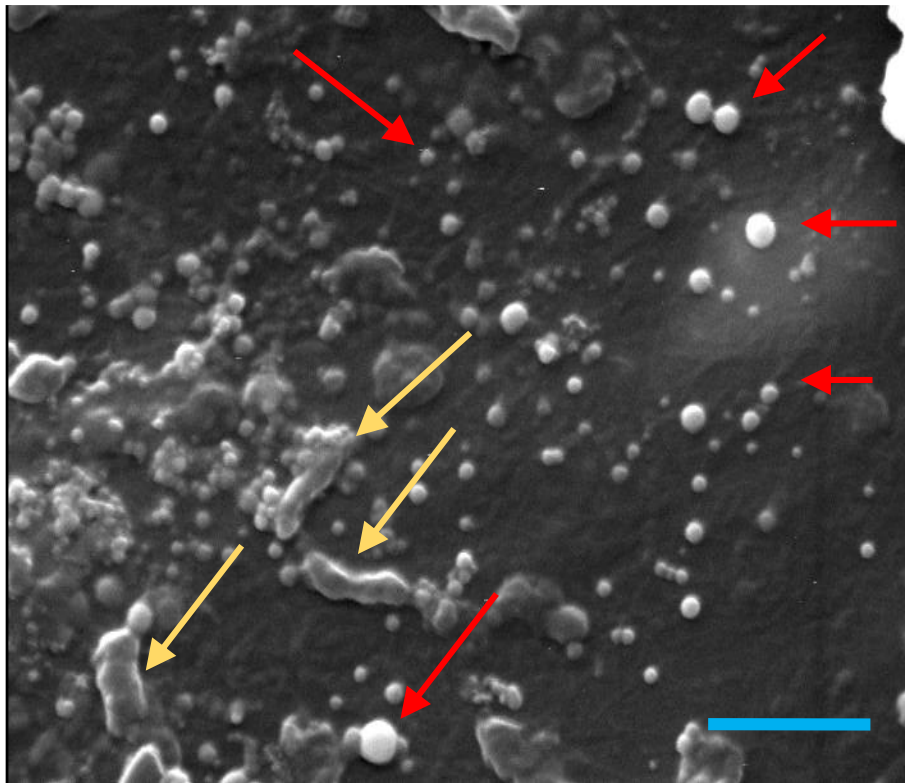


The heavy metal crisis and the microbial transformers

*Hey bro: What's all this worry about heavy metals?
Anything to do with dad's favourite music?*



Photograph of bacteria ('sausage'-shaped cells, indicated with yellow arrows) transforming the heavy metal selenium from a dissolved toxic form to a solid non-toxic form ('nanoparticle' ball shapes indicated with red arrows). The photograph was taken using a high-powered scanning electron microscope. For scale, the blue bar shows the length of a 1-micrometer ruler - that's one-millionth the length of a classroom metre stick or yardstick.

Tariq Siddique¹ and Julia M. Foght²

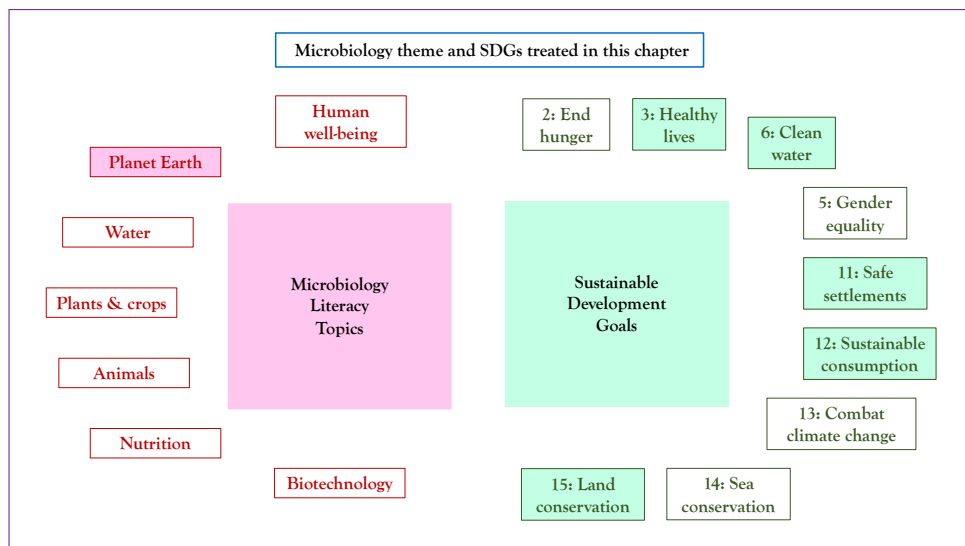
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The heavy metal crisis

Storyline

Environmental pollution by heavy metals has become a global concern in recent years due to its impact on public and environmental health. Metals have been extracted from ores and used in our daily lives for centuries; for example, in mercury thermometers, lead paints, aluminum foil food wrap, copper wiring, and nickel-cadmium batteries. Mining, industrial processes and waste generation lead to exposure, disposal and inadvertent release of heavy metals to the environment where receptors (e.g., microbes, plants and animals, including humans) can contact and/or ingest them, sometimes suffering toxic effects. Microbes are incredibly versatile and different types are widespread in environments ranging from freezing (polar regions) to boiling (hot springs), from acidic (acid mine drainage) to alkaline (soda lakes), and from aerobic (exposed to air) to anaerobic (zero oxygen). They also are versatile in what they do: for example, a few microbes cause diseases, some are used to produce food, some help plants grow, and some interact with metals in a variety of ways to generate energy for their growth and function. These microbe-metal interactions govern the cycling of elements in the environment when certain microbes generate energy in metal transformation processes and others obtain energy in the reverse process but under different environmental conditions. These microbial processes alter the chemical form (speciation) of metals, which increases or decreases their mobility in the environment and hence determines their pathways to environmental receptors. Speciation also affects the toxicity of the metals, making transformation processes very important to public safety and ecological health. Fortunately, many of these microbial processes can be harnessed by scientific technologies to clean up (bioremediate) both soil and groundwater at metal-contaminated sites. Environmental guidelines have been developed by different government jurisdictions to restrict the use of heavy metals in some products and to protect environmental receptors from heavy metal contamination.

The Microbiology and Societal Context

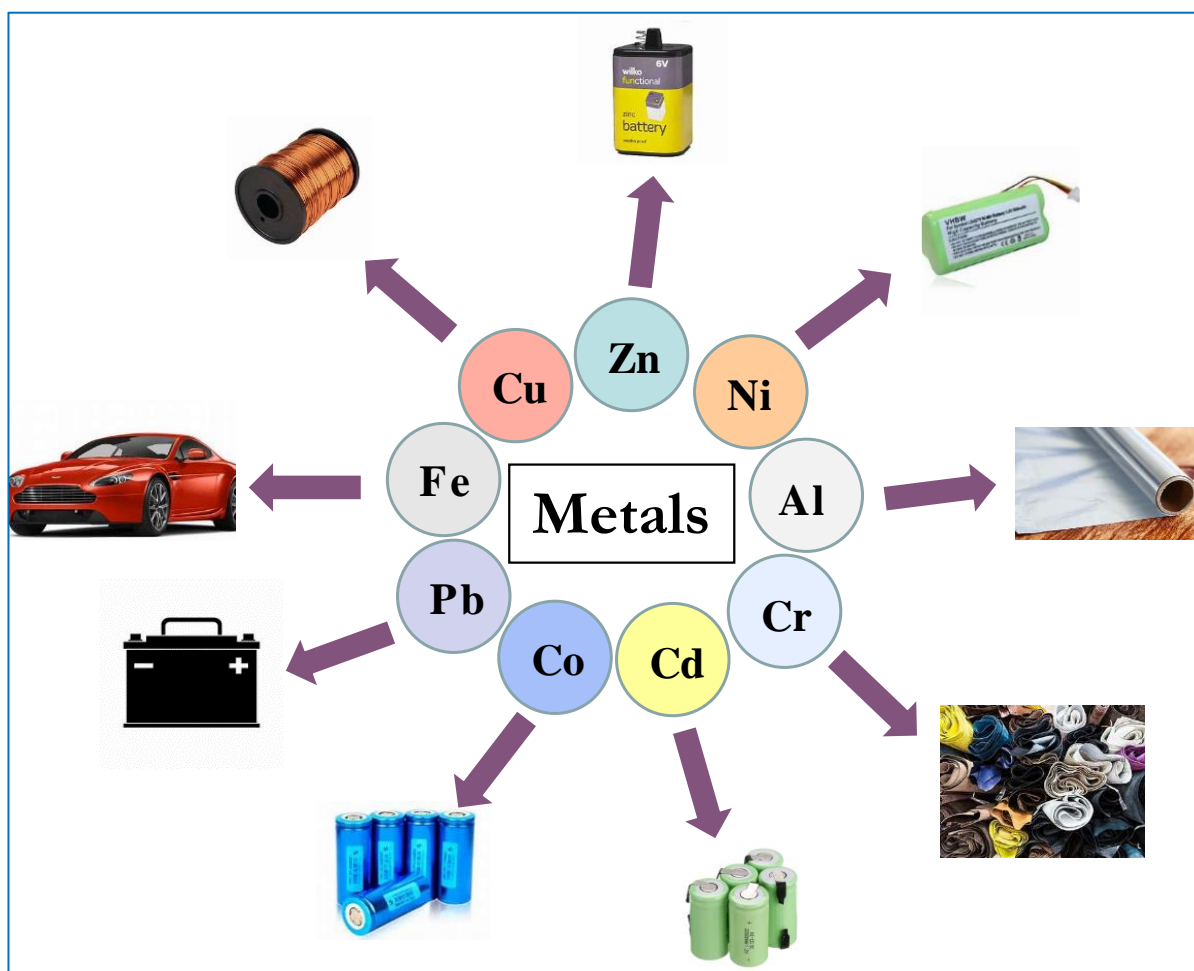


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The microbiology: Microbe-metal interactions; microbial metabolism; mechanisms of tolerance to metal toxicity; metal mobilization and immobilization; bioremediation; biomining and metal recovery. *Sustainability issues:* health; clean water; environmental pollution; sustainable production.

The heavy metal crisis: The Microbiology

1. **What are heavy metals?** “Heavy metal” is a vague and ambiguous term, having different definitions in metallurgy, physics, chemistry and biochemistry. Here, for simplicity, we use this term for all elements (as metals and metalloids fulfil some criteria of heavy metals) except non-metals, because any toxic metal or metalloid may be called a ‘heavy metal’ irrespective of atomic mass or density.



Box 1. Utilization of metals in daily life products.

Metals are naturally occurring elements that have the capacity to conduct electricity, are lustrous, have malleability and ductility, can form cations and form alkaline oxides. They are essential to our daily lives as they are used alone (in pure form) or combined with other metals (alloys) in a variety of tools, for food production, building construction, medical equipment,

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energy production, transport and communication. For example, steel (iron [Fe] alloyed with carbon and other metals) is the most widely used alloy in our homes, buildings, vehicles and factories. Thin aluminum (Al) foil is used in food packaging. Chromium (Cr)-containing chemicals are used in production of cement and leather tanning. Zinc (Zn), nickel (Ni), cadmium (Cd) and cobalt (Co)-containing chemicals are important in modern rechargeable battery technologies. Copper (Cu), having excellent electrical and thermal conductivity properties, is used in electrical cables. Lead (Pb) has been an important metal for thousands of years and its chemicals have been used in ceramic glazes, glass crystal, paint pigments, batteries and in gasoline additives (Box 1).

2. *We and other members of the biosphere need metals, but only in trace amounts.* Many metabolic reactions of organisms involve enzymes and other proteins with metal or metal-containing co-factors, additional components they need in order to effectively carry out their work. For example, via our blood circulatory system, haemoglobin in red blood cells transports oxygen we breathe into our lungs to all parts of our bodies; iron is an essential component of haemoglobin needed for binding oxygen. We need certain metals, so if we have too little of one we need we become ill. On the other hand, if we are exposed to too much, we also become ill. There is a fine line between what is essential and what is toxic!

Because some heavy metals can severely affect the behavior, growth, and health of plants and animals, many countries have established environmental limits for concentrations of heavy metals that are considered to be safe, and routinely monitor levels of heavy metals in drinking water and the environment. Countries have also reduced or restricted the use of heavy metals in some products (e.g., mercury [Hg] in thermometers, arsenic [As] in paints, As and Cr in agricultural pesticides and wood preservatives).

3. *Sources and environmental fates of heavy metals.* Stars create chemical elements such as metals that are distributed throughout space during supernova events (<https://spaceplace.nasa.gov/supernova/en/>). Metals became part of the Earth at the time of its formation 4.5 billion years ago. Metals in combination with non-metals (for example oxygen, hydrogen, carbon, sulfur, chlorine and phosphorus) form a variety of earth rock minerals such as oxides, silicates, carbonates, sulfates, sulfides, halides and phosphates, which constitute the Earth's crust.

“Weathering” processes, such as physical disintegration of rock into finer particles, and biogeochemical processes involving deterioration of minerals through interaction with living organisms, result in the formation of soil on the Earth's crust. These weathering processes (physical and biogeochemical) continue in the soil system and cause the transfer (movement) of metals from primary minerals (e.g., quartz, feldspar and mica) in sand and silt fractions of soil derived from rock to secondary minerals, such as phyllosilicates and oxide minerals in the clay fraction of soil, and eventually to the water coating soil particles (soil moisture). Once dissolved in soil water, metals can freely distribute and affect organisms in the biosphere.

Soil contains nearly all the elements in the periodic table, and the average elemental content of soils closely mirrors the mean values for the Earth's crust. Soil minerals and soil organic matter are ‘host phases’ of these elements. Soil organic matter is formed in soil mainly through decomposition of plant materials (those grown on soil) and is a major source of carbon

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(C), nitrogen (N), phosphorus (P) and sulfur (S), which are non-metals but essential for microbial (and plant and animal) growth.

4. **The key issue of bioavailability.** The bioavailability of an element or compound is the fraction of it that is accessible to an organism for uptake or absorption across its cellular membrane. A high concentration of a toxic metal in soil compared to its natural concentration in uncontaminated soil (background concentration range) does not indicate that a contamination problem or risk exists if the metal is part of a mineral crystal and is not **bioavailable**, i.e., if it cannot interact with living organisms. Conversely, a lower concentration of a toxic metal than background concentrations found in uncontaminated soil may pose an environmental risk if the metal is bioavailable and present in a toxic form (species).

5. **The key issue of speciation.** Mineral dissolution in soil water makes metals in their ionic forms (positively charged cations, negatively charged anions, oxyanions or oxycations) available for chemical/biochemical reactions and helps transport these 'free' metal **species** to environmental receptors such as plants, humans, wildlife etc. Therefore, knowledge of free ionic species of the elements in the environment is crucial to determine their mobility, toxicity, and bioavailability to the environmental receptors. Some metals can exist in several different forms (**elemental speciation**) and toxicities, depending upon the oxidation state of the element (indicated by Roman numeral superscripts), which varies with redox status of the soil containing the elements (aerobic versus anaerobic conditions) and its pH. **Box 2** shows speciation (different chemical forms) of elements and their potential toxicity in the environment.

Box 2: Elemental speciation and toxicity under aerobic and anaerobic environments

Elements	Aerobic environment ‡	Anaerobic environment *
Selenium (Se)	<u>Oxidation state: Se^{VI}</u> • Selenate (SeO ₄ ²⁻), soluble, toxic <u>Oxidation state: Se^{IV}</u> • Selenite (SeO ₃ ²⁻), soluble, toxic	<u>Oxidation state: Se⁰</u> • Elemental Se, solids, non-toxic <u>Oxidation state: Se^{II}</u> • Selenide (Se ²⁻), soluble, non-toxic • Dimethylselenide (CH ₃) ₂ Se, non-toxic volatile species
Chromium (Cr)	<u>Oxidation state: Cr^{VI}</u> • Chromate (CrO ₄ ²⁻), soluble, toxic	<u>Oxidation state: Cr^{III}</u> • Cr ³⁺ (low pH), cation • Cr(OH) ₃ (higher pH), solids
Uranium (U)	<u>Oxidation state: U^{VI}</u> • Uranyl (UO ₂ ²⁺), soluble, toxic	<u>Oxidation state: U^{IV}</u> • Uraninite (UO ₂), solids, non-toxic
Vanadium (V)	<u>Oxidation state: V^V</u> • VO ₂ ⁺ , soluble and toxic	<u>Oxidation state: V^{IV}</u> • VO ₂ ⁺ , solids, non-toxic
Arsenic (As)	<u>Oxidation state: As^V</u>	<u>Oxidation state: As^{III}</u>

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	<ul style="list-style-type: none"> • Arsenate (HAsO_4^{2-}), Less toxic, less soluble 	<ul style="list-style-type: none"> • Arsenite, HAsO_3^{2-}, more soluble, more toxic
Antimony (Sb)	<u>Oxidation state: Sb^{V}</u> <ul style="list-style-type: none"> • Antimonate, $\text{Sb}(\text{OH})_6^-$, less soluble, less toxic 	<u>Oxidation state: Sb^{III}</u> <ul style="list-style-type: none"> • Antimonite, $\text{Sb}(\text{OH})_3^0$, more soluble and more toxic
Iron (Fe)	<u>Oxidation state: Fe^{III}</u> <ul style="list-style-type: none"> • Iron oxide (Fe_2O_3), solid 	<u>Oxidation state: Fe^{II}</u> <ul style="list-style-type: none"> • Soluble cation, Fe^{2+}
Manganese (Mn)	<u>Oxidation state: Mn^{IV}</u> <ul style="list-style-type: none"> • Manganese oxide (MnO_2), solid 	<u>Oxidation state: Mn^{II}</u> <ul style="list-style-type: none"> • Soluble cation, Mn^{2+}
Sulfur (S)	<u>Oxidation state: S^{VI}</u> <ul style="list-style-type: none"> • Sulfate (SO_4^{2-}), soluble 	<u>Oxidation state: S^{II}</u> <ul style="list-style-type: none"> • Sulfide (H_2S, HS^-, S^{2-})

‡ Aerobic environments exist in the soil vadose (water-unsaturated) zone. Oxidized species (deficient in electrons) are present in aerobic conditions. These oxidized species become electron acceptors for chemoorganoheterotrophs under anaerobic conditions. * Anaerobic environments exist in wetlands, water-logged (saturated) soils, and groundwater. Reduced species (rich in electrons) are present in anaerobic conditions. These reduced species can serve as electron donors for chemolithoautotrophs under both aerobic and anaerobic environments.

6. *How do metals contaminate the environment? (natural and anthropogenic sources)*

Although weathering of minerals is the natural source of heavy metals in the soil, metal pollution in the environment has emerged mainly due to industrial and agricultural activities, as well as deposition from the atmosphere. Industrial sources include surface mining (that produces fluid tailings, acid mine drainage etc.), metal smelting (smelter waste), coal combustion in power plants, nuclear power stations and other industrial processes such as textile and paper processing plants.

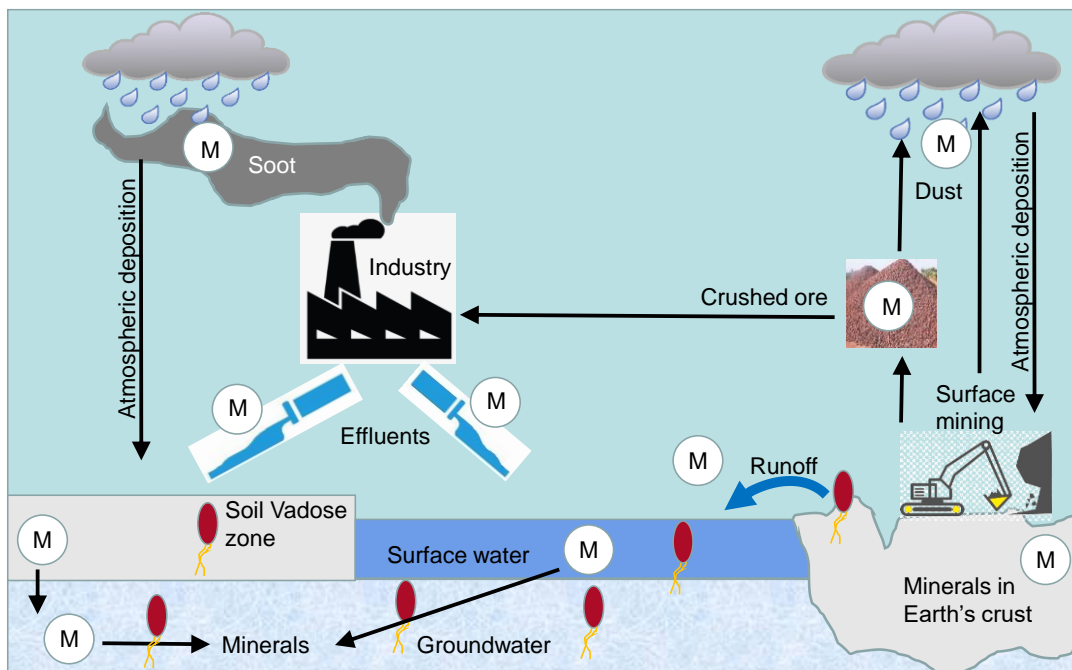
In agriculture, in addition to soil parent material (rock minerals) itself, the use of fertilizers, soil amendments such as limestone, pesticides, biosolids, and chicken and livestock manures contributes to heavy metal accumulation in soil. Landfarming of biosolids wastes for agricultural benefits (source of nutrients) and disposal, which has the tendency to concentrate heavy metals in agricultural soils, is a major potential source of heavy metal contamination. Industry and transportation (automobile exhaust) also emit heavy metals to the atmosphere. This atmospheric pollution not only affects the communities residing in the vicinity of these activities, but also affects the distant regions through atmospheric transport and deposition.

7. *How do we become exposed to heavy metals?* At sufficiently high concentrations in the soil, heavy metals can:

- a. be translocated into plants through their roots, and subsequently become accessible to animals and humans during food consumption;
- b. be leached into groundwater, a source of drinking water for many areas around the globe;
- c. contaminate surface water bodies through surface runoff, and bioaccumulate in other biota for subsequent translocation to higher trophic levels in the food web;

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d. be introduced into the atmosphere through dust blown from the soil surface. Therefore, metals may enter a human through food ingestion, inhalation, skin contact and drinking of contaminated water.



Life cycle assessment of metals (M). Metals originating from Earth's crust through anthropogenic activities passing through different environmental media end up in sediment minerals by the mediation of indigenous microbes.

Some metals (for example, vanadium [V], Cr, manganese [Mn], Fe, Co, Ni, Cu, Zn, selenium [Se], molybdenum [Mo], etc.) are considered essential for living organisms to maintain cell structure and function, but only at trace concentrations. Higher concentrations of these essential metals along with other nonessential metals can cause toxicity as these metals can affect cells and their organelles such as mitochondria, nuclei, lysosomes, cell membrane and enzymes. Metal ions can also interact with DNA and nuclear proteins and cause DNA damage that may cause cancer. Lead can cause cognitive and behavioural problems, especially in children.

Soil microbes greatly influence the mobilization and immobilization of heavy metals in the environment and therefore impact public health globally. Some of the important roles of microbes are briefly discussed in the following sections.

8. *How do microbes interact with metals/minerals to gain energy?* Life on Earth exists primarily due to redox reactions (transfer of electrons from one element to another element) carried out by living organisms, and these redox reactions involve transformation of elements that generate energy for cell growth and function. Microbes require three things for their metabolism: elements, primarily carbon along with small concentrations of N, P and S etc. for cell synthesis; electrons for energy generation when electrons are transferred through an electron transport chain (some microbes use light energy to initiate the electron transport chain); and a terminal electron acceptor that receives electrons at the end of the chain.

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Unlike humans, who use oxygen as a terminal electron acceptor, various microbes can utilize additional elements as electron acceptors if oxygen is not available in the environment (i.e., is anaerobic). Based on these metabolic needs, two major groups of microbes mobilize and immobilize elements/metals in the environment: chemolithoautotrophs and chemoorganoheterotrophs.

Chemolithoautotrophs do not require organic molecules for their carbon or electron needs. Instead, they use electrons from inorganic reduced (i.e., electron-rich) forms of redox-sensitive elements (e.g., Fe, Mn, S and N from the lithosphere) to generate energy through electron transport, and fix carbon dioxide gas (CO₂) into organic molecules to create biomass. These microbes are generally aerobic (use oxygen as terminal electron acceptor), although some live in anaerobic environments by using alternative electron acceptors, and are involved in elemental cycling in the environment.

Some facts about microbes in the subsurface

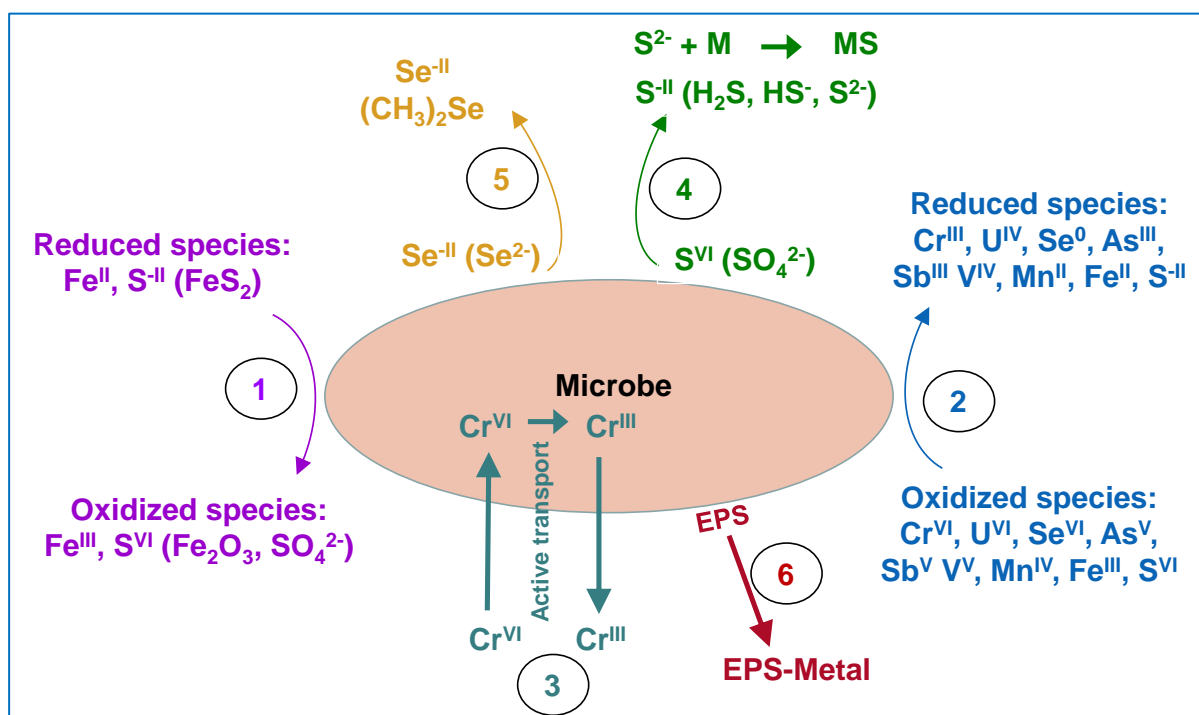
- Microbes live in many different environments including at and beneath the Earth's surface, perhaps as deep as 6.7 km underground and 10 km below the sea surface.
- Microbes can live under aerobic and anaerobic (oxygen-free) conditions; some specialize in one or the other condition and some can switch depending on access to air.
- Some microbes (chemolithoautotrophs) can live without needing any external organic compounds, needing only inorganic substrates like water, rocks and carbon dioxide.
- Some microbes (chemoorganoheterotrophs) get energy and nutrients for growth by converting organic molecules to water and carbon dioxide, i.e., breaking chemical bonds to make new compounds; this process is called **biodegradation**.
- Heavy metals, which are inorganic molecules, cannot be biodegraded because they cannot be broken down. Instead, some microbes get energy and nutrients through **biotransformation** of the metals, by changing the metals' oxidation state, making them **volatile**, and/or by precipitating or solubilizing the metals.
- Both biodegradation and biotransformation can be used to **bioremediate** contaminated environments, depending on whether the contaminants are organic or inorganic. Sometimes bioremediation can be stimulated by adding essential nutrients.
- Excessive concentrations of contaminants (whether metals or organics) can inhibit microbial growth. Conversely, microbial metabolism is not efficient at very low concentrations. That is, their bioremediation abilities are not limitless.

Chemoorganoheterotrophs also get energy through chemical reactions but their sources of carbon and electrons are organic carbon compounds (humans and other animals are also chemoorganoheterotrophs). This group of microbes is mainly responsible for the biodegradation of organic compounds in the environment. They can also play a role in the biotransformation (e.g., precipitation) of metals, particularly redox-sensitive metals (those that can change their oxidation states) such as hexavalent chromium (Cr^{VI}), hexavalent selenium (Se^{VI}), hexavalent

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uranium (U^{VI}) and pentavalent vanadium (V^V), along with other redox-sensitive metals under anaerobic conditions (**Box 2**). Chemoorganoheterotrophs can be aerobic and/or anaerobic and thrive in a wide diversity of environments. The metabolism of these two general groups of microbes contributes globally to mobilization and immobilization of heavy metals and hence their toxicity in the environment.

Keeping in mind these microbial metabolic needs, we can understand the microbial interactions with heavy metals or rock/soil minerals in the environment. The two major microbial processes, oxidation of reduced species as electron donors primarily under aerobic conditions (**Box 3, Process 1**), and reduction of oxidized species of redox-sensitive elements as electron acceptors under anaerobic conditions (**Box 3, Process 2**), are dominant and significant in elemental cycling (precipitation and dissolution) in the environment. Their implications in environmental biogeochemical cycling are described in following sections. The process of reducing oxidized redox-sensitive metals (as electron acceptors) by chemoorganoheterotrophs is also known as dissimilatory reduction, by which microbes generate energy through this reduction process.



Box 3. Microbe-metal interactions responsible for mobilization, immobilization, precipitation and/or volatilization of metals: (1) Oxidation (biobleaching); (2) Dissimilatory reduction; (3) Assimilatory reduction (a resistance mechanism); (4) Dissimilatory reduction plus chemical reaction (bioprecipitation); (5) Methylation and (6) Biosorption to extracellular polymeric substances (EPS).

9. *How do microbes interact with metals/minerals to counteract their toxicity?* In addition to these two major energy-generating microbial interactions with metals/minerals, microbes also interact with metals when they are coping with potentially toxic high concentrations of metals, to avoid or de-toxify those metals, using several different mechanisms.

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Some microbes produce extracellular polymeric substances (EPS, a type of slime) on the outer surfaces of their cells to sorb soluble metals and prevent their transport into the cell, thereby reducing toxicity (**Box 3, Process 6**).

Microbes also transport soluble toxic metals (generally the soluble oxidized species of metals) into their cells through active transport by consuming energy, transform them into a nontoxic form (i.e., into their reduced insoluble species) in the cell, then expel those insoluble forms of metals out of the cells. This reduction process, a resistance mechanism, is known as assimilatory reduction where microbes do not generate energy but rather consume energy for metal detoxification (**Box 3, Process 3**).

Some microbes use enzymes to add a methyl group ($-CH_3$) to metals, forming volatile metal species that evaporates away from the cells. This process is more common in anaerobic aquatic systems where reduced species of metals such as selenium (Se), arsenic (As), antimony (Sb) and mercury are methylated as a part of detoxification process or are formed as intermediates during microbial metabolism (**Box 3, Process 5**).

Microbes also indirectly precipitate some metals (transition metals) under anaerobic conditions, for example when they reduce sulfate (SO_4^{2-}) to sulfide (S^{2-}) that then chemically reacts with transition metals such as Zn, Cu, Fe and Mn to produce sulfide minerals that are not bioavailable (**Box 3, Process 4**).

10. How do microbes mobilize metals for environmental pollution and metal recovery from mineral ores? Microbes dissolve some minerals through oxidation and reduction reactions and release mineral-bound metals to the environment. A few prominent real-world examples of microbial transformations that increase mobility of metals and hence their toxicity in the environment are outlined below. Some effects are harmful to the environment and public health while others have benefits and biotechnology potential.

a. *Acid Mine Drainage from mine sites.* Human development over centuries is beholden to natural resources (fuels, metals, etc. for societal use) buried in the Earth's crust. Surface (open-pit) mining of these resources exposes buried rock material such as sulfide-bearing minerals to the atmosphere. Chemolithoautotrophs such as the sulfur-/sulfide-oxidizing bacteria (e.g., *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans* and *Sulfobacillus* spp.) generate energy by oxidizing reduced iron (Fe^{II}) and reduced sulfur (S^0 and S^{II}), using them as electron donors, in rock material in the presence of oxygen and water.

Oxidation of sulfide minerals, which can happen chemically but is accelerated by microbial activity, dissolves sulfide minerals, liberates iron/sulfide-bound heavy metals such as Zn, Cu, Cd, Fe, Mn, Ni, Pb, Hg, Cr, As, V and Se, and generates protons that drastically acidify the water to $pH < 2$ (**Box 3, Process 1**). The resulting water with extremely high acidity (acidic runoff) further dissolves heavy metals into groundwater or surface water when it comes in contact with sediments.

Globally, there are numerous abandoned mines that pose environmental threats, and rivers that are impacted by natural and mine-associated acidic drainage (e.g. the Rio Tinto river in Spain). The acidity and high metal content of such water bodies can be highly toxic to fish and other organisms downstream. Thus, run-off from uncontrolled metal oxidation, whether natural or from mines, can be detrimental to the downstream environment.

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On the other hand, this natural microbial activity can be exploited as a biotechnological process, using chemolithoautotrophs to extract metals from low-grade sulfidic ores in locations where conventional mining methods are not feasible or economical, for example, in Chile to extract copper from low-grade ores. In this biohydrometallurgical process, also known as bioleaching, bacteria oxidize sulfide in sulfidic ores or oxidize elemental sulfur (S^0) added to the ores, producing biogenic sulfuric acid (a source of protons) that solubilizes the metals. The leachate is collected and treated to recover the metals and recycled to continue the bioleaching process, thus preventing acidic runoff.

Bioleaching is also used to recover and recycle rare earth elements and precious metals from mining wastes and the waste from electrical and electronic equipment (WEEE). For example, spent lithium (Li) ion batteries are one type of WEEE stream containing significant concentrations of valuable metals such as Co, Li, Mn and Ni that can be recovered through a biohydrometallurgical process.

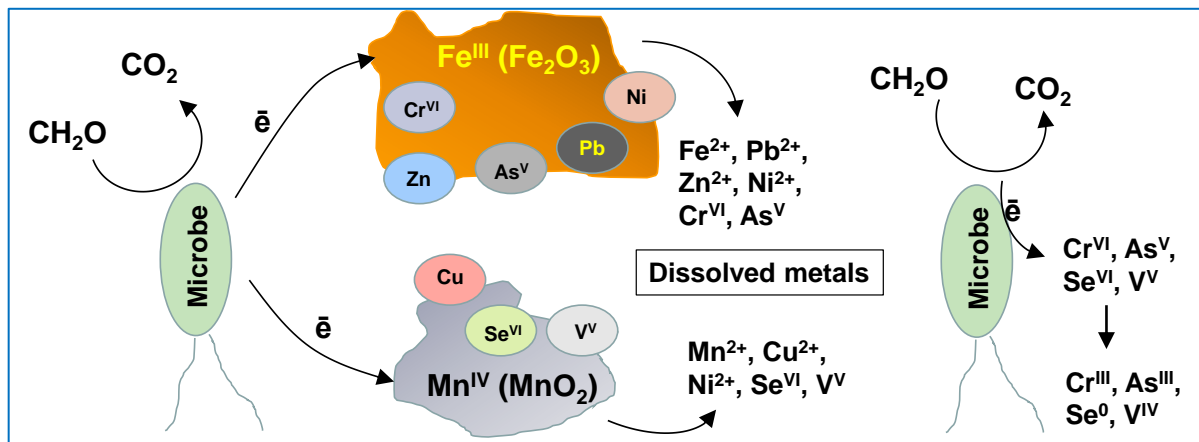
b. *Arsenic (As) poisoning in Bangladesh.* Globally, more than 2.5 billion people rely on groundwater for drinking and cooking, and provision of good quality drinking water has become a major global challenge. Prior to 1970, people in Bangladesh used to drink water from surface sources such as ponds and rivers but surface water contamination with sewage bacteria caused hundreds of thousands of infection-related deaths each year. In the 1970's, UNICEF initiated a clean water supply program and installed one million tube-wells to provide drinking water. Unfortunately, these wells started producing As-laden water that poisoned and affected the lives of millions of people in Bangladesh – the largest mass poisoning in human history.

There are two schools of thought describing As mobilization from soil into groundwater and both are associated with microbial processes. One process is microbial oxidation of sulfide minerals. Over-exploitation of groundwater (because of water removal through the wells) lowered the water table and exposed to oxygen (in air) the once-submerged (anaerobic) sulfide minerals containing As. Sulfur-/sulfide-oxidizing chemolithoautotrophs oxidized and dissolved the sulfide minerals (as described in “Acid Mine Drainage”, above), releasing sulfide-bound As to the drinking water. The second microbial process with more evidence for As poisoning of groundwater is “Reductive Dissolution” of iron oxide minerals in aquifer sediments (**Box 4**). Dissolved organic carbon (organic matter) in groundwater provides carbon and electrons to iron-reducing chemoorganoheterotrophs for their metabolism and growth. In the absence of oxygen, these microbes use iron oxide minerals (containing Fe^{III} that is deficient in electrons) as an electron acceptor during oxidation of organic matter and dissolve those iron oxide minerals to release soluble Fe^{2+} (Fe^{II}). Consequently, As and other heavy metals co-precipitated or adsorbed on iron oxide minerals are released to groundwater during reductive dissolution of iron oxide minerals (**Box 4**).

c. *Mine tailings dam disaster in Brazil.* In 2015, a large-scale mine tailings dam disaster occurred in Brazil releasing 43 million m^3 of iron and manganese oxide mineral-rich tailings that were transported 600 km into the Rio Doce, one of the country's largest river basins. Recent investigations reveal an imminent environmental disaster in the future when plants that colonize the deposited tailings-estuarine sediments will input organic matter to this anaerobic environment. It is expected that iron- and manganese-reducing chemoorganoheterotrophs will use the plant matter as a source of carbon and electrons for their metabolism and reductively dissolve Fe^{III} and Mn^{IV} (deficient in electrons and used as electron acceptors under anaerobic

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conditions) oxide minerals (**Box 4**), releasing a wide range of mineral-bound heavy metals to the estuarine environment and posing a toxicity risk for downstream organisms including marine organisms. This process is the dissimilatory reduction shown in **Box 3, Process 2**.



Box 4. Reductive dissolution (dissimilatory reduction) of oxide minerals of iron (Fe^{III}) and manganese (Mn^{IV}) by chemoorganoheterotrophs under anaerobic conditions, using organic matter (abbreviated as CH_2O) as the electron source and metals as the electron acceptors. Adsorbed/co-precipitated metals are released to the environment and redox-sensitive metals are further reduced by other chemoorganoheterotrophs that use them as electron acceptors.

11. *How do microbes bioremediate metal-contaminated environments?* Unlike organic pollutants such as hydrocarbons, pesticides, industrial organic solvents and other natural and synthetic organic compounds that are biodegraded (i.e., broken down into simpler compounds) by chemoorganoheterotrophs to obtain carbon, electrons and energy, metals cannot be biodegraded. Rather, metals are transformed from one form to another by microbial processes that increase or decrease metal mobility in the environment through dissolution and precipitation. Microbial immobilization of metals to reduce their bioavailability is an important bioremediation approach that environmental companies use to reduce groundwater contamination where anaerobic conditions prevail, as shown by the examples below.

a. *Selenium contamination in California, USA.* The Kesterson National Wildlife Refuge in central California is well-known for Se contamination: deaths and deformities of thousands of birds and fish due to Se toxicity were discovered in 1983. This widespread water contamination is still an unresolved environmental issue four decades later.

Seleniferous soils in western North America that developed on marine sedimentary rocks contain elevated levels of Se. Agricultural irrigation water dissolves Se as selenate (Se^{VI} ; SeO_4^{2-}) from soils, then drainage water from irrigation carries this soluble toxic species of Se (**Box 2**) to water storage ponds and other surface waterbodies, where it causes harm to wildlife.

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unlined ponds, despite knowing that it caused illness in the surrounding community. A law suit filed against the company finally concluded in 2008 with a total amount of \$648 million paid (1996 to 2008) by PG&E to affected residents of the town, the largest settlement in a direct-action lawsuit in the USA at that time.

Groundwater contamination with toxic redox-sensitive metals such as Cr^{VI} , Se^{VI} , uranium (U^{VI}), and V^{V} can be bioremediated by precipitating those metals out of the aqueous phase, and thereby reducing their bioavailability. If organic substrates such as molasses or emulsified vegetable oil are injected into groundwater contaminated with such metals, various metal-reducing chemoorganoheterotrophs will use the added organic substrates as carbon and electron sources and use those soluble toxic oxidized metals as electron acceptors to generate cellular energy for growth and function. Transferring the electrons to the metals (i.e., reducing them) causes the metals to precipitate out of the aqueous phase. This microbial approach (dissimilatory reduction) is used around the globe for remediating groundwater contaminated with the redox-sensitive elements (**Box 2; Box 3, Process 2**).

c. *Transition metal contamination in groundwater.* Soluble toxic “transition metals” such as non-redox-sensitive cobalt (Co^{2+}), Ni^{2+} , Zn^{2+} , Cu^{2+} , lead (Pb^{2+}), and cadmium (Cd^{2+}) including Mn^{2+} and Fe^{2+} can be bioremediated in anaerobic groundwater using a microbial process known as bioprecipitation (**Box 3, Process 4**). Bioprecipitation includes both biological and chemical processes in a sequence. Organic carbon substrates such as emulsified vegetable oil are injected into the groundwater along with sulfate (SO_4^{2-}), a soluble anion and oxidized species of S (S^{VI}). These amendments stimulate chemoorganoheterotrophic groundwater microbes such as sulfate-reducing bacteria (SRB) in groundwater sediments. SRB utilize organic substrates as carbon and electron sources, and use SO_4^{2-} as the electron acceptor under anaerobic conditions.

The dissimilatory reduction process (**Box 3, Process 2**) transforms soluble SO_4^{2-} to its reduced sulfide (S^{II}) species such as hydrogen sulfides (H_2S and HS^-) and sulfide (S^{2-}), depending on groundwater pH (acidic to slightly alkaline conditions favor hydrogen sulfide formation). These newly-produced sulfide species are very reactive and chemically react with transition metals to precipitate them out of the aqueous phase as sulfide minerals such as ZnS , FeS , MnS , CuS , PbS , NiS , CoS and CdS that become the part of groundwater sediments like other sediment minerals. These sulfide minerals are very stable under anaerobic conditions and no longer contribute to groundwater metal toxicity.

This microbial approach has been used in a biotechnological process that treats water contaminated with transition metals. A smelter operated by Budelco B.V. in southeastern Netherlands produces zinc (Zn) and its alloys, and the Zn-contaminated water from the smelter is treated using a high flow anaerobic bioreactor. Inflowing groundwater contains high levels of Zn and is supplemented with SO_4^{2-} . The bioreactor is fed with ethanol, providing a source of organic carbon and electrons for SRB to reduce the sulfate and precipitate the zinc. The bioreactor discharge outflow contains trace concentrations of Zn which meet environmental guidelines.

12. *Guidelines, bioremediation goals and challenges.* Guidelines for acceptable environmental concentrations of contaminants are developed by federal authorities for individual jurisdictions; for example, United State Environmental Protection Agency (USEPA), Canadian Council of Ministers for the Environment (CCME), European Environment Agency

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(EEA), and Australian Environment Agency (AEA) provide frameworks for the management of contaminated sites through remediation or exposure control. Typically, it is not practical to bioremediate the contaminants to their background concentrations; rather, a more pragmatic approach is used to lower contaminant concentrations to levels that do not pose risks to human and ecological health, while conserving the natural resources including soil. Guidelines are developed for different land uses including natural areas, agriculture, residential/parkland, and commercial and industrial sites, by defining exposure pathways and environmental receptors.

Bioremediation involves various microbial processes with the fundamental principle to stimulate microbes for optimum growth and enzyme activity. Under specific conditions, microbes degrade organic compounds (substrates)/contaminants and transform metals/elements to gain energy for growth and function. Environmental conditions such as nutrients (e.g., N, P and other trace elements), carbon sources, bioavailability of contaminants, temperature, pH, high (inhibitory) concentrations of contaminants, and redox (aerobic or anaerobic) conditions impact the rate of microbial degradation and transformation processes. Therefore, bioremediation goals set for the cleaning of contaminated sites are sometimes difficult to achieve, particularly if site conditions are not optimal for microbial growth and function. However, biotransformation of metals can be successfully used at a global scale to bioremediate metal-contaminated environments (converting bioavailable metals to their solid non-available states).

Summarizing the microbial processes discussed in this chapter and applying them to evaluating metals life-cycle assessment, we can confidently say that microbes on earth really are “Transformers”. Though they increase mobilization of some metals in the environment during anthropogenic activities such as mining, their ultimate processes return metals back to the sediments as solid minerals sustaining life on Earth.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Healthy lives:** Effective monitoring and mitigation strategies ensure healthy lives and promote well-being for the public of all ages. If we understand the circumstances/conditions in which microbial activity might promote contamination of drinking water and agricultural soil with heavy metals, we can better predict and monitor these potential sources and contamination pathways, and intervene before human health is affected.

- **Goal 6. Clean water:** Biotechnology approaches can be applied to contaminated drinking water, agricultural irrigation water and industrial effluents, either by exploiting natural microbes *in situ* or by treating water in bioreactors. The goals are to bioremediate water and soil that are currently contaminated with heavy metals in a cost-effective manner to achieve government safety guidelines for heavy metal remediation.

- **Goal 11. Healthy environments:** Industrial activities such as mining (coal, oil sands and precious metals like gold and uranium) and natural weathering processes accelerate natural processes of metal mobilization and create huge environmental footprints of industrial wastes such as tailings ponds, effluent storage lagoons etc., posing significant environmental threats to the biosphere. Employing microbial processes (such as dissimilatory reduction) in those anaerobic environments can reduce bioavailability of heavy metals and their transport to environmental receptors such as humans and wildlife. Furthermore, implementing biotechnology processes can prevent future metal pollution in the environment by treating

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natural sources of heavy metals where they may impact the environment, and by removing heavy metals from anthropogenic sources of this pollution.

- **Goal 12. Sustainable consumption:** Mining and metals are essential for human needs and progression. It is estimated that the world's population will grow to 9.6 billion in next 25 years, which will increase demand on Earth's resources, including metals. Sustainability will be a major challenge for our planet in coming years. Therefore, it has become increasingly important to use the planet's resources diligently and this can be achieved by improving industrial practices or processes for less waste production, proper waste management including recycling of metals and reclamation/remediation of metal-contaminated sites following evolving legislative measures. This integrative approach will foster a foreseeable healthy global economy and sustain health and the environment for future generations.

- **Goal 15. Land conservation:** Higher concentrations of heavy metals in soil associated with industrial activities produce stressed conditions not conducive to plant growth. Heavy metal toxicity in soil also inhibits seed germination. Plant growth promoting microbes in soil help plants by providing plant hormones, stabilizing metals in the root zone by decomposing organic matter and exposing functional groups for adsorption, and sometimes facilitating metal extraction by plants (microbially assisted phytoremediation of metal contaminated soil). Therefore, microbes play a great role in land conservation, reclamation and remediation.

Potential Implications for Decisions

1. *Individual*

- a. choose products that are certified to be low in heavy metals (e.g., non-lead-based paint; metal free deodorant/antiperspirant, sunscreen without titanium dioxide)
- b. dispose of heavy metal sources (e.g., batteries) in a responsible manner by returning them to appropriate recycling facilities
- c. reduce personal use of disposable heavy metal sources (e.g., single-use batteries)
- d. buy food (particularly vegetables) from reputable sources that do not irrigate with polluted water
- e. stay informed of pollution events by news reports

2. *Community policies*

- a. demand that appropriate recycling facilities are available to responsibly handle wastes that are potential sources of heavy metal pollution
- b. be aware of potential sources of heavy metals in the local environment (landfill sites, abandoned mines, landfarming of sewage treatment plant biosolids wastes)
- c. support companies that conduct their business in an environmentally responsible manner
- d. create public awareness about metal contamination and health effects

3. *National policies*

- a. establish, maintain and regularly update guidelines for acceptable levels of metal pollution in drinking water, groundwater, soil, and agricultural products

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- b. contribute to research to understand the sources, types and mobility of heavy metal pollution in the environment
- c. support innovation and research into reduction and prevention of natural and anthropogenic metal contamination

Pupil Participation

1. *Class discussion of environmental contamination associated with heavy metals*

- a. Discuss the relative costs to industry and consumers of remediating or preventing pollution compared with the cost of pollution to human well-being and healthy ecosystems.

2. *Pupil stakeholder awareness*

- a. Public awareness about heavy metal contamination in the environment caused by human activities such as mining of natural resources and industrial processes producing effluents and wastes.
- b. Awareness of individual contributions to heavy metal pollution, such as disposal of single-use batteries in landfills, use of personal hygiene products that contain metals.
- c. Development of stringent risk-based environmental guidelines for public and environmental health. More emphasis should be given to metal speciation as some chemical forms (species) are more toxic and more mobile as compared to other species of the same element. Therefore, guidelines developed to address total metal concentrations may be misleading and insufficient to protect public and environmental health.
- d. Finding innovative, environmentally friendly, science-intense remedial techniques for remediation of metal contaminated sites, instead of exposure control measures and translocation of contaminants to landfills for future liability.

3. *Exercises*

- a. What are the options to reduce environmental contamination of metals? Create plans for sustainable management of mining and industrial wastes relevant to an industry in your area.
- b. How can we mitigate metal contamination? What amendments can we use to neutralize acid mine drainage? What environmental conditions can we manipulate to prevent metal release to the environment?

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Anthropogenic: Created by people or caused by human activity.

Bioaccumulation: Accumulation of a contaminant in an organism from the surrounding environment, e.g., by ingestion, inhalation, absorption through the skin.

Bioavailability: The amount of an element or compound that is accessible to an organism for uptake or absorption across its cellular membrane.

Biodegradation: Breakdown of an organic contaminant into simpler, environmentally acceptable compounds plus cell biomass by the actions of a living microbe or its enzymes.

Biogeochemical: Relating to the movement of chemical elements and compounds between the living and nonliving components of an ecosystem.

Bioleaching: The process of using microorganisms to produce biogenic acid to dissolve ores for extracting metals.

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Bioprecipitation: Microbial production of metabolites/compounds/molecules that react with metals present in the wastewater and forms metal precipitates, i.e., it converts the metals from its aqueous phase into solid phase.

Bioreactors: An apparatus in which a biological reaction or process is carried out under controlled conditions, especially on an industrial scale.

Bioremediation: Using microbial processes to treat and improve a contaminated environment by removing or changing the pollutant(s).

Biotransformation: Conversion of a contaminant into another state or form by the actions of a living microbe or its enzymes

Chemolithoautotrophs: Microbes that utilize reduced form of elements from the bedrock (litho) for electron as an energy source for making their own (auto) food (troph) from carbon dioxide.

Chemoorganoheterotrophs: Microbes that utilize organic compounds made by autotrophs as sources of energy, electrons, and carbon.

Elemental speciation: Quantitative determination of individual chemical forms of an element. The toxicity and mobility of metals is dependent upon their chemical forms.

Groundwater: Water that exists underground in saturated zones beneath the land surface. The upper surface of the saturated zone is called the water table.

Lithosphere: Solid, outer part of the Earth, including the brittle upper portion of the mantle and the crust.

Metallurgy: The branch of science and technology that deals with the properties of metals and their production and purification.

Metalloid: An element whose properties are intermediate between those of metals and solid nonmetals.

Nanoparticle: An ultrafine particle of matter that is between 1 and 100 nanometres (nm) in diameter.

Receptors: Environmental receptors include natural areas such as national or state parks, forests, or monuments; officially designated wildlife sanctuaries, preserves, refuges, or areas; and Federal wilderness areas. In broader sense, environmental receptors also include groundwater, human beings, plants and animals.

Remediation: Application of physical, chemical or biological techniques to decrease concentrations of contaminants in a contaminated site to a level (permissible concentration) considered safe for the environmental receptors.

Species: A term used differently in metallurgy and microbiology: in metallurgy, it refers to the redox state of the metal; in microbiology, it is a taxonomic level defining a group of organisms that share common evolutionary and biochemical characteristics.

Transform: The conversion of a substance from one chemical form to another.

Transition metals: General description of a "transition metal" is that any element in the d-block of the periodic table, which includes groups 3 to 12 on the periodic table. The important transition metals in soil environment are Zn, Cu, Fe, Mn, Co, Ni, Pb, and Cd.

Vadose zone: Unsaturated zone of soil extending from the ground surface to the water table.

Weathering: Physical and chemical processes that break down and chemically alter solid rock minerals into sediments/soil clay minerals.