Review Article

Phosphate-Mediated Remediation of Metals and Radionuclides

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Worldwide industrialization activities create vast amounts of organic and inorganic waste streams that frequently result in significant soil and groundwater contamination. Metals and radionuclides are of particular concern due to their mobility and long-term persistence in aquatic and terrestrial environments. As the global population increases, the demand for safe, contaminant-free soil and groundwater will increase as will the need for effective and inexpensive remediation strategies. Remediation strategies that include physical and chemical methods (i.e., abiotic) or biological activities have been shown to impede the migration of radionuclide and metal contaminants within soil and groundwater. However, abiotic remediation methods are often too costly owing to the quantities and volumes of soils and/or groundwater requiring treatment. The *in situ* sequestration of metals and radionuclides mediated by biological activities associated with microbial phosphorus metabolism is a promising and less costly addition to our existing remediation methods. This review highlights the current strategies for abiotic and microbial phosphate-mediated techniques for uranium and metal remediation.

1. Introduction

The global population is predicted to reach 10 billion by the year 2100 [1]. To support the demand for increased food production and access to fresh water, human societies will be forced to employ less desirable (i.e., lower quality) soil and groundwater resources for crop production and drinking water [1–3]. Human activities associated with 20th century industrial-scale production of electrical components, fabrics, fertilizers, inks and dyes, mining, metal production, paints, paper products, pesticides, pharmaceuticals, rubber, and plastics contribute to the degradation of surface and subsurface sediments and water quality as evidenced by the production of more than 1 million metric tons of metal waste per year [4, 5]. Governmental activities have also contributed to the contamination of soils and groundwater throughout the United States, where the legacy of nuclear weapons research and development has resulted in the contamination of estimated 75 million cubic meters of sediment and more than 1.8 billion cubic meters of groundwater [6]. The devastating 2011 earthquake of the coast of Japan and the subsequent tsunami that destroyed three nuclear reactors at the Fukushima Daiichi Nuclear Power Plant complex highlight a

more recent challenge to remediation and disposal of large quantities of nuclear fuel materials including radionuclides. The scope of the remediation challenge is considerable, as deleterious effects to human health, surrounding environment, and food supply are well known due to metal and radionuclide exposure and ingestion [7–10]. Moreover, the presence of these contaminants negatively impacts ecosystem sustainability and contribute to the loss of biodiversity [11].

Lead (Pb), cadmium (Cd), and zinc (Zn) represent a subset of the most frequently reported metal contaminants in sediments and groundwater. These metals, many of which cooccur at the same site, are listed on the US EPA National Priorities List and are detected at many US EPA Superfund sites [12]. Uranium (U) waste, resulting from U.S. nuclear weapons production, is found in soils and groundwater at the Department of Energy (DOE) facilities [13] and in coal and phosphate mining/processing waste sites [14–19]. The fate and transport of metals and radionuclides in the environment are controlled by geochemical factors, including pH, adsorption, reduction/oxidation, and precipitation reactions. In natural environments, pH is one of the primary controlling variables for metal and radionuclide speciation [20]. Below pH ~5, most metals and radionuclides tend to exist primarily as

free divalent cations and as solid oxyhydroxides, carbonates, and oxides above pH 7 (Figures 1(a) and 1(b)). In the presence of phosphate, precipitation reactions control metal speciation through the formation of highly insoluble metal- and radionuclide-phosphate minerals that are stable over a wide pH range (Figures 2(a) and 2(b)). Though less studied to date than other methods, remediation approaches that promote phosphate immobilization of metals and radionuclides represent viable strategies for long-term *in situ* sequestration.

Strategies for the remediation of metal and radionuclide-contaminated soils and groundwater include physical and chemical (i.e., abiotic) and biologically mediated methods. Chemical and physical methods, including excavation and soil capping [21, 22], pump and treat technologies [23, 24], mineral adsorption [25–27], mineral precipitation [28, 29], complexation [30–32], adsorption to permeable zero-valent iron and hydroxyapatite reactive barriers [33–35], cement solidification [36, 37], and vitrification [38, 39], have all demonstrated efficacy in mitigating contaminant transport *in situ*. Remediation methods that depend upon chemical transformations for *in situ* sequestration of contaminants must first consider local geochemical parameters that include local geology, concentrations of soluble anions and cations, pH, and redox state. The influence of pH on a contaminated groundwater system (Figures 1(a) and 1(b)) highlights the importance of such considerations in order to predict contaminants speciation and bioavailability [40–44].

In addition to the chemically mediated methods for contaminant immobilization, bioremediation has demonstrated great promise as an additional strategy to promote *in situ* sequestration of contaminants [11, 45–48] by harnessing the metabolisms of plants and microorganisms to detoxify and/or affect the *in situ* mobility of a given contaminant. The use of prokaryotic and eukaryotic microorganisms has proven effective for metal and radionuclide remediation through the processes of biosorption, bioaccumulation, bioreduction, and biomineralization [49–61]. Recently, it has been shown that microorganisms, which can hydrolyze organophosphate compounds with a concomitant increase in the liberation of extracellular orthophosphate to the surrounding environment, represent a unique approach to promote subsurface *in situ* phosphate biomineralization of metals and radionuclides. The stability of phosphate minerals over a broad pH range (Figures 2(a) and 2(b)) provides an ideal insoluble phase for long-term contaminant sequestration within subsurface environments that experience changing local geochemistry (e.g., Eh, pH, oxidants).

The application of various phosphate compounds (e.g., orthophosphate solutions, soluble polyphosphates, and organophosphates) to immobilize contaminants (e.g., Cd, Pb, U, and Zn) in laboratory as well as field experiments detected contaminant sequestration via a combination of microbial-mediated mechanisms and abiotic reactions. The focus of this review will be on the chemical (abiotic) and microbial mechanisms promoting phosphate-mediated immobilization of uranium and cooccurring metals (Figure 3) within subsurface environments as well as highlighting the unique challenges that dynamic geochemical conditions have on long-term *in situ* sequestration strategies.

2. Chemical Approaches to Phosphate Immobilization of Metals and Radionuclides

The chemistry of phosphate is such that it facilitates reactions with over 30 elements and has resulted in the identification of 300 unique phosphate minerals [62]. On Earth, 95% of all phosphorus (P) is present within apatite minerals $[\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{OH}, \text{Cl})]$ [63, 64] and the biogeochemical interactions of this mineral not only play a role in global P cycling but also influence the mobility of soluble metals [65]. The use of orthophosphate solutions has been shown to immobilize contaminants (e.g., Cd, Pb, U, and Zn) via mineral precipitation over a broad pH range (Figures 2(a) and 2(b)) [66–69]. Additionally, phosphate minerals promote cocontaminant sequestration via mineral substitution reactions, coprecipitation reactions, adsorption, and ion-exchange reactions and provide a source of orthophosphate for dissolution-precipitation reactions [34, 70–76]. Various soluble and insoluble P compounds (e.g., orthophosphate solutions, soluble polyphosphate, phosphatidic clays, apatite reactive barriers, and vivianite) have demonstrated success in metal and radionuclide sequestration [34, 68, 77–85].

2.1. Soluble Phosphate Amendments. Soluble P, in the forms of phosphoric acid, phytic acid, and triphosphate, has been examined for the sequestration of Cd, Cu, Pb, U, and Zn in contaminated environments [78, 82, 84, 86–88]. Phosphoric acid and inorganic phosphate salts are examples of simple forms of reactive orthophosphate effective in forming stable minerals in surface soils (depth < 12.5 cm) contaminated with Cd, Cu, Pb, and Zn [87–89]. The use of orthophosphate solutions within surface soils and deep subsurface environments requires strategies that prevent (1) rapid precipitation of contaminants and other sediment components (e.g., Al, Ca, Fe, Mg, and Mn) that affects hydraulic conductivity, (2) leaching of metals (e.g., As, Se, and W), and (3) ecosystem eutrophication caused by excess P runoff [82, 89–91].

Compounds such as phytic acid (i.e., the acid form of inositol-6-phosphate) and polyphosphate have been examined for their ability to (1) act as sites for ion exchange, (2) facilitate the slow delivery of orthophosphate, and (3) act as chelating agents that minimize the bioavailability of cations within contaminated environments [92–95]. The use of these compounds will ideally minimize undesirable phosphate-sediment interactions at the site of delivery, allow for greater mobility beyond the point of injection within subsurface sediments, and promote greater sequestration of metals and/or radionuclides when compared to injections of orthophosphate-rich solutions.

Laboratory studies examining the immobilization of various cations (i.e., Ba, Co, Mn, Ni, Pb, U, and Zn) within contaminated sediments have shown phytic acid to be effective in reducing soluble contaminant concentrations. Specifically, concentrations of U(VI) were reduced from $2,242 \text{ g kg}^{-1}$ to 76 g kg^{-1} and of Ni from 58 mg kg^{-1} to 9.6 mg kg^{-1} in sediment batch incubations treated with calcium phytate (i.e., the salt of phytic acid) [92, 93]. However, the solubility of these metal-phytate complexes is highly dependent upon

FIGURE 1: Influence of changing pH on chemical speciation of (a) U(VI) and (b) metal mixture composed of Cd(II), Pb(II), and Zn(II). Calculated using MINEQL + v. 4.5 and the Nuclear Energy Agency's updated thermodynamic database for uranium [106, 107] as a function of pH in synthetic groundwater (SGW). The open system model at 22°C calculated aqueous (dotted lines) and solid phases (solid lines) at equilibrium using the concentrations of ions present in SGW, $\text{UO}_2^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{Cd}^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{Pb}^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{Zn}^{2+}(\text{aq}) = 500 \mu\text{M}$, and $P_{\text{CO}_2} = 10^{-3.5}$ atm. Chemical speciation calculations utilized SGW that consists of 2 μM FeSO_4 , 5 μM MnCl_2 , 8 μM Na_2MoO_4 , 0.8 mM MgSO_4 , 7.5 mM NaNO_3 , 0.4 mM KCl , 7.5 mM KNO_3 , and 0.2 mM $\text{Ca}(\text{NO}_3)_2$ [57].

FIGURE 2: Influence of soluble phosphate and changing pH on chemical speciation of (a) U(VI) and (b) metal mixture composed of Cd(II), Pb(II), and Zn(II). Calculated using MINEQL + v. 4.5 and the Nuclear Energy Agency's updated thermodynamic database for uranium [106, 107] as a function of pH in synthetic groundwater (SGW). The open system model at 22°C calculated aqueous (dotted lines) and solid phases (solid lines) at equilibrium using the concentrations of ions present in SGW, $\text{UO}_2^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{Cd}^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{Pb}^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{Zn}^{2+}(\text{aq}) = 500 \mu\text{M}$, $\text{PO}_4^{3-}(\text{aq}) = 1500 \mu\text{M}$, and $P_{\text{CO}_2} = 10^{-3.5}$ atm. Chemical speciation calculations utilized SGW that consists of 2 μM FeSO_4 , 5 μM MnCl_2 , 8 μM Na_2MoO_4 , 0.8 mM MgSO_4 , 7.5 mM NaNO_3 , 0.4 mM KCl , 7.5 mM KNO_3 , and 0.2 mM $\text{Ca}(\text{NO}_3)_2$ [57].

ionic strength, pH, ligand conformation, and metal-to-ligand ratio [96], which limits the efficacy of phytic acid for metal and radionuclide immobilization in dynamic geochemical conditions. More recent studies of phytic acid, sodium monophosphate, and sodium triphosphate (TPP) inter-

actions with U(VI) conducted in column experiments investigated sediments with basic porewater pH and geochemical conditions representative of metal- and radionuclide-contaminated environments in the western U.S. [82, 84]. In column studies, without soluble U, cation interactions

FIGURE 3: Flow-chart summary of chemical- and microbial-mediated reactions with phosphate that promote immobilization of metal and radionuclide contaminants within subsurface environments.

with phytic acid and sodium monophosphate resulted in a 30% decrease in hydraulic conductivity [82]. Conversely, TPP amendments did not affect hydraulic conductivity and allowed for a slow release of orthophosphate within the sediment column [82]. Within columns containing over 100 mg L^{-1} soluble U, TPP amendments were capable of promoting rapid precipitation of U, thereby decreasing U concentrations below U.S. EPA drinking water standards ($30 \mu\text{g L}^{-1}$) [84]. The use of TPP, rather than phytic acid or sodium monophosphate, demonstrates great promise within neutral-to-alkaline sediments as an agent for metal and radionuclide sequestration.

2.2. Solid Reactive Phosphate Amendments. An alternative to introducing soluble orthophosphate into contaminated sediment and groundwater is the application of apatite [$\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{OH}, \text{Cl})$] minerals (e.g., bone apatite, synthetic apatite minerals, and rock phosphate) as subsurface reactive barriers [97–102]. Numerous studies have demonstrated that *in situ* soluble metal and radionuclide concentrations can be greatly reduced by surface interactions, dissolution-precipitation reactions, and ion-exchange with apatite minerals [34, 70, 73, 103, 104]. Apatite minerals have been effective in decreasing soluble concentrations (i.e., a 1,000-fold reduction) of contaminants that include Cd, Co, Cu, Hg, Mn, Ni, Pb, Sb, Th, and U [73, 79, 105]. Apatite reactive barriers have been effective in decreasing soluble metal and radionuclide concentrations *in situ*; however, the reversibility of cation adsorption and possible changes to hydraulic conductivity must be considered with this approach [34, 80, 81, 100]. Thus, the use of apatite requires continuous monitoring of the barrier effluent under dynamic geochemical conditions (i.e., changing contaminant concentration and pH) to maintain optimal contaminant sequestration and to calculate barrier lifetime [80, 100].

Recent studies have demonstrated promising results in the use of phosphate mineral nanoparticles for the remediation of Cu, Cd, and Pb contaminants [83, 108, 109]. The

use of hydroxyapatite and vivianite [$\text{Fe}_3(\text{PO}_4)_2$] nanoparticles in the size range of 3–10 nm provides properties of both liquid and solid phosphate amendments by facilitating liquid injection of particles that have a high surface area. Additionally, the use of nanoparticles offers an approach that maximizes contaminant immobilization by minimizing (1) orthophosphate loss to runoff, (2) precipitation reactions at the injection site, and (3) leaching of oxyanions. The unique application of phosphate mineral phases in the nanoparticle size range not only offers an effective delivery system to promote contaminant adsorption, precipitation, and/or ion-exchange reactions, but also provides a source of P to deep subsurface microbial communities capable of catalyzing reactions that further contribute to contaminant sequestration. Due to the lack of studies that examine nanoparticle fate and transport as well as the difficulties in monitoring this class of material in the environment, the potential hazards posed to prokaryotes, plants, and animals must be evaluated prior to their implementation in any remediation strategies [110–112].

3. Biological Approaches to Phosphate-Mediated Immobilization of Metals and Radionuclides

Phosphorus in the form of inorganic phosphate (P_i) is essential for cellular energy conservation and proper structure/function of cellular macromolecules (i.e., nucleic acids, proteins, sugars, and lipids) in all living organisms [113]. Due to the essential requirement for P, the scarcity of this nutrient within terrestrial environments (i.e., average of $1 \mu\text{M}$ [114]) requires plants, fungi, and bacteria to employ P-scavenging strategies. Bacterial acquisition of P can occur through secretion of organic acids that solubilize phosphate minerals via expression of organophosphate hydrolases [115–124]. Conversely, microbial stress (i.e., low nutrients, low pH, and oxidizing agents) results in storage of P as intracellular polyphosphate [125–127]. The following sections will specifically examine microbial polyphosphate metabolism

and organophosphate hydrolase activity as they relate to metal and radionuclide immobilization.

3.1. Polyphosphate-Mediated Bioaccumulation. The biologically mediated polymerization of orthophosphate molecules as intracellular polyphosphate granules is among the most ancestral metabolic functions present in all domains of life [125]. In bacteria, this polymer has been shown to function as an energy source, P reserve, metal chelator, and a regulator of stress and development [125, 127]. Polyphosphate metabolism in *E. coli* and other bacterial species is driven by the genes polyphosphate kinase (*ppk*) and exopolyphosphatase (*ppx*) that catalyze intracellular inorganic phosphate polymerization and hydrolysis of polyphosphate granules, respectively [125]. Phenotypic analyses of *ppk* knock-out mutations have expanded the known functions of polyphosphates that contribute to virulence, motility, biofilm formation, and sensitivity to stressors (e.g., oxidative, heat, osmotic, pH, and nutrient) [125–127]. Within metal and radionuclide contaminated environments, low nutrient availability, high concentrations of oxidizing agents, and extremes in pH typify chemical stressors that pose challenges to microbial metabolism. In response to the local geochemistry of contaminated environments, production of intracellular polyphosphate provides microorganisms with a means to sequester toxic ions within the cell cytosol as well as the regulation of gene(s) expressed in response to cellular stress (e.g., DNA repair, RNA polymerase sigma factor, and pH extremes) [55, 127–131].

The reactivity of cytosolic polyphosphates has been shown to facilitate intracellular sequestration of Cd, Cu, Hg, Pb, U, and Zn in genetically engineered bacterial strains as well as naturally occurring archaeal and bacterial strains [52, 55, 132–136]. Electron microscopy analyses of cells exposed to these elements demonstrated intracellular localization with phosphate-rich granules, suggesting that contaminant sequestration may be achieved by polyphosphates and may protect sensitive cytosolic molecules from oxidative damage. In addition to polyphosphate chelation of metals and radionuclides, an engineered *Pseudomonas aeruginosa* strain overexpressing the *ppk* gene was shown to enhance intracellular phosphate concentrations when compared to the wild-type strain [133]. Upon nutrient starvation, polyphosphate depolymerization and efflux of phosphate into the media containing U(VI) promoted the removal of 80% of the soluble U(VI) as a uranyl phosphate precipitate. Thus, polyphosphate metabolism that promotes intracellular or extracellular sequestration of metals and radionuclides represents a remediation approach that harnesses the physiologies of extant microorganisms within contaminated environments.

3.2. Phosphatase-Mediated Biomineralization. Redox-independent biomineralization of metals and radionuclides, although not as extensively studied as reductive precipitation to date, could provide a complementary approach to the existing remediation strategies. Biogenically derived phosphate minerals result from the activities of microbial phosphatases, enzymes that are essential for microbial acquisition of C and P, regulation of cellular metabolism, and signal transduction [137–139]. Phosphatase enzymes are classified by pH

optima (acid or alkaline), molecular weight, and those that hydrolyze only phosphorylated serine or threonine residues [140]. Acid and alkaline phosphatases (either cytoplasmic or periplasmic) are fundamentally required for microbial nutrient acquisition [116, 119, 141–143].

Early work examining bacteria capable of phosphate-mediated biomineralization of metals and radionuclides focused on the acid phosphatase activity of *Serratia* sp. NCIMB 40259 (formerly *Citrobacter* species) [50, 144, 145]. Several studies that characterized Class A NSAP of *Serratia* sp. NCIMB 40259 reported that microorganisms with similar phosphatase activities could be exploited to promote mineralization of metals [50, 144–146]. Additionally, biofilms of *Serratia* sp. NCIMB 40259 that promoted the precipitation of $\text{H}_2(\text{UO}_2)_2(\text{PO}_4)_2$ (chernikovite) further removed 85% and 97% of cooccurring ^{60}Co and ^{137}Cs , respectively, via substitution of H^+ within chernikovite [147].

Prior to investigations into a possible bioremediation role for nonspecific acid phosphatases (NSAP), this class of enzymes had been examined for their role in microbial physiology and contributions to virulence [116, 148]. Although the exact physiological roles for these enzymes have yet to be discerned, the biochemical properties of NSAPs have been well studied. NSAPs are divided into three unique classes (Classes A, B, and C) based on catalytic domain motifs, comprised of low molecular weight monomeric subunits ranging from 25 to 30 kDa, and have a pH optima ranging from 5.0 to 6.5 and catalytic activities for a broad range of phosphomonoester substrates [116]. NSAPs can be localized to the outer membrane, periplasmic space and/or are secreted into the extracellular environment. As the physiological properties of bacterial NSAPs facilitate their activities at low pH conditions typical of many mixed waste sites, their contributions to *in situ* metal detoxification and metal immobilization activities may be greatly underappreciated.

Within mixed waste environments, the *in situ* activities of microbial phosphatases likely contribute to localized cation precipitation in addition to nutrient acquisition. Studies examining phosphatase activities (e.g., alkaline or acid) of genetically engineered and naturally occurring strains of Gram-positive and Gram-negative bacteria were shown to promote U immobilization (>90% precipitation of soluble U) via precipitation and coprecipitation reactions [50, 57, 60, 74, 149–153]. Interestingly, soil bacterial isolates demonstrated constitutive phosphatase activities that liberated comparable, if not greater concentrations, of reactive phosphate when compared to the phosphatase activities of genetically modified strains [57, 149]. The occurrence of naturally occurring bacteria with constitutive phosphate-liberating NSAP phenotypes isolated from radionuclide and metal contaminated subsurface soils supports the role of acid phosphatases in promoting microbial adaptation to *in situ* metal stresses.

To further highlight such adaptation, recent investigations of terrestrial and marine bacterial isolates belonging to the genera *Aeromonas*, *Bacillus*, *Myxococcus*, *Pantoea*, *Pseudomonas*, *Rahnella*, and *Vibrio* demonstrated that Cr, Pb, and U were removed from solution as phosphate minerals under both oxic and anoxic growth conditions [57, 60, 74, 152, 154–158]. Our recent work further examined lead and

uranium precipitates produced by *Rahnella* sp. Y9602, using X-ray diffraction (XRD), variable pressure scanning electron microscopy/energy dispersive X-ray spectroscopy (VP-SEM/EDX), and extended X-ray absorption fine structure (EXAFS). Hydrated lead precipitates that accumulated on the cell surface of *Rahnella* sp. Y9602 were identified as lead hydroxyapatite, $\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$ by XRD analysis (data unpublished). VP-SEM/EDX elemental mapping demonstrated U and P localization in precipitates generated by *Rahnella* sp. Y9602 grown in minimal media containing soluble U(VI). Subsequent synchrotron-based XRD and EXAFS analyses of these uranium phosphate precipitates identified the mineral as chernikovite $[\text{H}_2(\text{UO}_2)_2(\text{PO}_4)_2]$ [60].

4. Challenges for *In Situ* Immobilization of Metals and Radionuclides

The goal of metal and/or radionuclide remediation strategies that focus on *in situ* sequestration is to generate an insoluble precipitate that will be immobilized, sequestered, and stable within a given environment for effective long-term stewardship. The difficulty in maintaining precipitates in the solid phase arises from the dynamic nature of biogeochemical processes in the environment.

Approaches that employ reductive precipitation of metals and radionuclides through the use of zero-valent iron reactive barriers, mineral phase interactions, and microbial reduction activities face challenges for long-term sequestration that arise from oxidant (e.g., oxygen, nitrate, manganese, iron oxides, or humic substances) interactions that remobilize precipitated reduced contaminants to their oxidized valence states, thereby increasing their solubility [19, 45, 159–168]. Limitations to microbial reduction of metals and radionuclide also arise from low pH environments that must be neutralized to support growth of sulfate- and metal-reducing communities [163, 169, 170]. Additionally, microbial reduction in mixed waste environments must be carefully considered so as to minimize contaminant migration of elements such as As and Pu that demonstrate greater mobility in their reduced valence states [171, 172].

In contrast, the geochemical stability of insoluble metal- and radionuclide-phosphates allows for *in situ* sequestration within environments that undergo dynamic changes in redox conditions and pH. Such changes, in local geochemistry, however, do not support stable sequestration of metals and radionuclides in their reduced forms. Within uranium contaminated environments, phosphate mineralization of soluble U(VI) produces a wide array of uranyl phosphate minerals [173]. The formation of these minerals has been implicated in the control of U mobility in U.S. DOE contaminated sediments [174, 175], although the microbial contribution to *in situ* uranyl phosphate formation in these DOE sites has yet to be determined. With recent studies identifying autunite- and hydroxyapatite-precipitating capabilities of *Aeromonas*, *Bacillus*, *Pantoea*, *Pseudomonas*, and *Rahnella* spp. in both oxic and anoxic growth conditions, the synergistic properties of these minerals (i.e., ion-exchange reactions that sequester cooccurring metals) highlight an important role in not only stabilizing U contamination but also cooccurring metals [57,

60, 74, 157]. Furthermore, contaminated sites that are characterized by acidic to circumneutral porewater pH represent environments that can support stable mineral formation (Figures 2(a) and 2(b)), provided that carbonates are not present in significant concentrations (i.e., $\text{P}_{\text{CO}_2} < 10^{-3.5} \text{ atm}$) [176, 177]. Interestingly, investigations of microbial reduction of Cr, Np, Pu, and U have been shown to support subsequent phosphate precipitation reactions via thermodynamic modeling, chromatographic separation of actinides based on valence state, and X-ray analytical methods [154, 155, 172, 178, 179]. Unlike U, that is capable of forming phosphate minerals in both hexavalent and tetravalent states [50, 179], the reduction of Cr, Np, and Pu is initially required for these contaminants to participate in phosphate precipitation reactions [154, 155, 172, 178]. To date, only pure culture or coculture studies have identified such coupled microbial interactions that offer an additional approach to control contaminant toxicity and mobility that are perpetuated by valence state cycling. Due to the limited number of studies, future work is required to further understand proto cooperative interactions between extant subsurface metal reducing and phosphate solubilizing microbial communities that promote contaminant sequestration. Additionally, further examination of reduced valence state contaminants precipitated as phosphate minerals are required to understand the influence of changing geochemical parameters (e.g., Eh, pH, and oxidants) that can affect solubility of the *in situ* immobilized contaminants.

Overall, these studies highlight the need to consider contaminant physicochemical properties, redox state of the environment, pH, presence of complexing ligands, and the metabolic properties of the extant microbial community when developing an *in situ* sequestration strategy.

5. Summary, Challenges, and Future Directions

Prior to anthropogenic releases of metals and radionuclides into the environment, these elements had been (and continue to be) discharged into the environment through volcanic activity, hydrothermal vent sources, and the dissolution of metal-bearing minerals [180]. Prokaryotic and eukaryotic organisms play key roles in geochemical cycling through metabolic processes that scavenge, mobilize, and precipitate these elements in terrestrial, aquatic, and atmospheric environments [180–182]. The evolution of prokaryotic and eukaryotic organisms that influence the solubility of metal- and radionuclide-bearing minerals while tolerating changing concentrations of these elements has given rise to microbial metabolic diversity beneficial to passive and active environmental remediation efforts.

Passive remediation approaches such as monitored natural attenuation (MNA) and *in situ* sequestration of metals and radionuclides offer economical alternatives that minimize human exposure to contaminants. Regardless of the type of implemented remediation approach, successful *in situ* sequestration of metals and radionuclides requires the contaminant(s) remain immobilized as an insoluble species. Implementation of MNA of metals and radionuclides relies on natural physical, chemical, and biological processes to

FIGURE 4: Subsurface hydrobiogeochemical conceptual model. Important chemical and microbial mediated processes that control metal speciation and contaminant transport within subsurface sediments include (a) Fe-hydroxide surface (clays) adsorption; (b) sediment organic matter adsorption; (c) microbial detoxification mechanisms contributing to microbial survival, adaptation, and contaminant immobilization. Microbial metabolic processes that increase local concentrations of anions (X^{n-} ; e.g., PO_4^{3-} , S^{2-} , and CO_3^{2-}) promote inorganic contaminant (M^{n+}) mineralization [$MX_{(s)}$].

remediate a contaminated environment through the effects of dispersion, dilution, sorption, volatilization, radioactive decay, stabilization, and transformation [183]. Prior to adopting MNA as a sole strategy for metal and radionuclide remediation, the processes by which the contaminant(s) are immobilized must be shown to be irreversible [183]. Unfortunately, this approach relies on limited contaminant plume mobility as well as stable geochemical and hydrological conditions [183–187]. Therefore, contaminated subsurface environments with changing hydrobiogeochemical conditions (typical for most sites) will likely influence speciation chemistry and thus require an alternative strategy.

Active remediation strategies that promote metal- and/or radionuclide-phosphate formation can take advantage of *in situ* hydrobiogeochemical parameters (Figure 4) that support contaminant sequestration through the formation of (1) low solubility minerals that are unaffected by changes in redox, (2) mineral stability across a wide pH range, and (3) reactive mineral surfaces that can support sequestration of other cooccurring metals through adsorption, substitution, and precipitation reactions [60, 83, 109, 147, 154, 188]. Innovative bioremediation approaches that neutralize low

pH groundwater and maintain sufficient phosphate concentrations to complex soluble contaminants can enhance strategies that rely solely on abiotic approaches. By employing a phosphate-mediated pH buffering system, soluble U(VI) can be sequestered as insoluble uranyl hydroxide and uranyl phosphate species [157, 189]. Unlike uraninite (UO_2) mediated sequestration, uranyl phosphate species are not prone to dissolution in oxidizing environments. Thermodynamic modeling and recent anaerobic biomineralization assays utilizing *Rahnella* sp. Y9602 and U contaminated sediments have shown that uranium phosphate formation can be promoted by microbial hydrolysis of organophosphate substrates under reducing conditions [60, 190].

To date, the majority of metal and radionuclide immobilization studies have focused on bacterial physiology and ecology without considering concomitant archaeal and fungal community responses and/or contributions made by members of these two domains of life. Additional studies that include detailed analyses of archaeal, bacterial, and fungal activities that may be contributing to the mineralization of metals and radionuclides through phosphate-driven mechanisms are needed if we are to have a complete

understanding of microbial community responses as they relate to phosphate-mediated bioremediation strategies [122, 135, 191–193]. Interdisciplinary studies that combine analyses of microbial communities and geochemical dynamics are essential to provide a greater understanding of *in situ* processes that affect contaminant sequestration.

New and emerging methods, such as metagenomics and metaproteomics, which allow for a greater understanding of microbe-metal interactions, many of which were pioneered as a result of academic and U.S. Department of Energy National Laboratory collaborations, have yielded significant insights into subsurface microbial community dynamics and physiological responses within contaminated environments [194–206]. Additionally, synchrotron X-ray techniques (e.g., XRD, XANES, and EXAFS) have become tools for biogeochemical studies that enhance our understanding of *in situ* contaminant sequestration. Recent studies that incorporate X-ray techniques have elucidated U interactions with sediment surfaces and biomass as well as facilitated mineral identification [60, 153, 179, 207–209]. Combining results from such interdisciplinary studies need to be placed in the context of the whole “*in terra*” system for long-term field-scale applications [210]. Ultimately, the combined efforts of interdisciplinary research focused on microbial P cycling will support development of predictive models necessary to understand the challenges of long-term contaminant sequestration within geochemically dynamic environments.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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