Bioremedial potential of microbial mechanisms of metal mobilization and immobilization
Geoffrey Michael Gadd

Microorganisms play important roles in the environmental fate of toxic metals and radionuclides with a multiplicity of mechanisms effecting transformations between soluble and insoluble forms. These mechanisms are integral components of natural biogeochemical cycles and are of potential for both in situ and ex situ bioremedial treatment processes for solid and liquid wastes.

Addresses
Department of Biological Sciences, University of Dundee, Dundee, DD1 4HN, UK; e-mail: g.m.gadd@dundee.ac.uk

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Abbreviations
APCR air pollution control residues
DMSe dimethyl selenide
HP Gly–His–His–Pro–His–Gly
PCS phytochelatin synthase
SBR sulfate-reducing bacteria

Introduction
The mechanisms by which microorganisms effect changes in metal speciation and mobility are fundamental components of biogeochemical cycles for metals, as well as all other elements including carbon, nitrogen, sulfur and phosphorus, with additional implications for plant productivity and biogeochemical cycles [1,2]. Among all these interactions have been examined in the context of environmental biotechnology as a means for removal, recovery or detoxification of inorganic and organic metal or radionuclide pollutants [3,4••,5••]. Although much research is laboratory-based, there have been many developments to pilot/demonstration scale, with some processes apparently successful in commercial operation. It should also be noted that metal removal/transformation processes are intrinsic to the natural microbiota of most environments [6]. Molecular and genetic analysis is now furthering our understanding of microbial metal metabolism, including those aspects that have potential in biotechnology [7••,8••]. This review details recent advances in understanding of the most important mechanisms of microbial metal transformations within the contexts of bioremediation and environmental biogeochemistry.

Metal mobilization
Leaching
Leaching of mineral ores by acidophilic sulfur-oxidizing bacteria is an established biotechnology and although most interest arises from a hydrometallurgical perspective, leaching of contaminating metals from soils and other matrices is also possible [9••]. In fact, bioleaching using elemental sulfur as a substrate can be more efficient in terms of metal solubilization from contaminated aquatic sediments [10]. Iron- and sulfur-oxidizing bacteria, *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, respectively, were enhanced from contaminated soil and were able to leach >50% of the metals present (As, Cd, Co, Cu, Ni, V, Zn, B, and Be). Strains of *T. ferrooxidans* were also able to remove all of the Cd, Co, Cu and Ni [11]. *T. ferrooxidans* has also been used to treat air pollution control residues (APCR; fly ash and used lime). Although viability of the *Thiobacillus* was poor, removal of as much as 95% of Cd (from APCR of ~270 mg Cd kg–1) was achieved with ~69% Pb removal (from APCR of ~5 g Pb kg–1) and similar removals for Zn and Cu [12]. Note that the metals in such leachates can be subsequently removed using bacterial sulfate reduction where the soluble metals are precipitated as insoluble sulfides [9••].

The microbial production of organic acids, under the general heading of ‘heterotrophic leaching’, is also of developing interest in pollution treatment and metal recovery, and also in the context of mineral fertilization to increase plant productivity [1••,13,14]. In the soil, heterotrophic metal mobilization is of more importance than autotrophic leaching and solubilization of, for example, phosphate- and sulfate-containing minerals, as this leads to release of these important nutrients [1••]. Heterotrophic leaching may be particularly appropriate for wastes of high pH such as filter dust/oxides from metal processing, where as most *Thiobacillus* cannot solubilize effectively above pH 5.5. Fungi need good aeriation and a carbon source so their use is generally envisaged to be in bioreactors. In most fungi, leaching is mediated by the production of organic acids, which provide a source of protons and metal-complexing organic acid anions [1••,13,14]. *Trichoderma harzianum* can solubilize MnO2, Fe2O3, metallic zinc and rock (mostly Ca) phosphate with both chelation and reduction being involved in oxide solubilization [15]. Effective leaching of a variety of wastes and low-grade minerals has been demonstrated, for example, soils and muds, filter dust/oxides, lateritic ores, copper converter slag, fly ash and electronic waste materials [1••,16•]. For the latter, a two-stage process was proposed where initial growth of solubilizing organisms is followed by separate contact of electronic scrap with metal-rich-containing medium, which would overcome any inhibition of organism growth by high scrap concentrations [16•]. Another method for treatment of metal-contaminated sandy soil relies on siderophore-mediated metal solubilization by *Alcaligenes autotrophus*. Solubilized metals are adsorbed to the biomass and/or precipitated, with biomass separated from a soil slurry by a flocculation process. This process resulted in a large increase in the bioavailability of Cd, Zn and Pb [17••].
Heterotrophic solubilization can also have consequences for other remedial treatments for contaminated soils. Promising possibilities are the use of copper-tolerant wood and can form in urban and industrially contaminated soils. Such solubility reduces lead bioavailability and the formation of lead oxalate dihydrate was observed for the first time [18**]. Further, during the fungal growth with pyromorphite as a sole phosphorus source accumulate both P and Pb [18••]. Related to heterotrophic solubilization is fungal translocation of, for example, Cu, Zn and Cd, which can lead to concentration of metals in radiculodes and radiculocarps in specific regions of the mycelium and/or in fruiting bodies. Whether the concentration factors observed in vitro can be reproduced in the field and whether such amounts can contribute to soil bioremediation remains a topic for further investigation [19]. Some parallels may be drawn with the phytoremediatory approach to soil contamination. Other possibilities relate to the use of copper-tolerant wood decay fungi to degrade copper-treated wood products, although results obtained so far have shown only slight effects on the copper content of wood before or after decay [20].

Alkylolation and reduction

Volatile dimethyl selenide (DMSe) can form naturally from inorganic Se species in soils and this can regulate geochemical cycling of Se and therefore influence Se bioremediation. Many environmental and soil factors (e.g., organic amendments and frequent tillage) can be optimized to increase diffusive transport through soil and enhance volatilization [21–23]. In moist or flooded soil, however, less DMSe is lost to the atmosphere, with a significant proportion of DMSe being converted to non-volatile forms of Se. Oxidized dimethylated Se (OMDSe), including dimethyl selenoxide (DMSeO) and dimethyl selenone (DMSeO₂), was the dominant form of non-volatile Se, and accounted for up to 90% of Se in flooded soils. Such transformation of DMSe can be responsible for the low-Se volatilization rate in flooded soils and sediments [24••]. Microorganisms enhance arsenic mobilization from soil by up to 24%, compared to formaldehyde-treated controls, with only low formation of dissolved methylated arsenic species [25]. Other bacteria are capable of the oxidation of As(III) to As(V) [26].

Fe(III) and Mn(IV) oxides absorb metals strongly and this may hinder metal extraction from contaminated soils. Microbial reduction of Fe(III) and Mn(IV) could be one way for releasing such metals and this process can be enhanced with the addition of humic materials, or related to heterotrophic solubilization is fungal translocation of, for example, Cu, Zn and Cd, which can lead to concentration of metals in radiculodes and radiculocarps in specific regions of the mycelium and/or in fruiting bodies. Whether the concentration factors observed in vitro can be reproduced in the field and whether such amounts can contribute to soil bioremediation remains a topic for further investigation [19]. Some parallels may be drawn with the phytoremediatory approach to soil contamination. Other possibilities relate to the use of copper-tolerant wood decay fungi to degrade copper-treated wood products, although results obtained so far have shown only slight effects on the copper content of wood before or after decay [20].
La³⁺, Eu³⁺ and Yb³⁺ unaffected by K⁺, Ca²⁺, Cl⁻, SO₄²⁻, or NO₃⁻ but strongly inhibited by Al³⁺ [40]. As well as bacteria, fungi and algae also receive sustained biosorbptive examination. Zn biosorption to Rhizopus arrheni was predominantly to wall chitin and chitosan (maximum sorption capacity of 312 mg g⁻¹ dry wt compared to 213 mg g⁻¹ dry wt for the wall) [31]. Sorption activity for UO₂²⁺, Pb²⁺, Mn²⁺, and Co²⁺ of A. niger structure and function of chitin: chitosan [41]. Freeze-dried biomass of white-rot fungi was found to be the most efficient biomass type for Cd²⁺ biosorption [42], whereas dry biomass of Planorobustus chrysosporium sorbed mercury and alkylmercury species with an affinity of CH₃HgCl. The earlier commercial promise and development of Rhizopus oligosporus was more effective than freely suspended biomass for Cd²⁺ − a possible contribution to sorption by the immobilization matrix [34]. A reliable model based on Fick’s Law of Diffusion and the Langmuir adsorption model was able to predict experimentally determined kinetics of Ni²⁺ removal by fungal biosorbents [44]. Removal of Pb, Cd and Cu by alkali-treated A. niger biomass was greater than removal achieved by granular activated carbon (F-400) [45]. Benzaldehyde was an effective fixation agent for Saccharomyces cerevisiae biomass, which retained its original lead biosorption capacity [46]. For algae, in situ metal removal from the environment increased the metal capacity for nickel from ~70 to ~1096 mmol kg⁻¹. An increase in maximal binding capacity for Cd, Ni and Zn also occurred from 100–274, Ni 71–182, Zn 185–303 mmol kg⁻¹, respectively [52].

The earlier commercial promise and development of biosorption appears to have largely ceased and there is no adoption of biosorption as a commercially viable treatment method to date. The lack of commercial development is attributed to the biosorption of metals: specificity and lower robustness of biomass-based systems compared to ion exchange resins is often cited as a reason [59*]. Unless such reasons and the reluctance of industry to adopt and develop biosorption technology are overcome, the rationale for continued research activity is weak as few studies provide novel insights or understanding of the fundamental physics and chemistry of metal sorption mechanisms involved. This perhaps contrasts with a biosorption perspective where microbial metal sorption is contributing to an understanding of metal fate and cycling in the environment. For example, the application of surface complexation modeling or a linear programming approach to specific chemical and electrostatic interactions occurring at the solution−cell-wall interface can also be applied to biosorption phenomena. Such studies can relate variations in surface properties with variations in metal affinity in order to predict metal mobilities in complex natural systems [53,54,55*,56].

**Metal-binding molecules**

Eukaryotic metallothioneins and other metal-binding peptides have been expressed in E. coli as fusions to membrane or membrane-associated proteins such as LamB, an outer membrane protein that functions as a chloride surface receptor and is involved in maltose/maltodextrin transport. Such in vivo expression of metallothioneins provides a means of designing biomass with specific metal-binding properties [8**,57,58]. Metal-binding peptides of sequences Gly–His–His–Pro–His–Gly (HP) and Gly–Cys–Gly–Pro–Gly–Cys–Gly (CP) were engineered into one Chlorella strain exhibiting maximal removal of 39.4 mg Cd g⁻¹ dry cells from 50 µM Cd under marine conditions [47]. Cu was preferentially adsorbed to Zn by Chlamydomonas nivalis, a brown alga [48]. Little attention has been given to metal−organic mixtures, though equilibrium uptake of, for example, Ni and phenol are changed due to initial pH and other reaction components [49]. Bacterial, algal and fungal biomass were able to remove and concentrate gold from solutions in which it occurred as a gold−cyanide complex [50]. Note that extracellular polymers are intimately involved in Cd adsorption by activated sludges [51].

Chemical modification of biomass may create derivatives with altered metal-binding abilities and affinities. A. niger mycelium was modified by introducing additional carboxy- or ethyldiamino groups, with the latter substitution increasing the maximal capacity for nickel from ~70 to ~1096 mmol kg⁻¹. An increase in maximal binding capacities for Cd, Ni and Zn also resulted in carboxy-modified biomass (Cd 100–274; Ni 71–182; Zn 185–303 mmol kg⁻¹, respectively) [52].

Related to the application of metal-binding molecules is the identification of genes encoding phytochelatin synthases (PCSs) as phytochelatins play major roles in metal detoxification in plants and fungi. Heterologous expression of PCS genes dramatically enhanced binding of Cd²⁺, Cu²⁺, Hg²⁺, and Co²⁺ compared to cells lacking the peptide [57].
of other metal-binding molecules may have future potential for metal recovery. Culture filtrates from the engineered Desulfococcus multivorans exhibited copper-binding with 12 day old filtrates having a copper-binding capacity of 3.64 ± 0.33 mmol mol⁻¹ with a stability constant log_{10}K of 5.68 ± 0.64 (n = 4) [64]. A hollow-fibre reactor containing an engineered E. coli strain expressing Hg²⁺ transport and metallothionein accumulated Hg²⁺ effectively, reducing a concentration of 2 μg L⁻¹ to < 3 pg L⁻¹ [65]. It can be noted that biosensors for the detection of metal bioavailability have been developed based on the direct interaction between metal-binding proteins and metal ions. Here, capacitance changes of the proteins (e.g. a synchococcal metallothionein and a mercury resistance regulatory protein) were detected in the presence of femtomolar to millimolar metal ions. Furthermore, such biosensors have been used to detect metal binding in the presence of biopolymers, such as DNA, proteins, and leachates [3].

Reductive precipitation

Where reduction of a metal to a lower redox state occurs, mobility and toxicity can be reduced, thus offering potential bioremediation applications. Such processes can also accompany other indirect reductive metal precipitation mechanisms, for example, in sulfate-reducing bacterial systems where reduction of Cr(VI) can be a result of indirect reduction by Fe²⁺ and the produced sulfide. Aerobic or anaerobic reduction of Cr(VI) to Cr(III) is widespread in microorganisms and both ex situ reactor systems and in situ treatment approaches have been documented. Problems can occur with Cr(VI) toxicity as well as maintenance of anaerobic conditions for anaerobic Cr(VI) reducers. However, several aerobic and anaerobic Cr(VI) reducers are known with some being able to use sulfate as the electron acceptor [64]. D. desulfuricans can couple the oxidation of Cr(VI) to sulfate with the reduction of Fe(III) to Fe(II), thus providing a means of Pd recovery [67]. The incidental ability of some bacteria to reduce Pd(II) to cell-surface-bound Pd(0) with the hydrogen-dependent reduction of Fe(III) to Fe(II) has been observed [68]. Pd complexes can be synthesized with both sulfate- and metal-reducing groups of bacteria and can grow with Cr(VI), Fe(III) and U(IV) as sole electron acceptors [68].

Se(VI) reduction to elemental insoluble Se(0) has been employed in the remediation of contaminated waters and soils. Some bacteria can use such a reduction to support growth, making this a natural process for in situ applications. Though reduction of oxyanions of As and Se can occur by different mechanisms, the most environmentally significant process is dissimilatory reduction. Oxyanions of As and Se can be used in microbial anaerobic respiration as terminal electron acceptors providing enough energy for growth and metabolism. Their reduction can be coupled to a variety of organic substrates (e.g. lactate, acetate and aromatics) and the bacteria are found in a range of habitats and are not confined to any specific genus. These organisms, and perhaps even the enzymes themselves, may have applications for bioremediation of Se- and As-contaminated environments [69].

Sulfurospirillum barnesi can simultaneously reduce both NO₃⁻ and Se(VI). Kinetic experiments with cell membranes of S. barnesi suggest the presence of constitutive Se(VI) reduction capacity, which is also observed in nitrate-grown cells. Selenate and nitrate reduction as well as an inducible, high affinity nitrate reductase, which also has a low affinity for selenate, in nitrate-grown cells. Simultaneous reduction of micromolar Se(VI) in the presence of millimolar nitrate suggests a role for these organisms in bioremediating nitrate-rich seleniferous wastewater [70]. It has been shown that selenate does not sorb to soil components during transport in saturated soil columns, but rapidly reduces to forms that are strongly sorbed. Such findings suggest that Se will be retained near the soil surface even under extreme leaching conditions [71]. It should be noted that the reoxidation of Se(0) is now known to be under microbial control and where alteration in physico-chemical conditions leads to, for example, drying out of Se(0)-contaminated sediments or aerobic alteration of subsurface soils, Se(0) can be reoxidized to form toxic selenite and selenate [72]. The incidental ability of a variety of microorganisms from all major groups to reduce Se(VI) and Te(VI) by additional often uncharacterized mechanisms offers additional scope for bioreactor-based approaches [73]. Finally, rhizosphere bacteria increased the efficiency of Se and Hg phyto-remediation by promoting the accumulation of Se and Hg in tissues of wetland plants [74].
Sulfate reduction bacteria (SRB) are anaerobic heterotrophs utilizing a range of organic substrates and $\text{SO}_4^{2-}$ as a terminal electron acceptor. The sulfide produced from sulfate reduction plays a major role in metal sulfide immobilization in sediments but has also been applied to bioremediation of metals in waters and sediments [6-7]. In one integrated microbial process for the bioremediation of soil contaminated with toxic metals, sulfur-oxidizing bacteria were used to leach metals from contaminated soils by breakdown of minerals and liberation of acid-labile sorbed or chemical forms [9]. A large proportion of the acetic acid and almost the entirety of released metals were removed using bacterial sulfate reduction in an internal sedimentation reactor. The combination of soil bioleaching followed by separate bioprecipitation of leached metals by SRB proved to be effective in removing and concentrating a range of metals, including $\text{Zn}$, $\text{Cu}$ and $\text{Cd}$, from metal-contaminated soil. The resultant solids, once separated, represented a ~200-fold reduction in volume compared to the soil, whereas the low toxic metal content of the clarified liquor produced would permit discharge or allow recycling of the liquor to the bioleaching stage and permit water conservation during process operation. Where soil and water conditions are appropriate, the soil-leaching component of such an integrated process could possibly be carried out in situ, although conditions allowing this would be prone to uncertainty. In other cases, a range of ex situ options, including heap leaching or slurry bioprecipitation, could be used to solubilize metals and feed the bioprecipitation reactor [9]. Sulfate-reducing bacterial biofilm reactors may offer a means of process intensification and entrap or precipitate metals, for example, $\text{Cu}$ and $\text{Cd}$, onto the biofilm surface [73-76]. Mixed SRB cultures were more effective than pure cultures for metal removal, which was enhanced by greater production of exopolymers in the mixed culture [73-76]. Anaerobic solid substrate bio reactors have also been used in laboratory treatment of acid mine drainage with sulfate-reducing bacteria utilizing a range of organic substrates and $\text{SO}_4^{2-}$ as a terminal electron acceptor. The composition of these reactors, inoculated with various SRB, showed a mix of metals removal, which was enhanced by greater production of exopolymers in the mixed culture [73-76]. 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may have potential in bioremediation or biosensor applica-
tions [85]. Although the significance of extracellular polymeric substances in metal biosorption is well known, it has recently been demonstrated that the composition of cell-associated polymers and their ability to sorb toxic met-
als are functions of culture media and conditions, which offers manipulative possibilities for biosorption-type appli-
cations [86]. Citrate-utilizing strains of *Pseudomonas aeruginosa* and *Pseudomonas putida* have been isolated that
can remove Cd, Zn, Cu, Fe, Co and Ni from solution with incorporation of inorganic phosphate as a precipitant. Such
organisms may have potential in the treatment of metal-
citrate wastes: citrate is used as a complexing agent in
certain decontamination processes [87]. Sulfate-reducing
bacterial strains, isolated from uranium mine tailings, have been shown to be capable of Al biosorption with Al depo-
sition being exclusively associated with cell surfaces [88].

Further elucidation of the mechanisms of Se methylation has resulted from monitoring of volatile and dissolved DMSO and dimethylselenide, and dimethylselenonium compounds in soil spiked with selenomethionine. It is sug-
gested that selenomethionine undergoes a methylation pathway via dimethylselenomethionine and dimethylselenon-
ioseptinate to DMSO, and that oxidation of dissolved DMSO to dimethylselenoxide may occur in soil [89].

Aqueous phase flux can have an impact on the metabo-
Hemi (III)-reducing bacteria, which is important for lism of Fe(III)-reducing bacteria, is an important for predicting their influence on transport and fate of metals and predicting their influence on transport and fate of metals and radionuclides in the subsurface. It has been shown that removal of rhizogenic Fe(II) via aqueous phase trans-
port dramatically increased Fe(III) reduction and associated bacterial growth. As such organisms can reductively liberate or immobilize metal contaminants, as mentioned previously, these findings have implica-
tions for understanding the fate of metal, and organic, pollutants whose geochemical behaviour is linked to Fe(III) oxidation and the implementation of bioremediation strategies involving metal-reducing bact-
tera [90]. A diversity of hyperthermophilic microbes can reduce Fe(III), Mn(IV) and other metals at 100°C. Reduction was hydrogen-dependent with U(VI) reduced to U(IV) uraniumite, Te(VII) to Te(IV) or Te(V) or Cr(VI) to insoluble Cr(III), and Mn(IV) to Mn(II) with the forma-
tion of manganese carbonate. The metal reduction activities of these organisms or their enzymes may have potential for bioremediation of metal-laden water [91]. It has been shown for the first time that a mesophilic fac-

cultatively anaerobic Fe(III)-reducing bacterium, closely related to *Pantoea (Enterobacter) agglomerans*, can couple the oxidation of acetate or H₂ to dissimilatory reduction of Fe(III), Mn(IV) and Cr(VI) [92]. Some SRB strains have been shown to reduce arsenite. A *Dehalobacterium* sp. can use arsenate as a terminal electron acceptor in minimal medium in the absence of SO₄²⁻, the mecha-
nism of reduction being a c-type cytochrome or enzyme associated with such a cytchrome. A *Dehalococcoid* strain was unable to use arsenate as a terminal electron accep-
tor but possessed an arsenate reductase [93].

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• outstanding interest
2. Francis AJ: The biogeochemistry of citric and oxalic acid production in fungi and the intimate association of these acids and processes with metal speciation, physiology and mobility and their importance and involvement in key fungal-mediated processes, including metal biogeochem-
istry and biotechnology.
This review focusses on the bioremediation of mixed wastes and the recent developments designed to increase metal removal efficiency from contami-
nated sites e.g., for example, precipitation.
Key attributes and advantages of microbial systems for metal/radionuclide bioremediation are compared with proven physico-chemical technologies.
This review emphasises new approaches for genetically engineered microor-
genisms and enzymes for bioremediation of inorganic and organic pollutants.


This review highlights some of the recent advances and provides new insights into the taxonomy, physiology and biochemistry of arsenic and selenium-resisting bacteria.


51. The paper describes the simultaneous reduction of Fe(III) and nitrate by the selenium-resisting bacterium Sulfitobacillus barnesi and suggests a possible role in bioremediation of nitrate-rich arsenic-contaminated wastewaters.


55. This paper examines accumulation and transformation of tuffoles (TeO32–) by filamentous fungi. Reduction and precipitation of elemental Te within cultures and around biomass was demonstrated with only small amounts of Te being volatilized in a specie-dependent process.


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