

Developments in bioremediation of soils and sediments polluted with metals and radionuclides. 3. Influence of chemical speciation and bioavailability on contaminants immobilization/mobilization bio-processes

Eric D. van Hullebusch^{2,3,4,*}, Piet N.L. Lens² & Henry H. Tabak¹

¹US EPA, ORD, NRMRL, Cincinnati, OH, USA; ²Sub-department of Environmental Technology, Wageningen University, Wageningen, The Netherlands; ³Laboratoire des Géomatériaux, Université de Marne-la-Vallée, 77454 Marne La Vallée, Cedex 2, France; ⁴Laboratoire des Sciences de l'Eau et de l'Environnement, Université de Limoges, 87060, Limoges Cedex, France (*author for correspondence: e-mail: eric.vanhullebusch@univ-mlv.fr)

Received: 8 September 2005

Key words: bioreduction, bioremediation, speciation

Abstract

The biotransformation of metals is an exciting, developing strategy to treat metal contamination, especially in environments that are not accessible to other remediation technologies. However, our ability to benefit from these strategies hinges on our ability to monitor these transformations in the environment. That's why remediation of contaminated sediments and soil requires detailed *in situ* characterization of the speciation of the toxic substances and their transformations with respect to time and spatial distribution. The present paper gives an overview of the literature regarding research performed in the laboratory as well as in the field.

1. Introduction

Elements present in natural materials (soils or sediments) at concentrations of $< 1000 \text{ mg kg}^{-1}$ have been defined as trace elements (NRC 2003). Most elements of the periodic table are trace elements. Numerous trace metals have been identified as essential micronutrients for plants and/or micro-organisms (including iron, zinc, copper, selenium, and nickel) and as electron acceptors or donors in metabolic processes (e.g., iron). At elevated concentrations, however, many of these metals exhibit significant toxicity and trace metal pollution in sediment, soils and groundwater remains one of the most pressing issues in modern environmental science. Arsenic contamination of drinking water, cadmium, copper, lead, and zinc

pollution in soils, the transport of radionuclides over long time scales, and the cycling of mercury are a few examples of important research areas in this context. Contamination occurs from mine tailings, disposal of high metal wastes in improperly protected landfills, leaded gasoline and leaded based paints (NRC 2003), land application of fertilizer, animal manures, biosolids, composts, pesticides, coal combustion residues and atmospheric deposition (Adriano 2001).

Most of the inorganic contaminants listed in Table 1 bind strongly onto surfaces of soil and sediment components depending on the solution conditions, with pH and ionic composition being the primary determining factors.

Because metal and radionuclide contaminants are generally non-degradable except by

Table 1. Inorganic contaminants and their sources (modified from NRC 2003)

Chemical classes	Examples of contaminants	Sources or applications
Metals	Cr, Cu, Ni, Pb, Hg, Cd, Zn, As, Se	Mining, leaded gasoline, batteries, paints, fungicides, pesticides, irrigation drainage
Organo-metallics	Tributyltin, methylmercury	Paints, chemical manufacturing
Radionuclides	^{238,239,240} Pu, ^{235,238} U, ⁹⁰ Tc, ⁶⁰ Co, ¹³⁷ Cs, ⁹⁰ Sr	Nuclear reactors, weaponry, medicine, food irradiation

radioactive decay, remediation technologies must involve some form of mobilization or immobilization for removal or containment, respectively. The environmental impact of metallic contaminants in soils and sediments is dependent both on the chemical speciation of the metal and the response of the matrix to biological and physicochemical conditions. These factors are responsible for the mobilization of the metal from the solid into the aquatic phase and hence transport within the immediate vicinity, impacting on the rate of dispersal, dilution, uptake and transfer into living systems (Peijnenburg & Jager 2003). As a result of their multifaceted biological functions, the significance of metals in the environment will depend on the concentration at which they occur and on environmental factors that control their mobility and bioavailability (NRC 2003).

Soil and sediments are dynamic systems, subject to short term fluctuations, such as variations in moisture status in case of soils, pH and redox conditions and also undergoing gradual alterations in response to changes in management and environmental factors. These changes in soil and sediments properties affect the form and bioavailability of metals, and need to be considered in decisions on the management of polluted soils or sediments (Eggleton & Thomas 2004; Nowack et al. 2004; Basta et al. 2005).

Remediation of metallic contaminants can only be brought about by their removal from the site or by establishing conditions which favour their retention in the solid phase (Hursthouse 2001). In the context of soil and sediment contamination and remediation, we need to consider all the biogeochemical processes that control metal mobility and bioavailability, including sorption on mineral and plant surfaces, dissolution, (bio-)mineralization, redox processes, complexation by biogenic or non-biogenic

ligands, and biological uptake and derivatization. Some of these processes can effectively isolate heavy metals from the biosphere, whereas others cause their release or transformation to different species that may be more (or less) bioavailable and/or toxic to organisms (NRC 2003; Förstner 2004). This will impact on the influence of the contaminant source, reaction within the deposited environment, suitability and effectiveness of bioremediation processes and long-term behaviour of the remediation result (Hursthouse 2001).

2. Importance of metal speciation on immobilization/mobilization processes

An important aspect of metal-microbe/plant interactions, but one that is rarely addressed, is metal speciation and metal bioavailability rather than total metal concentration in the environment that determines the overall mobility and toxic effects on biological systems (Roane et al. 1996).

2.1. Definition of speciation

The mobility of metals and radionuclides depends in part on reactivity and solubility, which are determined by the speciation or chemical form of the element. The term "speciation," as used here, refers to (i) the identity of the element, (ii) its oxidation state, (iii) its physical state (i.e., phase association; presence in a liquid, gaseous, or solid phase (amorphous or crystalline), colloidal particle, animal or plant cell, or biofilm; presence as a surface coating or thin film on a solid, as a sorption complex (monomeric or polymeric) on a solid, colloidal particle, or an organic substance; etc.) (Brown et al. 1999; Templeton et al. 2000).

2.2. *Physicochemical interactions between inorganic contaminants and soil/sediments solid phases*

Radionuclides and metal ions can be retained in soil and sediment by (ad)sorption, precipitation and complexation reactions. Once deposited in soils or sediments, trace metals interact with the soil or sediment environment. These interactions depend on both soil and sediment properties and environmental factors (pH, redox potential...). The phase association of an element is very important in determining its mobility. The most important controlling factors defining metal speciation at a particular point in space and time are: pH; composition and amount of organic matter; clay minerals; the presence and nature of Fe/Mn/Al oxides and hydroxides; redox potential; concentrations of salts and complexing agents; anion and cation content of the soil/sediment solution. However, depending on the specific contaminant and site conditions, precipitation may play a large role in governing aqueous metal concentrations, particularly in anaerobic sediment environments where high concentrations of sulfide can result in the precipitation of metal sulfides.

Figure 1 shows the complexity and interplay of the chemical and biological processes that control element cycling in soils and sediments. Such processes range from dissolution of mineral particles in soils, which can release natural contaminants into pore waters, to the binding or sorption of metals (M) and organic ligands (L) to mineral surfaces, which can effectively immobilize contaminants and reduce their mobility and bioavailability (Warren & Haack 2001). Precipitation is another common means of sequestering a heavy metal if the precipitated phase is relatively insoluble. Some heavy metal contaminants such as lead normally exist in minerals in one dominant oxidation state, whereas others such as arsenic and selenium can exist in several oxidation states and can undergo oxidation or reduction when they interact with mineral surfaces or organic compounds, which act as oxidants or reductants. Microorganisms and plants can have a profound influence on chemical reactions involving contaminants. For example, microorganisms often play a major role in the degradation of organic contaminants and in the oxidation and reduction of heavy

metals (Ledin 2000). In the case of plants, the root–soil interfacial region, referred to as the rhizosphere (circled area in soil profile in Figure 1) is an area of particularly intense chemical and biological activity where organic acids, sugars, and other organic compounds are exuded by live plant roots. For instance, Krishnamurti et al. (1997) found that soil Cd might be mobilized by low molecular weight organic acids such as acetic, citric, oxalic, fumaric and succinic acids that are commonly formed in root exudates.

2.3. *Overview of basic biogeochemical processes in soils and sediments*

2.3.1. *Adsorption*

The most important chemical processes affecting the mobility and bioavailability of metals are those concerned with the adsorption of metals from the liquid phase onto the solid phase in sediments (Tessier et al. 1996; Chapman et al. 1998; Jackson et al. 1999) and in soils (Alloway 1995; Bradl 2004). Sorption on mineral surfaces is an important process that can bind and sequester heavy metals and other aqueous contaminant ions (Brown et al. 1999; Tessier et al. 1996). This process controls the concentrations of metal ions and complexes in the soil/sediment solution and thus exert a major influence on their interactions with bacteria and uptake by plant roots (Jackson et al. 1999; Ledin 2000; Bourg & Loch 1995; Huang 2004). Adsorption refers to an ion associated with a surface (organic or mineral) either by (1) chemical interactions through the sharing of electrons (covalent or ionic bonding) or (2) electrostatic attraction involving an ion and surface of opposite charge. The energy of adsorption includes contributions from both electrostatic and chemical interactions (Stumm & Morgan 1996; Warren & Haack 2001).

Both solid properties and interstitial water composition determine the equilibrium of metals between solution and solid phases. The effectiveness of sorption reactions in binding an ion is determined by a number of variables, including (i) pH (Lee & Saunders 2003), (ii) the charge on the mineral surface as a function of pH (Bertsch & Seaman 1999), (iii) the type of sorption complex formed (Sposito et al. 1999), (iv) competition between different ions for the same types of

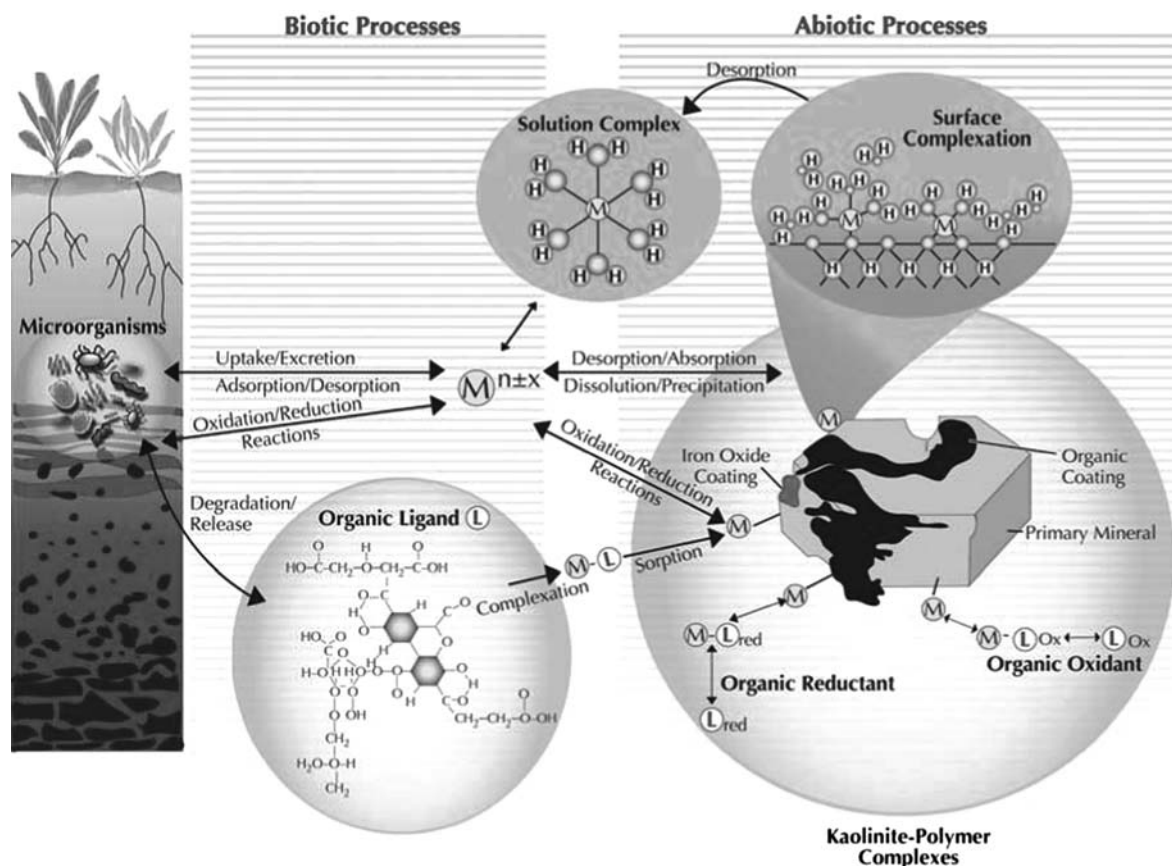


Figure 1. Schematic illustration of a variety of molecular environmental science processes affecting contaminant elements in soils (Reprinted with permission from Brown et al., copyright (1999) National Academy of Sciences, U.S.A.)

reactive surface sites (e.g., U(VI), Davis et al. 2004), (v) the presence of organic and/or inorganic ligands that can inhibit or enhance sorption of a metal ion (Tessier et al. 1996), and (vi) the presence of surface coatings such as biofilms that may block reactive sites and/or create new sorption sites (van Hullebusch et al. 2003; Templeton et al. 2003a, b).

The concentration of metals in solution is then largely influenced by the pH and the nature of both organic and inorganic anions. In soils and sediments, the effect of pH values >6 in lowering free metal cation activities is related to the increase in pH-dependant surface charges on oxides of Fe, Al and Mn, chelation by organic matter, or precipitation (Sauvé et al. 2000; Lee & Saunders 2003; NRC 2003; Adriano et al. 2004; Basta et al. 2005). Differences in adsorption and desorption isotherms typically show significant hysteresis and evidence that this process is not simply a

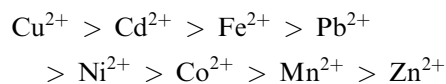
competitive ion exchange reaction between metal ions and protons or hydroxyls. Some adsorbed metal is strongly bonded and not readily desorbed from metal oxides surfaces (Gao et al. 2003; Basta et al. 2005). For instance, Qafoku et al. (2005) performed column experiments to investigate U(VI) desorption and sorption kinetics in a sand-textured, U(VI)-contaminated ($22.7 \text{ micromol kg}^{-1}$) capillary fringe sediment from the U.S. Department of Energy (DOE) Hanford site. Desorption was shown to be the slower process. Qafoku et al. (2005) suggested that U(VI) release and transport in the vadose zone and aquifer system from which the sediment was obtained are kinetically controlled.

2.3.2. Complexation

A series of complexation reactions in the soil solution affect metal transformation in the rhizosphere (Figure 1). Complexation reactions of

metals and radionuclides with ligands in the soil solution are significant in determining the chemical behaviour, availability, and toxicity of metals in the rhizosphere (Huang 2004).

Metals and radionuclides form both inorganic and organic complexes with a range of solutes. A number of studies have examined the effect of inorganic anionic complex formation on the adsorption of Cd^{2+} by soils. Weggler et al. (2004) showed that increasing chloride (Cl) concentrations in soil solution increase the cadmium (Cd) concentration in the soil solution and Cd uptake by plants. This suggests that soil-borne Cd is desorbed by chloride ligands in soil solution. Also, the microbial respiration causes the increase of the (bi)carbonate concentrations. Lately, the presence of carbonate has been shown to favour the reoxidation of bioreduced uranium under reducing conditions (Wan et al. 2005). These authors present evidence that bioreduced U(VI) is reoxidized under reducing conditions because carbonate accumulation promotes the formation of highly stable carbonate-U(VI) complexes under neutral to slightly alkaline conditions. This research outlined that one should be careful with the importance of the dosing of organic carbon (OC) into contaminated sediments or soil to stimulate microbial U(VI) reduction to U(IV) solids in order to minimize the carbonate microbial production. As one can also expect, the organic components of soil and sediments constituents has a high affinity for metal cations because of the presence of ligands or groups that chelate metals. With increasing pH, the carboxyl, phenolic, alcoholic and carbonyl functional groups in organic matter dissociate, thereby increasing the affinity of ligand ions for metal cations (Stumm & Morgan 1996; Warren & Haack 2001; Basta et al. 2005). The general order of affinity for metal cations complexed by organic is as follows (Adriano 2001):



In view of the occurrence of organic ligands in the rhizosphere due to root exudates and microbial metabolites and the stability constants of the complexes of metals with these ligands, a large fraction of the soluble metal ions in the soil solution may actually be complexed with a

series of organic ligands commonly present in the rhizosphere. For instance, Krishnamurti et al. (1997) found that soil Cd might be mobilized by low molecular weight organic acids such as acetic, citric, oxalic, fumaric and succinic acids that are commonly formed in root exudates. Moreover, bioremediation of heavy metal polluted soil by adding mulch has been shown to be a suitable method to treat a soil polluted by a copper–nickel smelter (Kiikkila et al. 2001). The concentration of complexed Cu was shown to increase and the free Cu^{2+} decreased in the soil solution following the mulch treatment. Hazen & Tabak (2005) give a detailed presentation of field study reporting metals immobilization with biosolids.

2.3.3. Precipitation

For many of the most abundant elements such as Al, Fe and Mn, precipitation of mineral forms is common and may control their solubility. Precipitation reactions result from a solution being oversaturated with respect to a solid phase. Solubility constants for precipitation in bulk solution are tabulated in many textbooks (e.g., Stumm & Morgan 1996). Moreover, precipitation appears to be the predominant process of metal immobilization in alkaline soils in the presence of anions such as sulfate, carbonate, hydroxide and phosphate, especially when the concentration of metal ions is high (Adriano et al. 2004). In reduced environments where the sulfide concentration is sufficiently high, precipitation of trace metals as sulfides may have a significant role in metal transformation (van den berg et al. 1998; Morse & Luther 1999).

A number of studies have examined the potential value of various soil amendments in immobilizing metals in soils, thereby reducing their bioavailability and mobility. The potential values of common soil amendments (phosphates, lime, biosolids and coal ash) relative to their ability to immobilize metals such as lead in soil or sediment are discussed by Traina & Laperche (1999), for attenuation of Cd and Zn availability in polluted soil amended with CaCO_3 and KH_2PO_4 (Hamon et al. 2002), for Pb, Cd, As and Hg immobilization with phosphates and lime (Porter et al. 2004). For the review of the implications of liming in relation to heavy metal

transformation and bioavailability in acidic soils see Bolan et al. (2003a) and Adriano et al. (2004).

The coprecipitation of contaminants such as uranium, cobalt and nickel with oxide minerals or other naturally occurring minerals such as the carbonates and silicates could influence the transport and the bioavailability (e.g., Duff et al. 2002; Zachara et al. 2001).

2.3.4. Importance of the redox potential on speciation and mobility

Redox reactions are important in controlling the chemical speciation of a number of contaminants metals, notably As, Se, Cr, Pu, Co, U, Pb, Ni and Cu (Alloway 1995). Considering the importance of the iron content in soils and sediments and the primary role played by iron oxides as sorbate of inorganic metal, the redox potential and microbial populations is expected to play a great role on the contaminants mobility. Many metal and radionuclide contaminants exist in the environment in multiple redox forms. The mobility of metallic contaminants depends strongly on the oxidation states as shown in Table 2. For instance Tc, Cr, U, and Pu exhibit multiple oxidation states, of which the reduced forms are quite insoluble in water. The oxidized forms Tc(VII) and Cr(VI) are both anions in water and generally sorb weakly to the negatively charged surfaces typically encountered in nature. If those metallic contaminants are reduced, their precipitation as oxide will be favoured (Table 2). For example, chromium is found as Cr(VI) (+6 oxidation state) under environmental conditions known as oxidizing conditions and as Cr(III) (+3 oxidation state) under reducing conditions.

The redox potential is related to the concentration of several redox pairs in the soil; the most important are given in Table 3. Oxygen is the first acceptor that plays a large role. Oxygen diffuses into the soil, but can also be produced by plants and leak into the soil by radial oxygen loss from roots (Jespersen et al. 1998; Aldridge & Ganf 2003). Simultaneously, other redox pairs can fluctuate in concentrations as well. Consequently, fluctuations in redox potential values measured in the soil can be very large (Mansfeldt 2003; Vorenhout et al. 2004). When studying redox behaviour *in situ*, the fluctuations can be of great interest to examine the metal availability and pollutant degradation.

Oxidizing conditions generally prevail in the absence of biodegradable organic matter and in near-surface environments. Reducing conditions generally prevail when an excess of biodegradable organic matter is present and the oxygen supply is limited. In anaerobic environments, microbial anaerobic respiration may promote mineral dissolution (Dassonville & Renault 2002). An example of this is the reductive dissolution of Fe(III) oxides, which liberates metalloids such as arsenic that are adsorbed to the oxides (Cummings et al. 1999; Bose and Sharma 2002). In contrast, such anaerobic conditions in sediments lead to sulfide production by microbially mediated reduction of sulfates in anoxic conditions, or as a direct product of organic matter decomposition (Neal et al. 2001). The major sulfide component found in most sediments is iron sulfide, due to the usually high abundance of iron oxyhydroxides, and their ease of reduction to Fe(II) in anoxic conditions. As amorphous iron sulfide (FeS) is the most soluble of the true metal sulfides, other metal cations can displace Fe to form a more stable

Table 2. Speciation of inorganic contaminants with multiple oxidation states (modified from NRC 2003)

Element	Oxidizing conditions	Reducing conditions
Tc	Tc(VII): TcO_4^- , high solubility, very weak adsorption	Tc(IV): $\text{TcO}_2 \cdot n\text{H}_2\text{O}_{(s)}$; low solubility
Cr	Cr(VI): CrO_4^{2-} , HCrO_4^- , $\text{Cr}_2\text{O}_7^{2-}$ depending on total Cr concentration and pH value; high solubility, weak adsorption	Cr(III): $\text{Cr}(\text{OH})_3_{(s)}$; low solubility
U	U(VI): UO_2^{2+} high solubility, moderate sorption; highly soluble, weakly sorbing anionic U(VI) carbonate complexes may predominate in waters with high carbonate concentrations	U(IV): $\text{UO}_2_{(s)}$; low solubility
Pu	Pu(VI), Pu(V), Pu(IV): Pu^{4+} , PuO_2^+ , PuO_2^+ complex, redox-active aqueous chemistry with moderate solubility and moderately sorbing species	Pu(IV): $\text{PuO}_2_{(s)}$, moderately low solubility

Table 3. The most important redox pairs and the approximate redox values at the occurrence of transitions at the reference pH of 7.0 (modified from Mitsch & Gosselink 1993; Dassonville & Renault 2002)

	Oxidized form	Reduced form	Approximate E _{hat} transformations, mV
Oxygen	O ₂	H ₂ O	+600 to +400
Nitrogen	NO ₃ ⁻	N ₂ O, N ₂ , NH ₄ ⁺	250
Manganese	Mn ⁴⁺	Mn ²⁺	225
Iron	Fe ³⁺	Fe ²⁺	+100 to -100
Sulfur	SO ₄ ²⁻	S ²⁻	-100 to -200
Carbon	CO ₂	CH ₄	Less than -200

sulfide in sediment (Chapman et al. 1998; Morse & Luther 1999) as well as in soils (Brennan & Lindsay 1996). Also in reduced conditions, As is mobilized as a result of reduction of Fe and Mn oxides and reduction of As(V) to As(III) (Cumings et al. 1999). However, in the rhizosphere in wetlands, As is immobilized because of the oxidation to As(V) and adsorption to FeOOH (Weiss et al. 2004). Therefore, As has been found to accumulate in the rhizosphere of many plants, but most of the As is likely to be retained on the root surface (Huang 2004; Liu et al. 2004). Table 4 summarizes the effect of redox condition on the mobility of trace metals in soils and sediments.

2.3.5. Transport and redox reactions

The fate of metal contaminants in soils and sediments is controlled by interdependent influences of transport and biogeochemical reactions (Tokunaga et al. 2001, 2003; Kaplan et al. 2004). Laboratory studies of biogeochemical processes are commonly conducted in well-mixed

suspensions and solution cultures (Fjeld et al. 2003; Kaplan et al. 2004). Limitations in applying results of laboratory studies to field environments often relate to the fact that the subsurface contains a broad spectrum of interconnected microenvironments, while individual laboratory batch systems represent specific isolated microenvironments. Soils are typically heterogeneous, composed of aggregates, and contain preferential flow paths, such that the early stages of a contamination event are usually in disequilibria with respect to transport and reactions (Hunter et al. 1998). In such structured soils, it is expected that contaminants rapidly move through a small subset of hydraulically active macropores and slowly diffuse into the adjacent soil aggregates. Such a scenario is the basis for applying two-region (mobile and immobile) models to describe solute transport, in which advection is limited to the mobile domain, and the immobile domain participates through diffusive mass transfer (Tokunaga et al. 2001). Individual soil aggregates can contain wide variations in chemical (Wilcke & Kaupenjohann 1998; Ilg et al. 2004) and microbiological (Tokunaga et al. 2003) composition, and sustain biogeochemical transformations through diffusion-controlled fluxes (Wang & Papenguth 2001).

Steep gradients in oxygen concentrations and redox potentials in soil aggregates that exhibit anaerobic interiors (Sierra & Renault 1995; Kremen et al. 2005) indicate that transformations experienced by redox-sensitive metal contaminants can occur within short distances (Figure 2). The distribution of metal contaminants such as chromium in soils or uranium in sediments can be strongly localized by transport limitations and

Table 4. Relative mobility and availability of trace metals (modified from Salomons 1995)

Metal species and association	Mobility
Exchangeable (dissolved) cations	<i>High.</i> Changes in major cationic composition (estuarine environment) may cause a release due to ion exchange
Metals associated with Fe–Mn oxides	<i>Medium.</i> Changes in redox conditions may cause a release but some metal precipitate is sulfide mineral present is insoluble
Metals associates with organic matter	<i>Medium/high.</i> With time, decomposition/oxidation of organic matter occurs
Metals associated with sulfide minerals	<i>Strongly dependant on environmental conditions.</i> Under oxygen-rich conditions, oxidation of sulfide minerals leads to release of metals
Metals fixed in crystalline phase	<i>Low.</i> Only available after weathering or decomposition

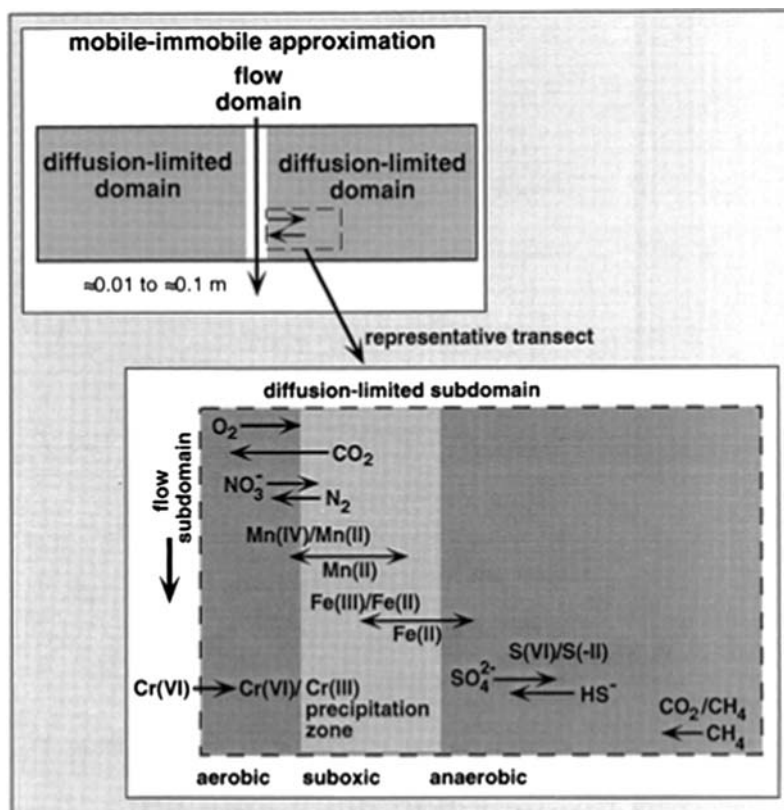


Figure 2. Conceptual model of flow and transport in structured subsurface environments. Most of the flow occurs within the advective domain, which is often a small fraction of the system volume. The remaining larger fraction of the subsurface exchanges chemical species primarily through diffusion. Microbial activity can cause large gradients in redox potentials within these diffusion limited domains, spatial stratification of redox processes, and localized precipitation of redox-sensitive contaminants (reprinted with permission from Tokunaga et al. 2003, Copyright © 2003, Journal of Environmental Quality by American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America).

redox gradients within soil aggregates (Tokunaga et al. 2001, 2005). Chromium contamination within soil aggregates can be strongly diffusion-limited, resulting in reduction to Cr(III) within short distances. In large diffusion-limited domains, the Cr-contamination can be restricted to outer regions in contact with preferential flow paths, leaving the deeper core region unaffected (Figure 2). Such aggregates contain microbial communities that have or have not been exposed to Cr(VI) residing within outer and core regions, respectively. Measurements of Cr(VI) diffusion and reduction to Cr(III) were obtained in soil columns representing transects into soil aggregates in order to quantify influences of OC and redox potentials on Cr transport distances and microbial community composition (Tokunaga et al. 2001). Outer surfaces of soil aggregates tend

to sustain oxidizing conditions favouring stability of Cr(VI), whereas interior regions are more reducing, promoting conversion to Cr(III). The level of microbial activity within aggregates is critical in controlling the extent of Cr(VI) reduction, whether directly through enzymatic pathways or through indirect influences involving control of intraaggregate redox potentials and resulting concentrations of abiotic reductants (Fe(II), S(-II), and OC). In general, the intra-aggregate domain may not be homogeneous with respect to microbial communities, redox potentials, and reactivity to contaminants.

These works showed the importance of intra-aggregate spatial relations for redox-sensitive contaminants as well as for the microbial communities responsible for redox gradients and reductants. By extension, similar stratification of

redox potentials, metal contaminants, and microbial communities might occur within larger sediment blocks, deeper in the subsurface (e.g., chromium in soil aggregates, Tokunaga et al. 2003).

3. Abiotic and biotic processes affecting the speciation metals and radionuclides and influencing their toxicity and transport

3.1. Role of abiotic processes affecting metals and radionuclides oxidation state

3.1.1. Role of iron and manganese speciation

Although some microorganisms can enzymatically reduce heavy metals and radionuclides directly (U(VI), Cr(VI), Mn(VI), Se(VI), Co(III) and Tc(VII)) (Kashefi & Lovely 2000), indirect reduction of soluble contaminants may be more feasible in natural sedimentary and subsurface environments. This indirect immobilization could be accomplished by metal-reducing and sulfate-reducing bacteria. This can be achieved by coupling the oxidation of organic compounds or hydrogen to the reduction of ferric iron Fe(III), Mn(IV), or sulfate (SO_4^{2-}). Iron(III) is reduced to iron(II), manganese(IV) to manganese (II), SO_4^{2-} to hydrogen sulfide (H_2S) (Lloyd et al. 1998).

However, Fe(III) and Mn(III/IV) oxides can retard the microbial reduction of contaminants via competition as a terminal electron acceptor (Wielinga et al. 2000) or by oxidizing biologically reduced metals or radionuclides (Fredrickson et al. 2002; Liu et al. 2002). Alternatively, the presence of crystalline Fe(III) oxides may have relatively little impact on the rate and extent of contaminant reduction by metal-reducing bacteria (Fredrickson et al. 2000; Wielinga et al. 2000) and may even facilitate reduction by forming reactive surface complexes with Fe(II) (Liger et al. 1999).

Whether metal oxides retard, promote, or have no impact on the reduction of contaminants largely depends on their mineralogy and free energy, surface area and surface properties, and physical distribution within sediments and soils (Burke et al. 2005).

3.1.1.1. *Manganese oxide.* Manganese (III/IV) oxides are common secondary phases in soils and sediments and electron acceptors for metal-

reducing bacteria. Manganese oxides are also relatively strong oxidants and can oxidize insoluble, reduced contaminants such as the mineral uraninite (UO_2)_(s), a common product of microbial uranium reduction (Liu et al. 2002). Fredrickson et al. (2002) studied the potential for Mn oxides to modify the biogeochemical behaviour of U during reduction by the subsurface bacterium *Shewanella putrefaciens* strain CN32 using synthetic Mn(III/IV) oxides (pyrolusite [$\beta\text{-MnO}_2$], bixbyite [Mn_2O_3] and K^+ -birnessite [$\text{K}_4\text{Mn}_{14}\text{O}_{27} \cdot 8\text{H}_2\text{O}$]). In the absence of bacteria, pyrolusite and bixbyite oxidized biogenic uraninite (UO_2 [s]) to soluble U(VI) species, with bixbyite being the most rapid oxidant. The Mn(III/IV) oxides lowered the bioreduction rate of U(VI) relative to rates in their absence. Differences in the solubility of oxidized Mn (insoluble) and U (soluble) challenge bacterium predictions of their biogeochemical behaviour during *in situ* bioreduction. Uraninite that precipitated as a result of microbial U(VI) reduction (Roden & Scheibe 2005) can be abiotically reoxidized to U(VI) by $\beta\text{-MnO}_{2(s)}$, with concomitant reduction to Mn(II) (Fredrickson et al. 2002; Liu et al. 2002). The oxidation of biogenic $\text{UO}_{2(s)}$ coupled with $\beta\text{-MnO}_{2(s)}$ reduction is well-described by an electrochemical model (Liu et al. 2002). The work of Liu et al. (2002) indicate that the presence of Mn(III/IV) oxides may impede the biological reduction of U(VI) in subsoils and sediments. However, the accumulation of U(IV) in the cell periplasm may physically protect reduced U from oxidation, promoting at least a temporal state of redox disequilibrium (Fredrickson et al. 2002).

Numerous studies have demonstrated a reduction of toxic Cr(VI) to non-toxic Cr(III) by various types of bacteria under both aerobic and anaerobic conditions (Guha et al. 2001b). In all these studies Cr(VI) was the electron acceptor. Experiments involving reduction of contaminant by bacteria in the presence of solid phase minerals are extremely rare (Guha et al. 2003; Guha 2004). Any successful prediction of microbially mediated metal contaminant containment in the field requires a thorough understanding of the geochemical processes that affect microbial reduction. Chromium is one such redox-sensitive contaminant whose transport in the subsurface depends on both geochemical and microbiological processes. Abiotic laboratory studies have

shown that in the presence of Fe(II)-bearing minerals and organic acids (acetic acids and mandelic acids), Cr(VI) is reduced to Cr(III) in the subsurface (Eary & Rai 1988). Similarly, Mn-oxide containing minerals have been shown to oxidize Cr(III) to Cr(VI) (Stepniewska et al. 2004; Guha 2004) influencing greatly the transport of this contaminant. Guha (2004) studied the chromium transport in water-saturated conditions with columns that appear to be homogeneously packed with either β -MnO₂-coated sand, or quartz sand in the presence of a facultative anaerobe *Shewanella alga* Simidu (BrY-MT) ATCC 55627. The results of this work indicate that Cr(VI) reduction occurs earlier in columns packed with quartz sand than in columns packed with β -MnO₂-coated sand. Experiments performed with columns packed with β -MnO₂-coated sand showed very little reduction of Cr(VI) within a time span of 57 h. The slow reduction rate in the presence of β -MnO₂ was attributed to a more rapid rate of Cr(III) oxidation by the manganese oxide relative to microbiological reduction. However, when the biomass content was high enough, the biological reduction of chromium was predominant. Pansar-Kallio et al. (2001) showed that the reactions between different soil components such as Fe(OH)₃, MnO₂, CaCO₃, kaolinite and natural organic matter (NOM) affect the oxidation/reduction ability of soils. As separate component MnO₂ oxidized Cr(III) to Cr(VI). However, in mixtures with NOM or Fe(OH)₃ the oxidation is hindered.

3.1.1.2. *Iron (III) oxides*. Fe(III) oxides are often in equal or greater abundance in soils and sediments than Mn(III/IV) oxides and may also affect the fate of metals and radionuclides during bioreduction. For instance, the presence of ferrihydrite, probably because of its poorly crystalline nature and relative availability as an electron acceptor, inhibits bacterial U reduction (Wielinga et al. 2000). However, the crystalline goethite has little effect (Fredrickson et al. 2000; Wielinga et al. 2000). The fact that Fe(III) and Mn(III/IV) oxides commonly occur in direct association even further complicates the prediction of in situ bioreduction processes. In addition to U(VI), Mn(III/IV) oxides can also oxidize Fe(II). The presence of Mn(III/IV) oxides has been shown to impede the formation of Fe(II) by

dissimilatory metal reducing bacteria (DMRB) (Myers & Nealson 1988), probably via the rapid oxidation of biogenic Fe(II) (Myers & Nealson 1988). Although the extent to which Mn(III/IV) and Fe(III) oxides may jointly affect the in situ microbial reduction of U(VI) is unknown, it is clear that metal reduction and mineral precipitation processes at the cellular level as described by Fredrickson et al. (2002) and Liu et al. (2002) can have an important role.

3.1.1.3. *Green rust*. Green rust (Fe₄(II)Fe₂(III)(OH)₁₂SO₄ · 3H₂O) is an intermediate phase in the formation of iron (oxyhydr)oxides such as goethite, lepidocrocite and magnetite; current thinking is that it occurs in many soil and sediment systems (Schwertmann & Fechter 1994). In addition, green rusts are a product of microbial degradation by dissimilatory iron reducing bacteria (Fredrickson et al. 1998). Due to their highly reactive surfaces and reduction potentials, green rusts may have strong influences on the fate of environmental contaminants such as selenium (Myneni et al. 1997; Johnson & Bullen 2003), chromium (Bond & Fendorf 2003; Lee & Batchelor 2003), uranium (O'Loughlin et al. 2003) and technetium (Pepper et al. 2003). In contrast, As(V) has shown not to be reduced in the presence of green rust (Randall et al. 2001).

Green rust is known to reduce selenate to Se(0), and it is the only naturally relevant abiotic selenate reduction pathway documented to date. Se reduction reactions, when they occur in nature, greatly reduce Se mobility and bioavailability. Myneni et al. (1997) showed that selenium reduced from an oxidation state of +VI to 0 in the presence of green rust at rates comparable with those found in sediments. However, it is quite difficult to quantify the contribution of such abiotic processes to the overall *in situ* selenium reduction that has been also shown to be biotically mediated (Oremland et al. 1998).

3.1.1.4. *Biogenic Fe(II)*. Some DMRB can reduce solid phase Fe(III) oxides and oxyhydroxides including poorly crystalline phases such as ferrihydrite (Lloyd et al. 2000) and crystalline phases such as goethite, hematite, and magnetite (Fredrickson et al. 2000; Behrends & van Cappellen 2005). The roles of biogenic Fe(II) in the reductive immobilization and potential use

for the *in situ* immobilization of redox-sensitive contaminants such as technetium (Fredrikson et al. 2004; Wildung et al. 2004), selenium (Zingaro et al. 1997), plutonium (Powell et al. 2004) and uranium (Fredrikson et al. 2000; Behrends & van Cappellen 2005) has been lately studied.

Technetium is a significant radioactive contaminant from nuclear fuel cycle operations. It is highly mobile in its oxic form (as Tc(VII)O_4^-) but is scavenged to sediments in its reduced forms (predominantly Tc(IV)). Wildung et al. (2004) studied the extent of pertechnetate ion [Tc(VII)O_4^-] reduction in sediments. The dominant electron donor in the sediments proved to be Fe(II) . Sediment $\text{Fe(II)}/\text{Tc(VII)}$ concentrations >4.3 were generally sufficient for complete reduction of Tc(VII) added [1-2.5 μmol (dry wt. sediment) g^{-1}]. At these Fe(II) concentrations, the Tc(VII) reduction rate exceeded that observed previously for Fe(II) -mediated reduction on isolated solids of geologic or biogenic origin, suggesting that sediment Fe(II) was either more reactive and/or that electron shuttles played a role in sediment Tc(VII) reduction processes. Nanocrystalline magnetite, as a product of microbially reduced ferrihydrite, has also been shown to reduce Tc(VII) to an insoluble form, and it has been suggested that the indirect reduction of Tc(VII)O_4^- via biogenic Fe(II) may be an environmentally important pathway (Lloyd et al. 2000). Fredrikson et al. (2004) investigated the biologic reduction of Fe(III) and Mn(III/IV) oxides in natural sediments as well. They determined the reactivity of these bioreduced sediments with regard to Tc(VII) reduction. The potential for reduction of $^{99}\text{TcO}_4^-$ to poorly soluble $^{99}\text{TcO}_2 \cdot n\text{H}_2\text{O}_{(s)}$ by biogenic sediment-associated Fe(II) was investigated with three Fe(III) -oxide containing subsurface materials and the dissimilatory metal-reducing subsurface bacterium *Shewanella putrefaciens* CN32. Subsurface materials that were bioreduced to varying degrees and then pasteurized to eliminate biological activity, reduced TcO_4^- at rates that generally increased with increasing concentrations of 0.5 N HCl extractable Fe(II) . These results suggest that Tc(VII) reduction and precipitation by biogenic Fe(II) is particularly efficient, and may offer a potentially useful mechanism for the remediation of Tc -contaminated sediments containing active concentrations of Fe(III) -reducing bacteria.

The reduction of U(VI) under iron reducing conditions was studied by Behrends & van Cappellen (2005) in a model system containing the dissimilatory metal-reducing bacterium *Shewanella putrefaciens* and colloidal hematite. They focused on the competition between direct enzymatic uranium reduction and abiotic reduction of U(VI) by Fe(II) , catalyzed by the hematite surface, at relatively low U(VI) concentrations ($<0.5 \mu\text{M}$) compared to the concentrations of ferric iron ($>10 \text{mM}$). Under these conditions surface catalyzed reduction by Fe(II) , which was produced by dissimilatory iron reduction, was the dominant pathway for uranium reduction. Reduction kinetics of U(VI) were identical to those in abiotic controls to which soluble Fe(II) was added. Strong adsorption of U(VI) at the hematite surface apparently favoured the abiotic pathway by reducing the availability of U(VI) to the bacteria. In control experiments, lacking either hematite or bacteria, the addition of 45 mM dissolved bicarbonate markedly slowed down U(VI) reduction. Biogenic magnetite produced as a result of dissimilatory iron reduction may be an important agent of uranium immobilization in natural environments. Fe(II) is shown to exhibit thermodynamic potential to function as abiotic reductant of U (Fredrikson et al. 2000). The reduced form then chemically interacts with the contaminants and forms separate or multicomponent insoluble species. Liger et al. (1999) recently reported the reduction of U(VI) by the Fe(II) complexes, $\equiv\text{FeOFe}^+$ and $\equiv\text{FeOFeOH}^\circ$, on the surface of hematite. The hydroxo complex $\equiv\text{FeOFeOH}^\circ$ was the rate determining species and the initial rate of U(VI) reduction exhibited a first order behaviour with respect to the sorbed uranyl concentration. Fredrikson et al. (2000) suggested that sorbed Fe(II) on goethite is a powerful and kinetically facile reductant as well.

3.1.1.5. *Iron sulfide.* The formation of iron sulfide minerals takes place in recent sediments via sulfate-reducing bacteria (Neal et al. 2001; Lin et al. 2002). The biologic process is the result of the oxidation of organic matter that occurs through bacterial reduction of sulfate, resulting in the production of hydrogen sulfide. Hydrogen sulfide then reacts with iron species from detritus or other sources to form an amorphous

precipitate, which within days crystallises to the more stable mackinawite (FeS). A direct precipitation mechanism between iron and sulfide species is also encountered in hydrothermal sulfide deposits associated with volcanic activity. Mackinawite is the first crystalline iron sulfide phase that is formed in aqueous systems (Wolthers et al. 2005) and is a precursor in sedimentary pyrite (FeS₂) formation (Wang & Morse 1996). Recent studies have demonstrated that mackinawite is an important natural reductant in soils and sediments for organic and inorganic contaminants (Moyes et al. 2000; Moyes et al. 2002; Lee & Batchelor 2003; Arias and Tabo 2003; Livens et al. 2004; Bruggeman et al. 2005).

Selenium (Se) in the environment exists in four different oxidation states (-II, 0, IV, and VI) and a variety of organic compounds. The speciation of Se is largely dependent on the oxidation-reduction potential and pH of soil and sediment (Masscheleyn et al. 1990; Dhillon & Dhillon 2003). In oxidized conditions, selenate (SeO₄²⁻) and selenite (SeO₃²⁻), are the two predominant forms (oxyanions) of Se. These species are soluble in water and possess toxic effects to living cells at low concentrations (Wu 2004). In reduced conditions, elemental Se [Se(0)] has been commonly considered as unavailable form of Se because of its insolubility. The most reduced form of selenium, selenide (Se(-II)), can exist as insoluble sulfide and selenide minerals (e.g., ferroselite, FeSe₂), substituted for S in pyrite (Belzille et al. 2000). Bruggeman et al. (2005) studied the Se speciation and solubility in reducing Boom clay geochemical environments. Upon introduction of SeO₃²⁻ to FeS₂-containing samples, adsorption of SeO₃²⁻ occurred at the FeS₂ surface, and led to a reduction and precipitation of a Se⁰ solid phase (after 60 days). Upon introduction of SeO₃²⁻ to Boom clay suspensions (equilibration up to 9 months), the competing adsorption process onto clays minerals (illite), and the presence of humic substances, decreased the kinetics of reduction with respect to FeS₂ samples. Bruggeman et al. (2005) demonstrated that selenite reduction by FeS₂ is kinetically controlled, with clay minerals and organic matter playing an important role.

3.1.2. Role of natural organic matter

NOM consists of a mixture of organic compounds with different structures and functional

groups. These groups include aromatic and phenolic moieties, carboxylic and heteroaliphatic hydroxyl functional groups, and free radicals (Dudal & Gérard 2004). NOM contain redox-active functional groups, such as quinones, and have the ability to form NOM-metal complexes (Haack & Warren 2003). NOM, or humic substances, are known to be redox reactive with elements in soil (Lovley et al. 1998; Struyk and Sposito 2001) and therefore capable of reducing redox-sensitive metals such as Cr(VI) (Wittbrodt & Palmer 1995; Nakayasu et al. 1999; Gu & Chen 2003; Zhilin et al. 2004; Banks et al. 2005), Mn(IV) (Sunda & Kieber 1994), V(V) (Lu et al. 1998), As(V) (Redman et al. 2002), U(VI) (Gu & Chen 2003; Gu et al. 2005) and Hg(II) (Alberts et al. 1974; Matthiessen 1996).

An important implication of these studies is the effect of NOM on the potential transport of the metals by either increasing or decreasing their redox states and solubility and thereby causing their mobilization or immobilization in the subsurface soil (Lovley et al. 1998; Fredrickson et al. 2000; Wildung et al. 2000). Because of the much smaller size of humic molecules as compared to the size of bacteria, humic substances could allow access to locations from which bacteria are excluded due to size or nutrient limitations and therefore transfer the microbial reducing power to contaminants at such isolated locations. However, because of the complexity of NOM, the reaction mechanisms and functional groups that participate in metal reduction or electron-transfer reactions are not well known.

NOM was found to greatly enhance the reduction of a key element in soil and sediment such as Fe(III) metals or Fe(III) oxides by a variety of microorganisms (Lovley 1996; Lovley et al. 1998; Nevin & Lovley 2000; Royer et al. 2002; Chen et al. 2003; Kappler et al. 2004). Lovley (1996) postulated that humic substances were likely acting as electron mediators or shuttles between microorganisms and Fe(III) or Fe(III)-oxide minerals. They found that certain anaerobic microorganisms could reduce NOM (as an electron acceptor), which then donates electrons to reduce Fe(III) or Fe(III)-containing minerals to release soluble Fe²⁺. Iron-reducing microorganisms such as *Shewanella putrefaciens*, *G. metallireducens*, *Shewanella alga*, and a variety of fermenting bacteria have all been shown to

use humic substances as terminal electron acceptors. By incubating NOM with *S. putrefaciens*, the equivalent Fe(III)-reducing capacity of NOM was reported to range from 0.1 to 0.6 mol/kg (Royer et al. 2002; Chen et al. 2003). In addition, Chen et al. (2003) showed that NOM was able to reduce Fe(III) abiotically. The reduction was pH dependant and varied greatly with different fractions of NOM. The polyphenolic-rich NOM fraction exhibited the highest reactivity and oxidation capacity at a low pH (<4) as compared with the carbohydrate-rich NOM fraction and a soil humic acid in reducing Fe(III).

Under oxic conditions, chromium and uranium are commonly present as CrO_4^{2-} and $\text{UO}_2(\text{CO}_3)_2^{2-}$ oxyanions (with CO_2 or carbonates) in the environment. These oxidized forms of Cr(VI) and U(VI) are soluble and highly mobile in soil because they are poorly sorbed by soil minerals carrying a negative surface charge. On the other hand, the reduced forms of Cr(III) and U(IV) are only sparingly soluble and are strongly retained by soil and sediments (Fendorf 1995; Anderson and Lovley 2002; Rifkin et al. 2004). While many studies to date have focused on direct microbial reduction of Cr(VI) and U(VI) (Lovley et al. 1991; Shen et al. 1996; Chen and Hao, 1998; Abdelouas et al. 2000; Fredrickson et al. 2000), few studies have examined the effect of NOM on the enhanced microbial reduction of Cr(VI) or U(VI), as has been observed for the reduction of Fe(III) or Fe(III)-oxide minerals (Lovley 1996; Royer et al. 2002; Chen et al. 2003). Therefore, of particular interest is the possibility that NOM-mediated reduction of Cr(VI) and U(VI) may lead to more rapid immobilization of these metals in soil and thus the remediation of a contaminated site (Gu & Chen 2003; Zhilin et al. 2004; Banks et al. 2005). Gu & Chen (2003) showed that the chemical and structural properties of NOM components may play different roles in enhancing the bioreduction of Cr(VI) and U(VI) by microorganisms. For instance polycondensed aromatic humic materials may be particularly useful in mediating the bioreduction and rapid immobilization of these contaminant metals in soil. Zhilin et al. (2004) also demonstrated that the origin of humic substances may greatly influence the kinetic of Cr(VI) abiotic reduction. For instance,

the capacity of leonardite humic substances to reduce Cr(VI) was lower than that of peat humic substances. The presence of Fe(III) accelerated the reduction of Cr(VI) by peat humic substances. The different mechanisms of Cr(VI) reduction on account of the irreversible oxidation of carbohydrate units, whereas leonardite humic substances reduce Cr(VI) owing to reversible transformations of quinoic-hydroquinoic units. The leonardite humic substance were concluded to be advantageous for an application of Cr(VI) contaminated sites due to their long-lasting capabilities for reducing Cr(VI).

The presence of natural humics was found to be beneficial for the enhancement of the bioreduction of U(VI) in the presence of metal ions such as Ca^{2+} and Ni^{2+} , which are known to inhibit the biological reduction of U(VI). Laboratory experiments showed that under strict anaerobic conditions, the presence of humic materials enhanced the U(VI) reduction rates (up to 10 fold) and alleviated the toxicity effect of Ni^{2+} on microorganisms. Humic acid was found to be more effective than fulvic acid in enhancing the reduction of U(VI). Such an enhancement effect is attributed to the ability of these humics in facilitating electron-transfer reactions and/or in complexing Ca^{2+} and Ni^{2+} ions (Gu et al. 2005). Gu et al. (2005) showed that humics were also found to form complexes with reduced U(IV) and increased the oxidation of U(IV) when exposed to oxygen.

3.2. Role of biotic processes affecting metals and radionuclides mobility

In addition to physico-chemical metal processes into the solid phase, microorganisms can also contribute to the immobilization or mobilization of metals and radionuclides. Microorganisms have a high surface area-to-volume ratio because of their small size and therefore provide a large contact area that can interact with metals in the surrounding environment. Microbe-metal interactions have received much attention in the last years due to the potential use of microorganisms for cleaning metal-polluted environments (Gadd 1993, 1996, 1999, 2000, 2002, 2004; Gadd & White 1993; White et al. 1997, 1998; Lloyd & Macaskie 2000; Lloyd & Lovley 2001; McLean

et al. 2002; Tabak et al. 2005). The ability of microorganisms to affect metal speciation relates to their ability to effect and/or mediate mobilization or immobilization processes that influence the balance of metal species between soluble and insoluble phases (Wiatrowski & Barkay 2005). Mobilization of metals can be achieved by, e.g., protonation, chelation, and chemical transformation while immobilization can occur by precipitation or crystallization of insoluble organic or inorganic compounds or by sorption, uptake and intracellular sequestration. Redox reactions can mobilize or immobilize metals depending on the metal species involved. As well as being an integral component of biogeochemical cycles for metals, these processes may be exploited in bioremediation of soils and sediments polluted by metals and radionuclides (Gadd 1993, 1996, 1999, 2000, 2002, 2004; Gadd & White 1993; White et al. 1997, 1998; Lloyd & Macaskie 2000; Lloyd & Lovley 2001; McLean et al. 2002; Tabak et al. 2005). Almost all metal-microbe interactions have been examined as a means for removal, recovery or detoxification of inorganic and organic metal or radionuclide pollutants (Lovley & Coates 1997; Stephen & MacNaughton 1999; Eccles 1999). In such a context, solubilization may enable removal from solid matrices, such as soils and sediments.

The main mechanisms by which microorganisms and plants affect changes in the speciation and mobility of metals and radionuclides are listed Table 5, including some examples (Gadd et al. 2001). These mechanisms have been reviewed in details in the literature (e.g., Lloyd 2003). Microbial biotransformation of metals or metalloids may alter their oxidation state or lead to their alkylation and subsequent precipitation or volatilization. Metabolic pathways leading to precipitation of heavy metals as metal sulfides, phosphates or carbonates place microorganisms in the forefront for possible biotechnological application. They sequester metals from soils and sediments or solubilize them to aid their extraction. Moreover, the possibility of altering the microbes used in heavy metal remediation and constructing chimeric organisms possessing desirable features using genetic engineering is now under study (Valls & de Lorenzo 2002). These processes can be summarized in a scheme as depicted in Figure 3 (modified from Toes et al. 2004).

4. Influence of metals and radionuclides bioavailability on inorganic contaminants bioremediation

Bioavailability of metals and radionuclides has emerged as an important paradigm in ecological as well as human health risk assessment (National Research Council, NRC 2003 and Adriano 2001), replacing an old belief that biological response by receptor organisms could be predicted by the total concentrations of the chemicals of concern. However, the term bioavailability can mean differently to different disciplines. A rather generic definition of bioavailability offered by the National Research Council (2003) states that "bioavailability processes" describe the complex mass transfer and uptake processes of contaminants into soil or sediment organisms conditioned by substance properties, soil or sediment characteristics and the biology of organisms (microorganisms or plants in the frame of bioremediation technology) under varying environmental conditions. Thus in both soil and sediment, processes that determine exposure to contamination include (A) release of a solid-bound contaminant and (B) subsequent transport, (C) transport of bound contaminants (e.g., contaminants bound to colloids, Cantwell & Burgess 2001; Vignati et al. 2005), (D) uptake across a physiological membrane (Simkiss & Taylor 1995), and (E) incorporation into a living system. The NRC report defines A, B, C, and D to be bioavailability processes, but not E, because soil and sediment no longer play a role (NRC 2003; Ehlers & Luthy 2003).

However, the definition of bioavailability and the concepts on which it is based are still unclear, the methods adopted for its measurement vary and as such there is no single standard technique for the assessment of either plant availability of contaminants or their ecotoxicological impacts on soil biota (Peijnenburg & Jager 2003). "Improving risk assessment and remediation rests on better understanding bioavailability" quoted from Ehlers & Luthy (2003). To do so, more consistent knowledge regarding the metal speciation are required as reviewed by Nolan et al. (2003). The determination of species concentration or distribution of trace elements in soils/sediments is more challenging than determination of total

Table 5. Main mechanisms by which microorganisms and plants affect changes in the speciation and mobility of metals and radionuclides

<i>Enzymatic detoxification mechanisms</i>	
Oxidation of AsO_2^- to AsO_4^{3-}	Bruneel et al. (2003), Macur et al. (2004)
Reduction of CrO_4^{2-} to Cr^{3+}	Kamaludeen et al. (2003)
Reduction of Hg^{2+} to Hg^0 and methylation	Wagner-Döbler et al. (2000), Wagner-Döbler et al. (2003), Barkay & Wagner-Dobler (2005)
<i>Utilization as electron donor or acceptor</i>	
<i>Oxidation by chemolithotrophs</i>	
Fe^{2+} to Fe^{3+}	Lack et al. (2002)
AsO_2^- to AsO_4^{3-}	Culen & Reimer (1989)
<i>Reduction by heterotrophs or autotrophs</i>	
Fe^{3+} to Fe^{2+} , Fe_3O_4 , FeCO_3	Nealson et al. (2002)
MnO_2 to Mn^{2+} , MnCO_3	Nealson et al. (2002)
SeO_4^{2-} and SeO_3^{2-} to Se^0 or Se^{2-}	Herbel et al. (2000, 2003)
MoO_4^{2-} to a lower oxidation state	Tucker et al. (1997)
TcO_4^- to $\text{TcO}_2 \cdot n\text{H}_2\text{O}$	Wildung et al. (2000, 2004)
CrO_4^{2-} to Cr^{3+}	Nealson et al. (2002), Cheung & Gu (2003)
<i>Non-enzymatic processes</i>	
<i>Binding or accumulation of metal cations to the cell surface</i>	
U(VI) sorption by <i>Shewanella putrefaciens</i>	Beveridge (1989), Huang et al. (2005)
U(VI) sorption by <i>Pseudomonas fluorescens</i>	Haas et al. (2001)
Promotion of leaching by metabolite products (acids or ligands)	Bencheikh-Latmani & Leckie (2003)
Sulfuric acid produced by sulfur oxidizing bacteria	White et al. (1998)
Siderophores produced by <i>R. metallidurans</i> CH34 which can solubilize metals in soil	Diels et al. (1999)
Bacterial extracellular polymer that mobilized adsorbed copper and lead from naturally aged soil	Jensen-Spaulding et al. (2004)
Microbial siderophores (desferrioxamine-B) that promote dissolution of UO_2 under reducing conditions	Frazier et al. (2005)
Plant roots exudates	Krishnamurti et al. (1997), White et al. (1997), Wenzel et al. (2003)
Precipitation of metals by metabolic products as sulfide, carbonate or phosphate ions:	For review see Ehrlich (1999)
CdS	Sharma et al. (2000)
Sr^{2+} , UO_2^{2+} or Co^{2+} coprecipitation with calcium carbonate	Fugita et al. (2000)
CoS precipitation in subsurface sediment	Krumholz et al. (2003)

elemental content due to (i) difficulty associated with isolating the compounds of interest from complex matrixes, such as soils/sediments; (ii) most of the currently available speciation techniques disturb the equilibria existing between the various chemical species present in a system; (iii) for those species which are present at ultra-trace levels, few analytical procedures allow the degree of sensitivity required; and (iv) suitable standard references materials are often not available to achieve analytical quality assurance.

In the forthcoming subsections the findings of recent studies regarding (i) the effect of metal bioavailability on toxicity toward micro-organ-

isms involved in bioremediation of inorganic pollutants, (ii) the effect of inorganic pollutant bioavailability on bacterial redox radionuclide biotransformation and (iii) the influence of inorganic pollutants bioavailability on plants and consequences on phytoremediation will be shortly presented.

4.1. Bioavailability and toxicity toward micro-organisms

Target pollutants rarely occur as sole contaminants *in situ*; rather they exist as components of complex mixtures of organic and inorganic

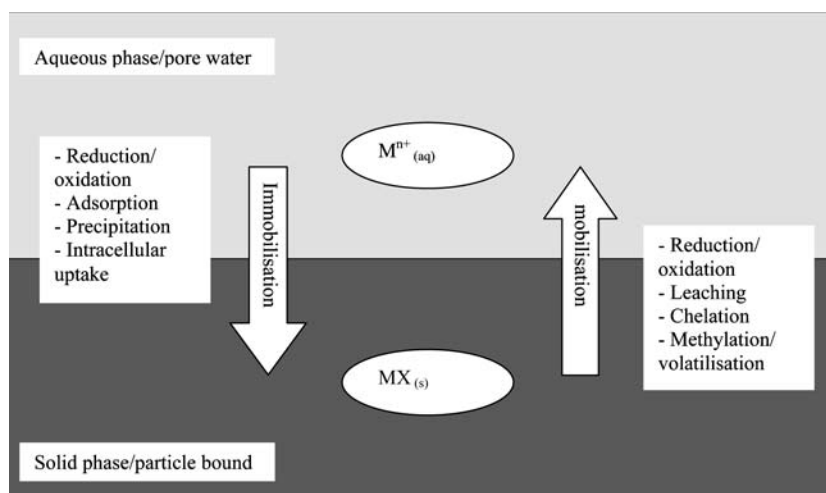


Figure 3. Schematic presentation of microbial influences on processes leading to either mobilization or immobilization of heavy metals in polluted soil or sediment (modified from Toes et al. 2004).

compounds. The presence of heavy metals, which are not destroyed biologically but are only transformed from one oxidation state to another, interfere with the bioremediation processes.

The bioavailability of metals and radionuclides and associated toxicity to soil biota vary with time, soil type, speciation, ageing, contaminant source, organisms and the environmental factors (Lock & Janssen 2003a,b; NRC 2003; Ruggiero et al. 2005). For example, Lock et al. (2000) found that the toxicity of Zn and Cd to the earthworm (*Enchyraeus albidus*) varied by two orders of magnitude for a range of different soils. The principal influences affecting bioavailability were pH and cation-exchange capacity. Moreover, due to long-term processes, called ageing, soil metal (bio)availability decreases with time. Lock & Janssen (2003a, b) showed that pH is the most important parameter determining the effect of ageing on zinc partitioning in soils, with the effect of ageing becoming more important with increasing pH. Plette et al. (1999) examined copper binding to maize root cells and to fungal and yeast cells in a sandy soil, as well as cadmium binding to bacteria in a clay and a sandy soil. They found that although pH was the most important factor, calcium concentration (as a competitor for the same adsorption sites as the metal ions) was also important. An additional problem arises with combination of metals. For example, van Gestel & Hensbergen (1997) found

that the water solubility of Cd was substantially increased by the presence of Zn, whereas Cd did not affect the water solubility of Zn.

Bacteria may be beneficial for alleviating actinides (short life radionuclides) contaminants migration through processes such as bioaccumulation or metal reduction. However, sites with radioactive contamination often contain multiple additional contaminants, including metals and organic chelators. Bacteria-based bioremediation requires that micro-organism functions in the presence of the target contaminant, as well as other contaminants. Ruggiero et al. (2005) evaluated the toxicity of actinides, metals and chelators to two different bacteria proposed for use in radionuclide bioremediation, *Deinococcus radiodurans* and *Pseudomonas putida*, and the toxicity of Pu(VI) to *Schewanella putrefaciens*. Ruggiero et al. (2005) showed that Pu is less toxic than U and that actinides are less toxic than other types of metals (Cd, Ni, Cr, Co, Pb, Zn), which suggest that actinide toxicity will not impede bioremediation using naturally occurring bacteria such as *Pseudomonas putida*, *Deinococcus radiodurans* and *Shewanella putrefaciens*. However the toxicity data were given on the total metal content and these authors did not perform any speciation analysis of the metal present in the growth medium, which do not allow to draw conclusion regarding the bioavailability of the metals. Given the potential environmental and financial

benefits of bioremediation, few studies on radionuclides and metal toxicity to microorganisms have been reported (Ruggiero et al. 2005).

4.2. Bioavailability and bacterial redox biotransformation

Dissimilatory microbial reduction of soluble U(VI) to relatively insoluble U(IV) has been proposed as a potentially viable mechanism by which U contamination in natural waters may be ameliorated via precipitative sequestration. Most studies of microbial U(VI) reduction have focused on dissolved U(VI) species, but in anoxic sedimentary environments, U(VI) species adsorbed onto organic and mineral surfaces are likely to dominate the chemical speciation of uranium (Liu et al. 2005). Despite this expectation, few studies have addressed the bioavailability to metal-reducing bacteria of U(VI) adsorbed onto sedimentary aquifer materials. Ortiz-Bernard et al. (2004) investigated the effect of U(VI) adsorption to sediment on microbial reduction of the actinide, using uranium-contaminated sediments from Rifle, Colorado. When sediment was incubated with acetate, U(VI) and Fe(III) reduction was stimulated and the concentration of U(VI) in the groundwater decreased. However, most of the uranium associated with the sediment was U(VI), and this was not reduced, suggesting that sorbed U(VI) was not bioavailable for microbial reduction.

Jeon et al. (2004) investigated the microbial (*Geobacter sulfurreducens*) reduction of 0.1 mM U(VI) in the presence of synthetic Fe(III) oxides and natural Fe(III) oxide-containing solids was investigated in pH 6.8 artificial groundwater containing 10 mM NaHCO₃. In most experiments, more than 95% of the added U(VI) was sorbed to solids, so that U(VI) reduction was governed by reactions at the solid–water interface. The rate and extent of reduction of U(VI) associated with surfaces of synthetic Fe(III) oxides (hydrated ferric oxide, goethite, and hematite) was comparable to that observed during reduction of aqueous U(VI). In contrast, microbial reduction of U(VI) sorbed to several different natural Fe(III) oxide containing solids was slower and less extensive compared to synthetic Fe(III) oxide systems. Addition of the electron shuttling agent anthraquinone-2,6-

disulfonate (AQDS; 0.1 mM) enhanced the rate and extent of both Fe(III) and U(VI) reduction. These findings suggest that AQDS facilitated electron transfer from *G. sulfurreducens* to U(VI) associated with surface sites at which direct enzymatic reduction was kinetically limited. These findings verify the predictions by Fredrickson et al. (2000) and Finneran et al. (2002) that the presence of AQDS should stimulate U(VI) bioreduction in Fe(III) oxide-reducing systems and indicate that addition of electron shuttling compounds, such as natural humic substances, could enhance the overall effectiveness of in situ U(VI) bioremediation. However, other recent studies indicate that humic complexation of U(VI) may inhibit microbial reduction of soluble U(VI) (Haas and Northup 2004). Additional studies are required to evaluate the extent to which natural or synthetic electron shuttles may be able to facilitate U(VI) reduction in subsurface sediments. Jeon et al. (2004) demonstrate that association of U(VI) with diverse surface sites in natural soils and sediments has the potential to limit the rate and extent of microbial U(VI) reduction and thereby modulate the effectiveness of *in situ* U(VI) bioremediation.

Moreover, the bioavailability and the bioreduction rate of metals and radionuclides associated with intraparticle regions of porous media is constrained by mass transfer processes in long-term contaminated sediments or soil. Recent characterization of U(VI) speciation and physical location in 30-year contaminated Hanford Site sediments demonstrated that U(VI) primarily resides as a U(VI) microprecipitate in small fractures, cleavages, or dead-end voids within sediment particle grains exhibiting pore sizes of a few microns or less (Catalano et al. 2004). The U(VI) microprecipitates dissolved into undersaturated pore water, but the rate of dissolution and transport out of intraparticle regions was limited by diffusive processes (Qafoku et al. 2005). These results indicated that most of the sorbed U(VI) pool was not physically accessible to metal-reducing bacteria due to size restrictions of the grain porosity. The overall kinetic rates and extent of microbial U(VI) reduction under such conditions would be therefore limited by the bioavailability and mass transfer rates of U(VI) out of intraparticle regions.

4.3. Bioavailability and plants

For most metals and metalloids, uptake into roots takes place from the aqueous phase. A major factor limiting metal uptake into roots is the slow transport from soil particles to root surface. Indeed, strong binding to soil particles and/or precipitation renders a significant soil metal fraction insoluble, and largely unavailable for plant uptake. Low soil bioavailability is therefore a major factor limiting the potential for phytoextraction of significant metal contaminants (Nolan et al. 2003). Thus, increasing metal solubility in the soil is an important prerequisite to enhance the potential of metal phytoextraction. The success of phytoextraction, as an environmental cleanup technology, depends on several factors including the extent of soil contamination, metal availability for uptake into roots, and plant ability to intercept, absorb, and accumulate metals in shoots (Ernst 1996, for cadmium, see review from Tudoreanu & Phillips 2004). In order to be bioavailable, metals have to come in contact with the plant (i.e., physical accessibility). Moreover, metals need to be in a particular form (i.e., chemical accessibility) to be able to enter a plant root (Adriano et al. 2004).

The different natural remediation-bioavailability processes are depicted in Figure 4. In the initial phase (A), the metals partitioning between the aqueous phase and solid phase are regulated by various biogeochemical processes. Some of these processes include basic ones like desorption/adsorption, precipitation/dissolution, complexation,

redox reactions, etc (see Section 2). The nature of these processes determines the kinetics of natural remediation that can be parameterized by bioavailability. The role of the rhizosphere in metal transformation and partitioning can be very important by secreting root exudates which enhanced the solubility of metals (Wenzel et al. 2003; Adriano et al. 2004).

The next phase (B, B') involves the transport of metals to organisms. The metal can be transported in soluble (B), colloidal, and/or particulate (B') form. From the aqueous phase (B) metals can be mobilized either in soluble or colloidal form, the latter being viewed as facilitated transport. Usually inorganic and OM-based colloids are highly reactive, thus they characteristically contain much higher metal concentration in comparison with those in the solution. The next phase (C) involves passing through a biological membrane, which in many instances can serve as a biofilter for contaminants. In plants, this is represented by the root membrane. The last phase (D) involves circulation and assimilation in the metabolic machinery of the organism, culminating in some form of biological response Figure 4.

The assessment of metal bioavailability and uptake by plants is an important parameter in several respects. Firstly, it allows a rapid, cost-effective and early assessment of the potential risk of heavy metal transfer linked to crop species grown on metal enriched soils. Secondly, a correlation between heavy metal bioavailability and plant uptake data can be used for modeling and in predicting the applicability of different

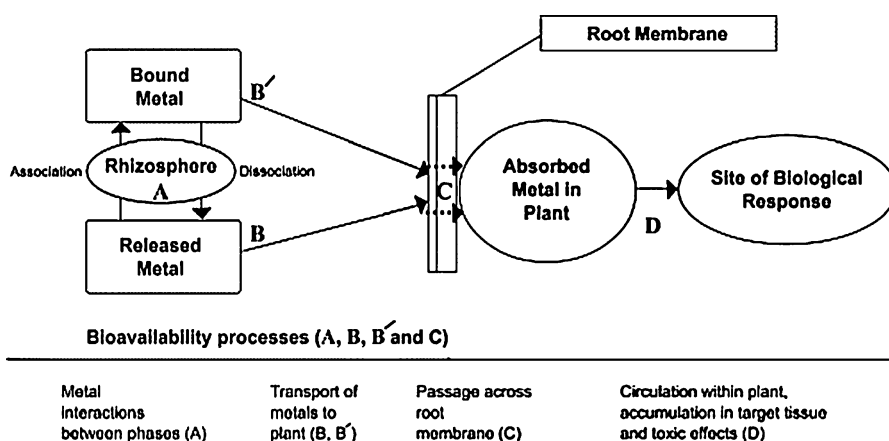


Figure 4. Bioavailability processes in the soil-plant system (Reprinted from Geoderma, vol. 122, Adriano et al. Role of assisted natural remediation in environmental cleanup, 127-142, Copyright (2004), with permission from Elsevier.

phytoremediation techniques as efficient remediation strategies for heavy metal polluted soils. This is of particular relevance for both *in situ* immobilization, aiming at reducing the plant-available heavy metal fraction and its uptake by plants, and phytoextraction, where information on the plant-available fraction is a prerequisite to predict the efficiency of the remediation process (Robinson et al. 1999; Tibazarwa et al. 2001; Nowack et al. 2004).

4.4. Bioavailability and phytoremediation

This section does not intend to review in details the phytoremediation technology to remediate polluted matrixes. For this, the readers are referred to the following review papers (Kamnev & van der Lelie 2000; Lasat 2002; Schmidt 2003; Vassilev et al. 2004; Alkorta et al. 2004a,b; Datta & Sarkar 2004).

In general, there are two approaches to the phytoextraction of heavy metals: continuous or natural phytoextraction and chemically enhanced phytoextraction. The former approach uses natural hyperaccumulating plants with extremely high metal-accumulating abilities to accumulate exceptionally high specific metal content in the shoots, which are harvestable, but such plants are usually slow-growing with a low biomass yield (Alkorta et al. 2004b). It has been estimated that such plants would need several years to remove all the metal from contaminated soil (Alkorta et al. 2004a). However, chemically enhanced phytoextraction has been shown to overcome the above problems. Common crop plants with high biomass can be triggered to accumulate vast amounts of metals when their mobility in soil is enhanced by chemical chelating agents. In addition, the effectiveness of phytoextraction for metals is highly dependent on the availability of metals for plant uptake (Garbisu and Alkorta 2001; Alkorta et al. 2004a).

Plant uptake of metals shows a marked dependence on the chemical speciation of the metal in solution. For most metals, uptake into roots takes place from the aqueous phase. Plant response generally correlates best with the activity of the free, uncomplexed metal ion in solution. Strong binding to soil particles and/or precipitation renders a significant soil metal fraction insoluble, and largely unavailable for plant uptake.

Low soil bioavailability is a major factor limiting the potential for phytoextraction of significant metal contaminants such as, lead (Cooper et al. 1999). A major objective of current phytoremediation research is for instance to induce heavy metals and metalloids (zinc, cadmium, lead and arsenic) desorption from the soil matrix into solution, and increase propensity for uptake into roots (Alkorta et al. 2004b). However, there are numerous observations that chelating agents are taken up by plants. A direct analytical determination of an intact metal-EDTA complex inside plants proved that the metal and the chelating agent form a complex (Vassil et al. 1998). Therefore, it is suggested that plants are not only able to take up free metal ion, but are also able to take up intact chelates (complexes). As an alternative to synthetic chelators, widespread natural sources, such as humic substances, could be used. The term humic substances refers to a category of naturally occurring organic materials found in soils, sediments, and natural waters. Humic acids contain acidic groups such as carboxyl and phenolic OH functional groups (Dudal & Gérard 2004) and, therefore, provide organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals (Halim et al. 2003). Humic acids were applied to a cadmium-contaminated soil at various dosages, and the uptake of cadmium into *Nicotiana tabacum* SR-1 was determined in relation to the amounts of total and bioavailable cadmium in the soil (Evangelou et al. 2004). It was found that the theoretical bioavailability of cadmium, as determined by diethylenetriaminepentaacetic acid (DTPA) extraction, did not change, but its plant uptake was enhanced significantly, in some cases up to 65%. Humic acids added at a rate of 2 g kg⁻¹ soil increased the cadmium concentration in the shoots from 30.9 to 39.9 mg kg⁻¹. A possible reason for this enhancement is the decrease in pH, resulting in higher cadmium availability. Another possibility taken into account is that plants may take up cadmium complexes with humic acid fragments, which result from microbiological degradation or, self-dissociation.

However, chelate-induced phytoextraction increases the risk of adverse environmental effects due to metal mobilization during extended periods of time (Wu et al. 2004). In order to minimize these problems associated with the use of

chelating agents, nowadays research is being focussed on the gradual application of small doses of the chelating agent during the growth period. However, EDTA utilization in the future will most likely be limited to *ex situ* conditions where control of the leachates can be achieved (Zeng et al. 2005). There are other mobilizing agents which are much less harmful to the environments such as citric acid, NTA, and particularly EDDS (Alkorta et al. 2004a, b; Tandy et al. 2004; Luo et al. 2005; Hauser et al. 2005).

In some cases, the bioavailable metal content in soil or sediment should be lowered in order to reduce the phytotoxicity through organic and inorganic amendements (Adriano et al. 2004, Clemente et al. 2005). In efforts to remediate soils contaminated with low levels of Cr(VI), it has been proposed to use plants to extract or immobilize the metal. However, the contamination of soil and ground water due to the use of Cr in various anthropomorphic activities has become a serious source of concern to plants (Shanjer et al. 2005). In case of heavy contamination with chromium, organic amendment can be used to decrease the phytoavailability of chromate known to be the more toxic. Bolan et al. (2003b) showed that addition of organic amendements (biosolid compost, farm yard manure, fish manure, horse manure, spent mushroom, pig manure, and poultry manure) enhanced the rate of reduction of Cr(VI) to Cr(III) in the soil. The effect of biosolid compost on the uptake of Cr(VI) from the soil, treated with various levels of Cr(VI) (0–1200 mg Cr kg⁻¹ soil), was examined with mustard (*Brassica juncea* L.) plants. Increasing addition of Cr(VI) increased Cr concentration in plants, resulting in decreased plant growth (i.e., phytotoxicity). Addition of the biosolid compost was effective in reducing the phytotoxicity of Cr(VI). The redistribution of Cr(VI) in various soil components was evaluated by a sequential fractionation scheme. In the unamended soil, the concentration of Cr was higher in the organic-bound, oxide-bound, and residual fractions than in the soluble and exchangeable fractions. Addition of organic amendements also decreased the concentration of the soluble and exchangeable fractions, but especially increased the organic-bound fraction in soil. However, research focusing on the interaction between

plants and chromium in the rhizosphere soil and the understanding of chromium bioavailability is relatively limited due to the complexity of chromium chemistry. To optimize phytoremediation as a potential remediation strategy and to assess potential secondary environmental and health hazards with this process, it is helpful to understand the mechanisms of Cr uptake, translocation, tolerance, and bonding by plants and the conditions under which Cr is maximally absorbed and/or immobilized by using for instance microanalytical spectroscopic techniques (Howe et al. 2003; Gardea-Torresdey et al. in press).

Soil micro-organisms have been shown to possess several mechanisms capable of altering metal bioavailability for uptake into roots. For example, microbes have been documented to catalyse redox reactions leading to changes in metal mobility in soil and propensity for uptake into roots.

The bacteria associated with plant roots may have profound effects on plant growth and nutrition through a number of mechanisms such as N₂ fixation, production of phytohormones and siderophores, and transformation of nutrient elements. Although many soil bacteria are tolerant to heavy metals and play important roles in mobilization or immobilization of heavy metals (Ledin 2000), only a few attempts have been made to study the rhizosphere bacteria of metal accumulating and hyperaccumulating plants and their role in the tolerance to and uptake of heavy metals by the plants. However, improvement of the interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals, and are considered to be an important component of phytoremediation technology (Glick 2003). Belimov et al. (2005) isolated several cadmium-tolerant bacterial strains from the root zone of Indian mustard (*Brassica juncea* L. Czern.) seedlings grown in Cd-supplemented soils as well as highly Cd contaminated sewage sludge and mining waste. The isolated strains, *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* sp., were capable of stimulating root elongation of *B. juncea* seedlings either in the presence or absence of toxic Cd concentrations. Some of the strains produced indoles or siderophores. The isolated bacteria offer promise

as inoculants to improve growth of the metal-accumulating plant *B. juncea* in the presence of toxic Cd concentrations and for the development of plant-inoculant systems useful for phytoremediation of polluted soils.

In addition, root mycorrhizal associations have been shown to affect the rate of metal uptake (Leyval et al. 1997; Khan 2005). It is well known that mycorrhizal fungi are a major component of the rhizosphere and form mutualistic associations (mycorrhizae) with most plant species. Of these, the arbuscular mycorrhizae (AM) are by far the most widespread. Benefits to the plant include for instance improved nutrition through phosphorus minerals solubilization. Note that this solubilization can result in release of the associated metals (Fomina et al. 2005). In addition, AM fungi have been shown to enhance tolerance of biotic and abiotic stresses, including heavy metals (Leyval et al. 1997): as they are a direct link between soil and roots, they can be very important for heavy metal availability and toxicity to plants (Leyval et al. 1997). The AM symbiotic status changes the chemical composition of root exudates and influences the soil pH, thus quantitatively and qualitatively affecting the microbial populations in the rhizosphere. All these factors, alone or in combination, can influence metal mobility or availability. Nevertheless, the role of AM fungi in the uptake and in the transfer of heavy metals to the plant is still poorly understood and literature results are conflicting (Citterio et al. 2005). Many fungi can survive and grow in high concentrations of toxic metals (Gadd 1993). The mechanisms by which they are able to deal with these metals are numerous and varied in their action, e.g. extracellular metal sequestration and precipitation, metal binding to the fungal cell walls, intracellular sequestration and complexation, compartmentation or volatilization (Gadd 1993). Recently, González-Chávez et al. (2004) showed that an insoluble glycoprotein, glomalin, produced in copious amounts on hyphae of arbuscular mycorrhizal fungi (AMF) was able to sequester potentially toxic elements in the rhizosphere. Glomalin may be considered for biostabilization leading to remediation of polluted soils.

In spite of the growing public and commercial interest and success, more fundamental research is still needed to better exploit the metabolic diversity of the plants themselves, but also to

better understand the complex interactions between metals (bioavailability), soil, plant roots and micro-organisms (bacteria and mycorrhiza) in the rhizosphere (Vassilev et al. 2004; Alkorta et al. 2004b; Kahn 2005).

5. Perspectives and recommendations

While many analytical approaches exist to address dissolved species of toxicants, there is a need to characterize sediments and soils *per se* in terms of materials or particles which bind toxicants and modulate their bioavailability and rate of burial as well as obtaining information on the activities of specific microbial taxa in communities that inhabit the treated site (Lloyd & Renshaw 2005; Wiatrowski & Barkay 2005). Several approaches have been discussed recently by NRC (2003), Nolan et al. (2003) and Wiatrowski & Barkay (2005).

- Microbial community analysis as evidence of biotic metal transformation. Monitoring spatial distribution of microbial communities is an essential part of metal bioremediation by applying molecular techniques (analysis of macromolecules such as DNA, RNA or phospholipids fatty acids, targeting the presence and expression of functional genes). This has been demonstrated during chromium attenuation in marine sediment (Arias et al. 2004).
- Solid phase metal speciation techniques that allow the investigation of metal distribution, surface reaction, and solid-phase speciation at the molecular level. Using these methods the physical and chemical forms and distribution of contaminants can be investigated *in situ* in soil and sediments (Nolan et al. 2003). There are generally spectroscopic techniques such as X-ray microanalyses (Leppard & Droppo 2003). Lately, X-ray absorption spectroscopy (XAS), which makes use of intense X-rays generated at synchrotron facilities, have improved greatly our ability to gain information regarding oxidation and coordination states, number and type of near neighbours and bond distances of the elements of interest (Sparks 2001; Prange & Modrow 2002). Moreover, third-generation synchrotron facilities offer greatly enhanced spatial resolution (in the order of a few micrometers) that allow analysis at the

microscale level and improved detection limit (Sparks 2001). These techniques can potentially provide an understanding of what controls bio-availability processes (Nolan et al. 2003).

- The monitoring of metal microbial transformations with stable isotope analysis holds great promise. The true potential of stable isotopes as a tool for monitoring transformations of specific elements in the environment is the use of isotopic ratios to distinguish sources and pathways. Because both biological and chemical transformations show a preference for specific isotopes, sources may exhibit isotopic ratios that reflect the process by which they were formed rather than the natural abundance of the isotope. These ratios can therefore be used as signatures to identify a specific source or transformation pathway (Herbel et al. 2000; Wiatrowski & Barkay 2005).

Thus, the spatial *in situ* speciation of inorganic contaminants (i.e., in the solid phase as well as in the bulk soil or sediment solution, Williams et al. 2005) has a pivotal role to play in risk assessment as well as in the improvement of the implementation of bioremediation technologies of contaminated soils and sediments.

References

- Abdelouas A, Lutze W, Gong W, Nuttall E, Strietelmaier BA & Travis BJ (2000) Biological reduction of uranium in ground-water and subsurface soil. *Sci. Total Environ.* 250: 21–35
- Adriano DC, Wenzel WW, Vangronsveld J & Bolan NS (2004) Role of assisted natural remediation in environmental cleanup. *Geoderma* 122: 121–142
- Adriano DC (2001) Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals, 2nd edition. Springer, New York
- Alberts JJ, Schindler JE & Miller RW (1974) Elemental mercury evolution mediated by humic acid. *Science* 184: 895–896
- Aldridge KT & Ganf GG (2003) Modification of sediment redox potential by three contrasting macrophytes: implications for phosphorus adsorption/desorption. *Marine Freshwater Res.* 54: 87–94
- Alkorta I, Hernandez-Allica J, Becerril JM, Amezcaga I, Albizu I, Anandia M & Garbisu C (2004a) Chelate-enhanced phytoremediation of soils polluted with heavy metals. *Rev. Environ. Sci. Bio/Technol.* 3: 55–70
- Alkorta I, Hernandez-Allica J, Becerril JM, Amezcaga I, Albizu I & Garbisu C (2004b) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. *Rev. Environ. Sci. Bio/Technol.* 3: 71–90
- Alloway BJ (1995) Heavy metals in soils. In: Alloway BJ (ed.) Blackie Academic & Professional, London, UK
- Anderson RT & Lovley DR (2002) Microbial redox interactions with uranium: an environmental perspective In: Keith-Roach MJ & Livens FR (Eds) Interactions of Microorganisms with Radionuclides (pp 205–223). Elsevier Science Ltd,
- Arias YM, Obraztsova A, Tebo BM & Green-Ruiz C (2004) Natural Attenuation of Cr(VI) Contamination in Laboratory Mesocosms. *Geomicrobiol. J.* 20: 389–401
- Barkay T & Wagner-Dobler I (2005) Microbial transformations of mercury: potentials, challenges, and achievements in controlling mercury toxicity in the environment. *Adv. Appl. Microbiol.* 57: 1–52
- Basta NT, Ryan JA & Chaney RL (2005) Trace element chemistry in residual-treated soil: key concepts and metal bioavailability. *J. Environ. Qual.* 34: 49–63
- Behrends T & Van Cappellen P (2005) Competition between enzymatic and abiotic reduction of uranium(VI) under iron reducing conditions. *Chemical Geology* 220: 315–327
- Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S & Glick BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern). *Soil Biol. Biochem.* 37: 241–250
- Belzile N, Chen Y-W & Xu R (2002) Early diagenetic behaviour of selenium in freshwater sediments. *Appl. Geochem.* 15: 1439–1454
- Bencheikh-Latmani R & Leckie JO (2003) Association of uranyl with the cell wall of *Pseudomonas fluorescens* inhibits metabolism. *Geochim. Cosmochim. Acta* 67: 4057–4066
- Bertsch PM & Seaman JC (1999) Characterization of complex mineral assemblages: implications for contaminant transport and environmental remediation. *Proc. Natl. Acad. Sci. USA* 96: 3350–3357
- Beveridge TJ (1989) Metal ions and bacteria In: Beveridge TJ & Doyle RJ (Eds) Metal Ions and Bacteria (pp 1–29). Wiley, New York
- Bolan NS, Adriano DC & Curtin D (2003a) Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Adv. Agron.* 78: 215
- Bolan NS, Adriano DC, Natesan R & Koo B-J (2003b) Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil. *J. Environ. Qual.* 32: 120–128
- Bond DL & Fendorf S (2003) Kinetics and structural constraints of chromate reduction by green rusts. *Environ. Sci. Technol.* 37: 2750–2757
- Bose P & Sharma A (2002) Role of iron in controlling speciation and mobilization of arsenic in subsurface environment. *Water Res.* 36: 4916–4926
- Bourg ACM & Loch JPG (1995) Mobilization of heavy metals as affected by pH and redox conditions In: Salomons W & Stigliani WM (Eds) Biogeochemistry of Pollutants in Soils and Sediments (pp 87–102). Springer, Berlin Heidelberg NY
- Bradl HB (2004) Adsorption of heavy metal ions on soils and soils constituents. *J. Coll. Interf. Sci.* 277: 1–18
- Brennan EW & Lindsay WL (1996) The role of pyrite in controlling metal ion activities in highly reduced soils. *Geochim. Cosmochim. Acta* 60: 3609–3618
- Brown GE Jr, Foster AL & Ostergren JD (1999) Mineral surfaces and bioavailability of heavy metals: a molecular-scale perspective. *Proc. Natl. Acad. Sci. USA* 96: 3388–3395
- Bruggeman C, Maes A, Vancluysen J & Vandemussel P (2005) Selenite reduction in Boom clay: effect of FeS₂, clay minerals and dissolved organic matter. *Environ. Pollut.* 137: 209–221

- Bruneel O, Personné J-C, Casiot C, Leblanc M, Elbaz-Poulichet F, Mahler BJ, Le Flèche A & Grimont PAD (2003) Mediation of arsenic oxidation by *Thiomonas* sp. in acid-mine drainage (Carnoulès, France). *J. Appl. Microbiol.* 95: 492–499
- Burke IT, Boothman C, Lloyd JR, Mortimer RJG, Livens FR & Morris K (2005) Effects of progressive anoxia on the solubility of technetium in sediments. *Environ. Sci. Technol.* 39: 4109–4116
- Cantwell MG & Burgess RM (2001) Metal-colloid partitioning in artificial interstitial waters of marine sediments: influences of salinity, pH, and colloidal organic carbon concentration. *Environ. Toxicol. Chem.* 20: 2420–2427
- Catalano JG, Heald SM, Zachara JM & Brown GE Jr (2004) Spectroscopic and diffraction study of uranium speciation in contaminated vadose zone sediments from the Hanford site, Washington state. *Environ. Sci. Technol.* 38: 2822–2828
- Chapman PM, Wang F, Janssen C, Persoone G & Allen HE (1998) Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. *Can. J. Fish. Aquat. Sci.* 55: 2221–2243
- Chen J, Gu B, Royer RA & Burgos WD (2003) The roles of natural organic fractions in chemical and microbial reduction of ferric iron. *Sci. Total Environ.* 307: 167–178
- Cheung KH & Gu J-D (2003) Reduction of chromate (CrO_4^{2-}) by an enrichment consortium and an isolate of marine sulfate-reduction bacteria. *Chemosphere* 52: 1523–1529
- Citterio S, Prato N, Fumagalli P, Aina R, Massa N, Santagostino A, Sgorbati S & Berta G (2005) The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L. *Chemosphere* 59: 21–29
- Clemente R, Walker DJ & Pilar Bernal M (2005) Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): the effect of soil amendments. *Environ. Pollut.* 138: 46–58
- Cooper EM, Sims JT, Cunningham SD, Huang JW & Berti WR (1999) Chelate-assisted phytoextraction of lead from contaminated soils. *J. Environ. Qual.* 28: 1709–1719
- Cullen WR & Reimer KJ (1989) Arsenic speciation in the environment. *Chem. Rev.* 89: 713–764
- Cummings DE, Caccavo F, Fendorf S & Rosenzweig RF (1999) Arsenic mobilization by the dissimilatory Fe(III)-reducing bacterium *Shewanella alga* BrY. *Environ. Sci. Technol.* 33: 723–729
- Dassonville F & Renault P (2002) Interactions between microbial processes and geochemical transformations under anaerobic conditions: a review. *Agronomie* 22: 51–68
- Datta R & Sarkar D (2004) Effective integration of soil chemistry and plant molecular biology in phytoremediation of metals. An overview. *Environ. Geosci.* 88: 53–63
- Davis JA, Meece DE, Kohler M & Curtis GP (2004) Approaches to surface complexation modelling of uranium(VI) adsorption on aquifer sediments. *Geochim. Cosmochim. Acta* 68: 3621–3641
- Dhillon KS & Dhillon SK (2003) Distribution and management of seleniferous soils. *Adv. Agron.* 79: 119–184
- Diels L, De M Smet, Hooyberghs L & Corbisier P (1999) Heavy metals bioremediation of soil. *Mol. Biotechnol.* 12: 149–158
- Dudal Y & Gérard F (2004) Accounting for natural organic matter in aqueous chemical equilibrium models: a review of the theories and applications. *Earth-Sci. Rev.* 66: 199–216
- Duff MC, Urbanik Coughlin J & Hunter DB (2002) Uranium co-precipitation with iron oxide minerals. *Geochim. Cosmochim. Acta* 66: 3533–3547
- Eary LE & Rai D (1988) Chromate removal from aqueous wastes by reduction with ferrous ion. *Environ. Sci. Technol.* 22: 972–977
- Eccles H (1999) Treatment of metal-contaminated wastes: why select a biological process?. *Trends Biotechnol.* 17: 462–465
- Eggleton J & Thomas KV (2004) A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environ. Internat.* 30: 973–980
- Ehlers LJ & Luthy RG (2003) Contaminant bioavailability in soil and sediment. *Environ. Sci. Technol.* 37: 295A–302A
- Ehrlich HL (1999) Microbes as geologic agents: their role in mineral formation. *Geomicrobiol. J.* 16: 135–153
- Evangelou MWH, Daghan H & Schaeffer A (2004) The influence of humic acids on the phytoextraction of cadmium from soil. *Chemosphere* 57: 207–213
- Fendorf SE (1995) Surface reactions of chromium in soils and waters. *Geoderma* 67: 55–71
- Finneran KT, Anderson RT, Nevin KP & Lovley DR (2002) Potential for bioremediation of uranium-contaminated aquifers with microbial U(VI) reduction. *J. Soil Contamin.* 11: 339–357
- Fjeld RA, Serkiz SM, McGinnis PL, Elci A & Kaplan DI (2003) Evaluation of a conceptual model for the subsurface transport of plutonium involving surface mediated reduction of Pu(V) to Pu(IV). *J. Contam. Hydrol.* 67: 79–94
- Fomina MA, Alexander IJ, Colpeart JV & Gadd GM (2005) Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biol. Biochem.* 37: 851–866
- Förstner U (2004) Sediment dynamics and pollutant mobility in rivers: an interdisciplinary approach. *Lakes Reservoirs: Res. Manage.* 9: 25–40
- Fortin D (2004) Geochemistry. What biogenic minerals tell us. *Science* 303: 1618–1619
- Frazier SW, Kretzschmar R & Kraemer SM (2005) Bacterial siderophores promote dissolution of UO_2 under reducing conditions. *Environ. Sci. Technol.* 39: 5709–5715
- Fredrickson JK, Zachara JM, Kennedy DW, Duff MC, Gorby YA, Li SW & Krupka KM (2000) Reduction of U(VI) in goethite ($\alpha\text{-FeOOH}$) suspensions by a dissimilatory metal-reducing bacterium. *Geochim. Cosmochim. Acta* 64: 3085–3098
- Fredrickson JK, Zachara JM, Kennedy DW, Dong H, Onstott TC, Hinman NW & Li S-M (1998) Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta* 62: 3239–3257
- Fredrickson JK, Zachara JM, Kennedy DW, Kukkadapu RK, McKinley JP, Heald SM, Liu C & Plymale AE (2004) Reduction of TcO_4^- by sediment-associated biogenic Fe(II). *Geochim. Cosmochim. Acta* 68: 3171–3187
- Fredrickson JK, Zachara JM, Kennedy DW, Liu C, Duff MC, Hunter DB & Dohnalkova A (2002) Influence of Mn oxides on the reduction of uranium(VI) by the metal-reducing bacterium *Shewanella putrefaciens*. *Geochim. Cosmochim. Acta* 66: 3247–3262
- Fugita Y, Ferris FG, Lawson RD, Colwell FS & Smith RW (2000) Calcium carbonate precipitation by ureolytic subsurface bacteria. *Geomicrobiol. J.* 17: 305–318
- Gadd GM (1993) Interactions of fungi with toxic metals. *New Phytol.* 124: 25–60
- Gadd GM & White C (1993) Microbial treatment of metal pollution – a working biotechnology?. *Trends Biotechnol.* 11: 353–359

- Gadd GM (1996) Influence of microorganisms on the environmental fate of radionuclides. *Endeavour* 20: 150–156
- Gadd GM (1999) Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. *Adv. Microb. Physiol.* 41: 47–92
- Gadd GM (2000) Bioremediation potential of microbial mechanisms of metal mobilization and immobilization. *Curr. Opin. Biotechnol.* 11: 271–279
- Gadd GM (2002) Interactions between microorganisms and metals/radionuclides: the basis of bioremediation. In: Keith-Roach MJ & Livens FR (Eds) *Interactions of Microorganisms with Radionuclides* (pp 179–203). Elsevier, Amsterdam
- Gao Y, Kan AT & Tomson MB (2003) Critical evaluation of desorption phenomena of heavy metals from natural sediments. *Environ. Sci. Technol.* 37: 5566–5573
- Gardea-Torresdey JL, Peralta-Videa JR, de la Rosa G & Parsons JG (2005) Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. *Coordination Chem. Rev.* 249: 1797–1810
- Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* 21: 383–393
- González-Chávez MC, Carrillo-González R, Wright SF & Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environ. Pollut.* 130: 317–323
- Groudev SN, Spasova II & Georgiev PS (2001) In situ bioremediation of soils contaminated with radioactive elements and toxic heavy metals. *Internat. J. Min. Process.* 62: 301–308
- Gu B & Chen J (2003) Enhanced microbial reduction of Cr(VI) and U(VI) by different natural organic matter fractions. *Geochim. Cosmochim. Acta* 67: 3575–3582
- Gu B, Yan H, Zhou P, Watson DB, Park M & Istok J (2005) Natural humics impact uranium bioreduction and oxidation. *Environ. Sci. Technol.* 39: 5268–5275
- Guha H (2004) Biogeochemical influence on transport of chromium in manganese sediments: experimental and modeling approaches. *J. Contam. Hydrol.* 70: 1–36
- Guha H, Jayachandran K & Maurrasse F (2001b) Kinetics of chromium(VI) reduction by a type strain *Shewanella* alga under different growth conditions. *Environ. Pollut.* 115: 209–218
- Guha H, Saiers JE, Brooks SC, Jardine PM & Jayachandran K (2001a) Chromium transport, oxidation, and adsorption in manganese-coated sand. *J. Contam. Hydrol.* 49: 311–334
- Guha H, Jayachandran K & Maurrasse F (2003) Microbiological reduction of chromium(VI) in presence of pyrolusite-coated sand by *Shewanella alga* Simidu ATCC 55627 in laboratory column experiments. *Chemosphere* 52: 175–183
- Haack EA & Warren LA (2003) Biofilm Hydrous Manganese oxyhydroxides and metal dynamics in acid rock drainage. *Environ. Sci. Technol.* 37: 4138–4147
- Haas JR & Northup A (2004) Effects of aqueous complexation on reductive precipitation of uranium by *Shewanella putrefaciens*. *Geochem. Trans.* 5: 41–48
- Halim M, Conte P & Piccolo A (2003) Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere* 52: 265–275
- Hamon RE, McLaughlin MJ & Cozens G (2002) Mechanisms of attenuation of metal availability in in-situ remediation treatments. *Environ. Sci. Technol.* 36: 3991–3996
- Hass JR, Dichristina TJ & Wade R Jr (2001) Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. *Chem. Geol.* 180: 33–54
- Hauser L, Tandy S, Schulin R & Nowack B (2005) Column extraction of heavy metals from soils using the biodegradable chelating agent EDDS. *Environ. Sci. Technol.* 39: 6819–6824
- Hazen TC & Tabak HH (2005) Developments in bioremediation of soils and sediments polluted with metals and radionuclides 2. Field research on bioremediation of metals and radionuclides. *Reviews Environ. Sci. Bio/Technol.* 4: 157–183
- Herbel MJ, Blum JS, Oremland RS & Borglin SE (2003) Reduction of elemental selenium to selenide: experiments with anoxic sediments and bacteria that respire Se-oxyanions. *Geomicrobiol. J.* 20: 587–602
- Herbel MJ, Johnson TM, Oremland RS & Bulen TD (2000) Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochim. Cosmochim. Acta* 64: 3701–3709
- Howe JA, Loeppert RH, DeRose VJ, Hunter DB & Bertsch PM (2003) Localization and speciation of chromium in subterranean clover using XRF, XANES, and EPR spectroscopy. *Environ. Sci. Technol.* 37: 4091–4097
- Huang PM (2004) Soil mineral-organic matter-microorganism interactions: fundamentals and impacts. *Adv. Agro.* 82
- Huang Q, Chen W & Xu L (2005) Adsorption of copper and cadmium by Cu- and Cd-resistant bacteria and their composites with soil colloids and kaolinite. *Geomicrobiol. J.* 22: 227–236
- Hunter KS, Wang Y & Van Cappellen P (1998) Kinetic modeling of microbially-driven redox chemistry of subsurface environments: coupling transport, microbial metabolism and geochemistry. *J. Hydrol.* 209: 53–80
- Hursthouse AS (2001) The relevance of speciation in the remediation of soils and sediments contaminated by metallic elements—an overview and examples from Central Scotland, UK. *J. Environ. Monit.* 3: 49–60
- Ilg K, Wilcke W, Safronov G, Lang F, Fokin A & Kaupenjohann M (2004) Heavy metal distribution in soil aggregates: a comparison of recent and archived aggregates from Russia. *Geoderma* 123: 153–162
- Jackson TA, West MM & Leppard GG (1999) Accumulation of heavy metals by individually analysed bacterial cells and associated non living material in polluted lake sediment. *Environ. Sci. Technol.* 33: 3795–3801
- Jensen-Spaulding A, Shuler ML & Lion LW (2004) Mobilization of adsorbed copper and lead from naturally aged soil by bacterial extracellular polymers. *Water Res.* 38: 1121–1128
- Jeon B-H, Kelly SD, Kemner KM, Barnett MO, Burgos WD, Dempsey BA & Roden EE (2004) Microbial reduction of U(VI) at the solid–water interface. *Environ. Sci. Technol.* 38: 5649–5655
- Jespersen DN, Sorrell BK & Brix H (1998) Growth and root oxygen release by *Typha latifolia* and its effects on sediment methanogenesis. *Aquat. Bot.* 61: 165–180
- Johnson TM & Bullen TD (2003) Selenium isotope fractionation during reduction by Fe(II)–Fe(III) hydroxide-sulfate (green rust). *Geochim. Cosmochim. Acta* 67: 413–419
- Kamaludeen SP, Megharaj M, Juhasz AL, Sethunathan N & Naidu R (2003) Chromium–microorganism interactions in soils: remediation implications. *Rev. Environ. Contam. Toxicol.* 178: 93–164

- Kamnev AA & van der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Biosci. Rep.* 20: 239–258
- Kaplan DI, Powell BA, Demirkanli DI, Fjeld RA, Molz FJ, Serkiz SM & Coates JT (2004) Influence of oxidation states on plutonium mobility during long-term transport through an unsaturated subsurface environment. *Environ. Sci. Technol.* 38: 5053–5058
- Kappler A, Benz M, Schink B & Brune A (2004) Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol. Ecol.* 47: 85–92
- Kashefi K & Lovley DR (2000) Reduction of Fe(III) and toxic and radioactive metals by *Pyrobaculum* species. *Appl. Environ. Microbiol.* 66: 1050–1056
- Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace Elements Med. Biol.* 18: 355–364
- Kiikkilä O, Perkiomäki J, Barnette M, Derome J, Pennanen T, Tulisalo E & Fritze H (2001) In situ bioremediation through mulching of soil polluted by a copper–nickel smelter. *J. Environ. Qual.* 30: 1134–1143
- Kremen A, Bear J, Shavit U & Shaviv A (2005) Model demonstrating the potential for coupled nitrification denitrification in soil aggregates. *Environ. Sci. Technol.* 39: 4180–4188
- Krishnamurthy GSR, Cieslinsky G, Huang PM & Rees KCJvan (1997) Kinetics of cadmium release from soils as influenced by organic acids: implication in cadmium availability. *J. Environ. Qual.* 26: 271–277
- Krumholz LR, Elias DA & Suffita JM (2003) Immobilization of cobalt by sulphate-reducing bacteria in subsurface sediment. *Geomicrobiol. J.* 20: 61–72
- Lack JG, Chaudhury SK, Kelly SD, Kemmer KM, O'Connor SM & Coates JD (2002) Immobilisation of radionuclides and heavy metals through anaerobic bio-oxidation of Fe(II). *Appl. Environ. Microbiol.* 68: 2704–2710
- Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. *J. Environ. Qual.* 31: 109–120
- Ledin M (2000) Accumulation of metals by microorganisms—processes and importance for soil systems. *Earth-Sci. Rev.* 51: 1–31
- Lee M-K & Saunders JA (2003) Effects of pH on metals precipitation and sorption: field bioremediation and geochemical modelling approaches. *Vadose Zone J.* 2: 177–185
- Lee W & Batchelor B (2003) Reductive capacity of natural reductants. *Environ. Sci. Technol.* 37: 535–541
- Leppard GG & Droppo IG (2003) The need and means to characterize sediment structure and behaviour prior to the selection and implementation of remediation plans. *Hydrobiologia* 494: 313–317
- Liger E, Charlet L & Cappelen Pvan (1999) Surface catalysis of uranium (VI) reduction by Fe(II). *Geochim. Cosmochim. Acta* 63: 2939–2955
- Lin S, Huang K-M & Chen S-K (2002) Sulfate reduction and iron sulfide mineral formation in the southern East China Sea continental slope sediment. *Deep Sea Research Part I* 49: 1837–1852
- Liu C, Zachara JM, Fredrickson JK, Kennedy DW & Dohnalkova A (2002) Modeling the inhibition of the bacterial reduction of U(VI) by beta-MnO_{2(s)}. *Environ. Sci. Technol.* 36: 1452–1459
- Liu C, Zachara JM, Zhong L, Kukkadupa R, Szecsody JE & Kennedy DW (2005) Influence of sediment bioreduction and reoxidation on uranium sorption. *Environ. Sci. Technol.* 39: 4125–4133
- Liu W-J, Zhu Y-G, Smith FA & Smith SE (2004) Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L) grown in solution culture?. *J. Exp. Bot.* 55: 1707–1713
- Livens FR, Jones MJ, Hynes AJ, Charnock JM, Mosselmans JFW, Hennig C, Steele H, Collison D, Vaughan DJ, Patrick RAD, Reed WA & Moyes LN (2004) X-ray absorption spectroscopy studies of reactions of technetium, uranium and neptunium with mackinawite. *J. Environ. Radioact.* 74: 211–219
- Lloyd JR, Sole VA, Van Praagh CVG & Lovley DR (2000) Direct and Fe(II)-mediated reduction of technetium by Fe(III)-reducing bacteria. *Appl. Environ. Microbiol.* 66: 3743–3749
- Lloyd JR & Macaskie LE (2000) Bioremediation of radioactive metals. In: Lovley D.R. (Ed) *Environmental Microbe–Metal Interactions* (pp 277–327). ASM Press
- Lloyd JR, Nolting H-F, Solé VA, Bosecker K & Macaskie LE (1998) Technetium reduction and precipitation by sulphate-reducing bacteria. *Geomicrobiol. J.* 15: 43–56
- Lloyd JR & Renshaw JC (2005) Bioremediation of radioactive waste: radionuclide–microbe interactions in laboratory and field-scale studies. *Curr. Opin. Biotechnol.* 16: 254–260
- Lloyd JR (2003) Microbial reduction of metals and radionuclides. *FEMS Microbiol. Rev.* 27: 411–425
- Lloyd JR, Mabbett AN, Williams DR & Macaskie LE (2001) Metal reduction by sulfate-reducing bacteria: physiological diversity and metal specificity. *Hydrometallurgy* 59: 327–337
- Lloyd JR & Lovley DR (2001) Microbial detoxification of metals and radionuclides. *Curr. Opin. Biotechnol.* 12: 248–253
- Lloyd JR & Macaskie LE (2000) Bioremediation of radionuclide containing wastewaters. In: Lovley DR (ed.) *Environmental Microbe-metal Interactions*. Washington, DC: ASM Press; 277–327.
- Lock K & Janssen CR (2003a) Influence of ageing on zinc bioavailability in soils. *Environ. Pollut.* 126: 371–374
- Lock K & Janssen CR (2003b) Influence of aging on metal availability in soils. *Rev. Environ. Contam. Toxicol.* 178: 1–21
- Lock K, Janssen CR & De Coen WM (2000) Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to *Enchytraeus albidus*. *Environ. Toxicol. Chem.* 19: 2666–2671
- Lovley DR (1996) Humic substances as electron acceptors for microbial respiration. *Nature* 382: 445–448
- Lovley DR & Coates JD (1997) Bioremediation of metal contamination. *Curr. Opin. Biotechnol.* 8: 285–289
- Lovley DR & Coates JD (2000) Novel forms of anaerobic respiration of environmental relevance. *Curr. Opin. Microbiol.* 3: 252–256
- Lovley DR, Fraga JL, Blunt-Harris EL, Hayes LA, Phillips EJP & Coates JD (1998) Humic substances as a mediator for microbially catalysed metal reduction. *Acta Hydrochim. Hydrobiol.* 26: 152–157
- Lu X, Johnson WD & Hook J (1998) Reaction of vanadate with aquatic humic substances. *Environ. Sci. Technol.* 32: 2257–2263
- Luo C, Shen Z & Li X (2005) Enhanced phytoextraction of Cu, Pb, Zn, and Cd with EDTA and EDDS. *Chemosphere* 59: 1–11
- Macaskie LE, Yong M & Paterson-Beedle P (2004) Bacterial precipitation of metal phosphates In: Valsami-jones E (Eds) *Phosphorus in Environmental Technology: Principles and Applications* (pp 547–579). IWA publishing, London, UK

- Macur RE, Jackson CR, Botero LM, McDermott TR & Inskeep WP (2004) Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environ. Sci. Technol.* 38: 104–111
- Mansfeldt T (2003) In situ long-term redox potential measurements in a dyked marsh soil. *J. Plant Nutr. Soil Sci.* 166: 210–219
- Matthiessen A (1996) Kinetic aspects of the reduction of mercury ions by humic substances. I. Experimental design. *Fresenius J. Anal. Chem.* 354: 747–749
- McLean JS, Lee JU & Beveridge TJ (2002) Interactions of bacteria and environmental metals, fine-grained mineral development, and bioremediation strategies In: Huang PM, Bollag J-M & Senesi N (Eds) *Interactions Between Soil Particles and Microorganisms* (pp 227–261). Wiley, New York
- Mitsch WJ & Gosselink JG (1993) *Wetlands*. John Wiley, New York
- Morse JW & Luther GW III (1999) Chemical influences on metal-sulfide interactions in anoxic sediments. *Geochim. Cosmochim. Acta* 63: 3373–3378
- Moyes LN, Jones MJ, Reed WA, Livens FR, Charnock JM, Mosselmans JFW, Hennig C, Vaughan DJ & Patrick RAD (2002) An X-ray absorption spectroscopy study of neptunium(V) reactions with mackinawite (FeS). *Environ. Sci. Technol.* 36: 179–183
- Moyes LN, Parkman RH, Charnock JM, Vaughan DJ, Livens FR, Hughes CR & Braithwaite A (2000) Uranium uptake from aqueous solution by interaction with goethite, lepidocrocite, muscovite and mackinawite: an X-ray absorption spectroscopy study. *Environ. Sci. Technol.* 34: 1062–1068
- Myers CR & Nealson KH (1988) Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochim. Cosmochim. Acta* 52: 2727–2732
- Myneni SCB, Tokunaga TK & Brown GE Jr (1997) Abiotic selenium redox transformation in the presence of Fe(II,III) oxides. *Science* 278: 1106–1109
- Nakayasu K, Fukushima M, Sasaki K, Tanaka S & Nakamura H (1999) Comparative studies of the reduction behavior of chromium(VI) by humic substances and their precursors. *Environ. Toxicol. Chem.* 18: 1085–1090
- National Research Council (2003) *Bioavailability of Contaminants in Soils and Sediments: Processes, Tools and Applications*. The National Academies Press, Washington, DC
- Neal AL, Techkarnjanaruk S, Dohnalkova A, McCreedy D, Peyton BM & Geesey GG (2001) Iron sulfides and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria. *Geochim. Cosmochim. Acta* 65: 223–235
- Nealson KH, Belz A & McKee B (2002) Breathing metals as a way of life: geobiology in action. *Antonie van Leeuwenhoek* 81: 215–222
- Nevin KP & Lovley DR (2000) Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. *Environ. Sci. Technol.* 34: 2472–2478
- Nolan AL, Lombi E & McLaughlin MJ (2003) Metal bioaccumulation and toxicity in soils – Why bother with speciation?. *Aust. J. Chem.* 56: 77–91
- Nowack B, Koehler S & Schulin R (2004) Use of diffusive gradients in thin films (DGT) in undisturbed field soils. *Environ. Sci. Technol.* 38: 1133–1138
- O'Loughlin EJ, Kelly SD, Cook RE, Csencsits R & Kemner KM (2003) Reduction of uranium(VI) by mixed iron(II)/iron(III) hydroxide (green rust): formation of UO₂ nanoparticles. *Environ. Sci. Technol.* 37: 721–727
- Oremland RS, Stolz J & Lovley DR (1998) “Green rust” in the lab and in the soil. *Science* 281: 1111
- Otte S, Kuenen JG, Nielsen LP, Pearl HW, Zopfi J, Schulz HN, Teske A, Strotmann B, Gallardo VA & Jorgensen BB (1999) Nitrogen, carbon, and sulphur metabolism in natural Thioploca samples. *Appl. Environ. Microbiol.* 65: 3148–3157.
- Pantzar-Kallio M, Reinikainen S-P & Oksanen M (2001) Interactions of soil components and their effects on speciation of chromium in soil. *Anal. Chim. Acta* 439: 9–17
- Peijnenburg WJGM & Jager T (2003) Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. *Ecotoxicol. Environ. Safe* 56: 63–77
- Pepper SE, Bunker DJ, Bryan ND, Livens FR, Charnock JM, Patrick RAD & Collison D (2003) Treatment of radioactive wastes: an X-ray absorption spectroscopy study of the reaction of technetium with green rust. *J. Coll. Inter. Sci.* 268: 408–412
- Plette ACC, Nederhof MM, Temminghoff EJM & Riemsdijk WHvan (1999) Bioavailability of heavy metals in terrestrial and aquatic systems: a quantitative approach. *Environ. Toxicol. Chem.* 18: 882–1890
- Porter SK, Scheckel KG, Impellitteri CA & Ryan JA (2004) Toxic metals in the environment: thermodynamic considerations for possible immobilization strategies for Pb, Cd, As, and Hg. *Crit. Rev. Environ. Sci. Technol.* 34: 495–604
- Powell BA, Fjeld RA, Kaplan DI, Coates JT & Serkiz SM (2004) Pu(V)O₂⁺ adsorption and reduction by synthetic magnetite (Fe₃O₄). *Environ. Sci. Technol.* 38: 6016–6024
- Prange A & Modrow H (2002) X-ray absorption spectroscopy and its application in biological, agricultural and environmental research. *Re/Views Environ. Sci. Bio/Technol.* 1: 259–276
- Qafoku NP, Zachara JM, Liu C, Gassman PL, Qafoku OS & Smith SC (2005) Kinetic desorption and sorption of U(VI) during reactive transport in a contaminated Hanford sediment. *Environ. Sci. Technol.* 39: 3157–3165
- Randall SR, Sherman DM & Vala Ragnarsdottir K (2001) Sorption of As(V) on green rust (Fe₄(II)Fe₂(III)(OH)₁₂SO₄·3H₂O) and lepidocrocite (γ-FeOOH): surface complexes from EXAFS spectroscopy. *Geochim. Cosmochim. Acta* 65: 1015–1023
- Redman AD, Macalady DL & Ahmann D (2002) Natural organic matter affects arsenic speciation and sorption onto hematite. *Environ. Sci. Technol.* 36: 2889–2896
- Rifkin E, Gwinn P & Bouwer E (2004) Chromium and sediment toxicity. *Environ. Sci. Technol.* 38: 267A–271A
- Roane TM, Miller RM & Pepper IL (1996) Microbial remediation of metals In: Crawford RL & Crawford DL (Eds) *Bioremediation: Principles and Applications* (pp 312–340). Cambridge University Press, United Kingdom
- Roden EE & Scheibe TD (2005) Conceptual and numerical model of uranium(VI) reductive immobilization in fractured subsurface sediments. *Chemosphere* 59: 617–628
- Roig MG, Manzano T, Diaz M, Pascual MJ, Paterson M & Kennedy JF (1995) Enzymically-enhanced extraction of uranium from biologically leached solutions. *Int. Biodeter. Biodegrad.* 35: 93–127
- Royer RA, Burgos WD, Fisher AS, Jeon B-H, Unz RF & Dempsey BA (2002) Enhancement of hematite bioreduction by natural organic matter. *Environ. Sci. Technol.* 36: 2897–2904

- Salomons W (1995) Environmental impact of metals derived from mining activities: processes, predictions, prevention. *J. Geochem. Expl.* 52: 5–23
- Sauvé S, Hendershot W & Allen HE (2000) Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden and organic matter. *Environ. Sci. Technol.* 34: 1125–1131
- Schmidt U (2003) Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. *J. Environ. Qual.* 32: 1939–1954
- Schwertmann U & Fechter H (1994) The formation of green rust and its transformation to lepidocrocite. *Clay Min.* 29: 87–92
- Shanker AK, Cervantes C, Loza-Tavera H & Avudainayagam S (2005) Chromium toxicity in plants. *Environ. Internat.* 31: 739–753
- Sharma PK, Balkwill DL, Frenkel A & Vairavamurthy MA (2000) A new *Klebsiella planticola* strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. *Appl. Environ. Microbiol.* 66: 3083–3087
- Sierra J & Renault P (1995) Oxygen consumption by soil microorganisms as affected by oxygen and carbon dioxide levels. *Appl. Soil Ecol.* 2: 175–184
- Simkiss K & Taylor MG (1995) Transport of metals across membranes. In: Tessier A & Turner DR (Eds) *Metal Speciation and Bioavailability in Aquatic Systems*. IUPAC Series on Analytical and Physical Chemistry of Environmental Systems (pp 2–44). Wiley, New York
- Smith WL & Gadd GM (2000) Reduction and precipitation of chromate by mixed culture sulphate-reducing bacterial biofilms. *J. Appl. Microbiol.* 88: 983–991.
- Sparks DL (2001) Elucidating the fundamental chemistry of soils: past and recent achievements and future frontier. *Geoderma* 100: 303–319
- Sposito G, Skipper NT, Sutton R, Park S-H, Soper AK & Greathouse JA (1999) Surface geochemistry of the clay minerals. *Proc. Natl. Acad. Sci. USA* 96: 3358–3364
- Stephen JR & Macnaughton SJ (1999) Developments in terrestrial bacterial remediation of metals. *Curr. Opin. Biotechnol.* 10: 230–233
- Stepniwska Z, Bucior K & Bennicelli RP (2004) The effects of MnO₂ on sorption and oxidation of Cr(III) by soils. *Geoderma* 122: 291–296
- Stumm W & Morgan JJ (1996) *Aquatic Chemistry*. Wiley, New York
- Sunda WG & Kieber DJ (1994) Oxidation of humic substances by manganese oxides yields low-molecular-weight organic substrates. *Nature* 367: 62–65
- Tabak HH, Lens PNL, van Hullebusch ED & Dejonghe W (2005) Developments in bioremediation of soils and sediments polluted with metal and radionuclides 1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport. *Reviews Environ. Sci. Bio/Technol.* 4: 115–156
- Tandy S, Bossart K, Mueller R, Ritschel J, Hauser L, Schulin R & Nowack B (2004) Extraction of heavy metals from soils using biodegradable chelating agents. *Environ. Sci. Technol.* 38: 937–944
- Templeton AS, Trainor TP, Spormann AM & Brown GE Jr (2003a) Selenium speciation and partitioning within *Burkholderia cepacia* biofilms formed on α -Al₂O₃ surfaces. *Geochim. Cosmochim. Acta* 67: 3547–3557
- Templeton DM, Ariese F, Cornelis R, Danielsson L-G, Muntau H, van Leeuwen HP & Lobinsky R (2000) Guidelines for terms related to chemical speciation and fractionation of elements: definitions, structural aspects, and methodological approaches. *Pure Appl. Chem.* 72: 1453–1470
- Templeton AS, Trainor TP, Spormann AM, Newville M, Sutton SR, Dohnalkova A, Gorby Y & Brown GE (2003b) Sorption versus biomineralization of Pb(II) within *Burkholderia cepacia* biofilms. *Environ. Sci. Technol.* 37: 300–307
- Tessier A, Fortin D, Belzile N, DeVitre RR & Leppard GG (1996) Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: narrowing the gap between field and laboratory measurements. *Geochim. Cosmochim. Acta* 60: 387–404
- Tibazarwa C, Corbisier P, Mench M, Bossus A, Solda P, Mergeay M, Wyns L & van der Lelie D (2001) A microbial biosensor to predict bioavailable nickel in soil and its transfer to plants. *Environ. Pollut.* 113: 19–26
- Toes ACM, Maas BA, Geelhoed JS, Kuenen JG & Muyzer G (2004) Interactions between microorganisms and heavy metals. In: Verstraete (Ed) *Proceedings of European Symposium on Environmental Biotechnology, ESEB 2004* (pp 55–59). Taylor and Francis Group, London
- Tokunaga TK, Wan J, Firestone MK, Hazen TC, Schwartz E, Sutton SR & Newville M (2001) Chromium diffusion and reduction in soil aggregates. *Environ. Sci. Technol.* 35: 3169–3174
- Tokunaga TK, Wan J, Pena J, Brodie EL, Firestone MK, Hazen TC, Sutton SR, Lanzirotti A & Newville M. (2005) Uranium reduction in sediments under diffusion-limited transport of organic carbon. *Environ. Sci. Technol.* in press
- Tokunaga TK, Wan J, Hazen TC, Schwartz E, Firestone MK, Sutton SR, Newville M, Olson KR, Lanzirotti A & Rao W (2003) Distribution of chromium contamination and microbial activity in soil aggregates. *J. Environ. Qual.* 32: 541–549
- Traina SJ & Laperche V (1999) Contaminant bioavailability in soils, sediments, and aquatic environments. *Proc. Natl. Acad. Sci. USA* 96: 3365–3371
- Tucker MD, Barton LL & Thomson BM (1997) Reduction and immobilisation of molybdenum by *Desulfovibrio desulfuricans*. *J. Environ. Qual.* 26: 1146–1152
- Tudoreanu L & Phillips CJC (2004) Modeling cadmium uptake and accumulation in plants. *Adv. Agron.* 84: 121–157
- Valls M & Lorenzo Vde (2002) Exploiting the genetic and biochemical capacities of bacteria for remediation of heavy metal pollution. *FEMS Microbiol. Rev.* 26: 327–338
- Van den Berg GA, Loch JPG & Winkels HJ (1998) Effect of fluctuating hydrological conditions on the mobility of heavy metals in soils of a freshwater estuary in the Netherlands. *Water Air Soil Pollut.* 102: 377–388
- Van Gestel CAM & Hensbergen PJ (1997) Interaction of Cd and Zn toxicity for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. *Environ. Toxicol. Chem.* 16: 1177–1186
- van Hullebusch ED, Zandvoort MH & Lens PNL (2003) Metal immobilisation by biofilms: mechanisms and analytical tools. *Re/Views Environ. Sci. Bio/Technol.* 2: 9–33
- Vassilev A, Schwitzguébel J-P, Thewys T, van der D Lelie & Vangronsveld J (2004) The use of plants for remediation of metal contaminated soils. *Sci. World J.* 4: 9–34
- Vignati DAL, Dworak T, Ferrari B, Koukal B, Loizeau J-L, Minouflet M, Camusso MI, Polesello S & Dominik J (2005) Assessment of the geochemical role of colloids and their impact

- on contaminant toxicity in freshwaters: an example from the Lambro-Po system (Italy). *Environ. Sci. Technol.* 39: 489–497
- Vorenhout M, van der Geest HG, van Marum D, Wattel K & Eijsackers HJP (2004) Automated and continuous redox potential measurements in soil. *J. Environ. Qual.* 33: 1562–1567
- Wagner-Döbler I (2003) Pilot plant for bioremediation of mercury-containing industrial wastewater. *Appl. Microbiol. Biotechnol.* 62: 124–133
- Wagner-Döbler I, Lünsdorf H, Lübbehüsen T, Canstein HF von & Li Y (2000) Structure and species composition of mercury-reducing biofilms. *Appl. Environ. Microbiol.* 66: 4559–4563
- Wan J, Tokunaga TK, Brodie E, Wang Z, Zheng Z, Herman D, Hazen TC, Firestone MK & Sutton SR (2005) Reoxidation of bioreduced uranium under reducing conditions. *Environ. Sci. Technol.* 39: 6162–6169
- Wang Q & Morse JW (1996) Pyrite formation under conditions approximating those in anoxic sediments I. Pathway and morphology. *Mar. Chem.* 52: 99–121
- Wang Y & Papenguth HW (2001) Kinetic modeling of microbially-driven redox chemistry of radionuclides in subsurface environments: coupling transport, microbial metabolism and geochemistry. *J. Contam. Hydrol.* 47: 297–309
- Warren LA & Haack EA (2001) Biogeochemical controls on metal behaviour in freshwater environments. *Earth Sci. Rev.* 54: 261–320
- Wegler K, McLaughlin MJ & Graham RD (2004) Effect of chloride in soil solution on the plant availability of biosolid-borne cadmium. *J. Environ. Qual.* 33: 496–504
- Weiss JV, Emerson D & Megonigal JP (2004) Geochemical control of microbial Fe(III) reduction potential in wetlands: comparison of the rhizosphere to non-rhizosphere soil. *FEMS Microbiol. Ecol.* 48: 89–100
- Wenzel WW, Bunkowski M, Puschenriter M & Horak O (2003) Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excludor plants on serpentine soil. *Environ. Pollut.* 123: 131–138
- White C & Gadd GM (1998) Accumulation and effects of cadmium on sulfate reducing bacterial biofilms. *Microbiology* 144: 1407–1415
- White C, Wilkinson SC & Gadd GM (1995) The role of microorganisms in biosorption of toxic metals and radionuclides. *Int. Biodeter. Biodegrad.* 35: 17–40
- White C, Sayer JA & Gadd GM (1997) Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiol. Rev.* 20: 503–516
- White C, Sharman AK & Gadd GM (1998) An integrated microbial process for the bioremediation of soil contaminated with toxic metals. *Nat. Biotechnol.* 16: 572–575
- White C & Gadd GM (2000) Copper accumulation by sulfate-reducing bacterial biofilms. *FEMS Microbiol. Lett.* 183: 313–318
- Wiatrowski HA & Barkay T (2005) Monitoring of microbial metal transformation in the environment. *Curr. Opin. Biotechnol.* 16: 261–268
- Wielinga B, Bostick B, Hansel CM, Rosenzweig RF & Fendorf S (2000) Inhibition of bacterially promoted uranium reduction: ferric (hydr)oxides as competitive electron acceptors. *Environ. Sci. Technol.* 34: 2190–2192
- Wilcke W & Kaupenjohann M (1998) Heavy metal distribution between soil aggregate core and surface fractions along gradients of deposition from the atmosphere. *Geoderma* 83: 55–66
- Wildung RE, Gorby YA, Krupka KM, Hess NJ, Li SW, Plymale AE, McKinley JP & Fredrickson JK (2000) Effect of electron donor and solution chemistry on products of dissimilatory reduction of technetium by *Shewanella putrefaciens*. *Appl. Environ. Microbiol.* 66: 2451–2460
- Wildung RE, Li SW, Murray CJ, Krupka KM, Xie Y, Hess NJ & Roden EE (2004) Technetium reduction in sediments of a shallow aquifer exhibiting dissimilatory iron reduction potential. *FEMS Microbiol. Ecol.* 49: 151–162
- Williams KH, Ntarlagiannis D, Slater LD, Dohnalkova A, Hubbard SS & Banfield JF (2005) Geophysical imaging of stimulated microbial biomineralization. *Environ. Sci. Technol.* 39: 7592–7600
- Wittbrodt PR & Palmer CD (1995) Reduction of Cr(VI) in the presence of excess soil fulvic acid. *Environ. Sci. Technol.* 29: 255–263
- Wolthers M, Charlet L, van der Linde PR, Rickard D & Weijden C.H.van Der (2005) Surface chemistry of disordered mackinawite (FeS). *Geochim. Cosmochim. Acta* 69: 3469–3481
- Wu L (2004) Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. *Ecotox. Environ. Safety* 57: 257–269
- Wu LH, Luo YM, Xing XR & Christie P (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agricult. Ecosyst. Environ.* 102: 307–318
- Zachara JM, Fredrickson JK, Smith SC & Gassman PL (2001) Solubilization of Fe(III) oxide-bound trace metals by a dissimilatory Fe(III) reducing bacterium. *Geochim. Cosmochim. Acta* 65: 75–93
- Zeng QR, Sauvé S, Allen HE & Hendershot WH (2005) Recycling EDTA solutions used to remediate metal-polluted soils. *Environ. Pollut.* 133: 225–231
- Zhilin DM, Schmitt-Kopplin P & Perminova IV (2004) Reduction of Cr(VI) by peat and coal humic substances. *Environ. Chem. Lett.* 2: 141–145
- Zingaro RA, Dufner DC, Murphy AP & Moody CD (1997) Reduction of oxoselenium anions by iron(II) hydroxide. *Environ. Internat.* 23: 299–304