Heavy Metal Pollutants: Environmental and Biotechnological Aspects

G M Gadd, University of Dundee, Dundee, Scotland, UK

© 2009 Elsevier Inc. All rights reserved.

Glossary

biosorption The removal of metal or metalloid species, compounds and particulates, radionuclides, and organometal(loid) compounds from solution by physicochemical interactions with microbial or other biological materials.

desorption Nondestructive elution and recovery of, for example, metal or metalloid species, radionuclides, and organometal(loid) compounds from loaded biological material by physicochemical treatment(s).

heavy metals An ill-defined group of biologically essential and inessential metallic elements, generally of density >5, exhibiting diverse physical, chemical, and biological properties with the potential to exert toxic effects on microorganisms and other life forms.

metal resistance The ability of a microorganism to survive the toxic effects of heavy metal exposure by means of a detoxification mechanism, usually produced in response to the metal species concerned.

metal tolerance The ability of a microorganism to survive the toxic effects of heavy metal exposure because of intrinsic properties and/or environmental modification of toxicity.

metallothioneins Low-molecular-weight cysteine-rich proteins capable of binding essential metals (e.g., Cu and Zn) as well as inessential metals (e.g., Cd).

organometallic compound A compound containing at least one metal–carbon bond, often exhibiting enhanced microbial toxicity. When such compounds contain 'metalloid' elements (e.g., Ge, As, Se, and Te), the term 'organometalloid' may be used.

phytochelatins Metal-binding γ-Glu-Cys peptides of general formula $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ (n generally 2–7); now designated as class III metallothioneins that are atypical, nontranslationally synthesized metal thiolate polypeptides. Cd-binding γ-Glu-Cys peptides from some yeasts are also called cadystins.

siderophores Low-molecular-weight Fe(III) coordination compounds excreted by microorganisms, which enable the accumulation of iron from the external environment.

Abbreviations

γ-Glu-Cys	γ-glutamyl-cysteinyl
DMSe	dimethyl selenide
EPS	extracellular polymeric substances
SRB	sulfate-reducing bacteria

Defining Statement

This article describes the roles of microorganisms in the transformation of heavy metals, as well as metalloids, organometals, and radionuclides, between soluble and insoluble phases, and the environmental and biotechnological importance of these transformation processes in biogeochemical cycles and in new biotechnologies for the treatment of metal, metalloid, and radionuclide pollution.

Introduction

Heavy metals comprise an ill-defined group of more than 60 metallic elements, of density higher than 5, with diverse physical, chemical, and biological properties, but generally having the ability to exert toxic effects toward microorganisms. Many metals are essential for microbial growth and metabolism at low concentrations (e.g., Cu, Fe, Zn, Co, and Mn); yet they are toxic in excess amounts, and both essential and nonessential metal ions may be

accumulated by the microbial cells by physico-chemical and biological mechanisms. Thus, 'toxic metals' and 'potentially-toxic metals' are useful general terms. In this article, the term 'heavy metal' is used in a broad sense, and the discussion will include actinides, metal radionuclides, and organometal(loid) compounds. All these substances have a common potential for microbial toxicity and bioaccumulation, and are of environmental significance as pollutants or because of introduction as biocides and other substances.

Environmental Aspects of Heavy Metal Pollution

Heavy Metals in the Environment

Although elevated levels of toxic heavy metals can occur in natural locations (e.g., volcanic soils and hot springs), average environmental abundances are generally low with most of that immobilized in sediments and ores being biologically unavailable. However, anthropogenic activities have disrupted natural biogeochemical cycles, and there is increased atmospheric release as well as deposition into aquatic and terrestrial environments. The major sources of pollution include fossil fuel combustion, mineral mining and processing, nuclear and other industrial effluents and sludges, brewery and distillery wastes, biocides, and preservatives including organometallic compounds. In fact, almost every industrial activity can lead to altered mobilization and distribution of heavy metals in the environment. Because of the fundamental microbial involvement in biogeochemical processes, as well as in plant and animal productivity and symbioses, toxic metal pollution can have significant short- and long-term effects and ultimately affect higher organisms, including humans, for example, by accumulation and transfer through food chains.

Effects of Heavy Metals on Microbial Populations

Metals exhibit a range of toxicities toward microorganisms, depending on physicochemical and biotic factors; while toxic effects can arise from natural activities, toxic effects on microbial communities are more commonly associated with anthropogenic contamination or redistribution of toxic metals. This can arise from aerial and aquatic sources, as well as agricultural practices, industrial activity, and domestic and industrial wastes. In some cases, microbial activity can result in remobilization of metals from other wastes and transfer into aquatic systems. It is commonly accepted that toxic metals (and their chemical derivatives and related substances) can have significant effects on microbial populations, and almost every index of microbial activity can be affected.

For toxicity to occur, heavy metals must directly interact with microbial cells and/or indirectly affect growth and metabolism by interfering with, for example, nutrient uptake, or by altering the physicochemical environment of the cell. A variety of nonspecific and specific mechanisms (e.g., biosorption and transport, respectively) determine the entry of mobile metal species into cells, and if toxic thresholds are exceeded, cell death will result unless mechanisms for detoxification are possessed. The plethora of intracellular metal-binding ligands ensures that many toxic interactions are possible. Thus, practically every index of microbial activity can be adversely affected by toxic metal concentrations, including primary productivity, methanogenesis, nitrogen fixation, respiration, motility, biogeochemical cycling of C, N, S, P, and other elements, organic matter decomposition, enzyme synthesis and activity in soils, sediments, and waters.

Despite potential toxicity, many microorganisms still survive, grow, and flourish in apparently metal-polluted locations, and a variety of mechanisms, both active and incidental, contribute to resistance and tolerance. However, general conclusions about heavy metal effects on natural populations are difficult to make because of the complexity of metal speciation, toxicity in the environment, and the morphological and physiological diversity encountered in microorganisms. Furthermore, environmental perturbations associated with industrial metal pollution (e.g., extremes of pH, salinity, and nutrient limitations) may also have adverse effects on microbial communities. Nevertheless, it is commonly assumed that microbes are able to respond to metal contamination and maintain metabolic activity through changes in microbial community structure and selection for resistance. Resistance and tolerance are arbitrarily defined, frequently interchangeable terms, and often based on whether particular strains and isolates can grow in the presence of selected heavy metal concentrations in laboratory media. It is probably more appropriate to use 'resistance' to describe a direct mechanism resulting from heavy metal exposure, for example, bacterial reduction of Hg^{2+} to Hg^0, metallothionein synthesis by yeasts. 'Tolerance' may be a result of intrinsic biochemical and structural properties of the host, such as possession of impermeable cell walls, extracellular slime layers or polysaccharide, metabolite excretion, as well as environmental modification of toxicity. However, distinctions are difficult in many cases because several direct and indirect mechanisms, both physicochemical and biological, can contribute to microbial survival. Thus, although heavy metal pollution can qualitatively and quantitatively affect microbial populations in the environment, it may be difficult to distinguish metal effects from those of environmental components, environmental influence on metal toxicity, and the nature of microbial resistance/tolerance mechanisms involved. Although some gross

generalizations are possible regarding toxic metal influence on microbial communities, individual cases are likely to be site-specific and potentially complex.

Environmental Modification of Heavy Metal Toxicity

The physicochemical characteristics of a given environment determine metal speciation and, therefore, chemical and biological properties of heavy metals. Concentration and speciation of metals in solution is governed by many processes, including inorganic and organic complexation, oxidation–reduction reactions, precipitation-dissolution, adsorption-desorption, some of these being mediated by microbial activities. Because major mechanisms of metal toxicity are a consequence of strong coordinating properties, a reduction in bioavailability may reduce toxicity and enhance microbial survival. Such parameters as pH, temperature, aeration, soluble and particulate organic matter, clay minerals, and salinity can influence heavy metal speciation, mobility, and toxicity. Metals can exist in solution as free cations (e.g., Cu^{2+}, Cd^{2+}, and Zn^{2+}), as soluble complexes with inorganic or organic ligands (e.g., $ZnCl^{+}$, $CdCl_3^{-}$, and metal citrates), or in association with colloidal material. Common inorganic ligands that can complex metals include SO_4^{2-}, Cl^{-}, OH^{-}, PO_4^{3-}, NO_3^{-}, and CO_3^{2-}. Organic complexing agents include humic and fulvic acids, aromatic and aliphatic compounds, and carboxylic acids. Acidic conditions may increase metal availability, although H^{+} may successfully compete with and reduce or prevent binding and transport. Environmental pH also affects metal complexation with organic components and inorganic anions (e.g., Cl^{-}). With increasing pH, there may be formation of hydroxides, oxides, and carbonates of varying solubility and toxicity. Some hydroxylated species may associate more efficiently with microbial cells than the corresponding metal cations. The oxidation state of several metals also determines solubility, for example, Cr(VI) being soluble and toxic and Cr(III) being immobile and less toxic. Such reductive transformations may be mediated by microbes, with accompanying consequences for survival. Colloidal materials of significance in affecting metal bioavailability and transport include iron and manganese oxides, clay minerals, and organic matter. Metals can precipitate as solid phases, for example, $CdCO_3$, $Pb(OH)_2$, ZnS, CuS, as well as mixed compounds. Toxic metals may also substitute for other metals in indigenous minerals, for example, Cd may substitute for Ca in $CaCO_3$. In addition, toxic metals may sorb onto preexisting minerals. A reduction in toxicity in the presence of elevated concentrations of anions such as Cl^{-}, CO_3^{2-}, S^{2-}, and PO_4^{3-} is frequently observed. Mono-, di-, and multivalent cations may affect heavy metal toxicity by competing with binding and transport sites. Other synthetic and naturally produced soluble and particulate organic substances, including microbial metabolites, may influence toxicity by binding and complexation. Anionic contaminants, such as arsenic, selenium, and chromium oxyanions like AsO_4^{3-}, AsO^{2-}, SeO_4^{2-}, SeO_3^{2-}, and CrO_4^{2-}, can sorb to positive charges on insoluble organic matter and iron, manganese, aluminum oxides, and carbonates. The removal of heavy metal species by intact living and dead microbial biomass, by physicochemical and/or biochemical interactions, may also be significant in some locations. In more general terms, microbial growth and activity is influenced by environmental parameters, including the availability of organic and inorganic nutrients, and this can clearly affect the responses to potentially toxic metals.

Mechanisms of Microbial Heavy Metal Detoxification

Extracellular metal complexation, precipitation, and crystallization can result in detoxification. Polysaccharides, organic acids, pigments, proteins, and other metabolites can remove metal ions from solution and/or convert them into less toxic species. Iron-chelating siderophores may chelate other metals and radionuclides and possibly reduce their toxic effects. The production of H_2S by microorganisms, for example, by *Desulfovibrio* sp., results in the formation of insoluble metal sulfides and also disproportionation of organometallics to volatile products as well as insoluble sulfides, for example:

$$2CH_3Hg^{+} + H_2S \rightarrow (CH_3)_2Hg + HgS$$

$$2(CH_3)_3Pb^{+} + H_2S \rightarrow (CH_3)_4Pb + (CH_3)_2PbS$$

Many other examples of metal crystallization and precipitation are known, and mediated by processes dependent and independent of metabolism. Some of these are of great importance in biogeochemical cycles and involved, for example, in microfossil formation, iron and manganese deposition, silver and uranium mineralization, and formation of stable calcareous minerals.

Decreased accumulation, sometimes as a result of efflux, and impermeability may be important survival mechanisms. Impermeability may be a consequence of cell wall and/or membrane composition, lack of transport mechanism, or increased turgor pressure. Bacterial plasmids have resistance genes to many toxic metals and metalloids, for example, Ag^{+}, AsO_2^{-}, AsO_4^{3-}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Sb^{3+}, TeO_3^{2-}, Tl^{+}, and Zn^{2+}. Related systems are frequently located on bacterial chromosomes, for example, Hg^{2+} resistance in *Bacillus*, Cd^{2+} efflux in *Bacillus*, and arsenic efflux in *Escherichia coli*. Copper tolerance genes are generally genome-located. General conclusions in bacterial metal resistance include the following: (1) plasmid-determined resistances are highly specific; (2) resistance systems have

been found on plasmids in all bacterial groups tested; and (3) resistance mechanisms generally involve efflux from the cells or enzymatic detoxification. However, other less-specific interactions, for example, sorption, may contribute to the overall response. Many bacterial metal resistance mechanisms, for example, Cd, Cu, and As, depend on efflux. Efflux pumps, determined by plasmid and chromosomal systems, are either ATPases or chemiosmotic systems, with mechanisms often showing similarity in different types of bacteria. Cd^{2+} resistance may involve (1) an efflux ATPase in Gram-positive bacteria, (2) cation-H^+ antiport in Gram-negative bacteria, and (3) intracellular metallothionein in cyanobacteria. Arsenic-resistant Gram-negative bacteria have an arsenite efflux ATPase and an arsenate reductase (which reduces arsenate [As(V)] to arsenite [(As(III)], which comprise the underlying biochemical mechanism. A Cd^{2+} efflux ATPase is widely found in Gram-positive bacteria, including species of *Bacillus*. Systems for Hg^{2+} resistance occur on plasmids from Gram-positive and Gram-negative bacteria with component genes being involved in the transport of Hg^{2+} to the detoxifying enzyme mercuric reductase, which reduces Hg^{2+} to elemental Hg^0. The enzyme organomercurial lyase can break the C-Hg bond in organomercurials. The large plasmids of *Alcaligenes eutrophus* have several toxic metal resistance determinants, for example, three for Hg^{2+}, one for Cr^{6+}, and two for divalent cations, *czc* (Cd^{2+}, Zn^{2+}, and Co^{2+} resistance) and *cnr* (Co^{2+} and Ni^{2+} resistance). Czc functions as a chemiosmotic divalent cation/H^+ antiporter. In *Enterococcus hirae* (previously *Streptococcus faecalis*), copper resistance is determined by two genes, *copA* and *copB*, which determine uptake and efflux P-type ATPases, respectively. Plasmid-determined Cu^{2+} resistance has been described in *Pseudomonas* sp., *Xanthomonas* sp., and *E. coli*. Chromosomal genes also affect Cu^{2+} transport and resistance by determining uptake, efflux, and intracellular Cu^{2+} binding. Bacterial arsenic resistance is plasmid-mediated in Gram-positive bacteria, and several mechanisms of plasmid-mediated tellurite resistance have been suggested, including reduction, reduced uptake, and enhanced efflux, although, as with Ag^+, resistance does not appear to depend on reduction to the elemental form (Te^0).

As with bacteria, intracellular metal concentrations in fungi may be regulated by transport, including efflux mechanisms. Such mechanisms are involved in normal metal homeostasis but also have a role in the detoxification of potentially toxic metals. Reduced heavy metal uptake has been observed in many tolerant microbes, including bacteria, algae, and fungi, although this is dependent on environmental factors, including pH and ion competition. However, some resistant strains may accumulate more metal than sensitive parental strains because of more efficient internal detoxification. Inside the cells, metal ions may be detoxified by chemical components, which include metal-binding proteins, or compartmentalized into specific organelles. Metal-sequestering organic and inorganic molecules, for example, polyphosphate, have been implicated in several microbial groups, whereas metal-binding peptides and proteins, including metallothioneins and γ-Glu-Cys peptides (phytochelatins, cadystins), have been detected in all microbial groups examined. Metallothioneins are small, cysteine-rich polypeptides that can bind essential metals (e.g., Cu and Zn), in addition to non-essential metals (e.g., Cd). Metal-binding γ-glutamyl-cysteinyl peptides are short peptides of general formula $(\gamma\text{-Glu-Cys})_n$-Gly. Peptides of $n = 2$–7 are most common, and these are important detoxification mechanisms in algae as well as several fungi and yeasts. In *Schizosaccharomyces pombe*, the value of n ranges from 2 to 5, whereas in *Saccharomyces cerevisiae*, only an n_2 isopeptide has been observed. Although $(\gamma EC)_nG$ can be induced by a wide variety of metal ions, including Ag, Au, Hg, Ni, Pb, Sn, and Zn, metal binding has only been shown for a few, primarily Cd and Cu. For Cd, two types of complexes exist in *S. pombe* and *Candida glabrata*. A low-molecular-weight complex consists of $(\gamma EC)_nG$ and Cd, whereas a high-molecular-weight complex also contains acid-labile sulfide. The $(\gamma EC)_nG\text{-}Cd\text{-}S^{2-}$ complex has greater stability and higher Cd-binding capacity than a low-molecular-weight complex, and consists of a CdS crystallite core and an outer layer of $(\gamma EC)_nG$ peptides. The higher binding capacity of sulfide-containing complex confers tolerance to Cd. In *S. pombe*, evidence has also been presented for vacuolar localization of $(\gamma EC)_nG\text{-}Cd\text{-}S^{2-}$ complexes. The main function of *S. cerevisiae* metallothionein (yeast MT) is cellular copper homeostasis. However, induction and synthesis of MT as well as amplification of MT genes leads to enhanced copper resistance in *S. cerevisiae*. The fungal vacuole also has an important role in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metal ions. Metals preferentially sequestered by the vacuole include Mn^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, Sr^{2+}, Ni^{2+}, and the monovalent cations K^+, Li^+, and Cs^+. The absence of a vacuole or a functional vacuolar H^+-ATPase in *S. cerevisiae* is associated with increased sensitivity and largely decreased capacity of the cells to accumulate Zn, Mn, Co, and Ni, the metals known to be mainly detoxified in the vacuole.

Chemical transformations of metal and metalloid species by microorganisms may also constitute detoxification mechanisms, for example, bacterial Hg^{2+} reduction to Hg^0. However, plasmid-determined chromate resistance appears unconnected with chromate [Cr(Vl)] reduction to Cr(III), resistance depending on reduced CrO_4^{2-} uptake. Similarly, plasmid-mediated Ag^+ resistance appears not to involve Ag^+ reduction to Ag^0. In addition

to these, other examples of reduction are carried out by bacteria, algae, and fungi (e.g., Au^{3+} to Au^0). Methylated metal and metalloid species may be volatile and lost from a given environment, for example, dimethyl selenide (DMSe). Methylation of Hg^{2+}, by direct and indirect microbial action, can result in the formation of CH_3Hg^+ and $(CH_3)_2Hg$. Arsenic methylation can be mediated by many organisms with compounds having the general structure $(CH_3)_nAsH_{3-n}$, and mono-, di-, and trimethylarsine ($n = 1$–3, respectively) being major volatile compounds. The reduction of arsenic oxyanions by reductase enzymes is also frequent and a determinant of As resistance. However, there appears no involvement of such reductases in biomethylation.

Organometallic compounds may be detoxified by sequential removal of alkyl or aryl groups. Organomercurials can be degraded by organomercurial lyase, whereas organotin detoxification involves sequential removal of organic groups from the tin atom:

$$R_4Sn \rightarrow R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4$$

It should be stressed that abiotic mechanisms of metal methylation and organometal(loid) degradation also contribute to their transformation and redistribution in aquatic, terrestrial, and aerial environments. The relative importance of biotic and abiotic mechanisms is often difficult to establish.

Biotechnological Aspects of Heavy Metal Pollution

Microbial Processes for Metal Removal and Recovery

Certain microbial processes can solubilize metals, thereby increasing their bioavailability and potential toxicity, whereas others immobilize them and thus reduce their bioavailability (**Figure 1**). The relative balance between

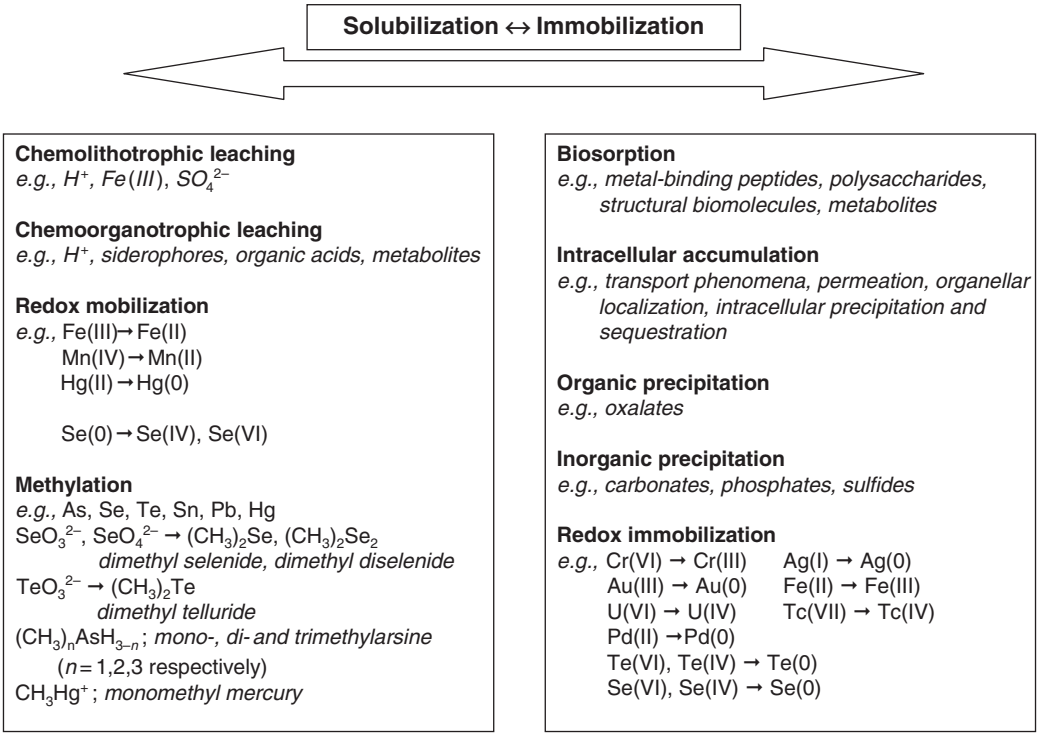

Figure 1 Diagram depicting the major mechanisms of microbial metal transformation between soluble and insoluble metal species. The relative balance between such processes will depend on the environment and associated physicochemical conditions, the microorganism(s) involved as well as relationships with plants, animals, and anthropogenic activities. Chemical equilibrium between soluble and insoluble phases is influenced by abiotic components, including dead biota and their decomposition products, as well as other physicochemical components of the environmental matrix, for example, pH, water, inorganic and organic ions, molecules, compounds, colloids, and particulates. Solubilization can occur by chemolithotrophic (autotrophic) and chemoorganotrophic (heterotrophic) leaching; siderophores and other complexing agents; redox reactions; methylation and demethylation; biodegradation of organoradionuclide complexes. Immobilization can occur by biosorption to cell walls, exopolymers, other structural components, and derived/excreted products; precipitation can be a result of metabolite release, for example, sulfide, oxalate, or reduction; transport, accumulation, intracellular deposition, localization and sequestration; adsorption and entrapment of colloids and particulates. The overall scheme is also affected by reciprocal interactions between biotic and abiotic components of the ecosystem, such as abiotic influence on microbial diversity, numbers and metabolic activity; ingestion of particulates and colloids (including bacteria) by phagotrophs; biotic modification of physico-chemical parameters including redox potential, pH, O_2, CO_2, other gases and metabolites, temperature, and nutrient depletion.

mobilization and immobilization varies depending on the organisms and their environment. As well as being an integral component of biogeochemical cycles for metals, these processes may be exploited for the treatment of contaminated solid and liquid wastes. Metal mobilization can be achieved by autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, and methylation, which can result in volatilization. Similarly, immobilization can result from sorption to cell components or exopolymers, transport and intracellular sequestration, or precipitation as insoluble organic and inorganic compounds, for example, oxalates, sulfides, or phosphates. In addition, microbiologically mediated reduction of higher valency species may effect either mobilization, for example, Mn(IV) to Mn(II), or immobilization, for example, Cr(VI) to Cr(III), and U(VI) to U(IV). In the context of bioremediation, solubilization of metal contaminants provides a means for removal of metals from solid matrices such as soils, sediments, and industrial wastes. Alternatively, immobilization processes enable metals to be transformed *in situ* and in bioreactors into insoluble, chemically inert forms. Biotechnological development of microbial systems may provide an alternative or adjunct to conventional physicochemical treatment methods for contaminated effluents and wastewaters. Growing evidence suggests that some biomass-related processes are economically competitive with existing treatments in mining and metallurgy.

Metal Solubilization

Chemolithotrophic (autotrophic) leaching

Most chemolithotrophic metal leaching is carried out by chemolithotrophic, acidophilic bacteria, which obtain energy from oxidation of Fe(II) or reduced sulfur compounds and solubilize metals because of the resulting production of Fe(III) and H_2SO_4. The microorganisms involved include sulfur-oxidizing bacteria (e.g., *Acidithiobacillus thiooxidans*), iron- and sulfur-oxidizing bacteria (e.g., *Acidithiobacillus ferrooxidans*), and iron-oxidizing bacteria (e.g., *Leptospirillum ferrooxidans*). As a result of sulfur and iron oxidation, metal sulfides are solubilized concomitant with the pH of their immediate environment being decreased, therefore resulting in solubilization of other metal compounds, including metals sorbed to soil and mineral constituents. Chemolithotrophic leaching of metal sulfides is well established for industrial-scale biomining processes, but it has also been used to solubilize metals from sewage sludge as well as remediate other metal-contaminated solid materials, including soil and red mud, the main waste product of Al extraction from bauxite. One two-stage soil treatment process used a mixture of sulfur-oxidizing bacteria to acidify metal-contaminated soil before treatment of the metal-loaded leachate with sulfate-reducing bacteria (SRB).

Chemoorganotrophic (heterotrophic) leaching

Many chemoorganotrophic (heterotrophic) fungi (and bacteria) can leach metals from industrial wastes, low-grade ores, and metal-bearing minerals. This occurs as a result of proton efflux, siderophores (for Fe(III)), and organic acids, for example, citric and oxalic. Organic acids provide a source of protons and a metal-complexing anion, for example, citrate, oxalate, with complexation being dependent on the metal/anion concentrations, pH, and metal complex stability constants. Organisms such as *Aspergillus niger* and *Penicillium simplicissimum* have been used to leach Zn, Cu, Ni, and Co from a variety of solid materials, including industrial filter dust, copper converter slag, lateritic ores, red mud, manganiferous minerals, and municipal waste fly ash. Citrate and oxalate can form stable complexes with a large number of metals. Many metal citrates are highly mobile and not readily degraded. Oxalic acid can act as a leaching agent for metals that form soluble oxalate complexes, including Al and Fe.

Siderophores are highly specific Fe(III) ligands (formation constant often $>10^{30}$) that are excreted by microorganisms to aid iron assimilation. Such assimilation may be improved by attachment to solid Fe oxides in soil. Although primarily produced as a means of obtaining iron, siderophores are also able to bind other metals such as magnesium, manganese, chromium (III), gallium (III), and radionuclides such as plutonium (IV).

Metal Immobilization

Biosorption

Biosorption (defined here as the microbial uptake of organic and inorganic metal, metalloid, and radionuclide species, both soluble and insoluble, by physicochemical mechanisms) may be influenced by metabolic activity (in living cells), and may also provide nucleation sites for the formation of stable minerals, including phosphates, sulfides, and oxides. Crystallization of elemental gold and silver may occur as a result of reduction, whereas the formation of hydrolysis products can enhance precipitation of U and Th. All biological macromolecules have an affinity for metal species with cell walls and associated materials being of the greatest significance in biosorption (**Table 1**). Moreover, mobile cationic species can be accumulated by cells via transport systems of varying affinity and specificity, and internally bound, transformed, precipitated, localized within organelles, or translocated to specific structures depending on the metal concerned and the organism.

Biosorption by cell walls and associated components

In bacteria, peptidoglycan carboxyl groups are the main cationic binding sites in Gram-positive species, with phosphate groups contributing significantly in Gram-negative species. Chitin, phenolic polymers, and melanin

Table 1 Examples of microbial metal and actinide accumulation to industrially significant levels. Data derived from a number of sources and presented without reference to important experimental conditions, for example, metal and biomass concentration, pH, and whether freely suspended, living, dead or immobilized, or the mechanism of accumulation. In most cases, highest uptake levels are due to general biosorptive mechanisms

Microorganism	*Metal*	*Accumulation (% dry weight)*
Bacteria		
Streptomyces sp.	Uranium	2–14
Streptomyces viridochromogenes	Uranium	30
Thiobacillus ferrooxidans	Silver	25
Bacillus cereus	Cadmium	4–9
Zoogloea sp.	Cobalt	25
	Copper	34
	Nickel	13
Citrobacter sp.[a]	Lead	34–40
	Cadmium	170
	Uranium	900
Pseudomonas aeruginosa	Uranium	15
Mixed culture	Silver	32
Cyanobacteria		
Anabaena cylindrica	Cadmium	0.25
Anacystis nidulans	Nickel	1
Spirulina platensis	Gold	0.52
Plectonema boryanum	Zirconium	0.16
Nostoc sp.	Cadmium	1
Algae		
Chlorella vulgaris	Gold	10
	Lead	8.5
Chlorella regularis	Uranium	15
	Zinc	2.8
	Manganese	0.8
Scenedesmus sp.	Molybdenum	2.3
Euglena sp.	Aluminum	1.5
Sargassum natans[b]	Gold	25
	Lead	8
	Silver	7
	Uranium	4.5
	Copper	2.5
	Zinc	2
	Cobalt	6
	Cadmium	8.3
Ascophyllum nodosum[b]	Gold	4
	Cobalt	15
	Cadmium	10
Fungi		
Phoma sp.	Silver	2
Penicillium sp.	Uranium	8–17
Rhizopus arrhizus	Lead	0.6
	Cadmium	3
	Lead	10
	Uranium	20
	Thorium	19
	Silver	5
	Mercury	6
Aspergillus niger	Thorium	19
	Uranium	22
	Gold	6–18
	Zinc	1–10
	Silver	10

(*Continued*)

Table 1 (Continued)

Microorganism	*Metal*	*Accumulation (% dry weight)*
Saccharomyces cerevisiae	Thorium	12
	Uranium	10–15
	Cadmium	7
	Copper	1–3
Ganoderma lucidum	Copper	1
Mucor miehei	Zinc	3.4
	Uranium	18

[a]Phosphatase-mediated metal removal.
[b]Macroalgae (seaweeds).

are important structural components of fungal walls, and these are also effective biosorbents for metals and radionuclides. Fungi can be efficient sorbents of metal ions over a wide range of pH values, and although they may take up less metal per unit dry weight than clay minerals (the most important metal-sorbing component in soil), they are more efficient sorbents per unit surface area. Variations in the chemical behavior of metal species as well as the composition of microbial cell walls and extracellular materials can result in wide differences in biosorptive capacities (**Table 1**). Extracellular polymeric substances (EPSs), a mixture of polysaccharides, mucopolysaccarides, and proteins, can bind significant amounts of potentially toxic metals and entrap precipitated metal sulfides and oxides. One process uses floating mats of cyanobacteria, the metal-binding process being due to large polysaccharides (>200 000 Da).

The ability of surface-associated macromolecules to effect the immobilization of aqueous metal(loid) species may be of great importance, particularly where organisms grow as surface-attached biofilms, enmeshed in a matrix of EPSs. The biofilm mode of growth is now widely accepted to be the predominant form in which natural bacterial populations occur, and it appears that natural mixed-species biofilms can act as sinks for precipitated minerals, including potentially toxic metals, in aqueous environments. The biofilm–EPS matrix can act as a direct adsorbent of dissolved metal ions, with the ionic state and charge density of EPS components determining the ionic binding and electrostatic immobilization properties. Bacterial EPSs are dominated by polysaccharides, but secreted polymers also include proteins, nucleic acids, peptidoglycan, lipids, and phospholipids. This heterogeneous matrix generally has a net negative charge, with polyanionic moieties acting as an ion-exchange matrix for metal cations. Well-characterized examples include the propensity of uronic acid-containing polysaccharides to bind with carboxyl groups and thus bind metals, whereas neutral carbohydrates can bind metals by the formation of weak electrostatic bonds around the hydroxyl groups. Cross-linking of extracellular polysaccharides by metal ions themselves may alter the mechanical and chemical properties of EPSs.

The biofilm growth mode appears to further enhance metal removal in various ways. Biosorption and bioprecipitation can be interrelated phenomena, such that ionic concentration by sorption at low-energy cellular, or EPS surface sites within biofilms can initiate mineral formation and immobilization within biofilms. Mineral precipitates formed in the bulk solution may also be physically entrapped or chemically adsorbed by the biofilm EPS matrix.

Biosorption by free and immobilized biomass

Both freely suspended and immobilized biomass from bacterial, cyanobacterial, algal, and fungal species have received attention with immobilized systems appearing to possess several advantages over 'free' biomass, including higher mechanical strength and easier biomass/liquid separation. Living or dead biomass of all groups has been immobilized by encapsulation or cross-linking using supports, which include agar, cellulose, alginates, cross-linked ethyl acrylate-ethylene glycol dimethylacrylate, polyacrylamide, silica gel, and cross-linking reagents, such as toluene diisocyanate and glutaraldehyde. Immobilized living biomass has mainly taken the form of bacterial biofilms (see "Biosorption by cell walls and associated components") on inert supports, and has been used in a variety of configurations, including rotating biological contactors, fixed-bed reactors, trickle filters, fluidized beds, and airlift bioreactors.

Metal desorption

Biotechnological exploitation of biosorption may depend on the ease of biosorbent regeneration for metal recovery. Metabolism-independent processes are frequently reversible by nondestructive methods, and hence can be considered analogous to conventional ion exchange. Most work has concentrated on nondestructive desorption, which should be efficient, cheap, and result in minimal damage to the biosorbent. Dilute mineral acids (~0.1 M) can be effective for metal removal, although

more concentrated acids or lengthy exposure times may result in biomass damage. It may be possible to apply selective desorption of metalloid species from a loaded biosorbent using an appropriate elution scheme. For example, metal cations (e.g., Cu^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, and Co^{2+}) were released from algal biomass using eluant at pH 2, whereas at higher pH values, anionic metal species (e.g., SeO_4^{2-}, CrO_4^{2-}, and MoO_4^{2-}) were removed. Au^{3+}, Ag^{+}, and Hg^{2+}, however, remained strongly bound at pH 2, and these were removed by the addition of ligands that formed stable complexes with these metal ions. Carbonates and/or bicarbonates are efficient desorption agents with potential for cheap, non-destructive metal recovery. Operating pH values for bicarbonates cause little damage to the biomass, which may retain at least 90% of the original uptake capacity.

Metal-binding proteins, polysaccharides, and other biomolecules

A diverse range of specific and nonspecific metal-binding compounds are produced by microorganisms. Nonspecific metal-binding compounds are metabolites or by-products of microbial metabolism and range from simple organic acids and alcohols to macromolecules, such as polysaccharides, humic and fulvic acids. Specific metal-binding compounds may be produced in response to external levels of metals. Siderophores are low-molecular-weight Fe(III) coordination compounds (500–1000 Da) excreted under iron-limiting conditions by iron-dependent microorganisms. Although specific to Fe(III), siderophores can also complex Pu(IV), Ga(III), Cr(III), scandium (Sc), indium (In), nickel, uranium, and thorium. Specific, low-molecular-weight (6000–10 000 Da) metal-binding metallothioneins are produced by animals, plants, and microorganisms in response to the presence of toxic metals. Metal-binding γ-Glu-Cys peptides (phytochelatins and cadystins) contain glutamic acid and cysteine at the N-terminal position, and have been identified in plants, algae, and several microorganisms. The metal-binding abilities of siderophores, metallothioneins, phytochelatins, and other similar molecules may have potential for bioremediation of waters containing low metal concentrations, although few examples have been rigorously tested.

Transport and accumulation

Microbial metal transport systems are of varying specificity, and essential and non-essential metal(loid) species may be accumulated. The rates of uptake can depend on the physiological state of cells as well as the nature of environment or growth medium. Integral to the transport of metal ions into cells are transmembrane electrochemical gradients, for example of H^{+}, resulting from the operation of enzymatic pumps (ATPases) that transform the chemical energy of ATP into this form of biological energy. ATPases are also involved in ion efflux in a variety of organisms and organellar ion compartmentation in eukaryotes via operation across vacuolar membranes. Metals may also enter (and leave) cells via pores or channels. With toxic heavy metals, permeabilization of cell membranes can result in exposure of intracellular metal-binding sites and increase passive accumulation. Intracellular uptake may result in death of sensitive organisms, unless a means of detoxification is possessed or induced (**Figure 2**). Other mechanisms of microbial metal accumulation include iron-binding siderophores and cotransport of metals with organic substrates.

Metal precipitation

Precipitation by redox processes: metal-reducing bacteria and iron oxidizers

A diverse range of microorganisms can use oxidized metallic species, for example, Fe(III), Cr(VI), or Mn(IV), as terminal electron acceptors. Many use more than one metal or anion, such as nitrate or sulfate. Fe(III) and Mn(IV) appear to be the most commonly utilized metals as terminal electron acceptors in the biosphere. However, since the solubility of both Fe and Mn is increased by reduction, other metals have been targeted in waste treatment, for example, molybdenum(VI) and Cr(VI). The reduction of, for example, Cr(VI) to Cr(III), by organisms including *Enterobacter cloacae* and *E. coli*, may facilitate removal by biosorption or (bio)precipitation. One potential application of dissimilatory biological metal reduction is uranium precipitation by reduction of soluble U(VI) compounds to U(IV) compounds, such as the hydroxide or carbonate, which have low solubility at neutral pH. Strains of *Shewanella* (*Alteromonas*) *putrefaciens* and *Desulfovibrio* sp. can produce a very pure precipitate of U(IV) carbonate. Such bacterial uranium reduction can also be combined with chemical extraction methods. The solubility of some other radionuclides, for example, Ra and Pu, may be increased by reduction, which may favor removal from, for example, contaminated soil.

Bacterial Fe oxidation is ubiquitous in environments with sufficient Fe^{2+} and conditions to support bacterial growth such as drainage waters and tailing piles in mined areas, pyritic and hydric soils (bogs and sediments), drain pipes and irrigation ditches, and plant rhizospheres. Iron oxidizers commonly found in acidic soil environments are acidophilic chemolithotrophs, such as *A. ferrooxidans*, significant for their role in generating acid mine drainage. Fungi, too, oxidize metals in their environment. Desert varnish is an oxidized metal layer (patina), of few millimeter thickness, found on rocks and in soils of arid and semi-arid regions, and is believed to be of fungal and bacterial origin.

Sulfate-reducing bacteria

SRB are strictly anaerobic heterotrophic bacteria found in environments where carbon substrates and sulfate are

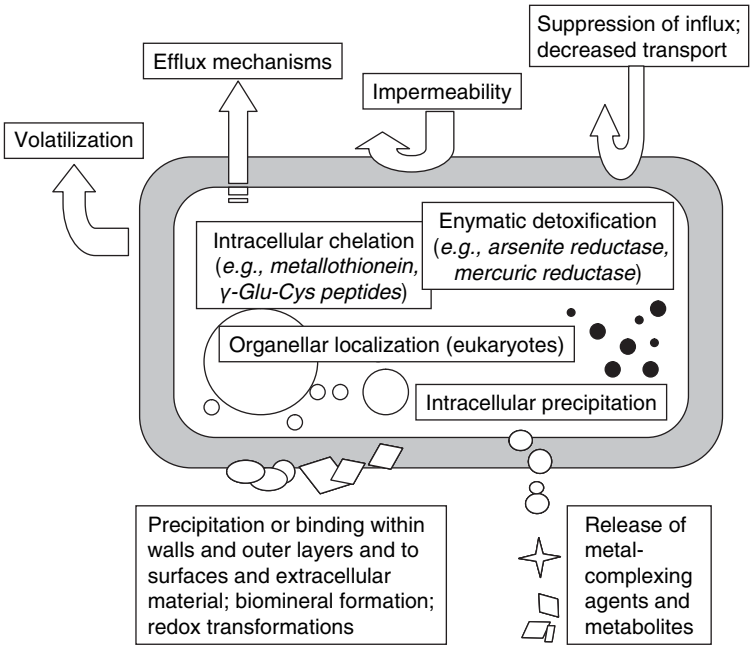

Figure 2 Mechanisms involved in the detoxification of metals including mechanisms that restrict entry into the cell and intracellular detoxification or organellar compartmentation, the latter occurring in some eukaryotes, for example, fungi. Operation of a number of mechanisms is possible depending on the organism and the cellular environment, some dependent and/or independent of metabolism. A variety of mechanisms may be involved in transport phenomenon contributing to decreased uptake and/or efflux. A variety of specific or nonspecific mechanisms may also affect redox transformations, intracellular chelation, and intracellular precipitation.

available. These utilize an energy metabolism in which the oxidation of organic compounds or hydrogen is coupled to the reduction of sulfate as the terminal electron acceptor, producing sulfide that often reach significant concentrations in sediments or bioreactors:

$$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$$

Sulfur in S(VI) oxidation state is stoichiometrically reduced to S(-II) and, under circumneutral conditions in which SRB are generally encountered, the main product is bisulfide (HS^-), with a small proportion of volatile H_2S. Bisulfide is a highly reactive species, with the propensity to bind with metal cations in solution-forming metal sulfide solids. This is the main mechanism whereby SRB remove toxic metals from solution, for example,

$$M^{2+} + SO_4^{2-} + 2CH_3CH_2OH \rightarrow 2CH_3COOH + 2H_2O + MS\downarrow$$

$$M^{2+} + SO_4^{2-} + 2CH_3CHOHCOOH \rightarrow 2CH_3COOH + 2CO_2 + 2H_2O + MS\downarrow$$

The solubility products of most heavy metal sulfides are very low, in the range of 4.65×10^{-14} (Mn) to 6.44×10^{-53} (Hg), so that even a moderate output of sulfide can remove metals to safer levels permitted in the environment. SRB can create extremely reducing conditions, which can chemically reduce metals such as uranium (VI). In addition, sulfate reduction partially eliminates acidity from the system, which can result in further precipitation of metals, for example, Cu, Al, as hydroxides as well as increasing the efficiency of sulfide precipitation.

Secondary metal(loid) removal by adsorption on SRB-produced metal sulfides deposited within, or immobilized by, the biofilm matrix can also contribute to overall removal. SRB-generated metal sulfides can adsorb a range of cations and anions. Fe(II) sulfides can bind a range of metals, allowing the level of metals in solution to be reduced from original concentrations in the order of $mg\,l^{-1}$ to $\mu g\,l^{-1}$.

Processes utilizing metal sulfide precipitation

Sulfate reduction can provide both *in situ* and *ex situ* metal removal from acid mine drainage and, together with other mechanisms such as biosorption, it contributes to the removal of metals and acidity in artificial and natural wetlands. Large-scale bioreactors have also been developed using bacterial sulfate reduction for treating metal-contaminated waters. The best known commercial application involving metal sulfide precipitation is the THIOPAQ technology, developed and marketed by Paques Bio Systems B.V., Balk, the Netherlands (http://www.paques.nl/), and first applied in 1992 for the treatment of contaminated groundwater at the Budelco zinc refinery in the Netherlands. The basic THIOPAQ system consisted of a two-stage biological process in series: anaerobic sulfate reduction to sulfide followed by aerobic

sulfide oxidation to elemental sulfur. Since the solubilities of most metal sulfides are much lower than those of their hydroxides, an advantage of the THIOPAQ system is that considerably lower effluent metal concentrations can be achieved than in the neutralization processes, which immobilize metals by hydroxide precipitation. In addition, the metal sulfide precipitate formed may be reprocessed in a smelter or a refinery.

Electron donors suitable for small-scale THIOPAQ installations were ethanol, various fatty acids, and organic waste streams. For large-scale applications, where more than 2.5 tons of hydrogen sulfide are produced per day, hydrogen gas is preferentially used as a reductant. Hydrogen gas can be produced on-site by cracking methanol or by steam-reforming natural gas or LPG. The main reaction that occurs in a reactor operated with H_2 is:

$$H_2SO_4 + 4H_2 \rightarrow H_2S + 4H_2O$$

Hydrogen sulfide in the reactor gas (3–15%, v/v) can be employed for metal precipitation. Compared to the addition of a NaHS or Na_2S solution, an advantage with the use of H_2S is that sodium is not introduced into the system. It is also possible that careful control of the pH and the redox potential of the process liquid may allow selective recovery of metals. Thus, sulfide precipitation makes it possible to separate copper from zinc, arsenic from copper, iron from nickel, and so on, in multiple reaction stages at different pH values. Alternatively, metals may also be precipitated as sulfides inside the anaerobic bioreactor.

At the Budelco zinc refinery in Budel-Dorplein, the Netherlands, a THIOPAQ system capable of processing approximately $300\,m^3\,h^{-1}$ of polluted groundwater has been in operation since 1992. Its products, a metal sulfide sludge (mainly ZnS) and sulfur slurry, are fed back to the roasters in the refinery. The capacity of the installation was increased to $400\,m^3\,h^{-1}$ (in 1998), with the feed also including a mixture of groundwater and process water. The most recent THIOPAQ installation (called Budelco II, in operation since 1999) treats several bleed streams and process water: sulfate reduction occurs in a $500\,m^3$ bioreactor where hydrogen is used as the electron donor.

Another process, integrating bacterial sulfate reduction with bioleaching by sulfur-oxidizing bacteria, was developed to remove contaminating toxic metals from soils. In this process, sulfur- and iron-oxidizing bacteria are employed to release metals from soils by the breakdown of sulfide minerals and production of sulfuric acid, which liberates acid-labile forms such as hydroxides, carbonates, or sorbed metals. Metals are liberated in the form of an acid sulfate solution, which enables both the large proportion of acidity and almost the entirety of metals to be removed by bacterial sulfate reduction. Precipitation efficiency is further increased by the addition of flocculating agents.

In confined systems, SRB can also bring about significant increases in bulk pH, which can enhance sulfide precipitation and lead to precipitation of hydroxides and carbonates of transition metals. When an organic substrate acts as the electron donor, bicarbonate is also generated:

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+$$

This has useful implications for the use of SRB in the remediation of acidic metal-processing waters and mine wastes, particularly where they are active within suspended or surface-attached mesophilic biofilms. SRB are reported to contribute significantly to metal removal in constructed wetlands as well as in alkalization of acidic mine wastes. Both sulfide generation and pH-related precipitation appear to be important. However, some studies have questioned the contribution of SRB in these broad-scale systems, arguing that Fe(III)-reducing bacteria make greater contribution where carbon is limiting, in terms of both metal removal and ameliorating low pH.

There is also evidence that extremely reducing conditions that develop during sulfate reduction can lead to chemical conversion of oxyanions into cationic species, which can be more easily precipitated or biosorbed. The indirect chemical reduction of Cr(VI), as soluble chromate (CrO_4^{2-}), to much less soluble Cr(III) cationic species by sulfide and/or Fe^{2+} in SRB culture appears to be at least partially responsible for the removal of chromate from solution by SRB.

Phosphatase-mediated metal precipitation

In this process, metal or radionuclide accumulation by bacterial (*Citrobacter* sp.) biomass is mediated by a phosphatase enzyme induced during metal-free growth, which liberates inorganic phosphate from a supplied organic phosphate donor molecule, for example, glycerol 2-phosphate. Metal/radionuclide cations are then precipitated as phosphates on the biomass to high levels. In addition, metal precipitation by secreted phosphate generated from polyphosphate hydrolysis has also been suggested as a mechanism to remove metals and actinides from aqueous waste streams.

High-gradient magnetic separation

Metal ion removal from solution has been achieved using bacteria rendered susceptible to magnetic fields. 'Nonmagnetic' bacteria can be made magnetic by the precipitation of metal phosphates (aerobic) or sulfides

(anaerobic) on their surfaces, as described previously. For those organisms producing iron sulfide, it has been found that this compound is not only magnetic but also an effective adsorbent for metallic elements.

Metal, Metalloid, and Organometal Transformations

Microorganisms can transform certain metal, metalloid, and organometallic species by oxidation, reduction, methylation, or dealkylation. Biomethylated derivatives are often volatile and may be eliminated from a system by evaporation. The two major metalloid transformation processes described are reduction of metalloid oxyanions to elemental forms and methylation.

Microbial reduction and oxidation of metalloid oxyanions

The reduction of selenate (Se(VI)) and selenite (Se(IV)) to elemental selenium can be catalyzed by numerous microbes, which can result in a red precipitate deposited around the cells and colonies. Some bacteria use SeO_4^{2-} as a terminal e^- acceptor in dissimilatory reduction as well as reduce and incorporate Se into organic components, for example, selenoproteins (assimilatory reduction). Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) can be reduced to Se^0, with SeO_3^{2-} reduction appearing more ubiquitous than SeO_4^{2-} reduction. However, only SeO_4^{2-} can support bacterial growth under anaerobic conditions: SeO_4^{2-} reduction to Se^0 is a major sink for Se oxyanions in anoxic sediments. Anaerobic SRB like *Desulfovibrio desulfuricans* can reduce selenate/selenite to Se^0, but neither oxyanion could be used for respiratory growth. Reduction to Se^0 can be considered a detoxification mechanism. Reduction of TeO_3^{2-} to Te^0 is also a means of detoxification found in bacteria and fungi, with Te^0 being deposited in or around the cells, resulting in black colonies. The opposite process of Se^0 oxidation can occur in soils and sediments. It is possible that Se^0 oxidation is similar to S oxidation, and may be mediated by heterotrophs and autotrophs. In aerobic soil slurries, Se^{4+} is the main product with lower amounts of Se^{6+} being produced; heterotrophic and autotrophic thiobacilli were believed to be the active organisms.

The extreme reducing conditions that SRB create can also result in indirect chemical reduction of metal(loid) and radionuclide species. This appears to be the case for U(VI), which is reduced chemically to U(IV) under highly reducing, sulfidic conditions. The ability of SRB to enzymatically reduce uranium is now established, and there has been some debate as to the relative contribution of chemical and enzymatic uranium reduction, with chemical reduction rates appearing relatively low. Nevertheless, while enzymatic reduction is effective in nonmetabolizing cells, some studies appear to support a role for chemical reduction in the presence of growing cells.

Technetium (which, as ^{99}Tc, is a long half-life product of the nuclear fuel cycle) is present in many environments as Tc(VII) in the form of highly mobile pertechnetate ion (TcO_4^-). Chemical and enzymatic reductive precipitation of Tc has been demonstrated in SRB, in that chemical precipitation appears to be more efficient for uranium, with sulfide as a reductant. Under sulfidogenic conditions, chemical precipitation is operated in preference to enzymatic reduction, and *D. desulfuricans* was able to precipitate Tc extracellularly, probably as sulfide.

In contrast to reductive precipitation of metal(loid) described above, arsenic reduction frequently increases the solubility of this toxic element. Microbial dissimilatory reduction of As(V) to As(III) has been identified as an important route for increased As toxicity in the environment. This capacity appeared, for some time, to be phylogenetically and metabolically separate from dissimilatory sulfate reduction, but a *Desulfotomaculum* strain has the capability to simultaneously reduce arsenic and sulfate and to stimulate the precipitation of As(III) sulfide. The capacity of SRB to reduce and solubilize As and for soluble As(III) to precipitate with sulfide has further potential for bioremediation.

Methylation of metalloids

Microbial methylation of metalloids to yield volatile derivatives, for example, dimethyl selenide, dimethyl telluride, or trimethylarsine, can be effected by a variety of bacteria, algae, and fungi. Bacteria and fungi are the most important Se-methylaters in soil, with the most frequently produced volatile being dimethyl selenide. Selenium methylation appears to involve the transfer of methyl groups as carbonium (CH_3^+) ions via the S-adenosyl methionine system. Arsenic compounds such as arsenate (As(V), AsO_4^{3-}), arsenite (As(III), AsO_2^-), and methylarsonic acid ($CH_3H_2AsO_3$), can be methylated to volatile dimethylarsine($(CH_3)_2HAs$) or trimethylarsine ($(CH_3)_3As$). Environmental factors that affect microbial activity can markedly affect Se methylation, for example, pH, temperature, organic amendments, Se speciation; however, the addition of organic amendments can stimulate methylation. The opposite process of demethylation can also occur in soil and water systems. Anaerobic demethylation may be mediated by methylotrophic bacteria.

Microbial metalloid transformations and bioremediation

In situ immobilization of SeO_4^{2-}, by reduction to Se^0, has been achieved in Se-contaminated sediments. Microbial methylation of selenium, resulting in volatilization, has also been used for *in situ* bioremediation of selenium-containing land and water at Kesterson Reservoir in the

United States. Selenium volatilization from soil was enhanced by optimizing soil moisture, particle size, and mixing, while waters it was stimulated by the growth phase, salinity, pH, and selenium concentration. Se-contaminated agricultural drainage water was evaporated to dryness until the sediment selenium concentration approached 100 mg Se kg^{-1} dry weight. Conditions such as carbon source, moisture, temperature, and aeration were then optimized for selenium volatilization, and the process continued until selenium levels in sediments declined to acceptable levels. Some potential for *ex situ* treatment of selenium-contaminated waters has also been demonstrated.

Mercury and organometals

Key microbial transformations of inorganic Hg^{2+} include reduction and methylation. The mechanism of bacterial Hg^{2+} resistance is enzymic reduction of Hg^{2+} to nontoxic volatile Hg^0 by mercuric reductase. Hg^{2+} may also arise from the action of organomercurial lyase on organomercurials. Since Hg^0 is volatile, this could provide one means of mercury removal. Methylation of inorganic Hg^{2+} leads to the formation of more toxic volatile derivatives; the bioremediation potential of this process (as for other metals and metalloids, besides selenium, capable of being methylated, e.g., As, Sn, and Pb) has not been explored in detail. In addition to organomercurials, other organometals may be degraded by microorganisms. Organoarsenicals can be demethylated by bacteria, while organotin degradation involves sequential removal of organic groups from the tin atom. In theory, such mechanisms and interaction with bioremediation possibilities described previously may provide a means of detoxification.

Concluding Remarks

Microorganisms play important roles in the environmental fate of toxic metals, metalloids, and radionuclides with physicochemical and biological mechanisms effecting transformations between soluble and insoluble phases. Such mechanisms are important components of natural biogeochemical cycles for metals and associated elements, for example, sulfur and phosphorus, with some processes being of potential application to the treatment of contaminated materials. The removal of such pollutants from contaminated solutions by living or dead microbial biomass and derived or excreted products may provide a means for element recovery and environmental protection. Although the biotechnological potential of some of these processes has only been explored in the laboratory or pilot scale, some mechanisms, notably bioleaching, biosorption, and precipitation, have been employed at a commercial scale. Of these, chemolithotrophic leaching is an established major process in mineral extraction but has also been applied to the treatment of contaminated land. There have been several attempts to commercialize biosorption using microbial biomass but success has been short-lived, primarily due to competition with commercially produced ion exchange media. Bioprecipitation of metals as sulfides has achieved large-scale application, and this holds out promise for further commercial development. Exploitation of other microbiological processes will undoubtedly depend on a number of scientific, economic, and political factors.

See also: Heavy Metals, Bacterial Resistance; Heavy Metals Cycle (Arsenic, Mercury, Selenium, others); Industrial Biotechnology, (overview); Metal Extraction and Biomining; Wastewater Treatment (not infectious hazards); Water Treatment, Industrial

Further Reading

Burford EP, Fomina M, and Gadd GM (2003) Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineralogical Magazine* 67: 1127–1155.

Chasteen TG and Bentley R (2003) Biomethylation of selenium and tellurium: Microorganisms and plants. *Chemical Reviews* 103: 1–26.

Gadd GM (1993a) Interactions of fungi with toxic metals. *New Phytologist* 124: 25–60.

Gadd GM (1993b) Microbial formation and transformation of organometallic and organometalloid compounds. *FEMS Microbiology Reviews* 11: 297–316.

Gadd GM (2000a) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology* 11: 271–279.

Gadd GM (2000b) Microbial interactions with tributyltin compounds: Detoxification, accumulation, and environmental fate. *Science of the Total Environment* 258: 119–127.

Gadd GM (2001a) Accumulation and transformation of metals by microorganisms. In: Rehm H-J, Reed G, Puhler A, and Stadler P (eds.) *Biotechnology, A Multi-volume Comprehensive Treatise, vol. 10: Special Processes*, pp. 225–264. Weinheim, Germany: Wiley-VCH Verlag GmbH.

Gadd GM (ed.) (2001b) *Fungi in Bioremediation*. Cambridge: Cambridge University Press.

Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122: 109–119.

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* 111: 3–49.

Gadd GM and Sayer GM (2000) Fungal transformations of metals and metalloids. In: Lovley DR (ed.) *Environmental Microbe-Metal Interactions*, pp. 237–256. Washington, DC: American Society for Microbiology.

Gadd GM and White C (1993) Microbial treatment of metal pollution – a working biotechnology. *Trends in Biotechnology* 11: 353–359.

Karlson U and Frankenberger WT (1993) Biological alkylation of selenium and tellurium. In: Sigel H and Sigel A (eds.) *Metal Ions in Biological Systems*, pp. 185–227. New York: Marcel Dekker.

Lloyd JR, Lovley DR, and Macaskie LE (2004) Biotechnological applications of metal-reducing microorganisms. *Advances in Applied Microbiology* 53: 85–128.

Macaskie LE (1991) The application of biotechnology to the treatment of wastes produced by the nuclear fuel cycle – biodegradation and bioaccumulation as a means of treating radionuclide-containing streams. *Critical Reviews in Biotechnology* 11: 41–112.