

Metal Cycles

Metals are everywhere in the world! How on Earth do microbes deal with them and is that good for us and the environment?



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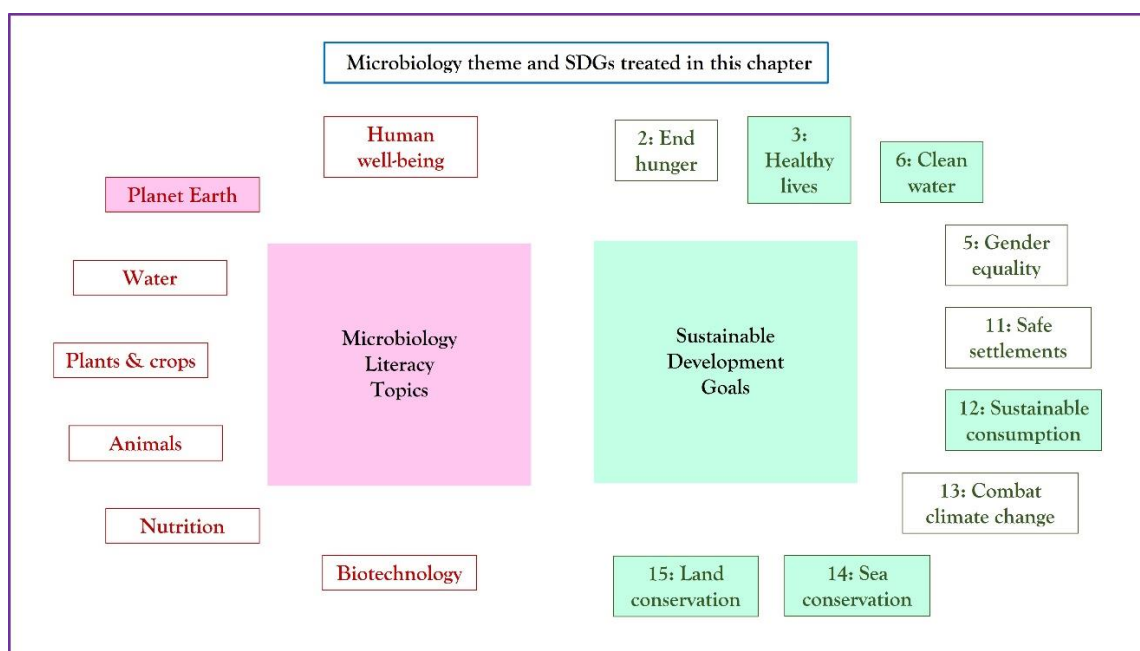
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Storyline

Metals are all around us in the **biosphere** – that part of planet Earth that supports life. In fact, most of the chemical elements in the Periodic table are metals and many of these are essential for life, as well as being important industrial and structural materials found in, for example, our homes, cars, computers and phones. For these applications, metals are obtained from the environment, usually by mining and metallurgical treatment of rocks and mineral ores that contain the desired metals. Metals are also found in different chemical forms in marine and freshwaters, and in soil, rocks and minerals. Metals that are essential to life such as iron, copper, zinc, magnesium, calcium, and potassium must be taken up by organisms from their environment and microorganisms and plants have special mechanisms by which they can accumulate metals in suitable chemical forms. Humans obtain essential metals from their food, as all biomass contains metals that have been accumulated from the environment or through the food chain by predation. When organisms die and decompose, all the elements in biomass are released and go back into the biosphere, to be taken up again by organisms or to react with other substances in the environment and form other organic and inorganic materials. Therefore, just like other important essential life elements like carbon, hydrogen, nitrogen, oxygen, sulfur and phosphorus, we can see that cycling of metals is very important for successful ecosystem functioning, plant productivity and human health.

The Microbiology and Societal Context

The microbiology: roles of microorganisms in metal cycling in the biosphere; physical, chemical and biological aspects of metal cycling; microbial metal solubilization; microbial metal immobilization; pollution treatment; **bioremediation**; clean environment. *Sustainability issues:* ecosystem and environmental health; environmental clean-up; metal recycling and recovery; land and water management and treatment.



Metal Cycles – the Microbiology

1. ***Some metals are essential to living organisms.*** Metals are directly and/or indirectly involved in all aspects of the growth and metabolism of microorganisms, as well as all other life forms, including humans. Essential metals include potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), and molybdenum (Mo), and they are involved in many different processes.

Cellular functions may reflect their chemical properties, e.g., charge, and redox properties. More than a third of all known enzymes contain a metal as a functional participant: these can also be called metalloenzymes. Other important roles for metal ions include the formation of charge and concentration gradients across membranes which are involved in transport processes, intracellular compartmentation, osmotic responses, and sensing. For example, intracellular Ca^{2+} is an important second messenger in microbial eukaryotes.

Other major functions of metals include stabilization of cellular structures, including cell walls, organelles and membranes, and biomolecules such as enzymes, proteins and nucleic acids. Several also function as redox catalysts in cytochromes, iron-sulfur proteins, blue-copper electron-transfer proteins as well as in, e.g. oxidases, oxygenases and hydrogenases. Deprivation of an essential metal ion will, by definition, cause impaired growth and metabolism and ultimately result in death.

2. ***Many metals are toxic to cellular organisms.*** All metals can be toxic to life, even those metals that are essential in low concentrations mentioned above. Some essential metals can be toxic even at low concentrations, e.g. copper. Above certain threshold concentrations, which will vary depending on the metal, essential metals will cause toxicity.

There are also many metals that are not essential for any biological functions but still can be accumulated by living organisms. These include aluminium (Al), silver (Ag), cadmium (Cd), caesium (Cs), tin (Sn), gold (Au), strontium (Sr), mercury (Hg), thallium (Tl), and lead (Pb). Some of these can be highly toxic even at low concentrations, e.g. Ag, Cd, Tl and Hg.

Some inessential metals are classed as major environmental pollutants because of their toxicity, e.g. Hg, Cd and Pb. Toxic effects caused by metals include blocking of functional groups in important molecules, e.g. enzymes, polynucleotides, disrupting transport systems for essential nutrients and ions, displacement and/or substitution of essential ions from cellular sites, denaturation and inactivation of enzymes, and disruption of cell- and organellar membranes. Almost every manifestation of microbial activity may be affected by metal pollution, e.g. primary productivity, nitrogen fixation, biogeochemical cycling of carbon (C), nitrogen (N), sulfur (S), phosphorus (P) and other elements (including metals), organic matter decomposition, enzyme synthesis and activity, in aquatic and terrestrial ecosystems.

Despite potential metal toxicity, microorganisms from all the major groups can exhibit a variety of mechanisms, both active and incidental, that contribute to metal resistance. Microorganisms are always found in metal-rich environments, whether natural or human-induced. It seems that most survival mechanisms depend on a change in metal speciation leading to decreased or increased mobility of the metal. These include redox transformations, the production of metal-binding peptides and proteins (e.g. metallothioneins, phytochelatins), organic and inorganic precipitation, active transport, efflux and intracellular compartmentalization, while cell walls and other structural components have significant metal-binding abilities.

3. *The importance of bioavailability, solubility and valency for microbe:metal interactions.* Bioavailability refers to the property of a substance to be available to interact with living organisms and is a term often used in the context of nutrient availability, and also to the interactions of pollutants, both organic and inorganic, with organisms. If a substance such as a metal, is in an inert or insoluble chemical form, or, tightly bound to an environmental component, e.g. clay minerals, then it cannot be used by an organism and therefore will have no nutritional value nor will it be able to exert toxic effects. It is said to be poorly bioavailable. Bioavailability of a metal is therefore closely related to its solubility, and so, in general, if a metal is soluble then it is available to interact with an organism, e.g. to be accumulated or exert toxicity.

Note that bioavailability is highly dependent on physical and chemical environmental conditions, and sometimes a metal may be soluble but still be unavailable to organisms. The best example of this is the effect of low pH where metals such as copper and zinc are very soluble in acidic conditions, yet do not affect organisms because cellular sites are protonated (H^+ = proton) and the positive charge "repels" positively charged metal cations. This is why many organisms can grow in highly acidic conditions, e.g. metal-containing ore leachates and even saturated copper sulfate.

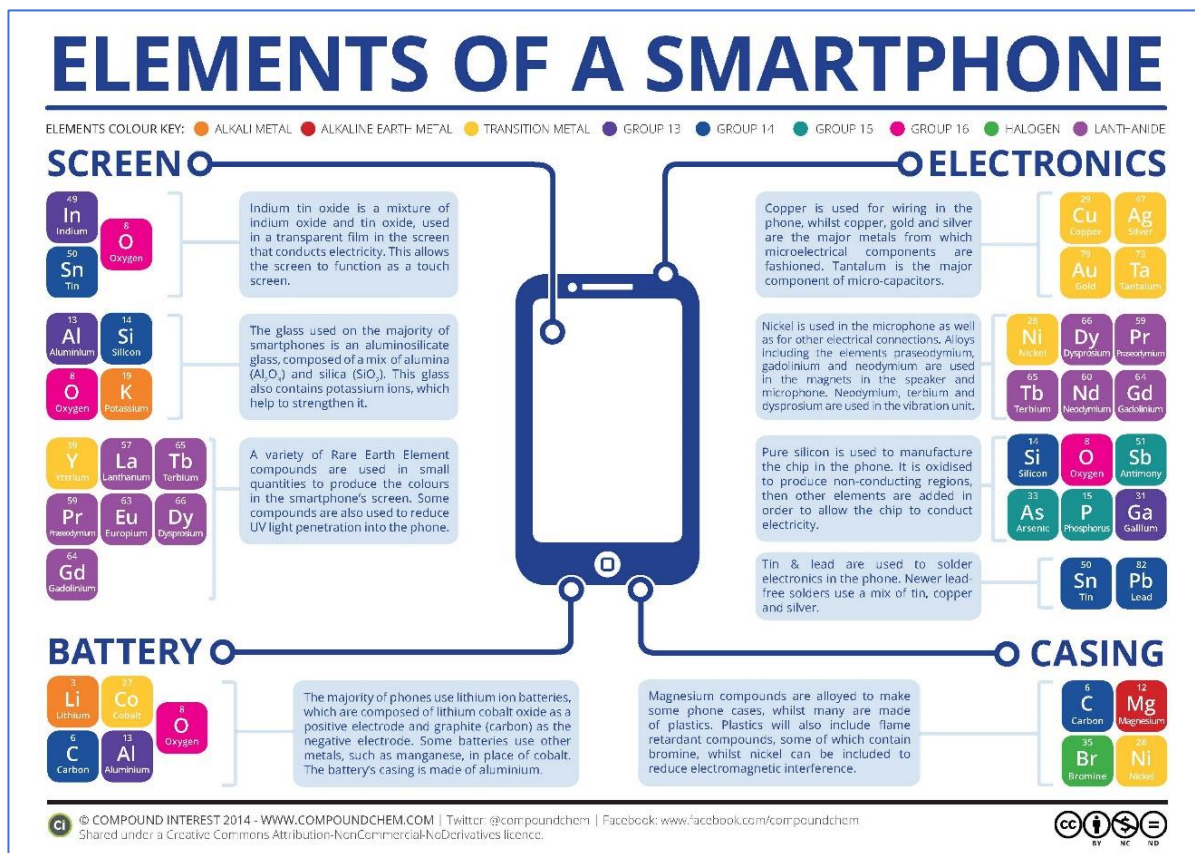
Bioavailability and solubility are also determined by the chemical state of a metal including valency. For example, iron(II) is soluble while Fe(III) is insoluble (similarly Mn(II) is soluble, Mn(IV) is insoluble). Such changes in valency can be brought about by microbial activities and are of key relevance to metal cycling in the environment. Therefore, the bioavailability, solubility and chemical state of a metal are very important for metal nutrition, potential toxicity and metal cycling in the environment. As described later, microorganisms possess mechanisms to solubilize metals, and other nutrients, from, e.g., mineral resources, as well as through organic matter decomposition.

4. *Metal cycling by microorganisms is important for the biosphere function, environmental biotechnology and human health.* The important roles of microorganisms in metal cycles is not only a part of natural Earth chemistry but can also combat threats to the environment from pollution by toxic metals. Although metal-rich environments occur naturally in the biosphere, many metal pollution problems arise from domestic and industrial activities, accidents and illegal dumping or discharge where high metal concentrations can enter terrestrial and aquatic ecosystems. Such metals can pass along food webs and ultimately affect human health. Radionuclides are predominantly metals and these can also enter the environment from nuclear waste and discharges, accidents (such as Chernobyl and Fukushima), military activities, weapons testing and medical waste.

5. *Metal cycling by microorganisms is important for biorecovery of valuable metals.* Many microbial processes involved in metal cycling can lead to immobilization and detoxification as well as their removal from solid materials such as soil and mining waste. In fact, these processes are now receiving additional interest because they can also be used for recovery of valuable metals. This is a very important topic since all electronic equipment, including computers, mobile phones, televisions, batteries, fridges, and microwaves, contains many valuable metals such as copper, cobalt, nickel and gold. A mobile phone can contain over 50 elements including rare Earth elements like cerium, dysprosium, and lanthanum, and there are current concerns about the supply of such elements from depleting Earth resources.

Reclamation and recycling will become essential to maintain the digital age and microbial bioprocessing can contribute to a solution. Therefore, metal cycling by microorganisms and the processes involved are of high importance to successful biosphere function, a clean safe

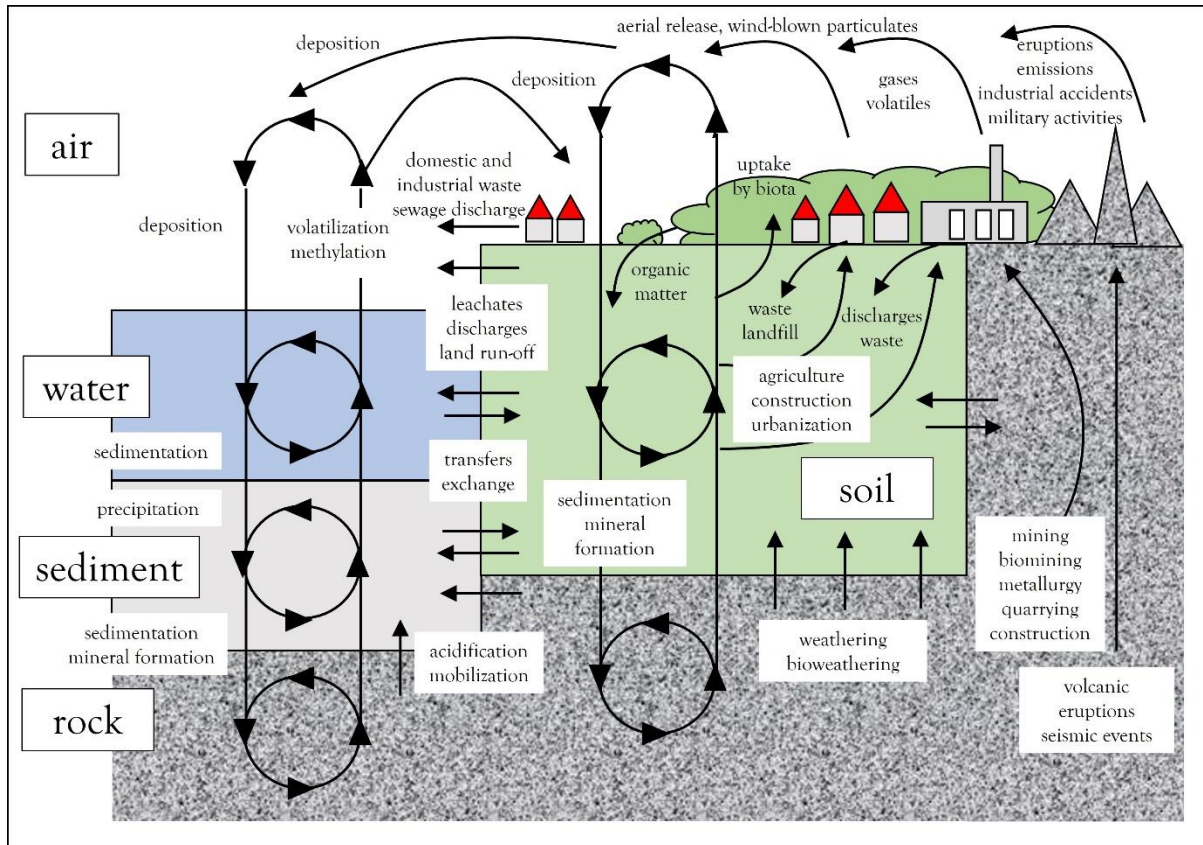
environment, and applications in environmental biotechnology for pollution treatment and metal **biorecovery**.



Elements in a mobile phone. The majority of elements are metals and there is concern that the supply of many of the valuable metals is under threat from depletion of natural resources, inadequate recycling, and geopolitical issues where economically-significant reserves are only found in a small number of countries. There is particular concern over rare earth elements that are also key components of computers, televisions and other electronic devices. There are many current initiatives worldwide to address this problem by improving methods for extraction from ore deposits and wastes that are more environmentally-friendly than traditional mining, and better methods for recycling and recovery from waste electronic materials. Many of the initiatives involve microbial bioleaching and biomineralization mechanisms. Bioprocessing using microbial systems is viewed as a significant contributor to the security of supply of important metals and minerals essential for the digital age. This diagram is shared under a Creative Commons Attribution-NonCommercial-NoDerivatives licence, see <https://www.compoundchem.com/2014/02/19/the-chemical-elements-of-a-smartphone/>

6. Metal cycles involve physical, chemical, and biological processes. Biological and non-biological factors interact in environmental metal cycling. Non-biological factors or events such as rainfall, wind, snow and ice, temperature, and desiccation, as well as volcanic eruptions, earthquakes, and geothermal activity (e.g. hot springs) can all affect rock and mineral weathering and distribution in the environment, and therefore the transport of metal-containing minerals and other substrates in and between environmental compartments, such as land and water. Atmospheric movement of metal-containing particulates by wind and industrial emissions is also significant.

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Main routes for metal cycling in the environment. Metals can exist in all states of matter, gases, liquids and solids, and are found in and pass between all environmental compartments - land, water and air. Within environmental compartments, e.g. water, sediment, soil, metal cycling can occur (arrowed circles) with changes in their chemical solubility and bioavailability depending on physico-chemical environmental factors and microbial populations. Similar reactions also lead to transfer between terrestrial and aquatic environments. Some of the main processes are indicated on the diagram as well as human influence. Every industrial activity leads to some kind of metal redistribution, and some are specifically concerned with extraction and application of important metals, e.g. mining and metallurgy. A by-product of industrial activity can be pollution of air, water and land by metals and organic substances. Domestic activities, urbanization, and construction also lead to significant metal redistributions and waste. Normal agricultural activities are also significant in metal cycles as well as geological and seismic events, e.g. volcanic eruptions. Rain is a major leaching agent and wind is very important for transfer of metal-containing particulates. Microbial activities can be involved in all aspects of metal cycling and in all environmental compartments.

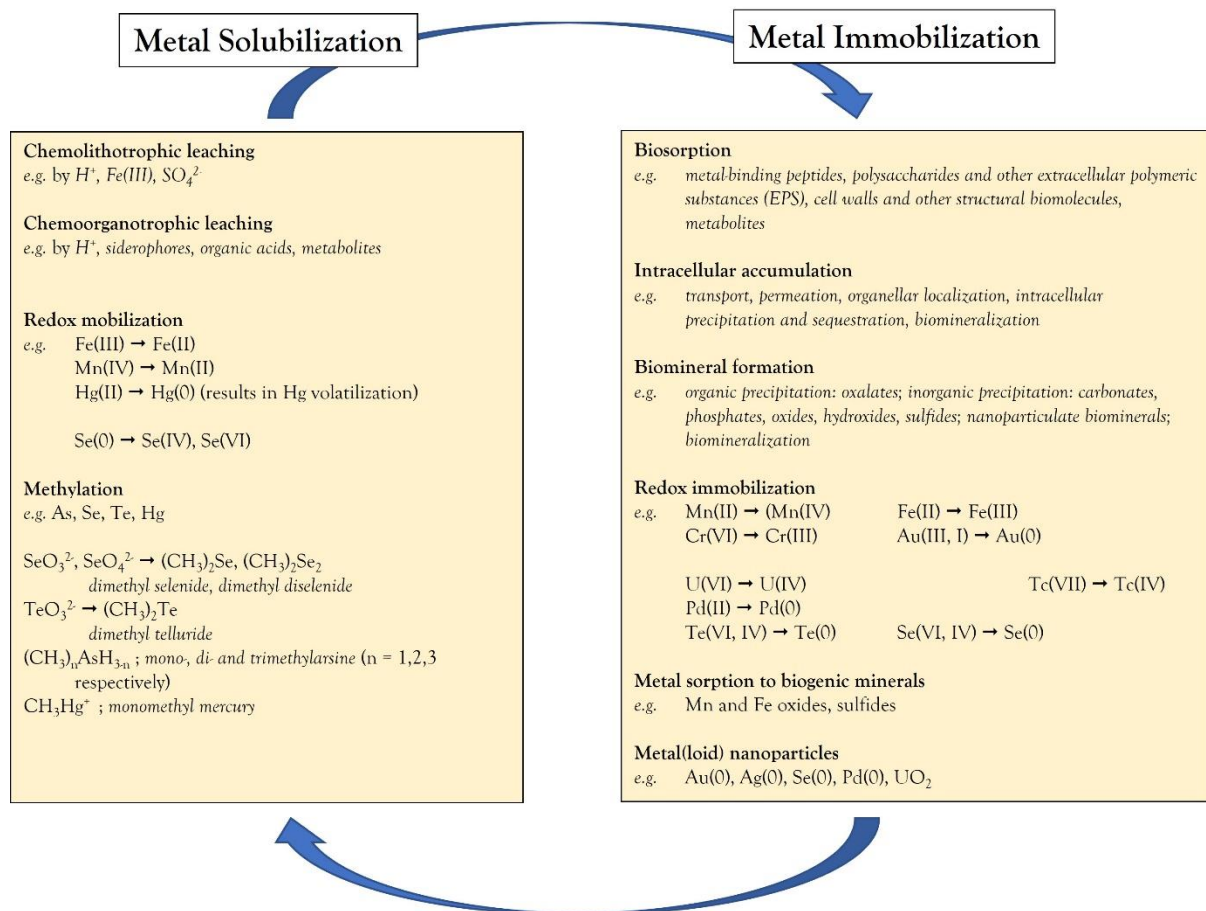
In addition to naturally-occurring metals, other localized disturbances and metal inputs lead to altered metal distributions in the environment such as mining, industrial pollution, fossil fuel utilization, domestic and industrial wastes, sewage discharge, fertilizers, nuclear emissions, and effluents. Some localized metal inputs may have damaging effects on living organisms and ecosystem function. Physical and chemical factors such as pH and temperature also affect the growth and metabolic activity of microorganisms that may be involved in metal cycling.

Whether naturally-occurring or introduced, soluble chemical forms of metals interact with environmental components and living organisms. Metals may bind to organic materials including organic detritus and microbial cells, sorb onto minerals including clay particles, or precipitate from solution after interaction with environmental precipitants or products of microbial metabolism.

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Soluble metals may also be taken up into cells by microorganisms and plants, and microorganisms possess a variety of **transport mechanisms** for essential metals, e.g. K, Na, Ca, Cu, Zn, Fe, Ni, Co, Mg, Mn, as well as inessential metals, e.g. Cd, Hg, Pb, and Cr. Naturally-occurring or introduced, insoluble chemical forms of metals, such as minerals, can be subject to biological activity that may result in solubilization of the contained metals.

Therefore, a key aspect of microbial metal cycling in the environment are the activities that lead to either solubilization of a metal from insoluble or bound forms, or immobilization in a solid or bound form. Since environmental factors, such as oxygen, and nutrient availability, etc affect the distribution and activity of microbial populations, the relative balance between metal solubilization and metal immobilization may vary significantly between different environmental compartments and locations.



The main mechanisms of microbial metal transformations between soluble and insoluble metal forms. The balance between these processes depends on the physical and chemical characteristics of the environment and the microbe(s) involved, as well as relationships with plants, animals and human activities. Important factors include dead biota and their decomposition products, as well as pH, water, inorganic and organic ions, molecules, compounds, colloids and particulates. The processes are also affected by interactions between biotic and abiotic components of the ecosystem which influence microbial diversity, numbers and metabolic activity, and biological effects on the environment that can alter redox potential, pH, O_2 , CO_2 , other gases and metabolites, temperature, and nutrient depletion.

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It should also be mentioned that as well as free-living microorganisms, symbiotic associations are also highly important. Lichens and mycorrhizas are significant geoactive agents. Lichens are fungi that exist in facultative or obligate symbioses with one or more photosynthesizing partners (algae, cyanobacteria) occurring in almost all surface terrestrial environments. Lichens play important roles in retention and distribution of nutrient (e.g. C, N) and trace elements, in soil formation, and rock **bioweathering**. Lichens can also accumulate many metals, e.g. Pb, Cu etc., including **metal radionuclides**, e.g. radiocaesium. They can also form a variety of metal-organic **biominerals**, e.g. oxalates, especially during growth on metal-rich substrates, that may result in a biomineralized crust.

The majority of terrestrial plants depend on symbiotic mycorrhizal fungi. Mycorrhizal fungi form a symbiotic association with plant roots. Mycorrhizal fungi can mediate metal and phosphate solubilization from minerals, extracellular precipitation of metal oxalates, and immobilize metals within biomass. Such activities lead to changes in the physico-chemical characteristics of the root environment and enhanced bioweathering of soil minerals, therefore influencing metal cycles.



Lichens are a fungal growth form consisting of a symbiosis between a fungus and a microalga or a cyanobacterium. A small number have both a microalga and a cyanobacterium, e.g. *Peltigera aphthosa*. Some lichens also have yeast partners, and all lichens have associate populations of other free-living microorganisms, such as bacteria and fungi. The fungal partner is responsible for physical and biochemical breakdown of rocks and minerals, and therefore directly involved in metal cycles. Lichens are regarded as “pioneer” colonizers of bare rock (A), and their activities are important in the initial stages of soil formation. They are also important in the colonization and biodeterioration of modern and ancient built environment structures (B) and cultural heritage, e.g. monuments and gravestones (C). Conversely, lichens can form biomineralized crusts through, e.g. oxalate deposition in the thallus, and these may provide a protective “umbrella” effect from weathering.

7. Microorganisms can solubilize metals from insoluble sources. Fungi and bacteria can convert insoluble metals into soluble forms through (a) dissolving metal compounds, including oxides, phosphates, and sulfides, and more complex minerals, (b) by desorption and removal from their binding sites on, e.g. clays and other minerals, colloids and organic matter, and (c) chemical **reduction**, e.g. reduction of insoluble ferric iron [Fe(III)] produces soluble ferrous iron [Fe(II)].

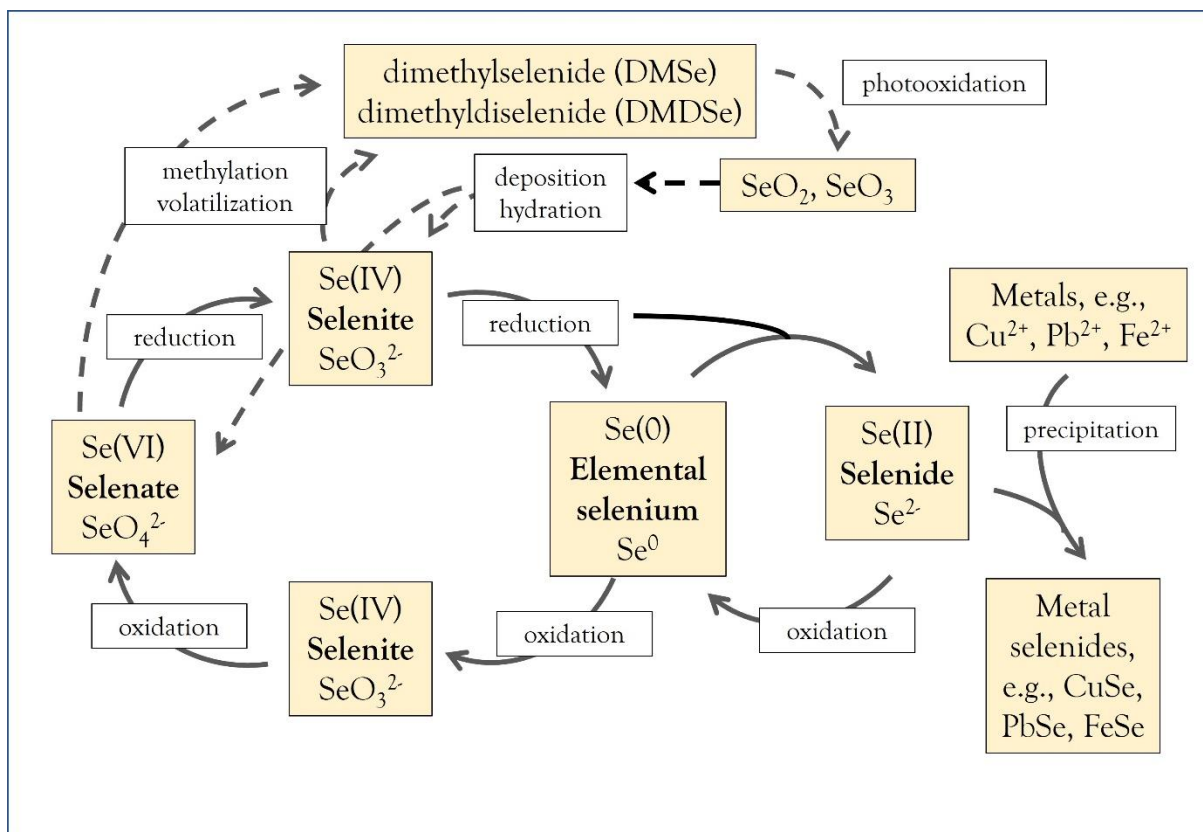
Many microorganisms can acidify their external environment through proton (H^+) excretion, CO_2 production from respiration which dissolves in the aqueous phase resulting in carbonic acid formation, and the production of other inorganic and organic acids. The action of H^+ (protonolysis) can be an important mechanism in mineral dissolution. Organic acids can also

be produced by many microorganisms with fungi particularly notable for this property. Organic acids such as citric, gluconic and oxalic acid can complex insoluble metals and release them as soluble metal-organic acid complexes from solid substrates as well as providing H^+ . Organic acids are important in the solubilization of metal phosphate minerals, and many other aspects of rock and mineral **biodeterioration**.

Siderophores are highly specific iron(III)-binding compounds that are excreted by a wide variety of fungi and bacteria to aid Fe assimilation. This is because insoluble Fe(III) predominates in the environment and is difficult to accumulate unless in the reduced ferrous (Fe(II)) form or as a soluble iron-complex. Iron is an essential metal for microorganisms and for other organisms.

Microbes can transform certain metalloids (elements with properties intermediate between those of metals and nonmetals). Elements included in this chemical classification of metalloids are boron (B), silicon (Si), germanium (Ge), arsenic (As), antimony (Sb), tellurium (Te) and polonium (Po). Selenium (Se) is also often considered as a metalloid.

Some metals and metalloids, e.g. Hg, As, Se, Sn, Te, and Pb can be methylated by a range of bacteria and archaea under anaerobic conditions, and several bacteria and fungi under aerobic conditions. **Methylation** of Se can occur in soils, sediments and water. Bacteria and fungi are the most important Se-methylators in soil.



Simplified selenium cycle in the environment. The chemical transitions caused by a variety of microorganisms, mainly bacteria and fungi, results in changes in solubility and toxicity, and transfers between air, land and water. Oxidations tend to occur in an aerobic environment, reduction is favoured under anaerobic conditions, especially selenide formation. However, reduction of selenium forms can also occur widely in aerobic environments. A similar cycle exists for tellurium.

Specific enzymes transfer methyl groups ($-CH_3$) to the metal, and these methylated metal compounds differ in their solubility, volatility, and toxicity. Some methylated compounds are volatile, e.g., dimethyl selenide $[(CH_3)_2Se]$ and dimethyl diselenide $[(CH_3)_2Se_2]$, and can be lost

from the soil to the atmosphere, reducing selenium concentrations in the soil and toxicity to the biota. Some methylated metals, e.g. methylmercury (CH_3Hg), are highly toxic. Some mercury-resistant bacteria can enzymatically reduce inorganic mercuric ion (Hg^{2+}) to elemental mercury (Hg^0) which is volatile and can escape to the atmosphere.

Other examples of metal reduction leading to increased metal solubilization are Fe(III) reduction to Fe(II) and Mn(IV) reduction to Mn(II). These processes can be important in releasing such metals from soil, rocks and minerals.

8. Microorganisms can immobilize metals as bound or solid forms including various minerals. As discussed above, there are a number of microbial processes which immobilize metals. Mechanisms of metal immobilization include biosorption, intracellular accumulation, redox and chemical precipitation, and biomineralization.

a. *Biosorption.* Biosorption refers to metal binding to living and dead biomass and is a physico-chemical process, but may be influenced by microbial metabolism. Soluble metals can have a strong attraction to microbial cell walls and extracellular materials such as polysaccharides and pigments. The small size and large specific surface area of microorganisms, particularly prokaryotes, can be effective in removing large amounts of metals from solution and in fact has received considerable attention in the context of assessment of processes potentially useful for bioremediation of metal-containing liquid wastes.

Microbial cell walls contain a variety of polymers, polysaccharides, proteins and pigments that possess chemical groups, such as carboxyl and phosphate, that possess a negative charge and therefore attract positively-charged metal cations. In some cases, metal binding by cell walls can protect organisms from toxic effects. Biosorption and further metal deposition can also lead to the formation of metal-containing minerals that encrust the cells, which is also a metal immobilization process.

b. *Metal uptake by cells.* Metals are found in all parts of microbial (and animal and plant) cells. Apart from sorption to cell walls and extracellular materials as mentioned above, microorganisms possess transport systems to take up soluble essential metals, e.g. Cu, Zn, Mg, Mn, inside the cell for structural and metabolic purposes. Toxic metals may also be taken up inside cells “accidentally”, or where a toxic metal closely resembles an essential metal, e.g. Sr and Ca show several chemical similarities.

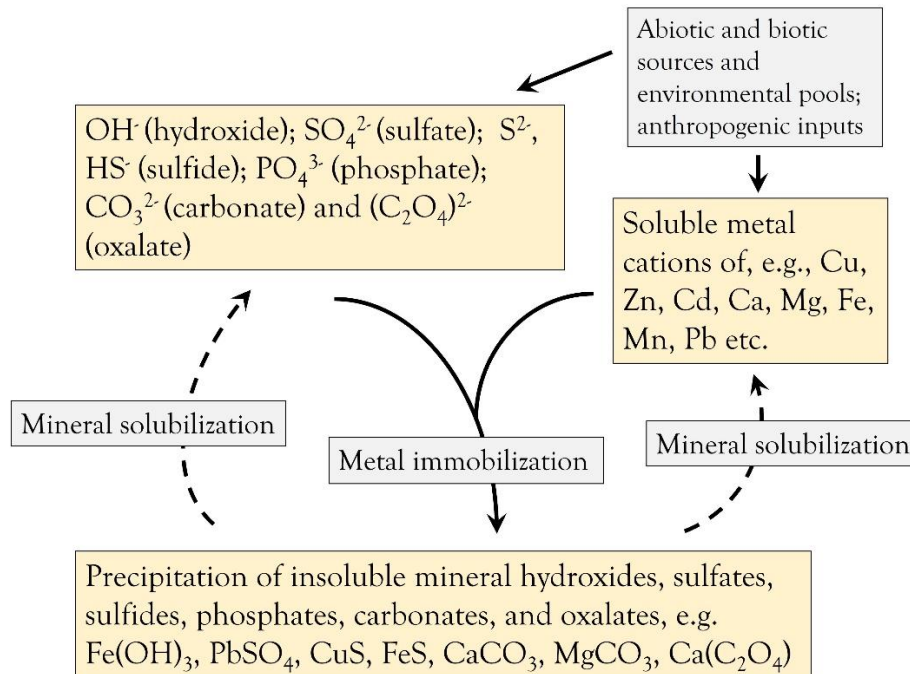
c. *Toxic metal resistance.* Microorganisms possess many mechanisms that provide resistance to toxic metals, which include intracellular precipitation, binding to specific molecules such as metallothioneins, or localization in cell compartments such as vacuoles in fungi. Accumulated metals in microorganisms will be released to the environment when organisms die and are decomposed (see later).

d. *Metal precipitation.* At sufficient concentrations, metal precipitates can develop on or within cells, or in the immediate extracellular environment. There are a number of mechanisms involved in external metal precipitation including those mediated by redox reactions (chemical oxidations or reductions), and excretion of organic or inorganic substances (metabolites) that precipitate metals.

i. Role of anions. The formation of extracellular and sorbed precipitates depends on the chemical composition of the extracellular environment and can be influenced by cellular processes. The precipitates can be copious and may completely enmesh the cells, effectively entombing them. Environmental anions, such as OH^- , SO_4^{2-} , S^{2-} , PO_4^{3-} , CO_3^{2-} and oxalate, provide counterions for precipitation of mineral hydroxides, sulfate and sulfides, phosphates, carbonates, and oxalates, respectively. Metal oxide and hydroxide minerals formed

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by abiotic reactions can attach to cells and this is another important mechanism by which cells concentrate metals. Phosphate may be released by microbial decomposition of organic matter and also by the microbial solubilization of mineral phosphates by proton and organic acid excretion. Cellular metabolism and excretion of some such anions, e.g. sulfide and oxalate, can also contribute to formation of minerals (see later).

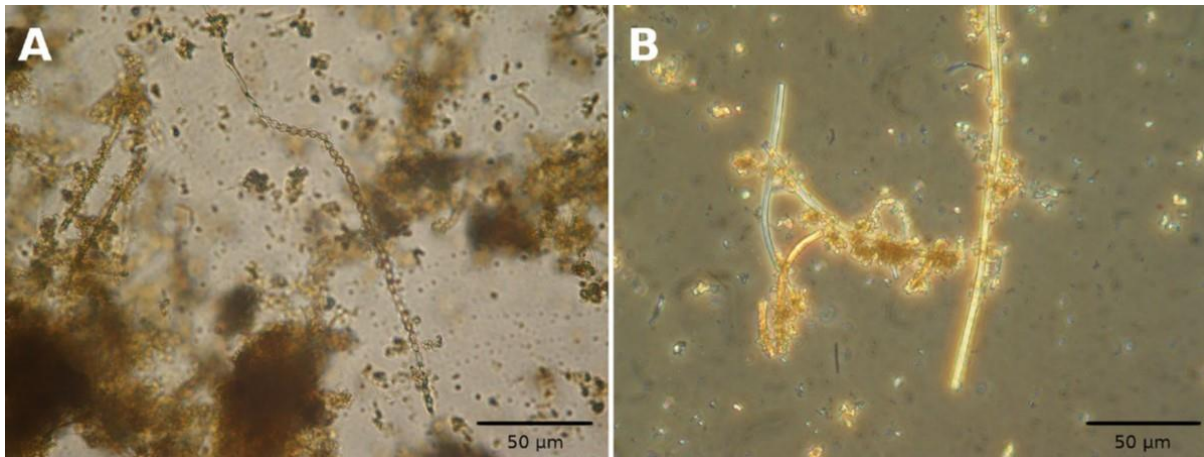


Metal precipitation as a result of interaction with various chemical anions that naturally occur in the environment, as well as being produced by microbial activity and introduced through human activity. Hydroxides, sulfates, sulfides, carbonates, phosphates, and oxalates are just some examples of the precipitated products. Microbial activities can also lead to recycling, degradation or solubilization of the insoluble compounds emphasising the cyclical nature of all the processes that may lead to metal solubilization or precipitation.

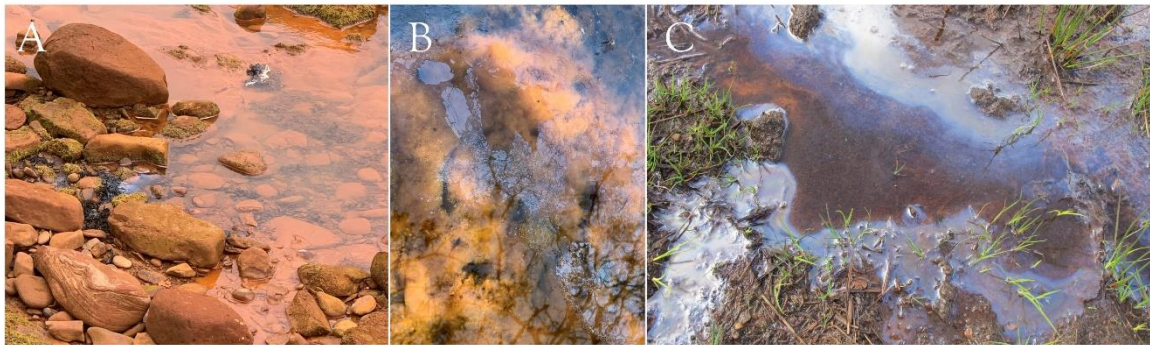
ii. Role of redox reactions.

1. Oxidation. A range of microorganisms can oxidize Fe and Mn if present in their soluble ferrous (Fe^{2+}) or manganous (Mn^{2+}) forms. Iron-oxidizers found in acidic soils are acidophilic chemolithotrophs (organisms that use inorganic sources of chemical energy), such as *Acidithiobacillus ferrooxidans*, which is important for its role in generating acid mine drainage and also for industrial **bioleaching** of copper and other important metals. Some iron-oxidizers, e.g. the bacterium *Gallionella ferruginea*, accumulate abundant Fe hydroxide precipitates on their filaments or stalk structures, and the red-orange deposits can be commonly seen in iron-rich waters. Other iron-oxidizers include *Leptothrix* species, e.g. *Leptothrix discophora*, and their activity can result in a blue film appearing on the surface of iron-containing waters. This film is composed of a very thin layer of iron oxide which appears blue because of light reflection and refraction, and is often mistaken for spilled oil. However, unlike oil, this film fragments if disturbed with, e.g. a stick.

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Images of the iron-oxidizing bacteria (A) *Gallionella* spp. and (B) *Leptothrix* spp. observed in mine drainage water. Image from Kisková et al. (2018) (<https://doi.org/10.1007/s00284-018-1472-6>) and reproduced under the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).



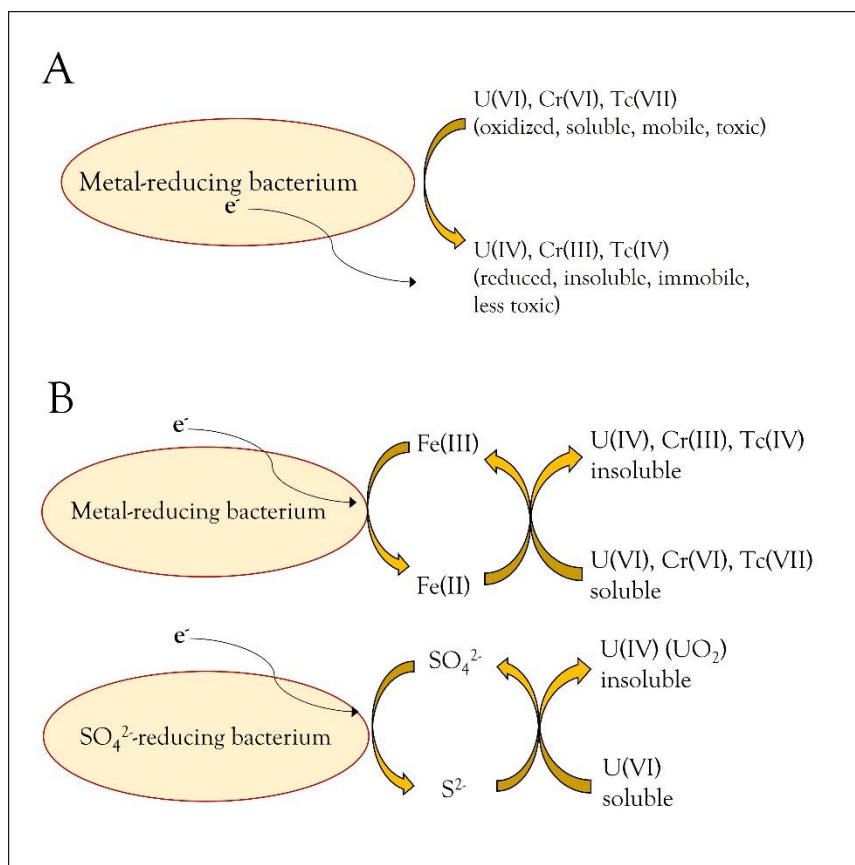
Iron oxidation in the environment. Iron naturally oxidizes to the ferric (Fe(III)) form under aerobic conditions but several bacteria can also oxidize soluble ferrous (Fe(II)) iron to hydrous Fe(III) oxides. (A) Red iron oxide precipitation can be frequently seen in and arising from waters where there is substantial iron available. The iron can be a result of leaching and run-off from the land. It can also be highly visible in leachates from disused mines – acid mine drainage. Iron oxidation by *Gallionella* spp. results in reddish precipitation of ferric oxide (B). In contrast, iron oxidation by *Leptothrix* spp. results in formation of a thin iron oxide layer on water surfaces which appears blue due to light refraction and reflection effects (C). The iron oxides can be closely associated with the bacterial stalks, sheaths and surfaces. In (B), there is a thin blue film produced by *Leptothrix* spp. on the water surface below which is reddish iron oxide precipitated by a combination of water chemistry and iron-oxidizers such as *Gallionella*.

After Fe, Mn is the second most abundant transition metal in the Earth's crust and is cycled in the same environments as Fe. Mn **oxidation** is carried out by many of the organisms that are implicated in Fe oxidation, including species of *Bacillus*, *Pseudomonas*, and *Leptothrix* bacteria. Several fungi can also oxidize Mn(II) to Mn(IV)O₂, e.g. *Acremonium* species through direct enzymic mechanisms. Mn oxides have high sorption capacities for numerous metals (e.g., Ni, Zn, Cu, Co, Mn, Pb, and Cd), and can also oxidize some metalloids [e.g., As(III) to As(V); Cr(III) to Cr(IV)] which results in element solubilization. Fungal and bacterial oxidations are believed to be involved in the formation of desert varnish, a dark oxidized layer (patina) a few millimeters thick found on rocks and in soils in arid and semiarid regions.

2. Reduction. Dissimilatory metal-reducing bacteria use relatively soluble metals such as Cr(VI) and U(VI) as electron acceptors in respiration, and their reduction

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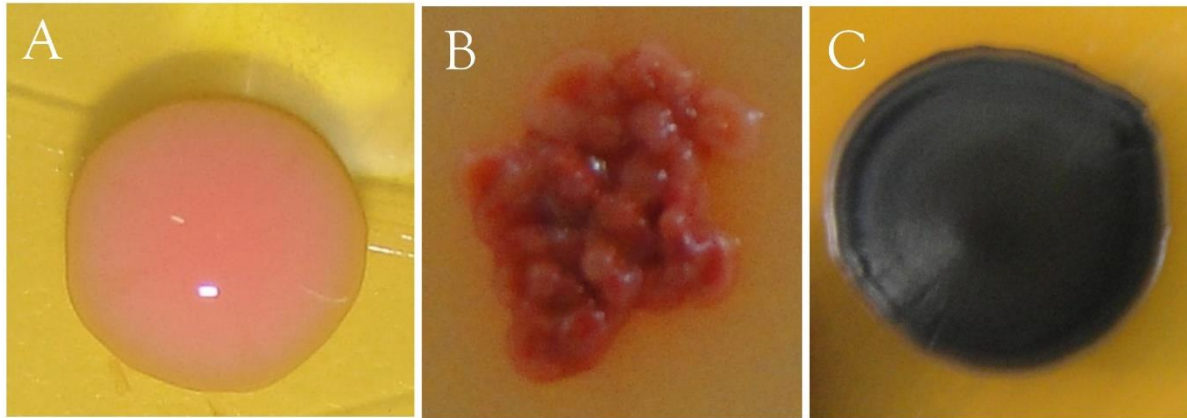
results in less-soluble Cr(III) and U(IV) species, which can sorb onto cells and/or be precipitated out of solution. U(IV) is deposited as a fine precipitate in the periplasm of Gram-negative metal-reducing bacteria. Soluble Fe^{2+} and Mn^{2+} , which can be generated by dissimilatory metal reduction of insoluble Fe(III) and Mn(IV) located in, e.g. iron or manganese-bearing minerals, can form extensive extracellular precipitates and nodules by precipitating with environmental anions, such as hydroxide and phosphate.



Reduction of certain metals (uranium (U), chromium (Cr), technetium (Tc)) by bacteria which leads to changes in solubility. (A) Direct enzymatic reduction of metals and radionuclides by metal-reducing bacteria that leads to immobilization and (B) Indirect immobilization of metals and radionuclides by metal-reducing and sulfate-reducing bacteria. Here, the bacterial products, Fe(II) and sulfide, act as reducing agents. The electrons are derived from organic compounds, e.g. lactate, acetate, ethanol, or H_2 that the bacteria can use in their metabolism.

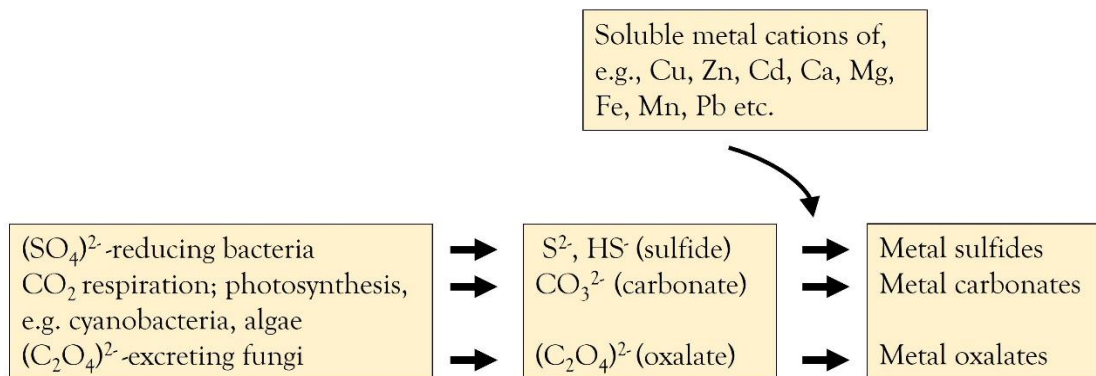
As well as methylation (described above), microbial reduction mechanisms influence the chemistry of metalloids and their mobility in the environment and toxicity. For example, reduction of metalloid oxyanions, such as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), or tellurite (TeO_3^{2-}), to amorphous elemental selenium (Se^0) or tellurium (Te^0) respectively, results in immobilization and detoxification. Selenate and selenite can be reduced to elemental selenium which is an important process in anaerobic sediments. Production of Se^0 or Te^0 results in red (Se) or black (Te) microbial colonies and extracellular and intracellular precipitation of the metalloids around cells. Some bacteria use selenate as a terminal electron acceptor in respiration and most microorganisms can accumulate and incorporate Se into organic components, e.g. selenoproteins.

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Reduction of selenite and tellurite to elemental selenium and elemental tellurium, respectively, by the pink yeast *Rhodotorula mucilaginosa*. Elemental Se(0) imparts a deep red colour; elemental Te(0) is black. (A) control colony (B) grown with 10 mM selenite (C) grown with 1 mM tellurite. The colonies are ~3-5 mm in diameter.

iii. Role of metabolite excretion. Metal bioprecipitation by excretion of inorganic and organic metabolites is widespread among microorganisms. It should be noted that the general metabolic activities of microorganisms alter the physical and chemical nature of their microenvironment, and such changes may influence whether a metal stays in solution or is precipitated. Obvious environmental changes resulting from microbial activity include changes in pH, O₂ and CO₂ levels, and redox potential.

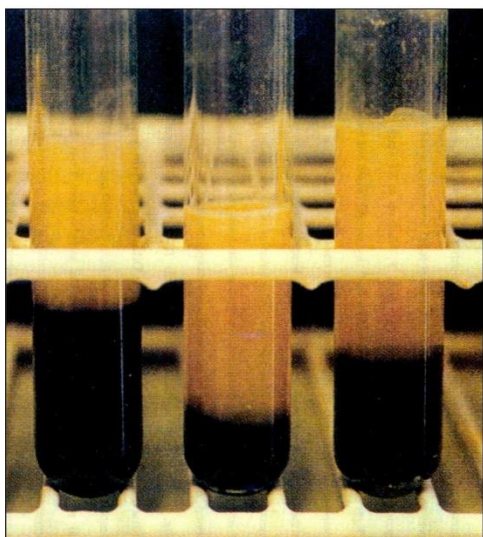


Metal bioprecipitation by excretion of inorganic and organic metabolites. Three examples are shown: sulfate-reducing bacteria that produce sulfide (HS⁻, S²⁻) by reduction of sulfate (SO₄²⁻); CO₂ production by many kinds of heterotrophic microorganisms that use O₂ for respiration and photosynthetic organisms that liberate CO₂ from photosynthesis, e.g. algae, cyanobacteria, the dissolved CO₂ leading to carbonate formation; many fungi excrete oxalic acid ((COOH)₂) and the oxalate anion ((C₂O₄)²⁻) is a powerful metal-complexing agent as well as a metal precipitant. On interaction with metals, these metabolites will form insoluble metal sulfides, carbonates and oxalates. Note that these reactions can be made reversible by other microorganisms. Many chemolithotrophic bacteria can oxidize metal sulfides; many bacteria and fungi can solubilize metal carbonates, and oxalotrophic (oxalate-eating) bacteria and fungi can use oxalates as a carbon source. The reversibility or otherwise of the precipitating reactions depends on the different environmental conditions and associated microbial populations.

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The formation of minerals by living organisms is called biomineralization, and in microorganisms this generally results from microbial alteration of the chemistry of their environment to that which favours mineral formation. As mentioned previously, sorption of metals may also precede mineral formation, and this can take place on dead as well as living organisms. Apart from physico-chemical changes in the microbial environment, metal bioprecipitation and biomineralization resulting from metabolite excretion is also widespread.

- Sulfate-reducing bacteria are strictly anaerobic **heterotrophic** bacteria found in environments where carbon substrates and sulfate are available. These metabolize organic compounds or hydrogen through coupling with the reduction of sulfate as the terminal electron acceptor for respiration, producing sulfide that can reach significant concentrations in sediments. Sulfide is highly reactive and precipitates soluble metal cations to form insoluble metal sulfides. Furthermore, sulfide can indirectly reduce some metal species such as U(VI) (soluble) to U(IV) (insoluble), leading to U precipitation. Metal sulfides can also adsorb free metal cations which results in metal removal from solution.



Sulfate reduction by sulfate-reducing bacteria (SRB) results in production of hydrogen sulfide:

$$\text{SO}_4^{2-} \rightarrow \text{H}_2\text{S}$$

Divalent metal ions, such as Cu^{2+} , Ni^{2+} , Pb^{2+} , Cd^{2+} , Fe^{2+} and Zn^{2+} , precipitate in the presence of sulfide forming insoluble metal sulfides:

$$\text{M}^{2+} + \text{S}^{2-} \rightarrow \text{MS}\downarrow$$

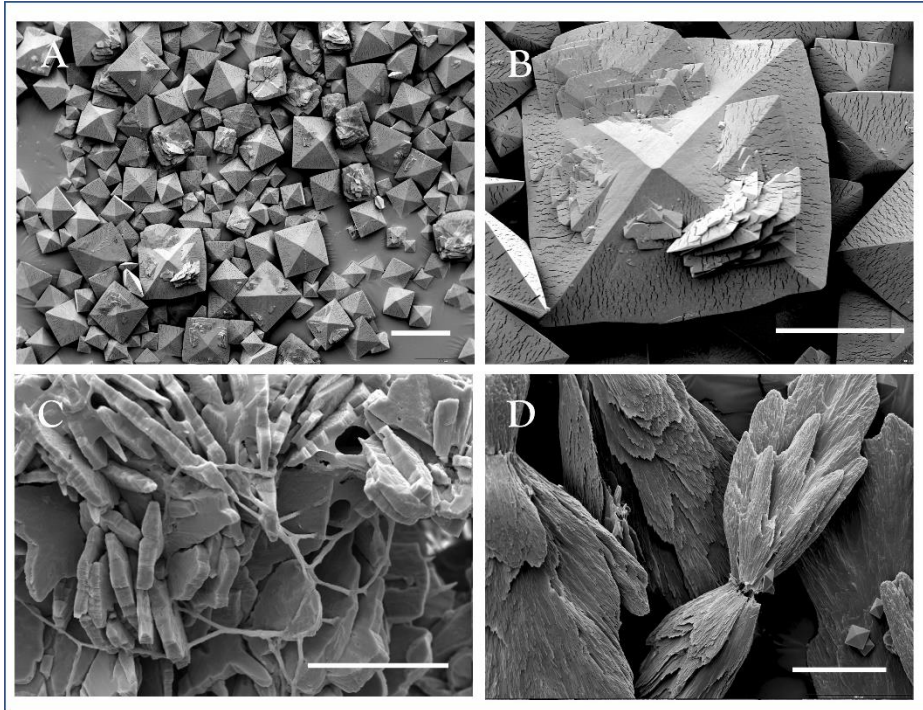
M = metal
 S^{2-} = sulfide
MS = metal sulfide

Iron and copper sulfide precipitation by sulfate-reducing bacteria (SRB). The tubes contain solid agar medium suitable for growth of SRB and contains iron and copper. The tubes were stab-inoculated so that anaerobic conditions were maintained at the bottom of the tube. The blackening reflects the growth of SRB and precipitation of iron and copper sulfide. Many bacteria and archaea can use sulfate (SO_4^{2-}) as a terminal electron acceptor. Most of these are obligate anaerobes, e.g. *Desulfovibrio* spp., and sulfate reduction results in production of hydrogen sulfide. Divalent metal ions, such as Cu^{2+} , Ni^{2+} , Pb^{2+} , Cd^{2+} , Fe^{2+} and Zn^{2+} , precipitate in the presence of sulfide forming insoluble metal sulfides. This is an important part of sulfur and metal cycles in the environment and metal sulfide precipitation has also been applied industrially for bioremediation of metal-containing effluents and process streams. They also cause corrosion of underground and underwater structures and are especially troublesome in petroleum refineries and sewage works, causing great economic loss. The main habitat of sulfidogens are sediments rich in organic nutrients and sulfate.

- Many fungal species are capable of oxalic acid excretion which removes metals from solution by precipitation as oxalates. Calcium oxalate is the most common form of oxalate encountered in the environment, mostly occurring as the dihydrate (weddelite) or the more stable monohydrate (whewellite). Fungi can also produce other insoluble metal oxalates on interacting with a variety of different metals and metal-bearing minerals, e.g. Cd, Co, Cu, Mg,

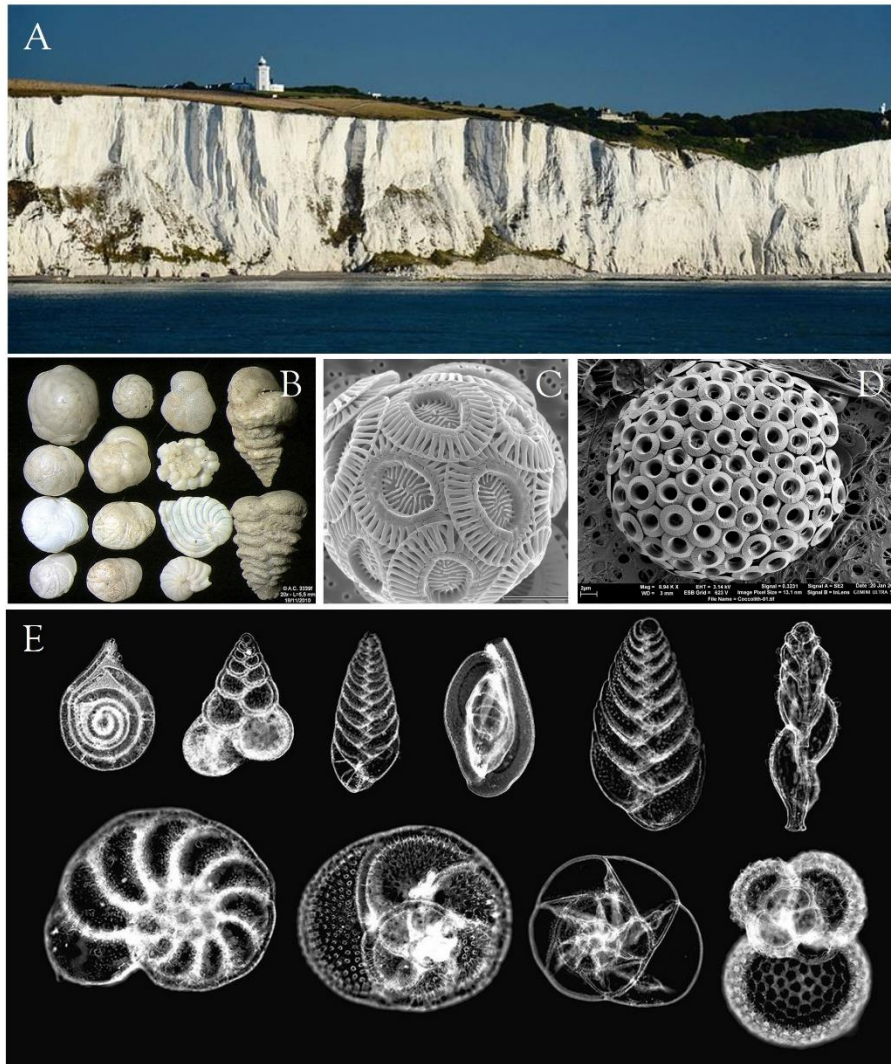
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Mn, Sr, Zn, Ni and Pb. The formation of metal oxalates enables fungi to tolerate environments containing high concentrations of toxic metals.



Fungal precipitation of metal oxalates. (A, B) calcium oxalate produced by *Beauveria caledonica* (C) manganese oxalate dihydrate produced by *Serpula himantioides*: some fungal hyphae can be seen growing over the crystal (D) crystal of manganese oxalate trihydrate produced by *S. himantioides*. Both fungi excrete large amounts of oxalic acid which precipitated with the Ca or Mn incorporated in the media. Bar markers are (A) 200 μm (B) 100 μm (C) 200 μm (D) 50 μm .

- Carbonate deposits. Much of the insoluble carbonate at the Earth's surface is of biogenic origin. Certain bacteria, cyanobacteria and fungi can deposit calcium carbonate extracellularly while some algae, such as coccolithophores, deposit calcium carbonate as their cell-surface structures, and some protozoa (foraminifera) use it for their "tests" or shells. Some biogenic carbonate deposits are massive, e.g. the White Cliffs of Dover, UK. Carbonate precipitation is a very important process that represents a 'carbon sink' and hence influences global carbon cycling. Insoluble carbonates may be broken down by microbial attack which is usually the result of organic and inorganic acid production.



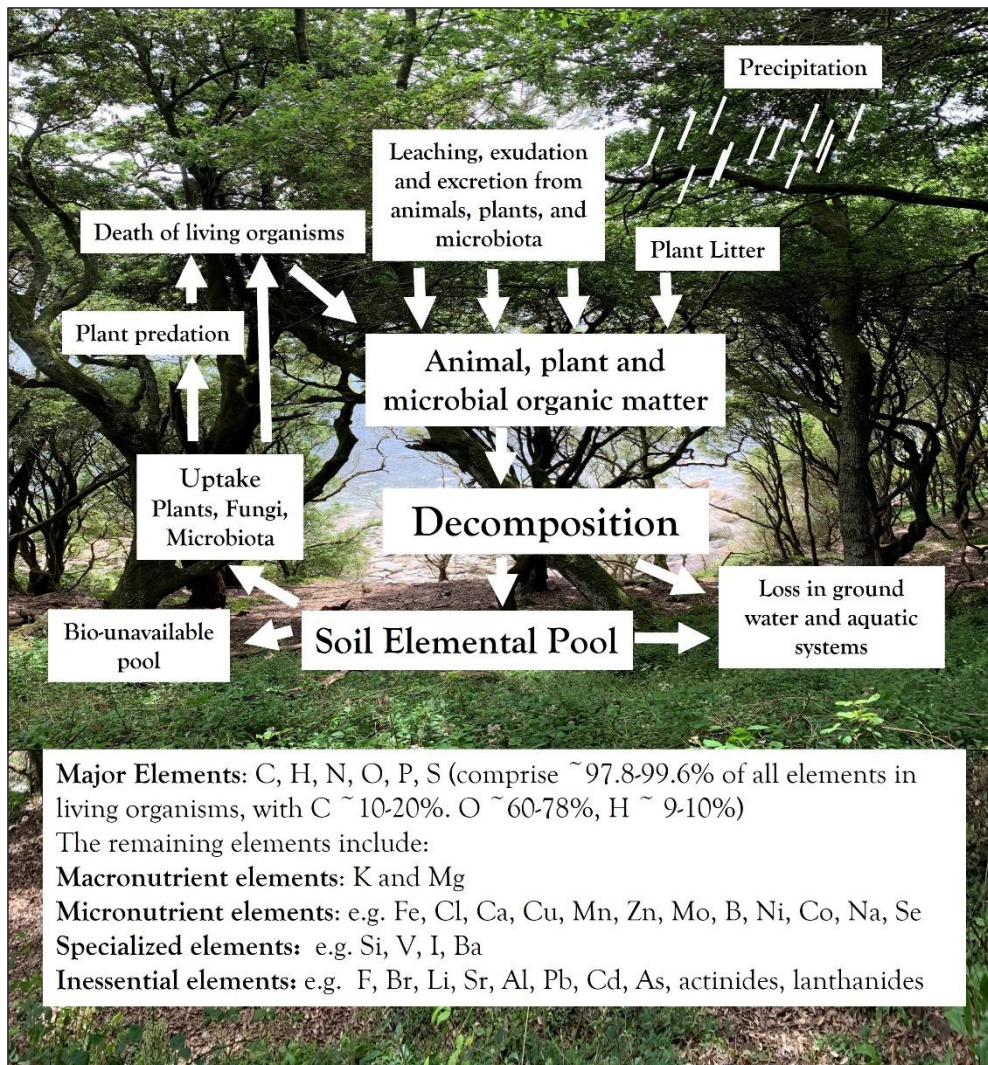
Chalk is a variety of limestone composed mainly of calcium carbonate derived from the shells of protists called foraminifera and from the calcareous remains of certain marine microalgae known as coccolithophores. Extensive bodies of chalk are found worldwide. The White Cliffs of Dover are a good example (A). (B) Some “shells” of different kinds of foraminifera; (C) The coccolithophore *Emiliana huxleyi* showing the patterned calcareous plates around the cell; (D) another coccolithophore; (E) Selection of different foraminifera showing the intricate chambered constructions in the calcareous shell. (A) <https://pixabay.com/images/search/chalk%20cliffs/> (B) "FORAMINIFERA" by fickleandfreckled is licensed under CC BY 2.0. (C) "File:Emiliana huxleyi coccolithophore (PLoS).png" by Alison R. Taylor (University of North Carolina Wilmington Microscopy Facility) is licensed under CC BY 2.5. (D) "Coccolithophore" by ZEISS Microscopy is licensed under CC BY-NC-ND 2.0. (E) "Foraminifera (10 Species)" by pali_nalu is licensed under CC BY-NC 2.0.

9. Organic matter decomposition is important for metal cycles. The decomposition of organic matter by microorganisms is of major importance in the biosphere, relevant to energy transfer through ecosystems, nutrient and elemental cycles and plant productivity. Fungi are particularly important organic degraders and, through the production of a suite of extracellular enzymes, can degrade many organic polymers particularly lignin-rich dead plant materials.

Apart from the essential elements C, H, N, O, P, and S found in the highest amounts in organic matter, other essential elements found at lower concentrations includes many metals, e.g. Na, K, Mg, Mn, Cu, Co, Zn, Ni, and Mo. In addition, living organisms accumulate many inessential or potentially toxic metals into their cells when encountering them in, e.g. a polluted

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environment. These could include Cd, and Pb, for example. Therefore, any decomposition process results in cycling of all the elements contained in the organic substrate and it can be easily appreciated that all these cycles are interlinked. Released metals from decomposition may be taken up by microorganisms and plants, bind to organic and inorganic environmental components, be subject to redox transformations and bioprecipitation mediated by microorganisms, and also precipitate with environmental anions, such as phosphate, itself being a product of organic matter decomposition and/or the microbial solubilization of phosphate-containing minerals.



A simple generalized element cycle based on the decomposition of organic materials. Apart from the major elements, C, H, N, O, P, and S, other important essential elements also occur in living organisms, many of them being metals. Inessential elements, that include many toxic metals, can also be accumulated by living organisms as a result of inhabiting a metal-rich environment, and natural or human-mediated pollution. Decomposition of organic matter therefore leads to cycling of all the elements associated with it. The cycle shown could refer to a copper, potassium or radio-caesium cycle for example.



Aquatic (A) cyanobacteria and (B) microalgae can produce extensive “blooms” in freshwater, especially when there is input of nitrogen and phosphorus from agricultural or industrial sources. Such blooms are significant in metals cycles since soluble metal species can be sorbed to cell walls and extracellular mucilage as well as being accumulated intracellularly. When the blooms die, subsequent decomposition can result in remobilization of accumulated metals which re-enter metal cycles in the water and sediment.

Relevance for Sustainable Development Goals and Grand Challenges

(<https://sdgs.un.org/2030agenda>)

- **Goal 3. Healthy lives and Goal 11. Healthy environments.** (*Planet Earth, water, plants and crops, human well-being*). Microbial roles in element cycling are essential for maintaining ecosystem function. Microorganisms are important in mobilizing essential elements including metals like Ca, Mg, Cu and Zn for uptake by other organisms including plants, and their interactions with organic matter and minerals can also release important elements like P. Metal cycling is important for transfer of essential elements through the food chain and for plant and crop productivity. Natural microbial processes can also detoxify or immobilize potentially toxic metals. For examples, many metals are locked up in marine sediments as sulfides because of the activities of sulfate-reducing bacteria. A healthy environment is important for human health.
- **Goal 6. Clean water** (*Planet Earth, water, human well-being, biotechnology*). Normal microbial processes carried out in water treatment systems like sewage treatment and reed beds remove many potentially toxic metals from the water preventing their discharge into the natural environment. Several microbial processes are used in other environmental biotechnologies to remove toxic metals from polluted waste streams, and industrial effluents.
- **Goal 12. Sustainable production, eliminate pollution, Goal 14: Sea conservation and Goal 15: Land Conservation** (*Planet Earth, water, plants and crops, human well-being, biotechnology*). Traditional mining of metals is polluting and energy intensive, thus unsustainable. The use of microbes to extract metals for ores is less polluting and has a lower energy demand, so is more sustainable. Natural microbial process can also deal with organic and inorganic pollution. Metal cycling can result in metal transfers between

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different environmental compartments, e.g. from land to water; from water to sediments. Some aspects of metal cycling lead to natural immobilization of toxic metals in soils and sediments, while several processes using microorganisms are used to treat metal pollution of land and water. The natural involvement of microorganisms in the cycling of metals (and all other environmental elements) and their harnessing in environmental biotechnologies is also relevant to land, freshwater and marine conservation.

Potential Implications for Decisions

1. *Individual*

- a. Which everyday products do you use that contain potentially-toxic metals? How might you reduce your exposure to them?
- b. With which legacy products do you come into contact (e.g. *lead-containing paint; metal-based biocides and preservatives; mercury thermometers*)? How might you reduce your exposure to them?
- c. Understand the importance of a clean and healthy environment and how basic microbial processes can determine the health of an ecosystem and benefit plant growth and human well-being.
- d. Understand the importance of metal recycling in view of depleting environmental resources of valuable metals for modern technologies and the potential of microbial processes for recovery of valuable metals and reducing environmental harm caused by conventional mining technologies.
- e. Understand that metal pollution is widespread and do not ignore local issues but take steps to encourage legal action to enforce clean-up measures and punish illegal polluters: pay attention to the views and actions of political parties to the environment

2. *Community*

- a. Awareness that microbial element cycling is fundamental to a healthy environment, plant productivity, and combating pollution. Therefore monitoring, challenging, and counteracting environmental threats and disturbance is essential.
- b. Promotion of microbial solutions for environmental health, waste clean-up, and bioremediation and the potential of microbial metal and mineral interactions to enhance the security of supply of precious minerals and metal resources and reduce the environmental impact of traditional mining and metallurgical processes.
- c. Encourage and promote greater inclusion of the environmental impact and importance of microorganisms in school curricula.

3. *National Policies*

- a. The inclusion of microbial environmental processes in environmental proposals and initiatives concerned with, e.g. a clean environment, climate change, security of supply of metal and mineral resources, recycling and reclamation, pollution prevention and treatment, water treatment, safeguarding natural ecosystems, plant and crop productivity, soil health, and storage of nuclear waste.
- b. Increased support for environmental biotechnology to provide greener and sustainable alternatives to traditional and environmentally-damaging technologies used in metal, mineral and waste treatment industries.

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c. Improved inspection and regulation of polluting industries and harsher penalties for careless or illegal polluters of land and water through illegal discharges or non-adherence to environmental legislation

Pupil Participation

1. Class discussion of the issues associated with metal and element cycling in the environment

a. Beneficial aspects relating to natural ecosystem function, positive influence on plant productivity, regeneration, recovery or bioremediation of polluted habitats.

b. Detrimental aspects such as acid mine drainage and release of toxic metals from soil and rocks into water.

2. Pupil stakeholder awareness

a. Why is metal cycling important to the environment and to living organisms? Note that metal cycling is part of the overall cycling of all elements in the biosphere including carbon, nitrogen, phosphorus and sulfur, and most, if not all, cycles are interlinked in some way.

b. The beneficial aspects of metal-microbe interactions in environmental biotechnologies for biorecovery of valuable metals or bioremediation of metal pollution. Some microbial processes release metals in soluble forms while some processes immobilize metals in insoluble forms including minerals.

c. Understand that even decomposition of organic matter leads to cycling of metals between environmental compartments and between organisms. Many metals are essential for life, and microbial processes that release metals also release other essential elements like phosphorus and sulfur.

3. Exercises

a. Name as many metal elements as you can. Which of these are essential for life? Which metals are not essential for life and are usually toxic?

b. What are important metals found in computers, mobile phones and other electronic devices? How can microbial interactions with metals and minerals contribute to recycling and recovery of such metals?

c. On a walk in the countryside or city, identify where metal cycling may be taking place and what the effects of such cycling may be.

The Evidence Base, Further Reading and Teaching Aids

Gadd, G.M. (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* 111: 3-49.
Gadd, G.M. (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology* 156: 609 – 643.

Glossary

biodeterioration – the activities of living organisms in deterioration of substances. Usually refers to the industrial or built environment and the biodeterioration of materials like wood, rock and mineral-based building components, leather, paper etc.

bioleaching – the process of using microorganisms to solubilize and extract metals from, e.g. ores, industrial wastes, and polluted soil.

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biomethylation - a methylation reaction mediated by a living organism, e.g. the microbial biomethylation of selenium to give volatile methylated selenium compounds.

biomineralization - the formation of minerals by living organisms.

biominerals - minerals formed by living organisms or as a result of biological activity.

bioremediation - a biological process that is used to degrade, detoxify or remove organic and inorganic substances from contaminated land or water. The majority of bioremediation applications use microorganisms or plant-microbial systems.

biosorption - biosorption refers to metal binding to living or dead biomass, and their products, and is a physico-chemical process, but may be influenced by microbial metabolism.

biosphere - that part of planet Earth that supports life.

bioweathering - the erosion, decay or biodeterioration of rocks and minerals mediated by living organisms and their metabolic products.

heterotrophic - an organism that utilizes organic nutrient sources for carbon metabolism and energy generation.

intracellular accumulation - refers to the accumulation of, e.g. nutrients, metals, inside cells as a result of various transport systems.

metal biorecovery - the recovery of valuable metals from, e.g. wastes, process streams, and leachates, using a biological system.

metal radionuclides - an unstable form of a chemical element that releases radiation as it breaks down and becomes more stable. They are also called radioisotopes. Many radionuclides can be metals, e.g. caesium-137, strontium-90. Some occur naturally, while others are industrially produced for a variety of purposes or result as by-products of nuclear reactions such as those involved in the supply of nuclear energy.

methylation - a chemical reaction where a methyl group is attached to a molecule. In this article, the referral is to certain metals or metalloids.

microbial eukaryotes - those microorganisms that possess a nucleus and membrane-bound organelles, e.g. fungi, microalgae, protists.

oxidation - the process of being oxidized which results in an increase in the oxidation state of a chemical species, e.g. Fe(II) to Fe(III). Other definitions for oxidation include loss of electrons, loss of hydrogen or gain of oxygen (hence oxidized) but these do not apply in all cases.

redox reactions - also called reduction-oxidation reactions and are reactions where both oxidation and reduction are taking place. This results in a change in the oxidation state of the participating reactants.

reduction - the process of being reduced which results in a decrease in the oxidation state of a chemical species, e.g. Fe(III) to Fe(II). Can also be defined as the gain of electrons, gain of hydrogen, or loss of oxygen in a chemical reaction but these do not apply in all cases.

transport mechanisms - the processes by which organisms take up nutrients or ions from their external environment. Transport systems are located in the cell membrane and permit regulated intracellular accumulation of such substances.