

# Bioremediation of metals; the application of micro-organisms that make and break minerals

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Metals have played a pivotal role in the development of human civilizations. As early as 15000 BC gold and copper, which both occur as native metals, were worked into useful and desirable objects. By 4000 BC primitive smelting techniques were developed to extract copper from ores, and within a further 1,000 years other metals, including silver, tin, lead and zinc, were also being extracted, leading the way to the manufacture of alloys such as bronze (a blend of copper and tin). Iron was harder to obtain from rocks and was not worked successfully until about 1300 BC in Asia Minor. The development of the blast furnace many centuries later led to the large-scale production of iron and steel and ushered in the Industrial Revolution. Metals are not just part of our long industrial heritage, however, but are finding increasing use in areas as diverse as medicine, electronics, catalysis and the generation of nuclear power. Given our long and intimate association with metals, and our continued reliance on these important natural resources, it is not surprising that their use (and abuse) can lead to significant environmental problems that need to be addressed.

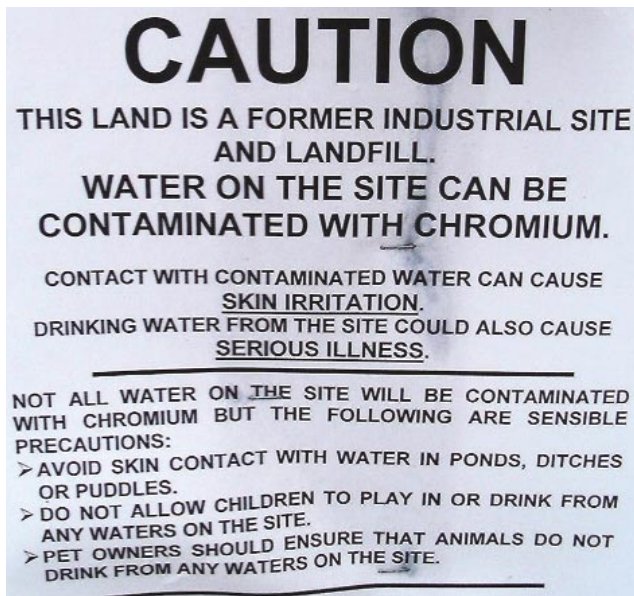
To put these environmental issues into perspective, the UK brownfield land area is estimated to cover about 360,000 hectares, half of which is expected to be contaminated with toxic metals (for an example, see Fig. 1). Estimates of the global market for the clean-up and prevention of metal contamination vary, but conservative calculations suggest that the current market for metal bioremediation may be about £20 billion per year, rising to £200 billion in the US alone by 2005. The emerging market for the clean-up of radioactive contamination may already be worth as much as £140 billion. Unfortunately, existing chemical

techniques are not cost-effective for the removal of metals from large areas of contaminated land. Current strategies rely on 'dig and dump' approaches that only move the problem to another site, and these are expensive and impractical for large volumes of soil or sediment. Likewise, soil washing, which removes the smallest particles that bind most of the metals, is useful but can be prohibitively expensive for most sites. 'Pump and treat' technologies rely on the removal of metals from the site in an aqueous phase which is treated *ex situ* (e.g. above land). These approaches can cut down on excavation costs but are still expensive, and metal removal can be inefficient. What is needed is a suite of low-cost techniques that can be used in the sediment or soil (*in situ*) to either extract the metals or stabilize them in forms that are immobile or non-toxic. There is also considerable interest in more effective techniques that can be used to treat metal-contaminated water from a range of industrial processes. Problems inherent in currently used chemical approaches include a lack of specificity associated with some ion exchange resins, or the generation of large quantities of sludge through treatment with alkali or flocculating agents. Biotechnological approaches that harness microbial activities may offer practical solutions to these problems, offering highly specific, potentially cost-effective alternatives that can be used at large scale in a range of settings, both *in situ* and *ex situ*. This article gives a very short overview of metal-microbe interactions, and describes how they could be harnessed to clean up metal-contaminated water and land.

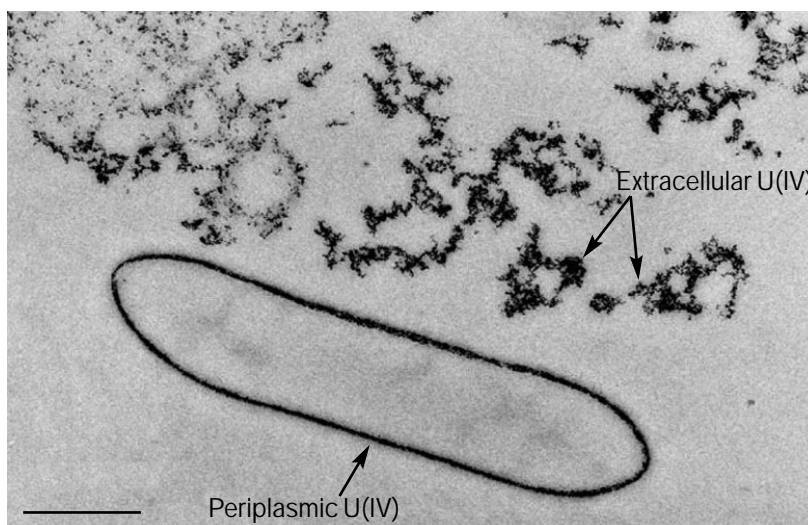
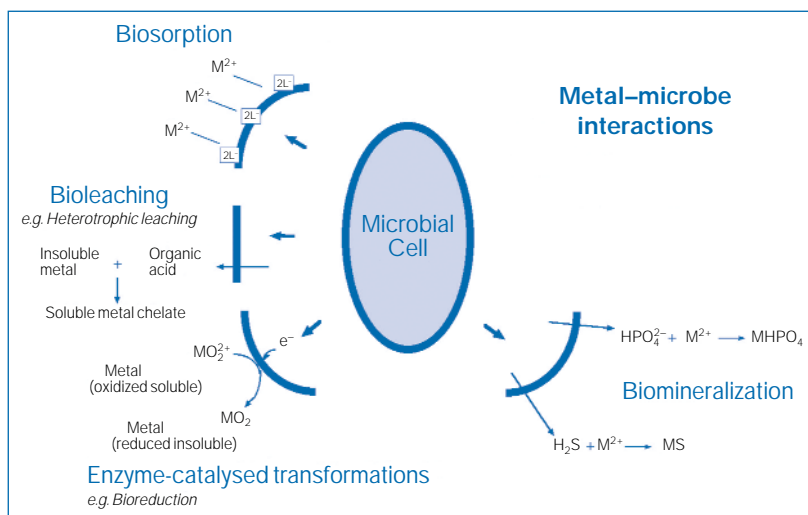
## ● Metal-microbe interactions

Although micro-organisms cannot destroy metals (they are not alchemists!) they can alter their chemical properties via a surprising array of mechanisms (see Fig. 2), some of which can be used to treat metal contamination. In some cases these processes involve highly specific biochemical pathways that have evolved to protect the microbial cell from toxic heavy metals. A good example here is the microbial reduction of mercury, a subject that is discussed in more detail below. Because these detoxification mechanisms are very specific, the biochemical components that recognize and detoxify the target metals may also prove useful for the design of biosensors for 'bioavailable' concentrations of toxic metals. In other examples, microbes can produce new mineral phases via non-specific mechanisms that result in the entrapment of toxic metals within soils or sediments. Other mechanisms of potential commercial importance rely on the production of biogenic ligands that can complex metals, resulting in their mobilization from contaminated soils. The mobilized metals can then be pumped out of the soil or sediment and trapped in a bioreactor on the surface.

Environmental pollution by metals is a serious problem. Jonathan Lloyd describes some ways in which microbes can be used in the clean-up process.



LEFT:  
Fig. 1. Contamination of land with metals such as chromate is a significant problem in both the developed and developing world. This site is in the North West of England.  
COURTESY GREATER MANCHESTER GEOLOGICAL UNIT



ABOVE TOP:  
**Fig. 2.** Mechanisms of metal-microbe interactions that can be harnessed for bioremediation applications.

ABOVE BOTTOM:  
**Fig. 3.** Transmission electron micrograph showing the reduction of soluble U(VI) to insoluble U(IV) by a subsurface metal-reducing bacterium *Geobacter sulfurreducens*. Insoluble U(IV), visible as an electron-dense mineral uraninite, is precipitated outside the cell and also in the periplasm. Bar, 0.5 µm.  
 COURTESY JONATHAN LLOYD

● **Metal-mobilizing micro-organisms**

Metals can be extracted from contaminated environments by two potentially useful mechanisms. First, some heterotrophic micro-organisms are able to mobilize metals via the production of organic acids. Alternatively, highly specialized autotrophic bacteria such as *Thiobacillus* species are able to generate significant quantities of metal-leaching sulfuric acid from the oxidation of elemental sulfur. This mechanism of metal mobilization has been used for many centuries to leach metals from low-grade ores, and currently supports a lucrative global market in mineral extraction. It can also be harnessed to remove metals from contaminated soils and sediments, and can be combined with a second *ex situ* step to remove the metals as insoluble sulfides using sulphate-reducing bacteria, which reverse the metal mobilizing step.

● **Using microbes as ion exchange resins**

Once metals are in solution, one of the simplest ways to remove them is through 'biosorption', which can be

defined as the metabolism-independent sorption of heavy metals and radionuclides to biomass. The cell surface carries a net negative charge at neutral pH due to the presence of carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups, and can adsorb appreciable quantities of positively charged cationic metals. Advantages of this type of metal-microbe interaction include the potential use of low-cost waste biomass sources (e.g. spent brewery yeast) and very rapid kinetics coupled with high adsorption capacities. Indeed, recent comparisons have suggested that biosorbents may be cheaper to implement than other commercially available ion exchange resins. Disadvantages include the perceived variation between batches of the biological product, a lack of specificity and sensitivity to changes in pH. Recent studies have suggested that it may be possible to increase uptake and specificity of biosorbents using the tools of molecular biology, for example by targeting engineered metal-binding proteins to the cell surface.

● **Enzyme-catalysed transformations**

Micro-organisms are ubiquitous and offer a potentially enormous gene pool to select from when looking for enzymes that can help treat metal contamination. Indeed, micro-organisms have evolved a wide range of biochemical tricks to protect themselves from potentially toxic metals and these activities can be useful for bioremediation applications. Many microbial detoxification processes involve efflux or exclusion of metal ions from the cell, which in some cases can result in high local concentrations of metals at the cell surface where they may react with biogenic ligands and precipitate. Alternative mechanisms involve redox transformations, for example the enzyme-catalysed reduction of the toxic mercuric ion ( $\text{Hg}^{2+}$ ) to non-toxic elemental mercury [ $\text{Hg}(0)$ ]. This approach has been used recently to treat chloralkali wastewaters contaminated with  $\text{Hg}^{2+}$  ions. Microbially reduced elemental mercury was trapped in a bioreactor containing a biofilm of mercury-resistant bacteria (pseudomonads).

In addition to the highly specific mercury reduction/detoxification pathway, some specialist subsurface bacteria are able to use high valence metals as electron acceptors for anaerobic growth. Metals that are reduced in this manner include Fe(III), Mn(IV), U(VI), Cr(VI), Se(VI) and As(V). In some cases the biological reduction of these metals can result in dramatic changes in solubility. For example, U(VI) is highly soluble and mobile, but U(IV), formed through enzymic reduction by a range of specialist anaerobic bacteria, is highly insoluble (see Fig. 3). This transformation, catalysed by a class of enzymes known as *c*-type cytochromes, can be used to stabilize uranium in contaminated groundwater. Although uranium is the priority contaminant in

nuclear waste, there are other less familiar isotopes that also cause considerable concern. These include technetium and neptunium, both of which normally exist as oxidized, soluble forms; Tc(VII) and Np(V), respectively. Thankfully, subsurface bacteria are also able to reduce these to less soluble forms [in these cases Tc(IV) and Np(IV)] and may therefore play a role in preventing their migration in contaminated soils and sediments. Another redox-active metal that can cause concern in the UK (see the sign in Fig. 1 from a site in the north west) and abroad is chromate. Film-goers will be familiar with health problems associated with chromate contamination raised in the recent Hollywood film *Erin Brockovich*. Metal-reducing bacteria are able, however, to reduce very toxic soluble chromate [Cr(VI)] to less toxic, less soluble Cr(III). These organisms may prove useful in the bioremediation of sediments contaminated by Cr(VI), or in the treatment of Cr(VI)-contaminated process waters.

#### ● Indirect mechanisms that build novel biominerals

In addition to reducing metals directly using 'metal reductases', anaerobic bacteria are also able to reduce and precipitate a range of metals via indirect mechanisms. For example, Fe(III)-respiring bacteria catalyse the formation of Fe(II)-bearing minerals that can in turn reduce and precipitate high valence metals abiotically. In many cases reduction is extremely efficient, driven by the very large surface area of biologically produced minerals. Examples here include Fe(II)-catalysed reduction of Cr(VI) and Tc(VII), with subsequent precipitation of Cr(III) and Tc(IV) respectively. Sulfate-reducing bacteria are also able to remove metals via indirect mechanisms. Here precipitation, sometimes with concomitant reduction, is driven via sulfide that is produced from respiration using sulfate as the terminal electron acceptor. A wide range of metals react to form insoluble sulfide minerals and this approach has been used successfully in several metal-treatment applications, including the bioremediation of water from a metal sulfide refining site in the Netherlands. Sulfate-reducing bacteria have also been used successfully to treat metal leachates generated by sulfuric-acid-producing *Thiobacillus* species as mentioned previously.

Metal phosphates, like the corresponding sulfides, are sparingly soluble and bacteria are also able to remove toxic metals as insoluble phosphate biominerals. A well studied model system here is a *Citrobacter* species that generates free inorganic phosphate from the degradation of glycerol 2-phosphate via a 'phosphatase' enzyme. This results in high local concentrations of metals and phosphate at the surface of the bacterial cell, driving the formation of an insoluble metal phosphate coat that can entrap significant quantities of toxic metals and radionuclides. Finally, both the sulfide and phosphate

biominerals described are able to remove a range of toxic metals via intercalation into the host mineral.

#### ● Conclusions

From this very brief overview it is clear that there are many microbial activities that may prove potentially useful for the bioremediation of metal-contaminated soils, sediments and waters. The challenge is now to implement these novel approaches in the field. This will require multidisciplinary studies encompassing a diverse scientific and technical community, including engineers, hydrologists and geochemists, as well as microbiologists. Although the application of biological approaches to treat metal contamination has been slow, tighter environmental legislation in combination with the inherent limitations of existing chemical approaches will surely mean that micro-organisms will play a very significant role in controlling metal contamination in the 21st century.

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#### Further reading

Lloyd, J.R. & Lovley, D.R. (2001). Microbial detoxification of metals and radionuclides. *Curr Opin Biotechnol* **12**, 248–253.

Lovley, D.R. (editor) (2000). *Environmental Microbe-Metal Interactions*. Washington, DC: ASM Press.

Macaskie, L.E., Empson, R.M., Cheetham, A.K., Grey, C.P. & Skarnulis, A.J. (1992). Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymically mediated growth of polycrystalline  $\text{HUO}_2\text{PO}_4$ . *Science* **257**, 782–784.

White, C., Sharman, A.K. & Gadd, G.M. (1998). An integrated microbial process for the bioremediation of soil contaminated with toxic metals. *Nat Biotechnol* **16**, 572–575.