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Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes

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Summary. Microbial activity in mixed wastes can have an appreciable effect on the dissolution or precipitation of toxic metals and radionuclides. Fundamental information on microbial dissolution and stabilization (immobilization) of toxic metals and radionuclides, in particular actinides and fission products, in nuclear wastes under various microbial process conditions, e.g., aerobic, denitrifying, iron-reducing, fermentative, sulfate-reducing, and methanogenic conditions is very limited. Microbial transformations of typical waste components such as metal oxides, metal coprecipitates, naturally occurring minerals, and metal organic complexes are reviewed. Such information can be useful in the development of 1) predictive models on the fate and long-term transport of toxic metals and radionuclides from waste disposal sites, and 2) biotechnological applications of waste treatment leading to volume reduction and stabilization as well as recovery and recycling of radionuclides and toxic metals.

Key words. Toxic metals; radionuclides; natural radioactive mineral deposits; metal oxides; carbonate complexes; organic complexes; coprecipitates; uranium; plutonium; low-level radioactive wastes; transuranic wastes; coal wastes.

Introduction

Microorganisms, which are ubiquitous throughout nature, have long been recognized for their ability to bring about transformations of organic and inorganic compounds. Such microbial processes have not been fully exploited in the treatment and management of nuclear and fossil-energy wastes, particularly in regulating the mobility of toxic metals and radionuclides or in the biodegradation of organic constituents to innocuous products. The contaminants in wastes may be present initially as soluble forms or they may be formed after disposal by chemical or microbiological processes. The organic compounds and inorganic elements (major and minor metals and radionuclides), depending upon their chemical forms, may react with each other to varying degrees in the waste. These include organic-inorganic complex formation, precipitation reactions, coprecipitation of metals and radionuclides with Fe- and Mn-oxides, and formation of minerals. In figure 1, the interactions between the organics, inorganics, radionuclides and microbes are depicted. Because of the complexity of the system, it is often difficult to elucidate clearly the exact mechanisms involved in the transformation of mixed wastes. Nevertheless, studies with pure or model compounds under defined conditions should provide information on the mechanisms involved in the transforma-

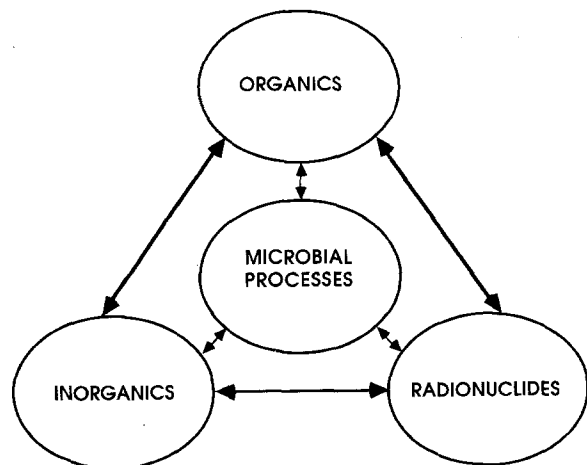


Figure 1. Interactions of organics, inorganics, radionuclides, and microbes in mixed wastes.

tions of such mixed wastes. There is a paucity of information on the fate and long-term transport of toxic metals and radionuclides present in energy-related wastes disposed of in subsurface environments. Of particular concern is the lack of information on specific microbial processes and the biochemical mechanisms involved in the dissolution (mobilization) or stabilization (immobi-

lization) of toxic metals and radionuclides in mixed wastes.

The microbial effects on low-level radioactive wastes disposed of in shallow-land burial grounds^{43,75} and the potential effects in the deep geological formations^{119,120} have been previously reviewed. A wide variety of microorganisms have been identified in coal wastes^{48,55,56,85,90,95} and in low-level radioactive wastes^{45,46} which can affect the integrity and the long-term stability of wastes. Leachate generation is a complex process involving physical, chemical, and biological actions. Microbial transformations of the waste at the disposal site may significantly influence the composition of leachates. Comprehensive information of the transformations of wastes under different microbial process conditions will be useful in developing predictive models on the fate and transport of toxic metals and radionuclides in the environment^{52,53,76}. It will also provide useful information for biotechnological applications in waste treatment such as recovery and recycling of elements, waste volume reduction and minimization, and waste stabilization as well as better waste management methods. The purpose of this paper is to review the relevant information available on the microbial processes involved in mobilization and immobilization of toxic metals and radionuclides present in mixed wastes.

1. Microbial processes

Mobilization and immobilization of radionuclides and toxic metals under aerobic conditions, by the activities of autotrophs in the case of inorganic wastes, and heterotrophs in mixed wastes containing organics, could be significant. Similarly, under anaerobic conditions heterotrophic microbial activity which is influenced by the presence and type of electron donors and acceptors in the waste can affect the mobility of radionuclides and toxic metals. Several of the key microbial processes which affect mobilization or immobilization of toxic metals and radionuclides under aerobic and anaerobic conditions are summarized in figure 2. Microorganisms under appropriate conditions bring about metal dissolution and mobilization or immobilization by one or more of the following mechanisms: 1) oxidation-reduction of metals which affect solubility; 2) changes in pH and Eh (which affect the valence or ionic state of the metals and enhance or retard their mobility in the subsurface environment); 3) solubilization and leaching of certain elements by microbial metabolites or decomposition products, alkylation, chelation or production of specific sequestering agents; and 4) immobilization leading to formation of stable minerals or biosorption by cells and biopolymers and, 5) release of biosorbed metals due to remineralization elsewhere in the environment.

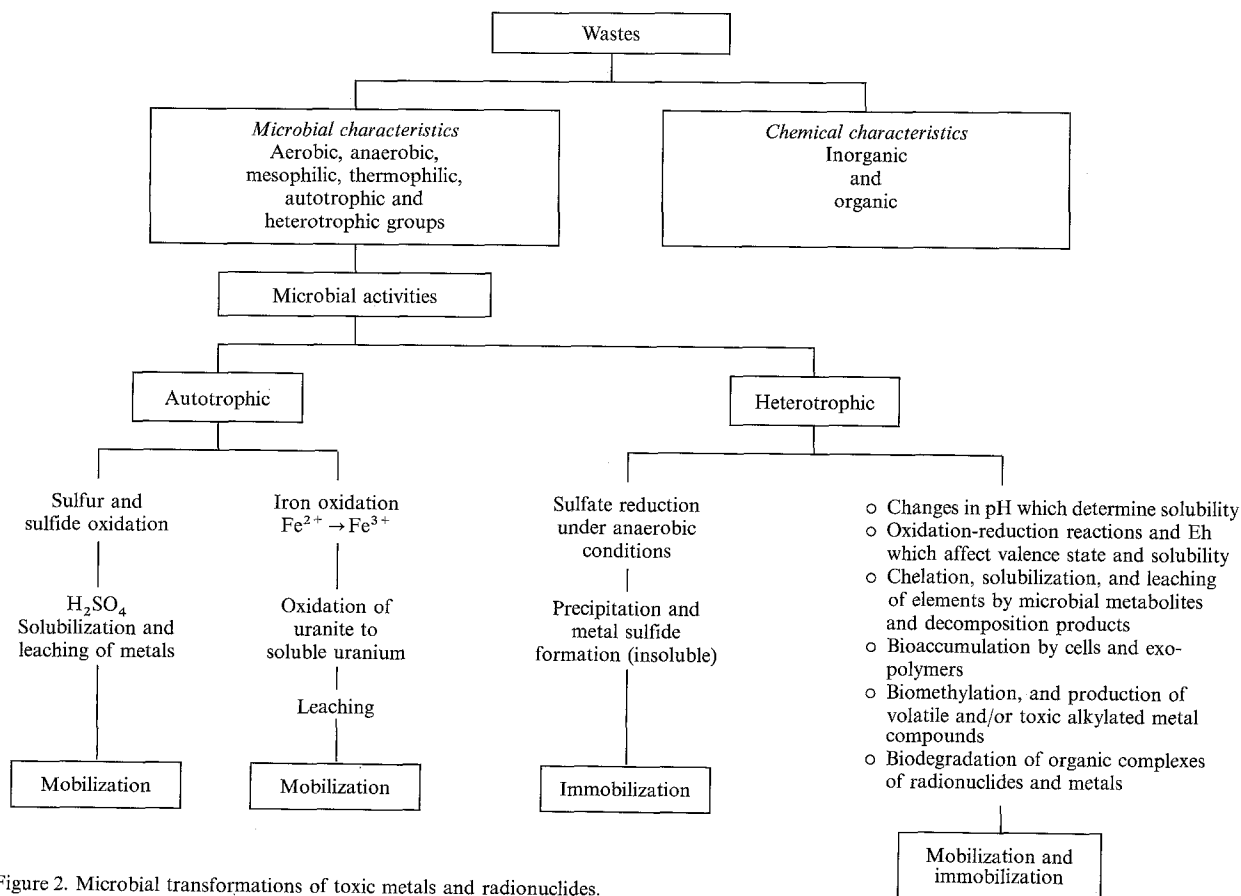


Figure 2. Microbial transformations of toxic metals and radionuclides.

The form in which the metal occurs (e.g., elemental, oxide, sulfide, ionic, inorganic complex, organic complex, or organometallics), the availability of electron donors and nutrients (carbon, nitrogen, phosphorus), the presence of alternate electron acceptors (Fe^{3+} , Mn^{4+} , NO_3^- , SO_4^{2-} organic compounds), and the environmental factors (pH, Eh, temperature, moisture) will affect the types, rates, and extent of microbial activity and microbial transformation of metals. The oxidizing and reducing conditions which prevail at the disposal sites to a large extent influence the mobilization and immobilization of radionuclides and metals. Eh levels have little direct influence on the mobility of the metal, but if the environment becomes reduced due to increased microbial activity, then certain metals can undergo reduction either chemically or enzymatically from higher oxidation state to lower oxidation state. For example, reduction of Fe^{3+} to Fe^{2+} increases the solubility of iron while reduction of U(VI) to U(IV) or Cr(VI) to Cr(III) decreases their solubility. Further, the mobility of soluble metal-organic complexes in the environment is dependent upon the biological stability of these complexes. Biodegradation of the metal organic complexes and subsequent release and fate of the metal are important but least understood^{21, 43, 44}.

Transformations of toxic metals and radionuclides in mixed wastes containing organic compounds by heterotrophic aerobic and anaerobic microorganisms could be significant. We have little information on either leachate production or the extent and mechanism of metal transformations due to the activities of the heterotrophs in mixed wastes. The presence of organic materials in low-

level radioactive waste is a major concern because they are responsible for most of the environmental contamination problems encountered in and around the disposal areas. Many of the organic compounds are capable of forming stable complexes with heavy metals and radionuclides or increasing their solubilization and leaching^{42, 43}. Likewise, microbial metabolites and waste degradation products or intermediates may be an important source of agents that affect the long-term solubility characteristics of heavy metals and radionuclides.

There are a few reported studies on microbial activity associated with the disposal of radioactive waste⁷⁵. For example, Francis et al.⁴⁵ studied the microbial activity of trench leachates collected from the commercial low-level radioactive waste disposal sites. Aerobic, anaerobic, denitrifying, methane-producing, and sulfate-reducing bacteria were detected in leachate samples (table 1). About 75 organic compounds consisting of several straight and branched chain aliphatic acids, aromatic acids, alcohols, aldehydes, ketones, amines, aromatic hydrocarbons, esters, ethers, and phenols were identified in the leachate samples⁴⁶. Some of the organic compounds were biodegraded under anaerobic conditions⁴⁴. At the shallowland, low-level radioactive waste disposal sites, in addition to synthetic chelating agents, the presence of solubilized organoradionuclide complexes, most probably of microbial origin, is a possible contributing factor to mobilization of radionuclides which might otherwise be expected to be precipitated or absorbed on clay and soil particles. Further, radioactive (tritiated and carbon-14) methane was produced by anaerobic microbial activity in the leachate⁴⁷. The levels of radioactivity and radio-

Table 1. Chemical and microbiological analyses of leachate samples collected from low-level radioactive waste disposal sites^{45, 122}

Sample	Chemical					Tritium (pCi/l)	Microbiological				
	pH	Eh (mV, NHE)	DOC (mg/l)	Gross alpha (pCi/l)	Gross beta (pCi/l)		Aerobes (CFU/ml)	Anaerobes (CFU/ml)	Denitrifiers (MPN/ml)	Sulfate- reducers (MPN/ml)	Methano- gens (MPN/ml)
Maxey Flats (5/78)											
Trench 19S	6.9	25	500	1.7×10^5	6.4×10^5	6.8×10^7	2.2×10^2	3.2×10^2	3.2×10^2	4.0×10^0	4.9×10^0
Well UB1-A	6.6	274	8	< 10	3.1×10^2	5.8×10^6	3.4×10^3	ND	4.6×10^2	ND	1.0×10^0
West Valley (10/78)											
Trench 3	7.3	-3.4	1700	7.3×10^2	2.9×10^6	4.8×10^8	5.4×10^3	4.0×10^3	1.3×10^4	7.0×10^1	2.3×10^1
Trench 4	6.5	54	630	1.4×10^3	1.7×10^7	3.0×10^8	2.3×10^3	3.3×10^3	2.3×10^3	4.9×10^2	1.7×10^0
Trench 5	6.7	40	2900	8.9×10^2	4.7×10^5	2.3×10^9	1.6×10^3	3.5×10^2	3.3×10^2	1.1×10^1	ND
Trench 8	6.9	-6.3	2900	1.3×10^5	4.7×10^5	3.7×10^9	1.4×10^3	7.6×10^2	1.7×10^2	1.0×10^0	1.0×10^0
Trench 9	6.7	18	1700	1.2×10^5	1.2×10^5	4.6×10^8	5.0×10^2	7.3×10^3	1.3×10^2	3.5×10^2	4.5×10^0
Barnwell (3/79)											
Trench 8D2	NA	308	170	< 9	7.9×10^2	1.0×10^2	2.0×10^8	1.0×10^4	2.3×10^5	1.1×10^0	0.8×10^0
Trench 6D1	5.9	358	2	1.6×10^1	< 28	5.7×10^5	2.3×10^3	1.3×10^2	1.1×10^3	ND	ND
Trench 25/21-D1	5.9	538	12	< 14	1.0×10^2	3.7×10^5	3.5×10^4	1.2×10^3	1.3×10^4	1.3×10^2	0.2×10^0
Trench 3D1	5.8	225	7	< 14	< 28	1.2×10^4	1.5×10^5	1.2×10^3	5.4×10^4	ND	ND
Sheffield (4/79)											
Trench 14A	5.0	143	100	8.6×10^2	5.6×10^4	6.2×10^5	1.7×10^5	4.4×10^4	2.4×10^5	ND	0.2×10^0
Trench 18	6.8	181	50	< 9	4.5×10^2	2.0×10^5	7.1×10^2	6.9×10^1	9.5×10^2	4.9×10^1	ND
Well 525	7.5	1.9	3	< 14	< 28	$< 6.2 \times 10^2$	6.3×10^2	4.2×10^2	1.7×10^3	2.3×10^0	ND

ND, not detected; NA, not analyzed. The radionuclides detected in the leachate include: ^{90}Sr , ^{238}Pu , $^{239,240}\text{Pu}$, ^{241}Am , ^{60}Co , ^{134}Cs , ^{137}Cs , ^{60}Co . Field measurements of redox potential (Eh) are reported relative to the normal hydrogen electrode.

nuclides present in the leachate samples did not have any significant effect on bacteria. These bacteria were able to bioaccumulate ^{60}Co , ^{134}Cs , ^{137}Cs , and ^{90}Sr to varying degree^{43, 45}.

Scientific information on the microbially catalyzed transformations (dissolution and immobilization) of radionuclides and metals in wastes in the subsurface environments is, however, very limited.

2. Dissolution of metals and radionuclides by autotrophs under aerobic conditions

Much of the information on the microbial dissolution of toxic metals deals with oxidation of inorganic compounds, primarily from ore leaching by autotrophic microorganisms under aerobic conditions. The responsible microorganisms, the chemical and the biochemical mechanism involved in the microbial leaching or biomining of metals, have been extensively studied^{18, 32, 64, 83, 108, 128}. Consequently, such microbiological processes are now being exploited on a commercial scale for extraction of copper and uranium from ores and recovery of strategic metals from wastes.

In many respects, the microorganisms and the basic principles involved in bioleaching would be operative in wastes containing inorganic compounds⁶⁹. In general, solubilization and leaching of metals from ores are principally brought about by the activities of autotrophic iron and sulfur-oxidizing bacteria. Bacterial oxidation of pyrite and metal sulfide minerals by *T. ferrooxidans* and *T. thiooxidans* has been extensively studied^{32, 128}. Torma and Sakaguchi¹¹² investigated the oxidation of several metal sulfides by *T. ferrooxidans* and found that the rate of metal dissolution was directly proportional to the initial surface area of the substrates. The highest rate was obtained with the substrate having the highest solubility product and they reported the following order of the rate of metal dissolution $\text{NiS} > \text{CoS} > \text{ZnS} > \text{CdS} > \text{CuS} > \text{Cu}_2\text{S}$.

Inorganic components in coal and nuclear wastes, including uranium mill tailings, may undergo a series of biochemical and chemical reactions which result in the solubilization of many minerals. This dissolution process may be brought about by direct or indirect bacterial action. Direct bacterial action entails an enzymatic attack by the bacteria on components of the mineral that are susceptible to oxidation. In the process of obtaining energy from the inorganic material, the bacteria cause electrons to be transferred from iron or sulfur to oxygen. In many cases, the more oxidized product is more soluble. On the other hand, indirect leaching is the oxidation of soluble ferrous iron to ferric iron which is a powerful oxidizing agent that reacts with other metals, transforming them into the soluble oxidized form in a sulfuric acid solution. In this reaction, ferrous iron is again produced and is reoxidized by the bacteria. Bacteria (*T. ferrooxidans*) can accelerate such an oxidation reaction by a fac-

tor of more than a million¹⁸. In nature, both direct and indirect leaching by bacteria take place.

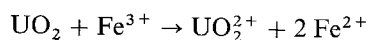
2.1 Dissolution of metals from coal waste

Microbial dissolution of toxic metals from two types of coal wastes, one high in pyrite and trace metals and low in organic carbon (fines fraction), and a second lower in trace metals and higher in organic carbon (filter cake) were investigated³⁸. Under aerobic conditions, native autotrophic bacteria solubilized varying amounts of As, Cr, Cu, Mn, Ni, Pb, and Zn from the filter cake and fines fraction. Dissolution of the above metals was increased by severalfold when the inorganic nutrients N and P were supplemented. Selective chemical extraction analyses indicated that most toxic metals were associated with pyrite, ferric oxides and a soluble phase, possibly ferric sulfate. The predominant mechanism of dissolution of metals from coal wastes under aerobic conditions was due to bacterial oxidation of pyrite. In a similar study, As, Cr, Cu, Mn, Ni and Zn were solubilized from core samples collected from a reclaimed coal mine site in West Virginia by aerobic autotrophic microbial activity. The concentration of solubilized metals varied with depth of the core sampled (Francis et al., unpublished results). Reduced sulfur (0.3 to 4.1) present in the ash/slag of the coal gasification waste were readily oxidized by autotrophic microorganisms but the extent of metal dissolution from the waste was not investigated¹⁰⁷.

Anaerobically or microaerophilically, *Sulfolobus* and *T. ferrooxidans* can oxidize sulfur with the coupled reduction of ferric to ferrous iron as an alternative to the reduction of oxygen. No anaerobic iron-oxidizing bacteria have yet been found. The chemoautotrophic thiobacilli, the facultative *T. denitrificans*, can oxidize sulfide, sulfite and thiosulfate anaerobically with nitrate as the electron acceptor. In leach dumps, the internal temperature rises as a result of bacterial and chemical mineral oxidation to between 54 °C and 80 °C.

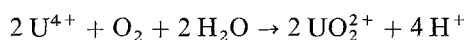
2.2 Dissolution of uranium

The iron and sulfur oxidizing bacteria play a significant role in the solubilization of uranium from ores, in mill tailings and coal wastes. The biogeochemistry of uranium has been intensively studied in light of the recovery of U from ores and bacterial leaching of pyritic uranium ore is both feasible and economical^{17, 32, 37}. The role of *Thiobacillus ferrooxidans* in the extraction of uranium from ore is primarily indirect and confined to the generation of the oxidizing agent, ferric sulfate, and the solvent sulfuric acid. The indirect involvement of $\text{Fe}^{2+}/\text{Fe}^3$ in cyclically mediating the oxidation of the insoluble uranium oxide (UO_2) has been documented:



It has now been demonstrated that *T. ferrooxidans* can directly oxidize reduced compounds of uranium (uranous sulfate and UO_2) to its hexavalent form without the

involvement of extraneous $\text{Fe}^{3+}/\text{Fe}^{2+}$ complex as the chemical electron carrier^{29, 116}.



In these studies, uranium sulfate which is soluble as tetravalent uranium was used as a substrate.

Jayram et al.⁵⁸ investigated the possible role of the microflora in the mobilization of uranium from rocks and its subsequent immobilization. The presence of bacteria (2.3×10^6 cells/g dry wt), higher radon values, elemental sulfur, soluble sulfates, and high leachable uranium led the authors to conclude that uranium was mobilized from the carbonaceous phyllites and was redeposited. Iron and sulfur oxidizing bacteria *T. ferrooxidans* and *T. thiooxidans* were isolated from several uranium ores^{10, 66}, and it has been suggested that microbial oxidation of pyrite is involved in the mobilization of U from the mine at Pocos de Caldas, Brazil¹²¹. The role of microorganisms in the solubilization of thorium is not known.

3. Dissolution of metals and radionuclides by heterotrophs under aerobic conditions

An increase in heterotrophic microbial activity due to biodegradation of organic constituents of the waste can affect the mobilization of toxic metals and radionuclides. Heterotrophic bacteria and fungi under aerobic and anaerobic conditions are able to release metals from various materials including copper-nickel concentrates, low-grade copper ore, uranium from granites, manganese ore and potassium from leucite. Several mechanisms for heterotrophic aerobic microbial solubilization of insoluble metals have been proposed. These include organic acid production³⁰, formation of chelating agents¹⁰⁰, and metabolism of the metal-associated anion⁹⁹. Leaching by heterotrophic organisms is entirely due to chemical reaction of excreted microbial metabolites and decomposition products¹³. In many cases, a combination effect is important, for example, when the organism secretes organic acids which may have a dual effect of increasing metal dissolution by lowering pH and increasing the load of soluble metal by complexation. Heterotrophic leaching could occur in an acid environment (pH 2–4) because of organic acid production or in an alkaline environment with no acid production.

3.1 Organic acid metabolites and complexing agents

Complexes of radionuclides and toxic metals may also be formed before waste burial as a result of laboratory procedures, process operation, decontamination activities due to microbial action, or at the burial site when species interact with buried or soil-derived organic matter. Microbially generated dicarboxylic acids, ketogluconic acid, polyhydroxy acids, and phenolic compounds such as protocatechuic acid, and salicylic acid are effective chelating agents of heavy metals and are known to accel-

erate the movement of metals in soils^{30, 96}. Cole²⁷ reported that several heterotrophic bacteria under aerobic conditions solubilized PbS, ZnS, CdS, and CuS but had no activity against PbSO₄, PbO, or Pb₃O₄. However, the solubilizing agent produced in the culture media was not identified. Bolter et al.¹⁴ found that organic acids from decaying leaf litter in soil increased the solubility of heavy metals deposited from smelters. Complexation of cadmium by organic components of sanitary landfill leachates was attributed to low- and high-molecular-weight compounds representing simple carboxylic acids and compounds containing hydroxyl groups⁶⁵. Wildung et al.¹²⁴ reported that many fungi produced low-molecular-weight ligands and extracellular complexes that substantially increased the solubility of Ni in soil. Similarly, Chanmugathas and Bollag²⁵ found that mobilization of strongly bound or fixed Cd was due to microbially mediated processes and was affected by soil environmental factors. Extraction of Zn from industrial waste by citric acid produced by a *Penicillium* sp. has been reported, suggesting use of such systems for recovery of metals from wastes⁹².

Several heterotrophic bacteria *Bacillus* sp., *B. luteus*, *B. subtilis*, *B. cereus*, *B. pumilis*, *Pseudomonas striata*, *P. viscosa*, *P. perolens*, *P. chlororaphis*, *Achromobacter xerosis*, *A. stoloniferum*, *A. healii*, and *Desulfovibrio desulfuricans* were identified in uranium ores¹⁰. Heterotrophic microorganisms are able to solubilize uranium from rock materials (granitic rock) where uranium is generally present as oxide^{6, 11}. Such solubilization is due to production of organic acid metabolites. Munier-Lamy⁸² has shown that microbial solubilization of uranium from rocks (syenite and aplite) was due to the formation of soluble low-molecular-weight organo-uranyl compounds. The metal-organic complexes were formed under aerobic (Eh + 300 to + 500 mv), acidic (pH 2.5–5) conditions by an association of metallic cations (UO_2^{2+} , Cu^{2+} , Fe^{2+} , Fe^{3+} , Al^{3+}) with simple organic acids, i.e., oxalic, isocitric, citric, succinic, hydroxybenzoic, and coumaric acids via their carboxylic and phenolic groups.

In addition to the use of synthetic chelating agents, complexing agents are also found in decomposing organic matter introduced as waste, and soil organic matter. The soil organic ligands humic and fulvic acids, and decomposing organic wastes contain complex mixtures composed of oxygenated organic degradation products. Humic substances have an appreciable exchange capacity due primarily to carboxyl and phenolic hydroxyl groups, and can form stable water soluble and insoluble complexes with metals. Soil organics are generally composed of amino acids, polysaccharides, polyfunctional aromatic compounds, and porphyrins. The most abundant and well-characterized fraction of soil fulvic acid is composed of a mixture of organic acids such as formic, acetic, benzoic, 3-hydroxy-5-methylbenzoic, p-hydroxybenzoic, protocatechuic, vanillic, gallic, propionic, citric, fumaric, malic, oxaloacetic, butyric, glycolic, lactic, and tartaric

acids^{77,93}. These compounds are generally excellent bi- and polydentate chelating ligands for a variety of metals.

3.2 *Siderophores and other specific chelators*

Specific chelating agents are produced by microorganisms that require iron or other essential metals for growth. When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators such as siderochrome and siderophore in the medium^{28,91}. Siderophore production by soil bacteria in soils has been reported^{67,87,94}. Since a variety of microbial types are active in the waste and waste leachate, it is possible that these microorganisms may elaborate specific or non-specific compounds capable of complexation with the radionuclides. Iron sequestering agents could play an important role in the complexation of Pu and other metals and thus increase their bioavailability. For example, dissolution of plutonium dioxide was enhanced in the presence of Desferol, a polyhydroxamate chelate produced by microorganisms³. Wildung and Garland¹²³ found that microorganisms grown in the presence of Pu produced complexing agents of higher molecular weight than that of diethylenetriaminepentaacetic acid (DTPA). Many of the cultures tested were capable of transporting Pu into the cell, and the role of complexing agents with such a transport has been suggested but was not identified^{5,123}. Likewise, *Pseudomonas aeruginosa* isolated from a plutonium-contaminated pond⁵⁹ known to bioaccumulate uranium¹⁰⁵ elaborated several new chelating agents for thorium and uranium when grown in the presence of these metals^{88,89}. This clearly indicates the potential of U, Th and Pu and other metals present in the waste for complexation by microbially produced chelating agents, and thus an increased bioavailability and mobilization of radionuclides and toxic metals due to microbial activity. Additional information on microbial complexing agents is discussed by Birch and Bachofen, this issue.

In addition to the metabolites, microbial exopolymers may form strong complexes with metals and radionuclides. Microbial polysaccharides which are present in biofilms often play a major role in the removal of metals from waste streams and in some instances form soluble complexes and enhance mobilization. The persistence of complexing agents in the disposal environment is a major concern because of the potential of increasing the transport and bioavailability of radionuclides and toxic metals.

4. *Dissolution of metals and radionuclides under anaerobic conditions*

Anaerobic conditions are common at most of the waste disposal sites and anaerobic microbial activities may significantly affect the transport and transformations of organic compounds, radionuclides, and toxic metals. There is a paucity of information on the anaerobic micro-

bial transformations of toxic metals and radionuclides under various process conditions such as denitrification, iron reduction, fermentation, sulfate-reduction, and methanogenesis. The behavior of radionuclides and toxic metals is significantly affected by anaerobic bacteria in organic matter-rich flooded soils and sediments, but little is known of the processes and mechanisms involved, in particular, of the range and nature of bacterial effects in anoxic environments^{12,57,62,63,86,126}. Increased solubilization of heavy metals from soils and sludges incubated under anaerobic conditions has been reported^{12,63}. Under anaerobic conditions, fermentation products such as organic acids and amino acids accumulate and may serve as ligands.

The predominant forms of toxic metals and radionuclides found in low-level radioactive and fossil-energy wastes can be divided into the following general categories: oxides (simple and complex oxides including ferrites), coprecipitates (metals coprecipitated with oxides of iron, aluminum), carbonate complexes, naturally occurring minerals, and organic and inorganic complexes. There is, however, very limited information on the specific characteristics of wastes disposed of in the subsurface. The available information on the anaerobic microbial transformations of the various forms of the metals and radionuclides is summarized in the following sections.

4.1 *Dissolution of lead compounds*

Lead is present in nuclear and coal wastes¹⁰⁹, and is also released into the atmosphere from fossil-fuel combustion and from lead smelters. Although the speciation of lead is not fully known, it is believed to be in the oxidized form, most probably as lead oxide, because of the oxidizing conditions. It has been estimated that 20% of the emitted lead is in the oxide form⁸⁴. Lead oxide is quite insoluble in water and the fate of PbO in wastes is not fully understood. Bruland et al.²⁰ and Burrows and Hulbert²³ noted that contaminated sediments released a greater fraction of their lead content under anaerobic conditions than unpolluted sediments. Wong et al.¹²⁵ reported that microorganisms in sediments from several Canadian lakes could transform certain inorganic and organic lead compounds into tetramethyl lead. Kee and Bloomfield⁶² found that lead dioxide (PbO₂) was mobilized from soils under anaerobic conditions when amended with plant residues. Francis and Dodge⁴¹ reported that the anaerobic bacterium *Clostridium* sp., isolated from coal cleaning waste, solubilized a significant amount of lead oxide (PbO) and to a lesser extent PbSO₄ but not Pb⁰, PbS, and galena. Dissolution of PbO by the bacteria was due to production of organic acids and lowering of pH of the growth medium. The solubilized metal was bioavailable to the organism as evidenced by lead associated with cell biomass as well as immobilization by a polymer-like substance produced by the organism. The solubilized lead affected the growth of *Clostrid-*

ium sp., as well as the metabolic end products of glucose fermentation shifting from acetic and butyric acid fermentation to predominantly lactic acid production. Soluble lead in the culture medium interfered with the iron metabolism of the organism, possibly affecting the synthesis of iron-containing proteins involved in the electron transport system⁴⁰.

4.2 Dissolution of metal oxides

Oxides of metals are present in soils, ores, and residues generated from fossil- and nuclear-fuel cycles. The metal oxides are usually in crystalline forms and are insoluble in water; their fate after disposal in the environment is not known. Organic compounds present in the energy residues and the natural environment can have a significant effect on the solubility and mobility of the metal oxides due to chemical and microbiological action.

Under anaerobic conditions, organic compounds can bring about reductive dissolution of metal oxides from a higher to a lower oxidation state^{101, 104}. This reduction has a dramatic impact on the solubility and speciation of metals. For example, oxides of Mn(III, IV), Fe(III), Co(III), and Ni(III), when reduced to divalent ions under anoxic conditions, show an increase in solubility by several orders of magnitude^{101, 102}. Humic substances, catechols, hydroxyquinones, methoxyphenols, resorcinols, ascorbate, pyruvic acid, oxalic acid, amines, anilines, and other naturally occurring organic compounds, including microbial metabolites, have been shown to have redox reactivity^{101-103, 110, 117}. Dissolution of cobalt, copper, lead, nickel, and zinc oxides by organic compounds from decomposing plant materials has been reported⁶².

Microorganisms also play an important role in the dissolution of metal oxides by direct or indirect action. Direct action involves enzymatic reductive dissolution of the metal oxide, wherein the oxide is used as the terminal electron acceptor; whereas indirect action involves dissolution due to production of metabolites, such as organic acids and chelating agents, and lowering of the pH of the medium. Microbial reduction and dissolution of iron and manganese oxides under anaerobic conditions have been extensively studied⁵¹. Most studies deal with the microbial dissolution of amorphous forms of oxyhydroxides of Fe and Mn, and studies with crystalline forms have been very limited^{60, 68, 80, 81, 115}. Anaerobic microbial dissolution of several crystalline, water-insoluble forms of metal oxides are summarized in table 2. An anaerobic N₂-fixing *Clostridium* sp. solubilized Fe₂O₃ and MnO₂ by direct enzymatic reduction; CdO, CuO, PbO, and ZnO were solubilized by indirect action due to the production of metabolites and the lowering of the pH of the growth medium. Although Mn₂O₃, Ni₂O₃, and PbO₂ may undergo reductive dissolution from a higher to a lower oxidation state, dissolution by direct or indirect action was not observed. Also, Cr₂O₃ and NiO were not solubilized by direct or indirect action. Significant amounts of

Table 2. Mechanism of dissolution of metal oxides by *Clostridium* sp.³⁹

Metal oxide	Oxidation state of metal used	Mechanism of action ^a Expected	Observed
CdO	2 ⁺	Indirect	Indirect
Co ₂ O ₃	3 ⁺	Direct (Co ³⁺ → Co ²⁺)	?
Cr ₂ O ₃	3 ⁺	Indirect	None
CuO	2 ⁺	Indirect	Indirect
Fe ₂ O ₃	3 ⁺	Direct (Fe ³⁺ → Fe ²⁺)	Direct
MnO ₂	4 ⁺	Direct (Mn ⁴⁺ → Mn ²⁺)	Direct
Mn ₂ O ₃	3 ⁺	Direct (Mn ³⁺ → Mn ²⁺)	None
NiO	2 ⁺	Indirect	None
Ni ₂ O ₃	3 ⁺	Direct (Ni ³⁺ → Ni ²⁺)	None
PbO	2 ⁺	Indirect	Indirect
PbO ₂	4 ⁺	Direct (Pb ⁴⁺ → Pb ²⁺)	None
ZnO	2 ⁺	Indirect	Indirect

^aIndirect, dissolution due to microbial metabolites or lowering of pH of medium; direct, enzymatic reductive dissolution of metals from higher oxidation state or lower oxidation state; none, no significant dissolution of metals detected.

solubilized Cd, Cu, and Pb were immobilized by the bacterial biomass.

4.3 Dissolution of metals coprecipitated with iron oxides

Iron oxides scavenge transition and heavy metals in soils, sediments, and energy wastes. Sorption and coprecipitation are the predominant processes by which most of the metals are retained by iron oxide. Toxic metals such as As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, U, and Zn from fossil- and nuclear-fuel cycle waste streams, geothermal fluids, and electroplating wastes are removed by coprecipitation with ferric iron. The removal of toxic metals from waste streams by coprecipitation with iron seems to be a very efficient and economically feasible method, but problems remain with the disposal of the coprecipitated metals in the environment. Significant dissolution of metals from the coprecipitate can be brought about by chemical and microbiological action. In general, solubility of iron oxide depends upon the degree of crystallinity. Amorphous iron oxides are orders of magnitude more soluble than a well-crystallized goethite or hematite. Metals Cd, Cr, Ni, Pb, and Zn coprecipitated with goethite (α -FeOOH) were solubilized by *Clostridium* sp.⁴⁹. Dissolution of the metals was caused by a) direct enzymatic reduction of ferric iron and the release of metals associated with iron, and b) indirect action due to the production of metabolites. The extent of dissolution depended upon the nature of the association of the metals with goethite. In the presence of bacteria, substantial amounts of Cd and Zn which were closely associated with iron were released due to reduction of ferric iron. The dissolution of Ni was due to direct and indirect actions, while a small amount of Cr was solubilized only by direct action. Lead was solubilized predominantly by indirect action. In the presence of bacteria, the concentration of Pb decreased due to biosorption. Silver and Ritcey⁹⁸ studied the effects of sulfur-oxidizing bacteria on the release of ²²⁶Ra²⁺ from uranium mine mill wastes which contained 0.72% pyrite. They showed

that bacterial oxidation of the pyrite increased the amount of sulfate and decreased the amount of $^{226}\text{Ra}^{2+}$ in the effluent. At many uranium mining and milling sites, soluble radium is removed as a coprecipitate with BaSO_4 by the addition of BaCl_2 to sulfate-rich tailing effluents. The resulting $(\text{Ba,Ra})\text{SO}_4$ precipitate is allowed to settle, yielding a supernatant which is sufficiently low in $^{226}\text{Ra}^{2+}$ for discharge to the environment and a radioactive sludge. The disposal of radioactive sludges must ensure that $^{226}\text{Ra}^{2+}$ does not leach into groundwaters because the stabilized radioactive wastes may be transformed into mobile compounds due to microbial activity. For example, radium coprecipitated with barium sulfate was solubilized by sulfate reducing bacteria under anaerobic conditions³³. Bolze et al.¹⁵ showed that under anaerobic conditions, BaSO_4 can be dissolved by microbial action. High Ba^{2+} concentrations in aquifers have been attributed to sulfate reduction by *Desulfovibrio vulgaris*⁷⁴. McCready et al.⁷³ prepared a $(\text{BaRa})\text{SO}_4$ sludge and showed that *D. vulgaris* could release H_2S , $^{226}\text{Ra}^{2+}$, and Ba^{2+} from the sludge. Studies of $(\text{Ba,Ra})\text{SO}_4$ sludges from two Canadian uranium mine and mill sites showed that with the addition of usable carbon, SO_4^{2-} was reduced to S^{2-} with a concurrent release of $^{226}\text{Ra}^{2+}$, Ba^{2+} , and Ca^{2+} due to enhanced anaerobic microbial activity. Levels of dissolved $^{226}\text{Ra}^{2+}$ reached approximately 400 Bq/l after 10 weeks of incubation. These results suggest that the ultimate disposal of these sludges must maintain conditions to minimize the activity of the indigenous sulfate-reducing bacteria to ensure that $^{226}\text{Ra}^{2+}$ is not released to the environment.

4.4 Dissolution of metals coal wastes

The extent of dissolution of metals from coal waste under aerobic and anaerobic conditions by the indigenous microflora is shown in figure 3. The metals As, Cd, Cr, Cu, Mn, Ni, Pb and Zn were associated with carbonates, Fe-Mn oxides, coal-organics, pyrite, and silicates to varying degrees. The mechanisms of dissolution of metals under aerobic conditions by autotrophic bacteria

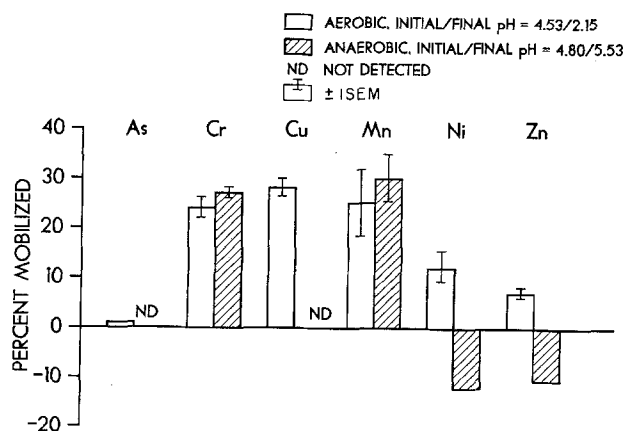


Figure 3. Microbial mobilization and immobilization of toxic metals from coal waste.

have been discussed in the previous section. Under anaerobic conditions, concentrations of Fe, Cr, and Mn increased due to native anaerobic bacterial activity from filter cake amended with carbon and nitrogen. Concentrations of soluble Ni and Zn in filter cake decreased due to sulfate-reduction and formation of insoluble metal sulfides. Also, dissolution of As, Cu, and Pb was not observed under anaerobic conditions. Similarly, core samples collected from a reclaimed coal mine site in West Virginia showed that, anaerobic microorganisms present in the core samples solubilized Fe^{2+} , Cr, Mn, and Zn. Addition of C and N to the cores enhanced the rate of dissolution of these metals (Francis et al., unpublished results). Under anaerobic conditions, dissolution of metals was due to the direct bacterial reduction of iron and manganese oxides and the release of trace metals coprecipitated with the oxides³⁸.

5. Immobilization of toxic metals and radionuclides

Immobilization of toxic metals and radionuclides in wastes and waste streams by microbial action is primarily due to bioaccumulation by microbial cells and biopolymers, reduction and/or precipitation of metals, formation of insoluble metal sulfides and minerals. There is a vast body of literature available on this topic and only the relevant information is summarized in this section. Of the various inorganic and organic metal complexing agents such as hydrous metal oxides, clays, and humic substances, microorganisms and their constituent polymers are among the most efficient scavengers of metallic ions. Bioaccumulation by microorganisms has been reported for such metals as lead^{1, 41, 113}, silver²⁶, platinum, palladium, gold, mercury²⁴, gallium⁷⁹, cadmium, copper, nickel by bacteria, fungi and algae; and radionuclides such as ^{60}Co , ^{137}Cs , ^{85}Sr ^{43, 54}, uranium^{97, 105, 106, 114}, thorium¹¹⁴, radium¹⁰⁶, americium⁵⁰, and plutonium^{5, 59}. The potential use of microorganisms in metals and radionuclide removal from waste process streams has been described^{19, 97, 106}. Such a biotreatment process has not received much attention at the commercial scale, most probably because of higher operating costs.

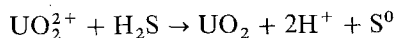
Microbial biofilms are not only capable of binding significant quantities of metallic ions under natural conditions but they also serve as templates for the precipitation of insoluble mineral phases. Ferris et al.³⁶ reported that Fe, Mn, Ni, Cu and Co were concentrated in biofilms and that the extracellular polymers often contained iron oxide precipitate resembling ferrihydrite ($\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$). The mechanisms and the biochemistry of the interactions of metal ions with the bacterial cell walls, extracellular biopolymers, and microfossil formations as means of immobilization of toxic metals, are described in detail by Beveridge and coworkers^{7-9, 34, 35}.

The microflora in the waste disposal sites and contaminated areas can indeed preferentially bioaccumulate cer-

tain radionuclides and toxic metals⁴³. It is possible then that these microorganisms, when transported by water and in the subsurface, may deposit the contaminants elsewhere in the environment upon cell lysis. Microbial transport processes in the subsurface environments are receiving much attention in light of the in situ bioremediation of contaminated aquifers.

Reduction of an element from higher oxidation state to lower oxidation state or to elemental forms affects the solubility of the metals. For example, conversion of selenate, selenite, tellurate, tellurite, to elemental forms, vanadate to a vanadyl compound, molybdate and molybdenum trioxide to a molybdenum blue compound, arsenate to arsenite, mercuric chloride to elemental mercury, chromate ions to chromic ions which precipitates at neutral pH, pentavalent and trivalent bismuth to elemental form, lead dioxide to divalent state, osmium tetroxide to osmate ion; osmium dioxide and trivalent osmium to the metal and uranyl uranium to tetravalent state by microbes have been reported^{4, 16, 127}.

Microbial reduction of sulfate in wastes containing toxic metals and radionuclides may result in the formation of metal sulfides. In general, most of the metal sulfides exhibit low solubility in aqueous medium. Reduction of uranium (UO_2^{2+} to UO_2) under anaerobic conditions by sulfate-reducing bacteria has been reported³¹.



West et al.¹²¹ noted that immobilization of uranium was due to reprecipitation of uranium in the reducing front of a uranium mine in Pocos de Caldas, Brazil. They attributed this to the activities of sulfate-reducers. Whether iron reducers play a role in the reduction of uranium is not known. The important parameters for the solubility of uranium species are Eh, pH, temperature, pressure, and uranium concentration. A change in redox potential can result in a change in valence of uranium. Percolation of uranium mine discharge water through soil lowered selenium, uranium, molybdenum and sulfate concentrations in the mine waters by the activities of *Clostridium* sp. and sulfate-reducing bacteria. The hydrogen sulfide produced by sulfate-reduction by *Desulfovibrio* reacted with uranyl and molybdate ions to form insoluble uranium and molybdenum species⁶¹. Uranium mill effluents containing toxic amounts of Mn^{2+} and ^{226}Ra were treated with *Arthrobacter* sp., which precipitated Mn^{2+} as hydrous oxide of manganese and along with it, ^{226}Ra was coprecipitated. About 92% of Mn^{2+} and 95% of ^{226}Ra were removed from the effluent by this biogenic treatment⁷². However, Mn and ^{226}Ra can be remobilized due to bacterial reduction of Mn^{4+} under anaerobic conditions.

Francis et al.³⁸ investigated anaerobic microbial transformations of uranium and toxic metals present in depleted uranium wastes. In addition to the high concentrations of uranium (3000 ppm), the wastes contained high levels of toxic metals As, Cd, Cr, Cu, Hg, Pb, Ni and Zn.

A substantial amount of uranium, associated with the exchangeable carbonate and iron-oxide fractions, was released into the medium due to enhanced indigenous anaerobic microbial activity, but little uranium was detected in solution. The authors suggest that the uranium released from exchangeable, carbonate, and iron oxide fractions was subsequently immobilized due to a) biosorption by bacterial biomass, and b) reduction and precipitation of uranium under reducing conditions brought about by microbial action. Under reducing conditions, U(VI) can be reduced to insoluble U(IV) by chemical reduction or by microbial enzymatic action. The mechanisms of chemical reduction of U(VI) have been documented in the literature, while microbial enzymatic reduction of U(VI) has not been clearly established.

6. Biodegradation of organic complexes of radionuclides and metals

Chelating agents are widely used in the nuclear industry for decontamination of reactors and equipment, and in cleanup operations. Several metal complexing agents, along with solvents such as decalin, diisopropylbenzene, are used in the separation chemistry of the radionuclides and metals. Examples of the types of complexing agents used are polyphosphates, mono-, di-, and tributyl-phosphates, amino carboxylic acids such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), and N-hydroxyethylenediaminetriacetic acid (HEDTA), dihexyl-N,N-diethylcarbonyl methylene phosphonate (DHDECMP), and carboxylic acids such as citric, hydroxy-acetic, oxalic, and tartaric acids. Although these multidentate chelates are effective in the decontamination of radionuclides by complexation, by virtue of the chemical reaction, they increase the solubility of the metals, are easily transported in the environment, and are readily bioavailable.

In general, information on microbial degradation of chelating agents is scarce. Many of these chelates either are poorly biodegraded under aerobic conditions or are found to undergo little biodegradation under anaerobic conditions^{22, 78, 111, 118}. Leachates from sanitary landfills incubated aerobically and anaerobically with clay loam, calcareous clay loam, and CaCO_3 , showed a significant reduction in chelation potential only in samples incubated under aerobic conditions. The chelating agents which had accumulated in the landfill as by-products of anaerobic fermentation seem to be stable in anaerobic systems, while under aerobic conditions relatively rapid decomposition of these compounds occurred². The degradation of citrate complexes of Cd^{2+} , Cu^{2+} , Mg^{2+} and Zn^{2+} by a *Klebsiella* sp. at naturally occurring low carbon (10 $\mu\text{g/l}$) concentrations was investigated by Brynhildsen and Rosswall²¹. A marked inhibition was observed when Cd^{2+} , Cu^{2+} or Zn^{2+} was bound to the organic anion. These results suggest that metals com-

plexed with low-molecular weight organic acids, such as citric acid, are persistent in the environment.

In addition to the organic acids, many of the microbial polymers form strong complexes with metals and radionuclides. Microbial polysaccharides which are present in biofilms often play a major role in the removal of metals from waste stream and in some instances form soluble complexes and enhance mobilization. Degradation of microbial polysaccharides has been studied under aerobic conditions⁷¹. The rate of decomposition of the metal-polysaccharide complexes or salts and the bacterial polysaccharide were determined in surface soils under aerobic conditions. Some or all of the metals exerted marked effects on the decomposition rate and percent decomposition of the polysaccharide tested. Specific effects of the metals depended on the polysaccharide. Zinc and Al exerted little influence on decomposition of *Azotobacter chroococcum* polysaccharide, for example, while Cu and Fe reduced decomposition by about 50%⁷⁰. Since these compounds can be utilized by microorganisms as carbon source and recycled, the metals associated with the polymers may also be affected.

Degradation of metal complexing agents may result in precipitation of the released ion as water-insoluble hydroxides, oxides, or salts, thereby retarding metal migration. The chemical and biological stability of the synthetic (decontamination agents), naturally occurring and microbiologically synthesized complexing agents of radionuclides and toxic metals are among the critical factors which determine the mobility of the radionuclides from the burial environment into the biosphere. Lack of such information often complicates studies that deal with prediction of soil retention characteristics of radionuclides.

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