovered using bioleaching.

There are two commercial-scale approaches for bioleaching. The first is used primarily for copper and involves recycling leach liquor through a copper sulfide ore body. As shown in Figure 18.16, this can be done *in situ*, or on ore heaps placed on pads on the ground. *In situ* bioleaching can occur either in a spent mine or can be applied to a new unmined ore body. However, *in situ* bioleaching requires suitable hydrologic conditions to allow efficient collection of the leachates, and also to ensure that leachates do not go off-site. Heap bioleaching or dump bioleaching usually involves mining the ore, crushing it and then placing it in piles on an irrigation pad. The leach liquor is applied to the top of the heap and percolates through the ore, collecting metals. The metal-laden leach liquor is collected from the bottom of the pile, processed to remove the metals, and then recycled onto the top of the pile.

The second commercial-scale approach for bioleaching involves the use of a series of continuous-flow bioreactors, a much more costly process. This process is usually used for high value metals such as gold. However, the principle is the same—the bioreactors are filled with ore and leach liquor is cycled through the bioreactors to remove the metals from the ore (Rawlings, 2002). Metals recovered by leaching can be concentrated by complexation with chelating agents or precipitation with lime. Bioleaching also has potential in the removal of metals from contaminated soils and metal-containing sludges. Unfortunately, this aspect of microbial leaching has received little attention.

Microorganisms can also increase metal solubility for recovery through the production of surfactants. Because of their small size, biosurfactants are a potentially powerful tool in metal remediation. Bacterial surfactants are water-soluble, low-molecular-weight molecules (<1500) that can move relatively freely through soil pores. In addition to their small size, biosurfactants have a high affinity for metals so that, once complexed, contaminating metals can be removed from the soil by soil flushing. Some surfactants, such as the rhamnolipid produced by *Pseudomonas aeruginosa*, show specificity for certain metals, such as cadmium and lead (Ochoa-Loza *et al.*, 2001). Biosurfactant specificity allows the optimization of removal of a particular metal. Related to biosurfactants, the higher molecular weight ($\approx 10^6$) bioemulsifiers such as emulsan, produced by *Acinetobacter calcoaceticus*, can also aid in metal removal and are increasingly being looked at as a potential application for metal recovery (Gutnick and Bach, 2000).

Like leaching, methylation of metals can increase metal bioavailability and toxicity. Some methylated metals are more lipophilic than their nonmethylated counterparts. In spite of the possible increased toxicity, many microorganisms still volatilize metals to facilitate their removal from the immediate environment. Because methylation enhances metal removal, methylation of certain metals has been used as a remediation strategy. The most famous example is the removal of selenium from contaminated soil in San Joaquin, California (see Case Study 18.1) by selenium volatilizing microorganisms. Mercury

is another metal commonly methylated by microorganisms. However, mercury is susceptible to bioaccumulation in the food chain, posing serious health risks to the human population, and therefore removal of mercury by volatilization would not be an acceptable approach.

Immobilization strategies include metal sequestration which takes advantage of the ability of some microorganisms to produce metal-complexing polymers (both extracellular and intracellular), or to convert metals to a less-soluble form. Recall that exopolymers have high affinities for various metals. The overall approach in microbial metal sequestration is to introduce the polymer-producing microorganism into the contaminated soil and allow the organism to grow and replicate, thereby increasing the amount of polymer present in the soil, and increasing the number of organisms producing the polymer. Microbial metal sequestration has been shown to be effective in laboratory studies but has yet to be proven effective in the field. A second immobilization strategy is to create reducing or anaerobic conditions which results in the reduction and precipitation of metals. For example, the reduction of sulfate to sulfide under anaerobic conditions can lead to the formation of metal sulfide precipitates that are immobile. Likewise, the reduction of uaramium (uranium(VI)) to a less-soluble form (uranium (IV)) has been demonstrated to dramatically lower concentrations of dissolved uranium in groundwater in field-scale studies (Case Study 18.2). It should be noted that this approach is very metal specific, since reduced forms of some metals (e.g., arsenic) are actually more soluble than their oxidized counterparts. Also, this approach would require that reducing conditions be maintained at the site in order to prevent reoxidation of the sequestered metals. Although immobilization strategies are generally more economical than removal strategies and appear to have tremendous potential for many sites, there is not yet sufficient evidence to confirm the long-term effectiveness of immobilization.

18.12 MICROBIAL APPROACHES IN THE REMEDIATION OF METAL-CONTAMINATED AQUATIC SYSTEMS

Microbially facilitated removal of metals from water is based on the ability of microorganisms to complex and precipitate metals, resulting in both detoxification and removal from the water column. Specific interactions for metal removal include metal binding to microbial cell surfaces and exopolymer layers, intracellular uptake, metal volatilization and metal precipitation via microbially facilitated metal redox reactions (Figure 18.17). Although these microbial mechanisms can effectively remove metals from contaminated aquatic systems, it is important to note that the metals are not destroyed and still have to be disposed of properly.

Wetland treatment is a cost-effective and efficient method for removal of metals from contaminated waters, such as acid mine drainage. Metal reductions are often greater than 90% (Scholz and Xu, 2002). Wetland remediation is based on microbial adsorption of metals, metal bioaccumulation, bacterial metal oxidation and sulfate reduction. The high organic matter content of wetlands provided by high plant and algal growth encourages both the growth of sulfate-reducing microorganisms and metal sorption to the organic material. Although these various processes contribute to the removal of toxic metals from the water column, the metals are not destroyed. Consequently, wetlands are constantly monitored for any environmental change that may adversely affect metal removal. For example, a decrease in pH may solubilize precipitated metals, or a disturbance of the wetland sediment may change the redox conditions and oxidize reduced metals. Wetlands are resilient systems, and as long as new vegetative growth and organic inputs occur, wetlands can effectively remove metals for an indefinite period of time.

The most common treatment for metal-contaminated waters is with microbial biofilms. Many microorganisms, including *Pseudomonas*, *Arthrobacter*, *Bacillus*, *Citrobacter*, *Streptomyces* and the yeasts *Saccharomyces* and *Candida*, produce exopolymers as part of their growth regime. Metals have high affinities for these anionic exopolymers. Microbial biofilms may be viable or nonviable when used in remediation. In general, the biofilm is immobilized on a support as contaminated water is passed through the support (Figure 18.18). Often, a mixture of biofilm-producing organisms grows on these supports, providing a constant supply of fresh biofilm. For example, live *Citrobacter* spp. biofilms are used to remove uranium from contaminated water. Both *Arthrobacter* spp. biofilms and biomass (nonliving) are used in recovery of cadmium, chromium, copper, lead and zinc from wastewaters. Nonliving *Bacillus* spp. biomass preparations effectively bind cadmium, chromium, copper, mercury and nickel, among other metals. The success of microbial biomass in metal recovery from contaminated waters has led to the commercial sale of several biomass products. For example, AMT-BIOCLAIM (*Bacillus* biomass) and AlgaSORB (*Chlorella vulgaris*) are commercially available immobilized, nonliving preparations for treating metal-contaminated water. Interestingly, microbial biofilms are also used in the treatment of metal-contaminated marine waters; however, marine bacteria such as *Deleya venustas* and *Moraxella* sp. are used. Microbial biofilms are likewise used in the removal of metals from domestic wastewater. In domestic waste treatment, the important biofilm-producing organisms include *Zoogloea*, *Klebsiella* and *Pseudomonas* spp. Complexed metals are removed from the wastewater via sedimentation before release from the sewage treatment plant.

Case Study 18.2 Bioremediation of Uranium in a Contaminated Aquifer

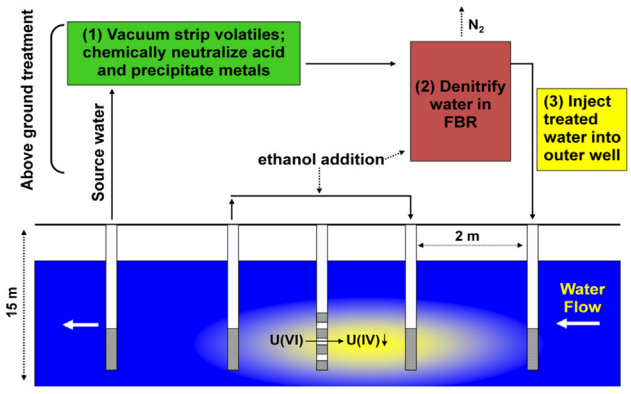
Many U.S. Department of Energy (DOE) facilities are highly contaminated as a result of post-WWII nuclear weapons programs. One of these sites is located at the Y-12 National Security Complex in Oak Ridge, TN. This site has extensive subsurface contamination due to the disposal of millions of liters of wastes containing uranium, other metals, organic solvents and acids in unlined disposal ponds from 1951 to 1983.

These wastes subsequently leached from the ponds, contaminating the underlying vadose zone and groundwater with a variety of contaminants including metals, radionuclides and organic solvents. In addition, this resulted in the site having a very low pH ($\approx$4) and extremely high levels of nitrate and sulfate due to the acids (e.g., nitric and sulfuric acids) used in processing.

A research group consisting primarily of scientists from Oak Ridge National Laboratory and Stanford University conducted a series of experiments to investigate the potential for *in situ* bioremediation of the site (Wu *et al*., 2006). Their goal was to decrease the potential for uranium to migrate offsite, and pollute nearby public waterways. Given the extent and depth of the contamination, the group decided that the best remediation option was to biologically reduce the metals thus decreasing their aqueous solubility and sequestering them *in situ*. However, in order to do this, the site had to be significantly modified. The pH was raised to $\approx$6 to facilitate greater biological activity. Also, in order to achieve stable reduction of uranium, the exceedingly high levels of nitrate (>2 g/L) first needed to be decreased. The logical process for decreasing nitrate levels was denitrification, but this could not be lone *in situ* since large amounts of cell biomass and gas produced during denitrification could potentially complicate the remediation process by clogging and/or changing flow paths in the aquifer. Also, denitrification by-products could possibly reoxidize reduced uranium. It was therefore decided to perform the denitrification aboveground and use a combined aboveground and belowground approach for the remediation. Groundwater was pumped aboveground, where volatiles such as TCE were stripped, and the water was chemically neutralized to precipitate out dissolved metals. The water was then pumped into a fluidized bed reactor, where ethanol was added as an electron donor for denitrification. The treated water was then pumped back into the aquifer, where ethanol was again added to serve as an electron donor to stimulate microbial reduction of uranium from the more soluble and mobile uranium (VI) form to the insoluble uranium (IV) form.

After approximately 70 days, aqueous uranium concentrations decreased ~80% from their initial levels (~50 mg/L), largely coinciding with the removal of residual nitrate via denitrification and a transition to sulfate-reducing conditions. After approximately 2 years of bioremediation, the groundwater uranium levels were further reduced to below 30 μg/L (the U.S. EPA Maximum Contaminant Level for uranium in drinking water). In other words, the water was improved so dramatically that it was considered safe for human consumption (at least in terms of uranium concentration)! X-ray absorption near-edge structure (XANES) spectroscopy confirmed that the uranium was reduced *in situ* with as much as 82% being U(IV) in sediment samples. The microorganisms and exact mechanisms responsible for the uranium reduction were not known, but microarray and DNA-sequencing results revealed that uranium reduction corresponded with large increases in the populations of iron-reducers, including *Geobacter* spp., and sulfate-reducers, including *Desulfovibrio* spp., related to organisms that have been reported to reduce uranium. Research is ongoing to determine the long-term stability of the immobilized uranium at the site.

S-3 Disposal Ponds



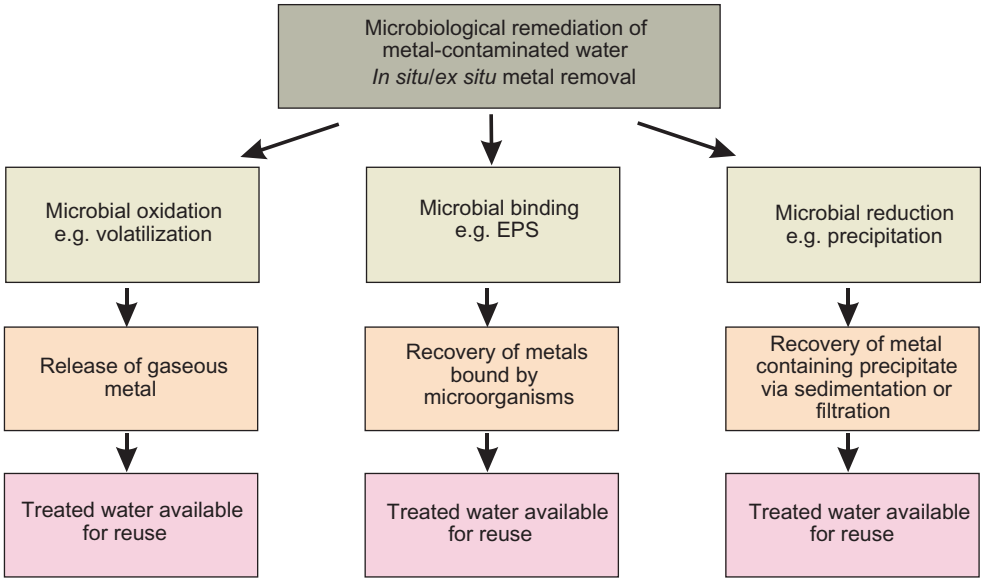


FIGURE 18.17 Microbial metal remediation approaches for metal-contaminated waters. In each method, the treated water is safe to release into the environment. Both metals and microorganisms can easily be recovered during treatment for proper disposal.

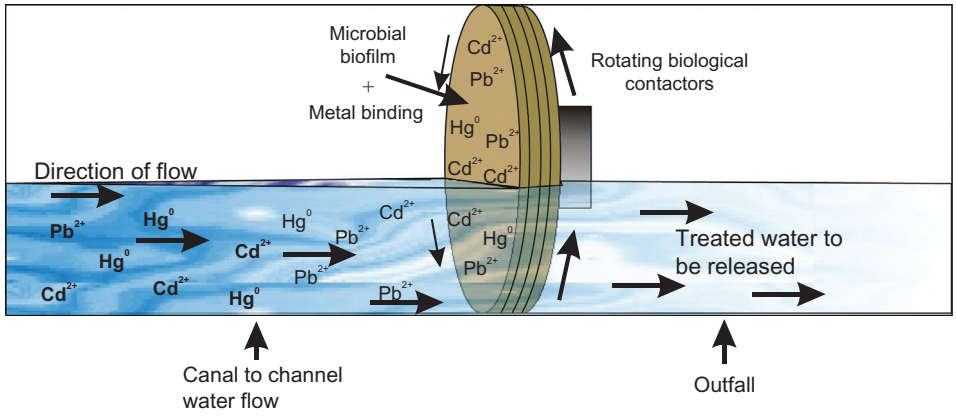


FIGURE 18.18 Schematic demonstrating how microbial biofilms are used in removing metals from contaminated waste streams. The biofilm located on the rotating drum accumulates metals as the water passes through the drum. The treated water can be safely released. The biofilm may either be viable or nonviable. When viable, the biofilm rarely needs to be replaced; however, nonliving biofilms need to be replaced periodically because their metal removal efficiency will decrease with time.

QUESTIONS AND PROBLEMS

1. Address the structural differences between prokaryotic and eukaryotic cells and how these differences influence cell resistance or sensitivity to metal toxicity.
2. In a metal contaminated lake, discuss metal bioavailability throughout the water column including the sediment.
3. Is dredging a viable option for removal of metal contamination in an aquatic system? What are the potential problems?
4. What factors need to be considered when bioaugmenting a metal-contaminated site with a metal-resistant microorganism?
5. Which chemical groups in proteins are most reactive with metals? In nucleic acids? In membranes?
6. Metal-resistant microorganisms are often isolated from noncontaminated environments (with no prior metal exposure). Discuss possible reasons why.
7. A metal-contaminated soil has been remediated using a metal-complexing microorganism. What factors need to be considered to ensure that the metal does not become "reavailable"?
8. Summarize the possible mechanisms of metal resistance in microorganisms and discuss which mechanism would be most effective in remediating a metal-contaminated surface soil, metal-contaminated soil in the vadose zone, a metal-contaminated stream and metal-contaminated groundwater?
9. Discuss the fate (both chemical and biological) of lead in (a) an acid soil, pH 4.0; (b) a neutral soil; (c) a basic soil, pH 8.5; (d) an anaerobic soil; and (e) an aerobic–anaerobic soil interface.

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